EMBO Conference on Meiosis 2017

27th August – 1st September 2017, Hvar, Croatia

Main organizer:

Iva Tolić, *Ruđer Bošković Institute, Croatia*

Co-organizers:

Franz Klein, *University of Vienna, Austria*
Christine Mézard, *Institut Jean-Pierre Bourgin, France*
Abby Dernburg, *University of California, Berkeley, USA*
Attila Toth, *TU Dresden, Germany*

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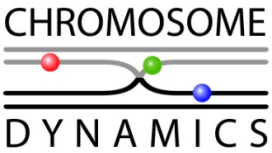

Scientific programme

DAY 1: Sunday, August 27, 2017

15:00 – 17:00	Arrival, registration and welcome get-together
17:00	Opening Remarks
17:10 – 20:00	**Session 1: Recombination** Chair: Daniel Camerini-Otero
17:10 – 17:32	**Raphael Mercier**, Institute Jean-Pierre Bourgin INRA, France *"What limits meiotic crossovers?"*
17:32 – 17:54	**Valérie Borde**, Institute Curie, CNRS, France *"The MutLβ complex limits the length of gene conversions in meiosis by interacting with the Mer3/HFM1 helicase"*
18:00 – 18:30	Break
18:30 – 18:52	**Monica Colaiacovo**, Harvard Medical School, USA *"Assessing the effects of germline exposure to environmental toxicants in C. elegans"*
18:52 – 19:14	**Silvia Prieler**, University of Vienna, Austria *"A novel mechanism for meiotic gene conversion"*
19:14 – 19:36	**Matt Neale**, University of Sussex, UK *"Regulation of meiotic recombination by Tel1/ATM"*
20:00 – 22:00	Dinner

DAY 2: Monday, August 28, 2017

08:00 – 09:00	Breakfast
09:00 – 13:05	**Session 2: Chromosome dynamics and structure** Chairs: Michael Klutstein, Ricardo Benavente
09:00 – 09:22	**Eric Greene**, Columbia University, USA *"Single molecule studies of condensin"*
09:22 – 09:44	**Leonid Mirny**, Massachusetts Institute of Technology, USA *"Chromosome folding by loop extrusion"*
09:44 – 10:06	**Vaishnavi Ananthanarayanan**, Indian Institute of Science, India *"Fission yeast Myo1 facilitates PI(4,5)P2-mediated anchoring of cytoplasmic dynein to the cortex"*
10:06 – 10:28	**Alfonso Fernandez-Alvarez**, National Institutes of Health, USA *"Telomeric control of Nuclear Envelope Disassembly in Meiosis"*
10:30 – 11:15	Coffee break
11:15 – 11:37	**Abby Dernburg**, University of California Berkeley, USA *"Evolutionary divergence in meiotic circuitry among nematodes: Pristionchus pacificus does things differently"*
11:37 – 11:59	**Lorant Szekvolgyi**, University of Debrecen, Hungary *"A Set1C-entric view of meiotic recombination"*
11:59 – 12:21	**Kevin Corbett**, University of California San Diego, USA *"Structural and functional dissection of yeast Hop1"*
12:21 – 12:43	**Talia Hatkevich** University of North Carolina, USA *"An Mcm5 mutation reveals a role for SMC1 enrichment at the centromere in early meiosis"*
12:43 – 13:05	**Gerben Vader**, Max-Planck-Institute of Molecular Physiology, Germany *"CRISPR/dCas9-driven ectopic targeting of kinetochore subunits reveals sufficiency in the local control of meiotic DNA break formation and recombination"*
13:05 – 14:30	Lunch
14:30 – 17:00	Poster session 1a, coffee at 16:00. Odd numbers present.
17:00 – 19:00	**Session 3: Spindle** Chair: Soni Lacefield
17:00 – 17:22	**Hiro Ohkura**, University of Edinburgh, UK *"Novel mechanisms to form the bipolar spindle only around chromosomes in oocytes"*
17:22 – 17:44	**Nenad Pavin**, University of Zagreb, Croatia *"The spindle is chiral due to torques generated by motor proteins"*
17:44 – 18:06	**Jan Brugues**, Max Planck Institute of Molecular Cell Biology and Genetics, Germany *"Autocatalytic microtubule nucleation determines the size and mass of spindles"*
18:06 – 18:28	**Sadie Wignall**, Northwestern University, USA *"Interplay between microtubule bundling and sorting factors ensures acentrosomal spindle stability during C. elegans oocyte meiosis"*
18:28 – 18:50	**Yoshinori Watanabe**, University of Tokyo, Japan *"Hierarchical regulation of centromeric cohesion protection by meikin and shugoshin in meiosis"*
19:00 – 20:30	Dinner
20:30 – 22:00	Poster session 1b. Odd numbers continue to present.

DAY 3: Tuesday, August 29, 2017

08:00 – 09:00	Breakfast
09:00 – 13:05	**Session 4: Chromosome segregation and aneuploidy** Chairs: JoAnne Engebrecht, Rolf Jessberger
09:00 – 09:22	**Eva Hoffmann**, University of Copenhagen, Denmark *"Towards combined gene conversion and crossover maps in the human meiosis"*
09:22 – 09:44	**Tomoya Kitajima**, RIKEN Center for Developmental Biology, Japan *"A unique role of kinetochores in mammalian oocytes"*
09:44 – 10:06	**Katja Wassmann**, Institute of Biology Paris Seine, France *"Mps1 kinase-dependent Sgo2 centromere localisation mediates cohesin protection in mouse oocyte meiosis I"*
10:06 – 10:28	**Adele Marston**, University of Edinburgh, UK *"Establishment of meiosis I-specific chromosome segregation by SPO13"*
10:30 – 11:15	Coffee break
11:15 – 11:37	**Melina Schuh**, Max Planck Institute for Biophysical Chemistry, Germany *"A method for the acute and rapid degradation of endogenous proteins in oocytes and other cell types"*
11:37 – 11:59	**Kikue Tachibana-Konwalski**, Institute of Molecular Biotechnology, Austria *"Wapl-mediated cohesin release from chromosomes contributes to maternal age-related egg aneuploidy"*
11:59 – 12:21	**Jan-Michael Peters**, Research Institute of Molecular Pathology, Austria *"Wapl and Pds5 proteins control cohesin-mediated chromosome axis and loop formation"*
12:21 – 12:43	**Anna Kouznetsova**, Karolinska Institutet, Sweden *"Chromosome dynamics during the second meiotic division"*
12:43 – 13:05	**Amira Sallem**, Cochin Institute, France *"Reducing human oocyte aneuploidy rate for assisted reproductive technologies"*
13:05 – 14:30	Lunch
14:30 – 17:00	Poster session 2a, coffee at 16:00. Even numbers present.
17:00 – 19:00	**Session 5: Cell cycle** Chair: Akira Shinohara
17:00 – 17:22	**Bela Novak**, University of Oxford, UK *"Cell cycle regulation by systems-level feedback controls"*
17:22 – 17:44	**Wolfgang Zachariae**, Max Planck Institute of Biochemistry, Germany *"Lessons from meiosis II"*
17:44 – 18:06	**Thomas Mayer**, University of Konstanz, Germany *"The role of calcineurin during exit from meiosis II"*
18:06 – 18:28	**Orlando Argüello-Miranda,** University of Texas Southwestern, USA *"A high-dimensional fluorescent microscopy system for quantitative prediction of cell fate during yeast meiosis"*
18:28 – 18:50	**Regis Meyer**, Oklahoma Medical Research Foundation, USA *"Phospho-regulation by Mps1 stabilizes force-generating kinetochore-microtubule attachment"*
19:00 – 20:30	Dinner
20:30 – 22:00	Poster session 2b. Even numbers continue to present.

DAY 4: Wednesday, August 30, 2017

08:00 – 09:00	Breakfast
09:00 – 12:45	**Session 6: Pairing and the synaptonemal complex** Chairs: Needhi Bhalla, Jeff Sekelsky
09:00 – 09:22	**Anne Villeneuve**, Stanford University, USA *"Promoting and limiting COs during C. elegans meiosis"*
09:22 – 09:44	**Denise Zickler**, University Paris-Süd, France *"Asy2/Mer2: an evolutionarily conserved mediator of the recombinosome/structure interface at every stage of meiosis"*
09:44 – 10:06	**Da-Qiao Ding**, Advanced ICT Research Institute, NICT, Japan *"RNA transcription and termination factors are important in meiotic homologous chromosome pairing in S. pombe"*
10:06 – 10:28	**Owen Davies**, Newcastle University, UK *"Structural basis of meiotic chromosome synapsis through SYCP1 self-assembly"*
10:30 – 11:15	Coffee break
11:15 – 11:37	**Vasily Zaburdaev**, Max Planck Institute for the Physics of Complex Systems, Germany *"Understanding the statistics of chromosomes during meiosis in fission yeast"*
11:37 – 11:59	**Monique Zetka**, McGill University, Canada *"A family of SUMO-E3 ligase-like proteins have distinct and essential functions in crossover formation in C. elegans"*
11:59 – 12:21	**Jiri Forejt**, Institute of Molecular Genetics of the ASCR, Czech Republic *"Prdm9-controlled asynapsis in sterile hybrid mice"*
12:21 – 12:43	**Arp Schnittger**, University of Hamburg, Germany *"The Arabidopsis Cdk1/Cdk2 homolog CDKA;1 controls the number and position of interference-sensitive cross-overs"*
12:45 – 14:00	Lunch
14:00 – 17:00	Social outdoors activities
17:00 – 19:00	**Session 7: Checkpoints and feedback controls** Chair: Diana E. Libuda
17:00 – 17:22	**Ewelina Bolcun-Filas**, The Jackson Laboratory, USA *"Meiotic Defects And Quality Control In Oocytes From Genetically Diverse Mice"*
17:22 – 17:44	**Attila Toth**, Technische Universität Dresden, Germany *"Controlling DNA Breaks in Mammalian Meiosis"*
17:44 – 18:06	**Andreas Hochwagen**, New York University, USA *"Regional control of meiotic DSB formation by the synaptonemal complex"*
18:06 – 18:28	**Martin Anger**, Central European Institute of Technology, Masaryk University, Czech Republic *"Functional correlation between Spindle Assembly Checkpoint and Anaphase Promoting Complex activity during mammalian meiosis I"*
18:28 – 18:50	**Philip Jordan**, Johns Hopkins University Bloomberg School of Public Health, USA *"Polo-like Kinase 4 Is Required for Homologous Recombination during Mouse Meiosis"*
19:00 – 20:30	Dinner
20:30 – 22:00	General poster session, scientific interactions

DAY 5: Thursday, August 31, 2017

08:00 – 09:00	Breakfast
09:00 – 13:05	**Session 8: Double-strand breaks, hotspots and recombination** Chairs: Bertrand Llorente, Ian Henderson
09:00 – 09:22	**Bernard De Massy**, Institute of Human Genetics, CNRS, France *"The control of meiotic DSB formation by Prdm9"*
09:22 – 09:44	**Alastair Goldman**, University of Bradford, UK *"Srs2 regulates Rad51 localisation during meiosis and protects from abnormal events."*
09:44 – 10:06	**Mathilde Grelon**, Institute Jean-Pierre Bourgin INRA, France *"Meiotic recombination initiation in A. thaliana"*
10:06 – 10:28	**Florencia Pratto**, National Institutes of Health, USA *"Cell-type specific genomics and in silico modelling of the crosstalk between meiotic replication and recombination in mammals"*
10:30 – 11:15	Coffee break
11:15 – 11:37	**Scott Keeney**, Memorial Sloan Kettering Cancer Center, USA *"Spo11: A "broken" topoisomerase"*
11:37 – 11:59	**Paula Cohen**, Cornell University, USA *"Elucidating the Role of Cyclin N-terminal domain containing-1 (CNTD1) in Crossover Designation During Mammalian Meiosis"*
11:59 – 12:21	**Ran Li**, University of Oxford, UK *"PRDM9 binding symmetry impacts crossover versus non-crossover recombination event resolution in mice"*
12:21 – 12:43	**Galina Petukhova**, Uniformed Services University of the Health Sciences, USA *"Extensive sex differences at the initiation of genetic recombination"*
12:43 – 13:05	**Maria Jasin**, Memorial Sloan Kettering Cancer Center, USA *"The mouse Shu complex SWS1-SWSAP1 is essential for meiotic recombination"*
13:05 – 14:30	Lunch
15:00 – 17:15	**Session 9: Pathway choice** Chair: Ayelet Arbel-Eden
15:00 – 15:22	**Neil Hunter**, University of California Davis, USA *"RNF212 Impedes DNA Break Repair to Enable Oocyte Quality Control"*
15:22 – 15:44	**Michael Lichten**, National Cancer Institute, NIH, USA *"Interplay between chromosome structure and meiotic recombination biochemistry"*
15:44 – 16:06	**Valentin Börner**, Cleveland State University, USA *"DNA Helicase Mph1FANCM Mediates Interhomolog Repair of Meiotic DSBs by Disrupting D-Loops between Sister Chromatids"*
16:06 – 16:28	**Joao Matos**, ETH Zurich, Switzerland *"Dynamic suppression of Holliday junction resolution enables meiotic crossover patterning"*
16:28 – 16:50	**Francesca Cole**, University of Texas MD Anderson Cancer Center, USA *"Temporally and spatially distinct meiotic recombination pathways in mouse spermatocytes"*
16:50 – 17:12	**Peter Donnelly**, University of Oxford, UK *"Repair delay is a key factor in the crossover/non-crossover decision in mouse meiosis"*
17:15 – 18:00	Business meeting
20:00 – 24:00	Gala dinner Entertainment

DAY 6: Friday, September 1, 2017

Breakfast

Departure

T1 What limits meiotic crossovers?

Raphael Mercier, Joiselle Fernandes, Mathilde Seguéla Arnaud, Rajeev Kumar

Institut Jean-Pierre Bourgin, INRA, France

Meiotic crossovers (COs) have two important roles, shuffling genetic information and ensuring proper chromosome segregation. Despite a large excess of precursors (i.e DNA double strand-breaks, DSBs), the number of meiotic COs is tightly regulated, typically one to three per chromosome pair [1]. Nevertheless, the mechanisms that ensure DSBs repair mostly as non-crossovers, and the evolutionary forces that impose this constraint, are poorly understood. Following a specific genetic screen, we identified three pathways that antagonize crossover formation in *Arabidopsis thaliana*. This involves very conserved proteins such the helicase FANCM and its co-factors [2,3], the BTR complex components -RECQ4/BLM, TOP3α [4] and RMI1- and the AAA-ATPase FIDGETIN-LIKE1 (FIGL1) [5].

Here, we will present the identification of FLIP, a partner of FIGL1. We propose that the conserved FLIP-FIGL1 complex constitute a novel regulator of homologous recombination, through a direct interaction with RAD51 and DMC1. Further, we will present the effect of the concomitant disruption of the RECQ4, FIGL1 and FANCM pathways on recombination along the genome.

1. *Mercier R, Mézard C, Jenczewski E, Macaisne N, Grelon M. The molecular biology of meiosis in plants. Annu Rev Plant Biol. 2015;66: 297–327.*
2. *Girard C, Crismani W, Froger N, Mazel J, Lemhemdi A, Horlow C, et al. FANCM-associated proteins MHF1 and MHF2, but not the other Fanconi anemia factors, limit meiotic crossovers. Nucleic Acids Res. 2014;42: 9087–9095.*
3. *Crismani W, Girard C, Froger N, Pradillo M, Santos JL, Chelysheva L, et al. FANCM Limits Meiotic Crossovers. Science (80-). 2012;336: 1588–1590.*
4. *Séguéla-Arnaud M, Crismani W, Larchevêque C, Mazel J, Froger N, Choinard S, et al. Multiple mechanisms limit meiotic crossovers: TOP3α and two BLM homologs antagonize crossovers in parallel to FANCM. Proc Natl Acad Sci U S A. 2015;112: 4713–4718.*
5. *Girard C, Chelysheva L, Choinard S, Froger N, Macaisne N, Lehmemdi A, et al. AAA-ATPase FIDGETIN-LIKE 1 and Helicase FANCM Antagonize Meiotic Crossovers by Distinct Mechanisms. PLOS Genet. 2015;11: e1005369.*

T2 The MutLβ complex limits the length of gene conversions in meiosis by interacting with the Mer3/HFM1 helicase

Dipti Vernekar[1], Yann Duroc[1], Céline Adam[1], Raphaël Guérois[2], Marie-Claude Marsolier Kergoat[2], Bertrand Llorente[3], Valérie Borde[1]

[1] *Institut Curie, CNRS, Paris, France*
[2] *I2BC, CEA, CNRS, Saclay, France*
[3] *CRCM, Inserm, CNRS, Marseille, France*

Gene conversion tracts in meiosis are shorter than those in vegetative cells, but how gene conversion length is regulated is unknown. Through a proteomic analysis of Mlh1 partners in meiotic cells, we have shown that in budding yeast meiosis, the MutLβ Mlh1-Mlh2 complex specifically interacts with Mer3, a conserved DNA helicase important for crossover formation. Like Mer3, the purified MutLβ complex preferentially recognizes D-loop intermediates precursors of crossovers, and MutLβ is recruited *in vivo* to recombination hotspots. Using a separation of function mutant of Mer3 that specifically affects its interaction with Mlh2, we show that Mer3 interaction is sufficient to recruit MutLβ to recombination hotspots, independently of any mismatch recognition. We further show that through its interaction with Mer3, MutLβ limits genome-wide the length of sequences involved in meiotic recombination intermediates, and thus limits the extent of gene conversion produced at each meiosis. Since purified MutLβ lacks endonuclease activity, we propose it plays a structural role, inhibiting the extension of recombination intermediates (Duroc et al, 2017).

The molecular actors promoting these longer gene conversion events that are normally inhibited by MutLβ are not known. Candidates involve DNA polymerases, or helicases that would stimulate D-loop extension. We used a candidate approach to identify the involved factor(s). Contrary to expectations, Mer3 helicase activity, proposed to extend D-loop recombination intermediate, does not influence the length of gene conversion events, and is not inhibited by MutLβ *in vitro*. We obtained similar results for Sgs1. By contrast, we found that the Pif1 helicase is required for long conversion tracts in the absence of MutLβ recruitment in meiotic cells. Pif1 has previously been involved in Break-Induced Replication in vegetative cells. Our results suggest that it is also acting at meiotic recombination sites.

The local features responsible for the determination of gene conversion lengths, in wild type or in the absence of MutLβ, are not known. We have set an experimental system to monitor genome wide DNA synthesis at recombination sites in large cell populations. We will present our first results at a subset of recombination sites in various mutants. This approach will provide the first genome-wide map of the tracts of gene conversion associated to recombination and should allow identify the local determinants of their extent.

1. *Duroc et al (2017)* ***eLife*** *6, e21900*

T3 Assessing the effects of germline exposure to environmental toxicants in *C. elegans*

Nara Shin, Monica Colaiácovo

Department of Genetics, Harvard Medical School, Boston, USA

Chemicals that are highly prevalent in our environment, such as phthalates and pesticides, have been linked to problems in reproductive health. However, identifying chemicals that impact reproductive health and understanding how they cause such deleterious effects, remains challenging. We have established a high-throughput screening system in *C. elegans* which allows us to sort live worms by using a fluorescent reporter construct for the identification of chemicals resulting in increased aneuploidy. We completed a screen of 44 chemicals, including pesticides, phthalates, chemicals used in crude oil processing and hydraulic fracturing, at concentrations correlating well with mammalian reproductive endpoints. Here, we focus on four chemicals which our screen revealed led to increased chromosome non-disjunction: dibutyl phthalate (DBP), a likely endocrine disruptor and frequently used plasticizer, as well as the pesticides 2-(thiocyanomethylthio) benzothiazole (TCMTB), permethrin, and N,N-diethyl-meta-toluamide (DEET). Our analyses have revealed increased germ cell apoptosis, altered DNA double-strand break repair progression, chromosome morphology defects in oocytes at diakinesis as well as defects in the first embryonic cell division after exposure to all four chemicals compared to vehicle alone. Current studies are focused on assessing whether any of these exposures may alter germline-specific gene expression. Taken together, these studies reveal that exposures to DBP, TCMTB, permethrin and DEET interfere with meiotic events and can lead to aneuploidy.

T4 A novel mechanism for meiotic gene conversion

Silvia Prieler, Doris Chen, Elisa Mayrhofer, Franz Klein

Department of Chromosome Biology, MFPL, University of Vienna, Vienna, Austria

Gene conversion describes the non-reciprocal segregation of appropriate markers close to recombination initiation sites and is known to accompany cross over (CO), as well as non-cross over (NCO) recombination in meiosis. It is currently thought, that these 3:1 type segregation events originate from mismatch-correction on a recombination intermediate, called heteroduplex and consequently require mismatch repair. In the absence of mismatch repair, such as in msh2 or pms1 mutants, indeed heteroduplex formation often leads to "post meiotic segregation", where apparently uncorrected mismatches segregate through meiosis and cause "split" progeny of meiotic products, giving rise to 5:3 segregation types. While evidence for this pathway is overwhelming, we have evidence for a distinct, novel pathway without heteroduplex formation.
Briefly, we have precisely characterized the generation of Spo11-flanked dsDNA fragments during wild type and mutant meiosis. These fragments leave behind gaps with a chance to be repaired from the homologous chromosome. We have set up genome wide-gene conversion mapping and will discuss to which extent the generation of dsDNA fragments contributes to gene conversion rates.

T5 Regulation of meiotic recombination by Tel1/ATM

Matt Neale

GDSC, University of Sussex, Brighton, UK

In sexually reproducing eukaryotes, genetic variation arises during meiosis via the formation and repair of numerous DNA double-strand breaks (DSBs) created by the evolutionarily conserved topoisomerase-like enzyme, Spo11. DSBs form preferentially within nucleosome-depleted regions of the genome termed 'hotspots'. Current views support the notion that meiotic DSBs are relatively evenly spread across the genome, with each isolated DSB giving rise to a discrete meiotic recombination event. Here we present evidence that challenges this view, and which instead suggests that a substantial fraction of recombination is initiated by a cluster of DSBs forming within a hotspot region—something that is exacerbated in the absence of Tel1, the yeast orthologue of mammalian ATM protein. Remarkably, loss of Tel1 kinase activity causes such DSB clusters to spread into the usually chromatinised regions flanking canonical DSB hotspots. Intriguingly, such spreading arises predominantly in the direction of local gene transcription—potentially due to a change in the stability of nucleosomes at the 5' end of genes. Complementary deep-sequencing of meiotic progeny identifies numerous recombination 'scars' that are consistent with initiation by DSB clusters. Our results revise current thinking about how genetic recombination arises, and allow us to revisit, with new eyes, the original concepts of meiotic recombination as a form of double-strand gap repair.

T6 Single molecule studies of condensin

Eric Greene

Columbia University, USA

Our group uses single-molecule optical microscopy to study fundamental interactions between proteins and nucleic acids, and our overarching goal is to understand the molecular mechanisms that cells use to repair, maintain, and decode their genetic information. Our research utilizes single molecule microscopy to answer questions about complex biological problems that cannot easily be addressed through more traditional biochemical or genetic approaches. We are particularly interested in determining the physical basis for the mechanisms that proteins use to survey DNA molecules for damage and initiate repair processes, and how these initial steps are coordinated with downstream events that lead to completion of repair. The advantages of the technologies developed in our group are that we can see what proteins are bound to DNA, where they are bound, how they move, and how they interact with and influence partner proteins – all in real-time, at the level of a single reaction.

T7 Chromosome folding by loop extrusion

Leonid Mirny

Institute for Medical Engineering and Science, and Department of Physics, Massachusetts Institute of Technology, Cambridge, USA

Earlier we suggested that loop extrusion by SMC complexes could be a universal mechanism responsible for formation of domains in interphase [1], and chromosome compaction and segregation in metaphase [2]. Our recent results [3, 4] show that cohesin plays a central role in the loop extrusion process, while CTCF modulates progression of loop extrusion and has an instructive function in domain formation. Loss of these factors leads to predictably different changes in Hi-C maps and the loss of chromosomal domains. We further demonstrate that spatial compartmentalization of the genome remains largely unaffected by cohesin and CTCF loss, demonstrating that compartmentalization is produced by an independent and yet to be understood mechanism. Recent single-nucleus Hi-C [5] allows examining chromosome structure in individual cells and provides additional support to loop extrusion activity during interphase. We also show that loop extrusion by SMCs with two different exchange rates, like condensin I and II, can lead to formation of an array of nested loops that, when modeled in 3D and arrange on spiral scaffold, reproduces Hi-C maps of prophase and mitotic chromosomes. I would love to see whether the mechanism loop extrusion can be useful in meiosis, particularly for search and alignment of homologous chromosomes and formation of meiotic synapsis.

1. *Fudenberg G, Imakaev M, et al Formation of Chromosomal Domains by Loop Extrusion. Cell Rep 15(9) (2016)*
2. *Goloborodko A, Imakaev MV, Marko JF, Mirny L. Compaction and segregation of sister chromatids via active loop extrusion. Elife 18;5 (2016)*
3. *Nora EP, et al Targeted degradation of CTCF decouples local insulation of chromosome domains from higher-order genomic compartmentalization. Cell 169(5) (2017)*
4. *Schwarzer W, et al. Two independent modes of chromosome organization are revealed by cohesin removal http://biorxiv.org/content/early/2016/12/21/095802*
5. *Flyamer IM, Gassler J, Imakaev M, Brandão HB, Ulianov SV, Abdennur N, Razin SV, Mirny LA, Tachibana-Konwalski K Single-nucleus Hi-C reveals unique chromatin reorganization at oocyte-to-zygote transition, Nature, 544(7648) (2017)*

T8 Fission yeast Myo1 facilitates PI(4,5)P2-mediated anchoring of cytoplasmic dynein to the cortex

Jerrin Mathew Thankachan, Stephen Sukumar Nuthalapati, Nireekshit Addanki Tirumala, Vaishnavi Ananthanarayanan

Centre for BioSystems Science and Engineering, Indian Institute of Science, Bangalore, India

Several key processes in the cell, such as vesicle transport and spindle positioning are mediated by the motor protein cytoplasmic dynein, which produces force on the microtubule. For the functions that require movement of the centrosome and the associated nuclear material, dynein needs to have a stable attachment at the cell cortex. In fission yeast, Mcp5 is the anchor protein of dynein and is required for the oscillations of the horsetail nucleus during meiotic prophase. While the role of Mcp5 in anchoring dynein to the cortex has been identified, it is unknown how Mcp5 associates with the membrane as well as the importance of the underlying attachment to the nuclear oscillations. Here, we set out to quantify Mcp5 organization and identify the binding partner of Mcp5 at the membrane. We employed confocal and total internal reflection fluorescence microscopy to count the number of Mcp5 foci and the number of Mcp5 molecules in an individual focus. Further, we quantified the localization pattern of Mcp5 in fission yeast zygotes and show by perturbation of phosphatidylinositol 4-phosphate 5-kinase that Mcp5 binds to phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$). Remarkably, we discovered that the myosin I protein in fission yeast Myo1, which is required for organization of sterol-rich domains in the cell membrane, facilitates the localization of Mcp5 and that of cytoplasmic dynein on the membrane. Finally, we demonstrate that Myo1-facilitated association of Mcp5 and dynein to the membrane determines the dynamics of nuclear oscillations and in essence, dynein activity.

T9 Telomeric control of Nuclear Envelope Disassembly in Meiosis

Alfonso Fernandez-Alvarez, Haitong Hou, Julia Promisel Cooper

National Institutes of Health, Bethesda, USA

Faithful chromosome segregation requires coordination between nuclear envelope (NE) breakdown, in which the NE is dissolved to allow the chromosomes to access their segregation vehicle, the spindle. We showed previously that loss of the telomere bouquet, the chromosomal configuration in which telomeres cluster together at the NE beneath the centrosome during meiotic prophase, compromises spindle formation in meiosis. Moreover, we demonstrated that this control of spindle formation by the bouquet is mediated by telomeric control of NE breakdown; contacts between telomeres and the NE-spanning LINC (linker of nucleoskeleton and cytoskeleton) complex are required for the NE disassembly that necessarily preceded spindle formation. This observation implies that specialized chromosome regions may control NE breakdown across eukaryotes by binding to LINC complexes and modifying local NE components. Indeed, we found that centromeres harbor this LINC-contacting and NE breakdown controlling activity in proliferative fission yeast cells. Here, we investigate the molecular mechanisms that govern signaling between the bouquet and NE disassembly. We found that centromeres and telomeres co-localize with nuclear CDK-1 during meiotic prophase. Moreover, CDK-1 recrutiment beneath the centrosome depends on telomere bouquet formation, implying that CDK-1 is actively concentrated at these chromosome regions. Interestingly, loss of CDK-1 at these regions in prophase compromises NE disassembly and consequently, meiotic spindle formation. Congruently, forced recruitment of CDK-1 to the NE fully rescued the NE disassembly defects incurred by bouquet disruption. Our opbservations suggest that telomeres might create a critical concentration of CDK-1 at the NE, inducing its disassembly and triggering the meiotic nuclear divisions.

T10 Evolutionary divergence in meiotic circuitry among nematodes: *Pristionchus pacificus* does things differently

Regina Rillo-Bohn[1,2], Baris Avsaroglu[1], Joshua Bayes[3], Clara Wang[4], Simone Köhler[1,2], and Abby F. Dernburg[1]

[1] *Department of Molecular and Cell Biology, University of California, Berkeley, USA*
[2] *Howard Hughes Medical Institute, USA*
[3] *California Institute for Quantitative Biosciences (QB3), USA*
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The nematode *Caenorhabditis elegans* is a well-established experimental model for investigating meiosis. Its experimental advantages include a small genome compared to most metazoans, a low number of chromosomes (6 pairs), powerful genetics, and germline organization that facilitates temporal analysis of meiotic progression. Our recent work has elucidated circuitry that acts downstream of homolog synapsis to regulate crossover formation, which appears to be widely conserved across eukaryotes. However, other aspects of meiotic regulation in *C. elegans* are unusual, including homolog pairing and synapsis in the absence of recombination, and the emergence of specialized chromosome regions – "pairing centers" - that mediate nuclear envelope attachment and microtubule-based chromosome movement.

To better understand how these innovations arose during evolution, we are developing the nematode *Pristionchus pacificus* as a comparative model. Like *C. elegans*, *P. pacificus* is a self-fertilizing hermaphroditic species that can be readily cultured in the laboratory. Genome editing with Cas9/CRISPR is fairly robust, allowing reverse genetics and epitope tagging, although large knock-ins have been problematic. While germline organization and meiotic progression appear superficially similar between these two nematodes, we have identified major differences in meiotic mechanisms and their interdependence. Unlike the genomes of Caenorhabditids, which have lost Dmc1 and its cofactors Mnd1 and Hop2, the *Pristionchus* genome has intact copies of these genes. Disruption of *Ppa_spo-11* or *dmc-1* blocks homolog pairing and synapsis. DMC-1 and RAD-51 localize sequentially to meiotic chromosomes; crossing-over and meiotic progression require DMC-1 but not RAD-51. Localization of COSA-1/CNTD1 indicates that a single obligate Class I crossover occurs per chromosome pair and directs asymmetric SC disassembly, as in *C. elegans*, although additional crossovers likely arise through the Class II pathway. Perhaps most surprisingly, CO designation occurs much earlier in *P. pacificus*, and synapsis depends on crossover designation by COSA-1 and ZHP-3, analogous to the situation in budding yeast but divergent from what is observed in *C. elegans* or mammals.

In my presentation I will likely speculate wildly about how these divergent patterns of meiotic progression in nematodes are functionally related.

T11 A Set1C-entric view of meiotic recombination

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Spp1 is the H3K4-trimethyl reader subunit of the Set1 histone methylase complex (Set1C) in budding yeast, having a recognized role in meiotic DNA break formation (Acqvaviva L *et al*. Science 2013). It has been proposed that Spp1 binds to Mer2 and H3K4-trimethylated nucleosomes through its Mer2-binding and PHD finger motifs, respectively, tethering DSB hotspots to the chromosome axes where they undergo Spo11-mediated DNA cleavage. In this study, we used genomic and biophysical approaches (competition ChIP – cChIP, fluorescent correlation spectroscopy – FCS) to assess the binding characteristics and chromosomal turnover of Spp1 in meiotic growth conditions. We found that i) the nuclear dynamics of Spp1 showed significant differences in comparison with Set1 (the catalytic subunit of Set1C), ii) Spp1 segregated into a slow- and fast diffusing kinetic compartment based on the measured diffusion times and diffusion coefficients, iii) the chromosome-bound fraction of Spp1 was either static or dynamic in nature, such that the dynamic sites gradually appeared or disappeared in the course of meiotic progression, iv) the dynamic and static sites had differential relationships and associations with recombinosome proteins and H3K4me3 and showed distinctive turnover rate characteristics (reflected by the average number of Spp1 replacements per genomic location per unit of time [1/min]). Finally, genetic analysis performed with various histone point-mutants and separation-of-function alleles of Spp1 suggested that the dynamic behaviour of Spp1 was dependent on three factors: 1) the PHD finger subunit of Spp1 (binding to H3K4me3), 2) the presence of modifiable histone residues (H3K4, H3R2), 3) the Mer2-binding motif of Spp1, anchoring the H3K4me3-Spp1 complex to the chromosome axis. These results shed more light on the potential molecular mechanism of tethered loop-axis structure formation in relation to meiotic DSBs.

T12 Structural and functional dissection of yeast Hop1

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In budding yeast, Hop1 and its binding partner Red1 are centrally-important components of the chromosome axis, controlling DNA double-strand break levels and the pathway of meiotic recombination. Here, we present a comprehensive dissection of Hop1, highlighting high functional conservation with meiotic HORMADs from other species, but also revealing a novel fungal-specific regulatory domain. Our prior work has shown that in both *C. elegans* and mammals, meiotic HORMAD proteins self-assemble through interactions between their N-terminal HORMA domains and short C-terminal "closure motifs" (Kim *et al*, 2014). Here, we show that *S. cerevisiae* Hop1 shares this self-assembly mechanism, with its HORMA domain able to bind related closure motifs in both its own C-terminus and in Red1. We further show that the Hop1 HORMA domain can adopt two different states in solution, hinting at functional parallels with the related Mad2 protein. A mutant Hop1 protein unable to adopt these two conformations shows defective binding to Red1 in cells, showing that conformational conversion of the Hop1 HORMA domain is necessary for its chromosome axis localization. Since the discovery of its primary amino acid sequence in 1990 (Hollingsworth *et al*, 1990), *S. cerevisiae* Hop1 has been theorized to contain a zinc-finger domain between its N-terminal HORMA domain and C-terminal closure motif. We isolated, purified, and determined the crystal structure of the Hop1 central region, and show that it contains not a zinc finger, but rather a PHD finger with two bound zinc ions. The PHD finger domain is folded tightly against a modified winged-helix domain (WHD), making up a novel "PHD-WHD" module. This module is conserved throughout fungi and is partially conserved plant meiotic HORMADs including *A. thaliana* ASY1, but is not found in *C. elegans* or mammalian HORMADs. Deletion of Hop1's PHD-WHD module in cells does not affect its localization to the chromosome axis or its phosphorylation by ATM/ATR, but nonetheless causes near-complete spore inviability. We propose that the Hop1 PHD-WHD module mediates protein-protein interactions to drive a key step in meiotic recombination and/or checkpoint control in meiosis I.

1. *Hollingsworth NM, Goetsch L & Byers B (1990) The HOP1 gene encodes a meiosis-specific component of yeast chromosomes. Cell 61: 73–84*
2. *Kim Y, Rosenberg SC, Kugel CL, Kostow N, Rog O, Davydov V, Su TY, Dernburg AF & Corbett KD (2014) The Chromosome Axis Controls Meiotic Events through a Hierarchical Assembly of HORMA Domain Proteins. Dev Cell 31: 487–502*

T13 An Mcm5 mutation reveals a role for SMC1 enrichment at the centromere in early meiosis

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In meiotic cells, sister chromatid cohesion acts along the arms of the chromosome as well as at the centromere. Both regulation and function of cohesion differ between the centromere and the arms. Arm cohesion functions primarily during prophase I, with cohesin proteins contributing to building the meiotic axis, preventing intersister recombination, and maintaining synaptonemal complex integrity. In stark contrast, centromere cohesion is thought to be essential for meiotic events succeeding prophase I, such bipolar attachment at metaphase I and maintaining connection of sisters following release of arm cohesion with homologs segregate at anaphase I. Although the biological relevance of centromere cohesion is only understood in processes following prophase I, cohesins are enriched around the centromere prior to the onset of meiosis and remain enriched throughout prophase I.

It has been difficult to directly assess the biological relevance of centromeric cohesion in early meiosis because no mutant that provides intact arm cohesion while disrupting centromeric cohesion has been described. We find that flies harboring $Mcm5^{A7}$, a missense mutation in the replicative *Mcm5* gene[1], exhibit intact arm cohesion during prophase I, as demonstrated by (1) absence of precocious arm separation; (2) stable association of SMC1 along the arm; (3) the lack of an increase in intersister exchange; (4) normal synaptonemal complex formation and maintenance. Although arm cohesion appears normal, $Mcm5^{A7}$ mutants lack SMC1 enrichment at the centromere prior to the onset of meiosis and throughout pachytene of prophase I, providing us with a unique genetic tool to investigate the direct role of centromeric SMC1 on early meiotic events.

Using $Mcm5^{A7}$ mutants, we find that flies that lack SMC1 centromeric enrichment prior to and during early prophase I exhibit aberrant homologous and nonhomologous centromere dynamics: Pairing of sister centromeres is decreased and the synaptonemal complex does not assemble at the centromere. It has been previously reported that $Mcm5^{A7}$ mutants exhibit a 90% decrease of crossovers on the *X* chromosome[1]; we confirm this result and further show that crossovers are substantially decreased on chromosome *2L*. To investigate whether SMC1 defects cause this decrease in crossovers, we reduced the expression of the SMC1 antagonist Wapl. Heterozygosity for a *wapl* mutation results in a 50% increase in crossovers on *2*L relative to $Mcm5^{A7}$ single mutants, suggesting that chromatin-bound SMC1 at the centromere may be required for normal levels of crossing over along the arms. Lastly, although centromeric SMC1 is severely reduced, the cohesion protein ORD remains present at apparently normal levels at the centromere. This uncoupling of ORD and SMC1 at the centromere suggests that SMC1 loading or maintenance differs from that of other cohesion proteins found at the centromere and is dependent on wildtype Mcm5 protein.

Overall, our $Mcm5^{A7}$ studies suggest that the importance of SMC1 enrichment at the centromere during early prophase may be to promote normal levels of crossing over along the arms, possibly by allowing proper centromere dynamics prior to the onset of meiosis and providing a foundation on which the centromere synaptonemal complex – and thus a continuous synaptonemal complex – can build.

References:

1. *Lake CM, Teeter K, Page SL, Nielsen R, Hawley RS. A genetic analysis of the Drosophila mcm5 gene defines a domain specifically required for meiotic recombination. Genetics. 2007;176:2151–2163. doi:10.1534/genetics.107.073551.*

T14 CRISPR/dCas9-driven ectopic targeting of kinetochore subunits reveals sufficiency in the local control of meiotic DNA break formation and recombination

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Controlled DNA breakage followed by crossover recombination is an essential part of meiosis. However, the formation of crossovers in the direct vicinity of kinetochores is associated with meiotic chromosome missegregation and developmental aneuploidy (*e.g.* Trisomy 21 in humans). We recently showed that in budding yeast, crossover recombination close to centromeres is actively suppressed by the kinetochore. Within the kinetochore assembly, we identified components of the conserved *CTF19*-complex that minimize pericentromeric DNA break formation. We could demonstrate a requirement for several subunits of this complex in controlling meiotic DNA breakage as well as repair. In our current work we have established targeting systems, based on a catalytically-inactive version of CRISPR-Cas9 (CRISPR-dCas9), to direct isolated *CTF19* complex subunits to ectopic regions away from kinetochores. Using these systems, we could demonstrate a sufficiency for specific kinetochore subunits in driving the establishment of DNA recombination suppressed regions. Our ongoing work is aimed at using these powerful systems to further dissect how kinetochore, and more specifically, the *CTF19* complex, is controlling pericentromeric recombination events.

T15 Novel mechanisms to form the bipolar spindle only around chromosomes in oocytes

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Oocytes lacks centrosomes and have a large volume, but the meiotic spindle forms only around chromosomes in oocytes. How does the meiotic spindle form around chromosomes, and not form in other part of the cytoplasm? We found two novel mechanisms responsible for spatially restricting microtubule assembly and microtubule cross-linking only to the proximity of chromosomes, respectively.

We discovered a novel microtubule nucleating pathway that are only active in oocyte and essential for assembling most of spindle microtubules in oocytes, redundantly with Augmin. This pathway is mediated by the kinesin-6 Subito/MKlp2, and recruits the gamma-tubulin complex to the spindle equator to nucleate microtubules in oocytes. Mis-regulation of Subito on its own can recruit the gamma-tubulin complex and trigger ectopic spindle assembly away from chromosomes.

We also found a novel mechanism to target a protein to the meiotic spindle. The phospho-docking protein 14-3-3 inhibits microtubule binding activity of the kinesin-14 Ncd (human HSET; kinesin-14) crucial for microtubule cross-linking in the cytoplasm. This inhibition is removed by chromatin-bound Aurora B kinase to allow Ncd to bind spindle microtubules for cross-linking.

The meiotic spindle is formed without centrosomes in a large volume of oocytes. Local activation of crucial spindle proteins around chromosomes is important for formation and maintenance of a bipolar spindle in oocytes. A critical 14-3-3 target is the minus-end directed motor Ncd (human HSET; kinesin-14) which has well documented roles in stabilising a bipolar spindle in oocytes. Phospho-docking by 14-3-3 inhibits the microtubule binding activity of the non-motor Ncd tail. Further phosphorylation by Aurora B kinase can release Ncd from this inhibitory effect of 14-3-3. As Aurora B localises to chromosomes and spindles, 14-3-3 facilitates specific association of Ncd with spindle microtubules by preventing Ncd from binding to non-spindle microtubules in oocytes. Therefore, 14-3-3 translates a spatial cue provided by Aurora B to target Ncd selectively to the spindle within the large volume of oocytes.

T16 The spindle is chiral due to torques generated by motor proteins

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Division of the genetic material relies on forces generated in the spindle, a micro-machine composed of microtubules and associated proteins. Forces are required for the congression of chromosomes to the metaphase plate and separation of chromatids in anaphase. However, torques may also exist in the spindle, yet they have not been investigated. Here we show that the spindle is chiral. Chirality is evident from the finding that microtubule bundles follow a left-handed helical path, which cannot be explained by forces but rather by torques acting in the bundles. STED super-resolution microscopy, as well as confocal microscopy, of human spindles shows that the bundles have complex curved shapes. The average helicity of the bundles with respect to the spindle axis is 1.2 °/μm. Inactivation of kinesin-5 (Eg5/Kif11) abolished the chirality of the spindle, suggesting that this motor generates the helical shape of microtubule bundles. To explain the observed shapes, we introduce a theoretical model for the balance of forces and torques acting in the spindle, and show that torque is required to generate the helical shapes. We conclude that torques generated by motor proteins, in addition to forces, exist in the spindle and determine its architecture.

T17 Autocatalytic microtubule nucleation determines the size and mass of spindles

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Regulation of size and growth is a fundamental problem in biology. A prominent example is the formation of the meiotic spindle, where protein concentration gradients around chromosomes are thought to regulate spindle growth by controlling microtubule nucleation. Previous evidence suggests that microtubules nucleate throughout the spindle structure. However, the mechanisms underlying microtubule nucleation and its spatial regulation are still unclear. Here, we developed an assay based on laser ablation to directly probe microtubule nucleation events in *Xenopus laevis* egg extracts. Combining this method with theory and quantitative microscopy, we show that the size of a spindle is controlled by autocatalytic growth of microtubules, driven by microtubule-stimulated microtubule nucleation. The autocatalytic activity of this nucleation system is spatially regulated by the availability of the active form of the small GTPase Ran, which decreases with distance from the chromosomes. Thus, the size of spindles is determined by the distance where one microtubule nucleates on average less than one new microtubule. This mechanism provides an upper limit to spindle size even when resources are not limiting and may have implications for spindle scaling during development.

T18 Interplay between microtubule bundling and sorting factors ensures acentrosomal spindle stability during *C. elegans* oocyte meiosis

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During mitosis, duplicated centrosomes nucleate microtubules and form the spindle poles. However, oocytes of most species lack centrioles, so spindles assemble using a different pathway. Recently, we reported that acentrosomal spindle assembly in *C. elegans* oocytes proceeds by: 1) formation of a cage-like microtubule array inside the disassembling nuclear envelope, 2) sorting of microtubule minus ends to the periphery of the array, and 3) focusing of these ends into nascent poles that coalescence until bipolarity is achieved. Moreover, we demonstrated that the kinesin-12 family motor KLP-18 acts during the sorting step to force the minus-ends outwards where they can be organized into the spindle poles (1). Now, we have uncovered additional insights into the principles underlying this specialized form of cell division, through studies of KLP-15 and KLP-16, two highly homologous members of the minus-end-directed kinesin-14 family. Fixed and live imaging of KLP-15/16-depleted oocytes revealed that following nuclear envelope breakdown, microtubules form a transient cage-like structure, but then microtubules collapse into a disorganized ball-shaped structure surrounding the chromosomes. These results suggest that KLP-15/16 bundle and organize microtubules during acentrosomal spindle assembly, and consistent with this proposed function, these proteins localize to spindle microtubules during the cage stage and remain microtubule-associated throughout the meiotic divisions. However, despite the severe spindle assembly defects observed following KLP-15/16 depletion, we were surprised to find that these disorganized microtubules were then able to reorganize into a spindle capable of segregating chromosomes during anaphase, revealing the existence of additional mechanisms that can act to bundle and organize spindle microtubules. This phenotype therefore enabled us to identify factors promoting microtubule organization and assembly during anaphase, whose contributions are normally imperceptible in wild-type worms. First, we discovered that SPD-1 (PRC1), which loads onto microtubules in early anaphase, is required for the formation of anaphase microtubule bundles in the absence of KLP-15/16. Moreover, we found that KLP-18, which sorts microtubules during spindle assembly, can then function during anaphase to sort these bundles into a functional orientation capable of mediating chromosome segregation. Therefore, our studies have revealed an interplay between distinct mechanisms that together promote spindle formation and chromosome segregation in the absence of structural cues such as centrioles.

1. *Wolff, et.al., (2016). Molecular Biology of the Cell.*

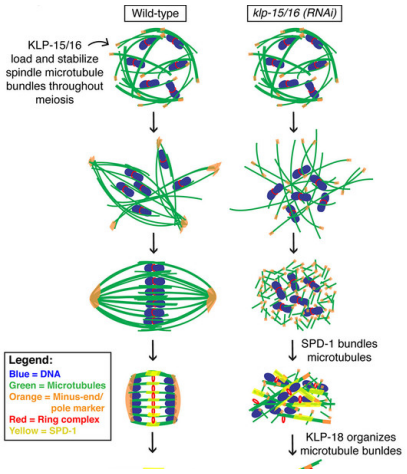

Figure 1 Model for acentrosomal spindle assembly during C. elegans oocyte meiosis and the interplay between microtubule bundling and sorting factors operating during this process.

T19 Hierarchical regulation of centromeric cohesion protection by meikin and shugoshin in meiosis

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The kinetochore is the crucial apparatus regulating chromosome segregation in mitosis and meiosis. Particularly in meiosis I, unlike in mitosis, sister kinetochores are captured by microtubules emanating from the same spindle pole (mono-orientation) and centromeric cohesion mediated by cohesin is protected in the following anaphase. Shugoshin, which is localized to centromeres depending on the phosphorylation of histone H2A by Bub1 kinase, plays a central role in protecting meiotic cohesin Rec8 from separase cleavage. Another key meiotic kinetochore factor Moa1 (Meikin), which was initially characterized as mono-orientation factor in fission yeast, also regulates cohesion protection. However, the underlying molecular mechanisms remain elusive. We show that Moa1, which associates stably with CENP-C during meiosis I, recruits Plo1 (polo-like kinase) to the kinetochores and phosphorylates Spc7 (KNL1) to accumulate Bub1. Consequently, in contrast to the transient kinetochore localization of mitotic Bub1, meiotic Bub1 persists at kinetochores until anaphase I. The meiotic Bub1 pool ensures robust Sgo1 (shugoshin) localization and cohesion protection at centromeres by cooperating with heterochromatin protein Swi6, which binds and stabilizes Sgo1. Further, our analyses indicate that Mo1-Plo1 phosphorylates Rec8 to facilitate the protection by shugoshin. Thus, our analyses reveal a hierarchical regulation of centromeric cohesion protection by meikin and shugoshin, which is important for establishing meiosis-specific chromosome segregation.

T20 Towards combined gene conversion and crossover maps in the human meiosis

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Reciprocal recombination (crossovers) and non-reciprocal gene conversion between homologous chromosomes (homologs) are critical for mediating pairing and correct segregation of homologs to opposite spindle poles in meiosis. Recombination and stable chromosome alignment are dynamic and interdependent as well as a prerequisite for meiotic progression. Meiotic prophase I in human females is protracted compared to males and is characterized by nearly twice as many crossovers. Gene conversion data from female meiosis are not available, but cytological studies of fetal oocytes suggest over 600 per meiosis (1).

To understand recombination features in human oocytes and their impact on chromosome segregation and aneuploidy, we and others recently developed single-cell genomics to map crossing over and homolog segregation in human oocytes (2,3). We have further advanced our single-cell technologies to detect gene conversions across all three products of single meioses (oocytes and polar bodies). Mapping gene conversions directly across all four DNA strands in human meiosis has been limited by obtaining all products from an individual meiosis and getting accurate genetic data from single-cell applications. We used high-fidelity single genome amplification together with machine-learning algorithms to detect high-quality calls and gene conversions directly. This has allowed us to generate a partial, first-generation gene conversion map across the human genome. We will discuss genomic features that shape the conversion landscape and conversely, how the maps allow us to infer stable interactions between chromosomes that were mediated by meiotic recombination.

T21 A unique role of kinetochores in mammalian oocytes

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Bipolar spindle formation is a prerequisite for chromosome segregation. Most animal somatic cells have two centrosomes, which determine the bipolarity of the spindle. Mammalian oocytes, however, lack centrosomes. In mice, oocytes have many cytoplasmic acentriolar microtubule-organizing centers (MTOCs), which relocate and cluster into foci at the poles of the forming bipolar spindle. This bipolar sorting of MTOCs likely contributes to the bipolarization of the acentrosomal spindle. In human oocytes, however, a bipolar acentrosomal spindle forms in the absence of MTOC foci. This raises a possibility that spindle bipolarization is driven by an MTOC-independent mechanism in mammalian oocytes. Here, we show a unique role of kinetochores in acentrosomal spindle bipolarization during meiosis I in mouse oocytes. Knockout of Hec1, a kinetochore component that directly binds to microtubules, results in failure of spindle bipolarization in oocytes. This phenotype is partially rescued by a Hec1 construct that lacks microtubule-binding domains. We find that Hec1's C-terminal domain physically interacts with anti-parallel microtubule cross-linkers and recruits them to kinetochores. These cross-linkers are indispensable for efficient spindle bipolarization. Based on these results, we discuss a potential model for a conserved kinetochore-based mechanism that drives acentrosomal spindle bipolarization during meiosis I in mammalian oocytes.

T22 Mps1 kinase-dependent Sgo2 centromere localisation mediates cohesin protection in mouse oocyte meiosis I

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Step-wise cohesin removal is a key feature of the two meiotic divisions that generate haploid gametes. Arm cohesin is removed in meiosis I, whereas centromeric cohesin is protected to maintain sister chromatids together until meiosis II. Protection of centromeric cohesin in meiosis I is brought about by Sgo2 in mammalian germ cells. Sgo2 is localised to the centromere region and brings about protection through the recruitment of PP2A-B56 phosphatase which is thought to keep the meiosis-specific cohesin subunit Rec8 unphosphorylated and thereby non-cleavable by Separase. In our study we decided to determine how Sgo2 localisation for centromeric cohesin protection is regulated in mammalian oocyte meiosis. Sgo1 in mitotic cells is recruited through the generation of a Histone mark by Bub1-dependent phosphorylation, for protection from prophase-dependent cohesin removal. But as Bub1 kinase-dead mice are not sterile, Sgo2 must be recruited additionally by some other mechanism in oocytes. Here we show that Sgo2 recruitment for centromeric cohesin protection in oocyte meiosis depends on Mps1 kinase activity. Using optimized high resolution confocal microscopy we demonstrate that at least two distinguishable pools of Sgo2 exist in the centromere region of bivalents, namely at the centromere and at the pericentromere between sisters. Mps1 kinase localises Sgo2 to the centromere, and it is the centromeric pool of Sgo2 that is foremost required for cohesin protection in meiosis I. Bub1 dependent Histone H2A T121 phosphorylation promotes Sgo2 accumulation at the pericentromere between sister chromatids, but this pool of Sgo2 is not essential for protection. Our work provides new insights into the molecular pathways required for the correct localisation of distinct Sgo2 pools in mammalian oocytes, which will help us better understand how chromosome segregation errors can arise also in human oocytes.

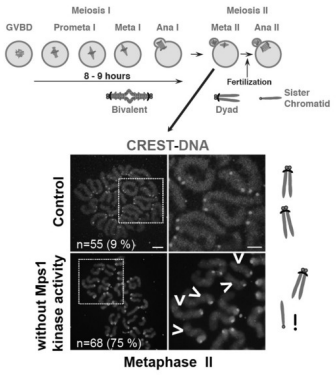

Figure 1 Oocytes that progress into meiosis II without Mps1 kinase activity harbour single sister chromatids that have been precociously separated, as shown here by chromosome spreads.

T23 Establishment of meiosis I-specific chromosome segregation by SPO13

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Meiosis is a specialised cell division process that allows the generation of four haploid gametes from a single mother cell where one round of DNA replication is followed by two consecutive rounds of chromosome segregation. While the second meiotic division is similar to mitosis, meiosis I features a number of adaptations to the chromosome segregation machinery that mediate the segregation of homologous chromosomes as opposed to sister chromatids. These include the mono-orientation of sister kinetochores, which allows sister chromatids to move to the same pole in anaphase I, and the protection of cohesin in the pericentromere, which ensures accurate chromosome segregation in the second meiotic division. In budding yeast, both of these processes require the meiosis I-specific protein Spo13, thought to be functionally orthologous to the recently identified mouse MEIKIN and fission yeast Moa1. However, the molecular functions of these proteins have long remained elusive. We investigated how Spo13 protects cohesin. During meiosis, cohesin cleavage by separase requires its prior phosphorylation. However, at the pericentromere, shugoshin (Sgo1) and its associated phosphatase, $PP2A^{Rts1}$, counteract cohesin phosphorylation, thereby protecting the pericentromeric pool of cohesin from cleavage. Our analysis indicates that Spo13 is not required for the localization or function of Sgo1-PP2A. Instead, our findings are consistent with a model whereby Spo13 aids cohesin protection by counteracting the activity of cohesin kinases. Furthermore, we find that recruitment of the polo-like kinase Cdc5 to kinetochores is an essential function of Spo13 in setting up the meiotic segregation programme. Together, our results suggest that the segregation of homologous chromosomes requires the manipulation of key chromosome segregation pathways by Spo13.

T24 A method for the acute and rapid degradation of endogenous proteins in oocytes and other cell types

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Methods for the targeted disruption of protein function have revolutionized science and greatly expedited the systematic characterization of genes. Two main approaches are currently used to disrupt protein function: DNA knockout and RNA interference, which act at the level of the genome or mRNA, respectively. A method that directly alters endogenous protein levels is currently not available. Here we present Trim-Away, a technique to degrade endogenous proteins acutely in mammalian cells without prior modification of the genome or mRNA. Trim-Away harnesses the cellular degradation machinery to remove unmodified native proteins within minutes of application. This rapidity minimizes the risk that defects caused by protein disruption are compensated and that secondary, non-specific defects accumulate over time. Because Trim-Away utilizes antibodies, it can be applied to a wide range of target proteins using off-the-shelf reagents. Trim-Away allows the study of protein function in diverse cell types, including non-dividing primary cells where genome- and RNA-targeting methods are poorly effective. It is also ideally suited to degrade endogenous proteins in mammalian oocytes, providing the basis for rapid mechanistic studies of meiosis.

T25 Wapl-mediated cohesin release from chromosomes contributes to maternal age-related egg aneuploidy

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Trisomic pregnancies leading to spontaneous abortions or developmental defects increase dramatically with maternal age (Hassold & Chiu, 1985). Most trisomies are caused by chromosome missegregation in oocytes (Hassold & Hunt, 2001). One cause of these homologue non-disjunction events is thought to be the loss of cohesin from chromosomes and weakened sister chromatid cohesion (Hodges *et al*., 2005; Storlazzi et al., 2008; Chiang et al., 2010; Lister et al., 2010; Tachibana-Konwalski et al., 2010; Nagaoka *et al*., 2012; Tsutsumi *et al*., 2014; Sakakibara *et al*., 2015; Zielinska *et al*., 2015; Burkhardt *et al*., 2016). Cohesin can be removed from mitotic chromosomes by the protein Wapl (Tedeschi *et al*., 2013). In meiosis, Wapl affects chromosome axis length in budding yeast (Challa *et al*., 2016), antagonizes COH3/4-mediated cohesion in worms (Crawley *et al*., 2016) and is indirectly regulated by Nek1 kinase in prophase I in mice (Brieno-Enriquez *et al*., 2016). Whether Wapl releases cohesin from bivalent chromosomes after meiotic recombination is poorly understood. By using a conditional genetic knockout approach in mice, we show that Wapl controls chromosomal cohesin levels in meiosis I oocytes long after meiotic DNA replication and recombination. In meiosis II, sister kinetochores are more closely associated in the absence of Wapl, implying that Wapl is removing cohesin complexes connecting sister centromeres. This interpretation is consistent with Wapl not merely regulating chromosomal cohesin levels but sister chromatid cohesion. If so, then we predict that depleting Wapl in oocytes with weakened cohesion can rescue bivalents that might otherwise disjoin into chromatids. To test this, we employed a novel premature cohesion ageing mouse model with these characteristics. Crucially, Wapl depletion delays precocious bivalent disjunction into chromatids both by chromosome spreads and live-cell imaging of oocytes. We propose that Wapl lowers chromosomal cohesin levels below a critical threshold and thereby contributes to maternal age-related egg aneuploidy and infertility.

T26 Wapl and Pds5 proteins control cohesin-mediated chromosome axis and loop formation

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Cohesins are protein complexes that are thought to embrace DNA inside a ring-structure that is formed by a heterodimer of two "structural maintenance of chromosomes" (SMC) proteins and a kleisin subunit that connects ATPase domains of the SMC subunits. These cohesin-DNA interactions are essential for chromosome segregation in mitosis and meiosis.

In meiotic chromosomes, cohesin complexes are located in axial structures and are required for synaptonemal complex formation, recombination, homolog bias of recombination and for cohesion between chromosome arms and centromeres, which is essential for chromosome bi-orientation on the meiosis I and II spindle, respectively (Klein *et al*., Cell 1999; Kim *et al*., Cell 2010). Cohesin-DNA interactions are highly regulated and can be reversed either by the kleisin protease separase, or by Wapl, a protein that is thought to open a DNA "exit gate" between the kleisin and one of the SMC subunits. Previous work in yeast has shown that Wapl inactivation causes shortening of meiotic chromosome axes, indicating that Wapl negatively controls this process by an unknown mechanism (Challa *et al*., Nuc. Acid. Res. 2016). However, the functions of Wapl in mammalian meiosis are poorly understood.

We have, therefore, genetically inactivated Wapl in mouse oocytes and analyzed meiotic progression and cohesin regulation in these cells in collaboration with the Tachibana-Konwalski lab (see abstracts by Mariana Salva and Kikue Tachibana-Konwalski). Interestingly, our previous work revealed that Wapl depletion also causes the formation of cohesin-containing axial chromosomal structures and chromatin compaction in somatic interphase cells (Tedeschi *et al*., Nature 2013). These structures, which we call "vermicelli", are reminiscent of meiotic chromosome axes, which contain cohesin, and the sister chromatid axes of mitotic chromosomes, which contain related SMC complexes, called condensins. Because both meiotic and mitotic chromosome axes are thought to organize chromatin loops, we proposed that Wapl depletion leads to the stabilization and/or *de novo* formation of chromatin loops by cohesin in interphase chromatin, and that the axial vermicelli structures represent the base of such loops (Tedeschi *et al*., Nature 2013).

Subsequent *in silico* modeling by the Mirny lab predicted that Wapl depletion causes chromatin compaction through the cohesin-dependent formation of extended loops by a hypothetical loop extrusion mechanism (Fudenberg *et al*., Cell Rep. 2016). This hypothesis predicts that cohesin is required for loop formation, that cohesin is able to translocate rapidly along DNA to be able to mediate loop extrusion, and that Wapl depletion causes the elongation of loops. We have tested these predictions by a combination of microscopic imaging and genome-wide chromatin immunoprecipitation (ChIP-seq) and chromatin conformation capture (Hi-C) techniques. All our results are consistent with the model proposed by Fudenberg et al. (2016). Unexpectedly, however, our observations indicate that Pds5 proteins, which are binding partners of Wapl and are thought to cooperate with it in releasing cohesin from DNA, have a function in chromatin loop formation that is distinct from the function of Wapl. Our results also imply that the shortening of meiotic chromosome axes observed in Wapl deficient yeast cells (Challa *et al*., Nuc. Acid Res. 2016) is caused by the formation of extended chromatin loops.

T27 Chromosome dynamics during the second meiotic division

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Chromosomes must establish stable attachments to the spindle microtubules prior to the anaphase. For the faithful segregation at the second meiotic division sister kinetochores should connect to the microtubules from the opposite spindle poles. The detailed process of attachment correction, chromosomes congression and segregation at the anaphase of the second meiotic division remains unclear.

The individual chromosomes were tracked in both mitotic cells and during the first meiotic division. Here, for the first time we tracked all the kinetochores of mouse oocytes throughout the second meiotic division by high-resolution imaging in live mouse oocytes. The obtained 4D datasets allow to establish a normal behavior pattern of the chromosomes. Then, we compared the behavior of the chromosomes in oocytes derived from young and aged mice, and movements of the error-producing single chromatids in the aneuploid oocytes.

The single chromatids cannot establish normal attachments to the meiotic spindle, as they possess only one kinetochore. Still, they do not delay the anaphase onset in mouse oocytes. We show that the single chromatids in about half of the observed cases align stably on the spindle equator, and in majority of the rest they oscillate between the spindle poles.

We observed a high rate of lagging chromatids at the second meiotic division in both young and old mouse oocytes with normal chromosome complement. Our analysis reveals that the affected chromosomes have abnormal attachments to the meiotic spindle and reduced inter-kinetochore tension, indicating merotelic attachments. Notably, the oocytes from aged mice have a higher level of abnormalities and a higher risk of nondisjunction in the second meiotic division.

Our analysis contributes to the understanding of the basic biology of the second meiotic division and helps to find the strategies to prevent aneuploidy in human embryos.

T28 Reducing human oocyte aneuploidy rate for assisted reproductive technologies

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Oocyte quality is the major pillar of female fertility and so assisted reproductive technologies (ART) success. In spite of tremendous improvements in managing infertility, oocyte quality remains poorly understood, diagnosed and so managed. The reserve of oocytes is formed before birth and meiosis process begin during fetal life and may last several decades because human oocytes are arrested at prophase I until the surge of luteineizing hormone in the middle of the menstrual cycle induces meiosis resumption and maturation of the ovulated oocyte. Hence, the ovulated oocyte may have approximately between 10 and 40 years of meiosis arrest which may negatively affect the chromosomal status. Moreover, meiosis resumption (germinal vesicle breakdown, chromosome alignment, meiotic spindle organization and first polar body extrusion) requires high levels of ATP which permanently decreases during women's life. The above mentioned reasons explain why oogenesis is prone to chromosomal segregation errors and oocyte aneuploidy is then supposed to be one of the major contributors to embryo aneuploidy and so embryo development arrest. Investigating gamete interaction, our research team at Cochin Hospital synthesized a peptide that increases mouse fertilization rate (Ziyyat *and al*., 2005). We validated peptide innocuity after IVF treatment in the mouse model (Barraud-Lange *and al.,* 2009). With the agreement of the French Biomedicine Agency, we tested this peptide in human IVF: a preliminary clinical trial was launched in 2013 with 66 couples (clinicaltrial.gov NCT02161861). The actually most striking result is a decreased spontaneous miscarriage rate in the treated group (from 30.8% to 15.4%). We hypothesize that peptide-exposure during IVF leads to this long term beneficial effect by improving embryo ploïdy. We suggest that the peptide acts directly on oocyte to reduce aneuploidy rate. Thus, we investigated the *in vitro* maturation of germinal vesicle oocyte model to assess meiosis I quality through performing array comparative genome hybridization (aCGH) on *in vitro* matured oocytes with or without peptide supplementation (a randomized study). Preliminary results (49 oocytes) have shown that the peptide increases both maturation and euploïdy rates of human oocytes. Aneuploidy rate decreased from 80% in control group to 62.5% in treated group. In women over 37 years old, all the oocytes were aneuploid in the control group vs 60% of aneuploidy among the treated group.
Pursuing this study by demonstrating the beneficial effect of the peptide on oocyte and embryo ploïdy and identifying its molecular signaling pathway(s) could help to manage women infertility.

T29 Cell cycle regulation by systems-level feedback controls

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In order to maintain genome integrity and an effective nucleocytoplasmic ratio from one generation to the next, cells carefully monitor progression through their replication-division cycle and fix any errors before they jeopardize the progeny of the cellular reproduction process. These error surveillance and correction mechanisms operate at distinct 'checkpoints' in the cell division cycle, where a growing cell must 'decide' whether it must wait for errors to be corrected or it may proceed to the next phase of the cell cycle. Once a decision is made to proceed, the cell unequivocally enters into a qualitatively different biochemical state, which makes cell cycle transitions switch-like and irreversible. These characteristics of cell cycle transitions are best explained by bistable switches with different activation and inactivation thresholds, resulting in a hysteresis effect. Almost 25 years ago, John Tyson and I proposed that the activity of the mitosis-inducing protein kinase, Cdk1:CycB, is controlled by an underlying bistable switch generated by positive feedbacks involving inhibitory phosphorylations of the kinase subunit. Numerous predictions of this model were experimentally verified by different groups, and bistability has become a paradigm of cell cycle transitions. The phosphorylation of mitotic proteins by Cdk1:CycB is counteracted by a protein phosphatase, PP2A:B55, which is inhibited during mitosis by a stoichiometric binding partner, ENSA-P, which is itself activated by Greatwall-kinase. Using mathematical modelling guided by biochemical reconstitution experiments, we showed recently that the BEG (B55-ENSA-Greatwall) pathway also represents a bistable, hysteretic switch controlled by the activity of Cdk1:CycB. Bistable regulation of the kinase (Cdk1:CycB) and the phosphatase (PP2A:B55) makes hysteresis a robust property of mitotic control, with suppression of futile cycling of protein phosphorylation and dephosphorylation during M phase. These considerations show that both entry into and exit from mitosis are controlled by bistable switches intimately connected to the activities of the major mitotic kinase, Cdk1:CycB, and phosphatase, PP2A:B55. Intriguingly, the 'design principle' of the BEG pathway is operative as well at two other cell cycle checkpoints, as will be discussed.

T30 Lessons from meiosis II

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The hallmark of meiosis is one round of DNA replication being followed by two rounds of chromosome segregation and a differentiation program to generate gametes. Whereas the mechanisms governing the segregation of homologous chromosomes in meiosis I have been studied extensively, comparatively little is known about the control of chromatid segregation in meiosis II. This is in part due to the perception of meiosis II as a "mitosis-like division". However, meiosis II differs from mitosis in fundamental aspects: in contrast to mitosis, meiosis II is preceded not by an S-phase but by an M-phase (meiosis I), and it is not followed by another S-phase but by gametogenesis. Furthermore, the meiosis II division is triggered by the cleavage of centromeric cohesin that has been protected from cleavage by the separase protease in meiosis I. We have developed tools to study meiosis II in budding yeast to provide insights into the following questions: (1) How and when is centromeric cohesin de-protected so that it becomes susceptible to separase at the onset of anaphase II? We have recently shown that in yeast, APC/C-Cdc20 de-protects centromeric cohesin by targeting for degradation the cohesin-protector shugoshin. Since APC/C-Cdc20 also triggers the degradation of Pds1/securin, de-protection of centromeric cohesin is linked to the activation of separase. Our data imply that at least in yeast, centromeric cohesin is de-protected by meiosis II-specific proteolysis mediated by APC/C-Cdc20 rather than sister kinetochore bi-orientation. (2) What mechanisms generate two, and only two, M phases after pre-meiotic S phase? Progression through successive M phases is thought to be driven by an oscillator containing Cdk1 and APC/C-Cdc20 at its core. We suspect that the oscillator is stopped by the special properties of the meiosis II exit-machinery. Indeed, our data show that the meiosis II exit-machinery differs from that of meiosis I or mitosis. This information is fed into a dynamic model that aims to describe, eventually, the entire, two-division meiosis and its coordination with gametogenesis.

T31 The role of calcineurin during exit from meiosis II

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In vertebrates, mature eggs await fertilization arrested at metaphase of the second meiotic division (MII). This metaphase arrest is maintained by the activity of the protein XErp1/Emi2, which binds to and inhibits the E3 ligase anaphase-promoting complex/cyclosome (APC/C), thus stabilizing the APC/C substrates cyclin-B and securin. XErp1/Emi2 needs to be in a hypophosphorylated state to be stable and bound to the APC/C and this is ensured by the meiotic Mos/MAPK/p90Rsk signaling cascade in combination with the phosphoprotein phosphatase P2A-B56. Thus, XErp1/Emi2 together with Mos/MAPK/p90Rsk/PP2-B56 constitute the so-called cytostatic factor mediating the MII arrest of mature eggs. Fertilization triggers a calcium wave resulting in activation of calcium/calmodulin-dependent kinase II (CaMKII), which mediates the destruction of XErp1/Emi2 and hence, APC/C activation and exit from MII.
Recently, it has been shown that the activity of the calcium-activated phosphatase calcineurin (CaN) is critical for proper activation of the APC/C at meiotic exit and inhibition of its function results in impaired degradation of cyclin-B. Yet, the molecular mechanism underlying the requirement of CaN activity for MII exit including the relevant substrates remained elusive. Our studies suggest that CaN contributes to the calcium-induced inactivation of XErp1/Emi2. Oocyte extract experiments indicate that CaN mediates the dephosphorylation of XErp1/Emi2 at a site phosphorylated by p90Rsk. As shown previously, p90Rsk phosphorylated XErp1/Emi2 recruits PP2A-B56, which protects XErp1/Emi2 from inactivation and destruction by removing phosphorylations mediated by multiple kinases involving cyclin-dependent kinase 1, Polo-like kinase 1, and casein kinase 1. Based on our data, we propose a model according to which CaN contributes to XErp1/Emi2 destruction upon fertilization by antagonizing the protective function of PP2A-B56 on XErp1/Emi2. Thus, the calcium wave - triggered by fertilization - branches into CaMKII and CaN activation, both of which mediate XErp1/Emi2 inactivation resulting in APC/C activation, destruction of the APC/C substrates securin and cyclin-B and, hence, exit from MII.

T32 A high-dimensional fluorescent microscopy system for quantitative prediction of cell fate during yeast meiosis

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The ability to differentiate or choose a fate/cellular state in response to external or internal cues is a crucial emergent property of many living systems. Although understanding how cells commit to- and maintain new cellular states is essential for development and meiosis, we still lack quantitative approaches to study single cells *in vivo* during the process of signal integration and cell fate determination.

Here, we describe a single-cell microfluidics based assay to study meiotic differentiation in budding yeast. Our system combines precise environmental control, single-cell tracking throughout the entire process of meiosis and the use of up to 6 distinct fluorescently labelled reporters. To determine how the intracellular signaling state affect meiotic commitment we used our 6-color strains to measure key metabolic, cell cycle and signaling markers simultaneously at the single cell level. Next, we used the method of *statistical evidence* to transform this data into probabilities in a mathematically stringent way. We show that the decision to commit to meiosis is highly deterministic over long periods of time and that it depends on specific well-defined metabolic states. The framework presented here is general and can be adapted for the study cell fate determination in other systems.

T33 Phospho-regulation by Mps1 stabilizes force-generating kinetochore-microtubule attachment

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Proper attachment of chromosomes on the spindle is an essential step to insure their proper segregation. During meiosis the centromeres of homologous chromosomes must attach to microtubules that radiate from opposite poles of the spindle. This interaction between centromeres and microtubules is mediated by a multi-protein structure called the kinetochore. Chromosomes become bi-oriented in a multi-step process that involves the attachment of microtubules to kinetochore with the release and re-attachment of connections that would lead to segregation errors. Using live cell imaging to follow chromosome movements, we have characterized the interactions of kinetochores with microtubules throughout meiosis I. Remarkably, we found that in budding yeast the 1st attachments of microtubules to kinetochores in pro-metaphase usually are incorrect. Two conserved kinases; Ipl1 and Mps1 were found to work sequentially to re-orient the mis-aligned chromosomes on the metaphase spindle. Ipl1 was necessary to release improper kinetochore-microtubule attachments whereas Mps1 acted at the next step, creating new force-generating attachments.
Over the years, several targets of Mps1 have been identified that could have roles in the formation of kinetochore-microtubule attachments, but whether these or other targets are central to its role in meiotic bi-orientation is unknown. Therefore to identify those targets, we have taken advantage of meiotic cells because they are highly sensitive to defects in the bi-orientation process and followed their segregation behavior using high-speed live cell imaging. We have been able to identify a key substrate of Mps1 that is critical to stabilize kinetochore-microtubule attachments. Interestingly, our preliminary data also suggest that the phosphorylation of this substrate might be negatively regulating the activity of the main destabilizer of kinetochore-microtubule attachment: the Ipl1/Aurora-B kinase.

T34 Promoting and limiting COs during *C. elegans* meiosis

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Because the DSBs that serve as the initiating events of meiotic recombination pose a danger to genome integrity, the success of genome inheritance during meiosis requires cells to maintain a balance between the beneficial effects of COs and the potential harmful consequences of the process by which they are generated. A major goal of our research is to understand the mechanisms that operate during meiosis to achieve this crucial balance. We are using the *C. elegans* experimental system to investigate the mechanisms that promote and limit formation of meiotic COs, both at the level of designating recombination sites for a CO or non-CO fate and at the level of enforcing reliable execution of these fates. I will present evidence suggesting that SC central region proteins function as pro-CO factors both by promoting stability/normal architecture of recombination complexes and by creating a local environment that protects recombination intermediates at CO-designated sites from being dismantled or resolved inappropriately. Further, I will present data implicating COSA-1-dependent phosphorylation of MSH-5 in a positive feedback mechanism that operates to recruit/stabilize association of pro-CO factors at prospective CO sites. Finally, I will discuss our recent work identifying a role for a conserved 14-3-3 protein in antagonizing or limiting the activity of pro-CO factors.

T35 Asy2/Mer2: an evolutionarily conserved mediator of the recombinosome/structure interface at every stage of meiosis

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Meiotic prophase chromosomes are organized as linear arrays of chromatin loops connected to a structural axis that ultimately defines the interaction side of the homologous chromosomes during pairing. Here, identification of Asy2/Mer2 in the fungus Sordaria macrospora reveals a new, evolutionary-conserved protein family. Asy2/Mer2 localizes as foci, prominently on axes and chromatin but also to the synaptonemal complex (SC) and to post-pachytene chromosomes. It localization on axes depends on cohesins but is independent of DSB initiation or SC formation. Via these localizations, Asy2/Mer2 plays critical roles in mediating chromatin/axis/SC interplay at every major stage of meiotic prophase. It mediates recombination initiation, recombination-mediated homologous recognition/pairing and thereby crossover patterning/interference, and transfer, maintenance plus release of recombination complexes to SC central regions. Moreover, after completion of recombination, Asy2/Mer2 mediates global chromosome compaction, dependent on SUMOylation. We propose that Mer2 proteins actively promote chromosome structural reorganization, first locally as activated by association with core recombination components and then globally at post-recombination stages.

T36 RNA transcription and termination factors are important in meiotic homologous chromosome pairing in *S. pombe*

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Pairing of homologous chromosome is an essential step in meiosis that ensures effective homologous recombination and reductional segregation in meiotic division. In fission yeast, the formation of telomere bouquet and accompany telomere-led chromosome oscillation align the homologous chromosomes to the same direction and effectively promote their contact and pairing. However, the mechanisms for homologous chromosome specific recognition remain undefined. We have found a double stand break independent pairing site, the *sme2* locus, shows extraordinary high and stable pairing activity: transcription of a long non-coding RNA, the meiRNA-L, and formation of a meiRNA-dot at the *sme2* locus is essential for the robust pairing (Ding *et al*., Science 2012). To further understand the molecular mechanisms of meiRNA promoted pairing, we screened for *sme2* locus-localization protein (smp). As a result, we found ten kinds of Smp and half of them are required for the robust pairing of the *sme2* locus. These proteins are RNA binding proteins and/or involved in transcription termination. The Smp proteins have two more distinct binding loci on chromosomes, in addition to the *sme2* locus. We propose that retaining of RNA transcripts on the chromosome play an important role in homologous chromosome recognition and pairing.

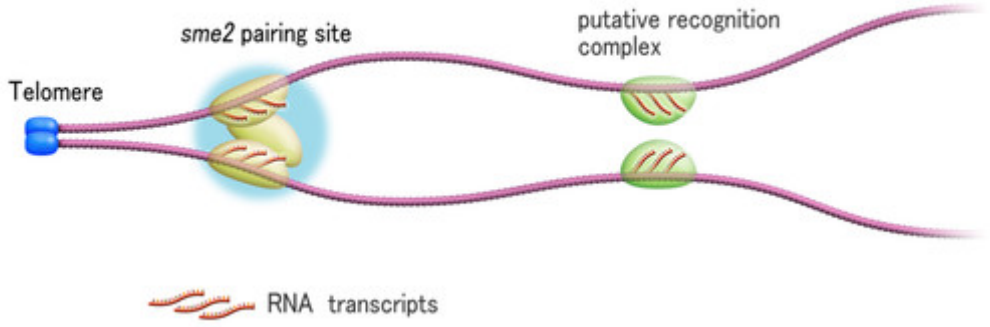

Figure 1. Homologous chromosomes may recognize its partner through RNA containing pairing sites.

T37 Structural basis of meiotic chromosome synapsis through SYCP1 self-assembly

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The process of reductive cell division by meiosis is essential for fertility in all sexually reproducing organisms. At its centre is the synaptonemal complex (SC), a zipper-like molecular structure that synapses homologous chromosome pairs and provides the three-dimensional architecture necessary for genetic exchange through crossing over. The underlying structure of the vertebrate SC is provided by transverse filament protein SYCP1, of 976 amino acids in humans, which bridges between the midline central element and chromosome-bound lateral element, with its N- and C-termini located within each of these regions respectively. Here, we report the structure of SYCP1 and the molecular basis of its self-assembly through sites within its N- and C-terminal regions. Biophysical analyses through small angle X-ray and light scattering experiments reveal that SYCP1 adopts an elongated tetrameric structure. This obligate assembly is composed of an N-terminal tetrameric bundle that bifurcates at its C-terminal end into two elongated dimeric coiled-coils of sufficient length to bridge between the central and lateral elements of the SC. We have further elucidated the X-ray crystal structures the N- and C-terminal self-assembly sites of SYCP1 in 'assembled' conformations. In combination with solution biophysical studies, these reveal distinct mechanisms of dynamic head-to-head and back-to-back assembly at both termini of the central structured region of SYCP1. The C-terminus undergoes pH-dependent self-assembly into an anti-parallel tetramer that is competent for DNA-binding. We propose that self-assembly occurs concomitantly with DNA-binding to achieve the processive looping back-to-back assembly of SYCP1 along the chromosome axis. In contrast, the N-terminus undergoes head-to-head self-assembly into a dimer-of-dimers structure. This self-assembly mechanism is highly cooperative between adjacent SYCP1 molecules, thus ensuring a continuous midline synapsis between perfectly aligned homologous chromosomes. Overall, our data suggest a model in which self-assembly of SYCP1 through its N- and C-termini lead to its coating of the chromosome axis and midline zipper-like assembly into a lattice-like array that is continuous along the chromosome axis. This nascent structure provides the molecular underpinning of meiotic chromosome synapsis and is likely locked in place through stabilisation by assembly of SC central element proteins SYCE1, SYCE3, SIX6OS1 and SYCE2-TEX12. Our data thus provide a full model for the structure of SYCP1 and how its self-assembly underlies the cooperative and continuous synapsis achieved along the length of homologous chromosome pairs during meiosis.

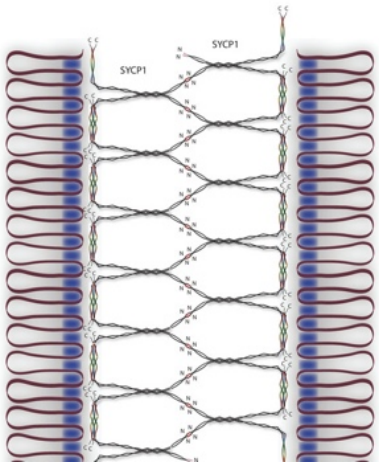

Figure 1 Meiotic chromosome synapsis is achieved by self-assembly of SYCP1 tetramers, through sites within their N- and C-termini, into a continuous lattice-like molecular array.

T38 Understanding the statistics of chromosomes during meiosis in fission yeast

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In meiosis of fission yeast, the nucleus performs pronounced oscillatory motion. It is believed that these oscillations are necessary for proper chromosome pairing and recombination. In this work, we show how the statistics of chromosome configurations during meiosis in fission yeast can be quantified based on theoretical models of polymer physics. With the help of the model we can explain how the pulling of chromosomes through the viscous nucleoplasm suppresses fluctuations and brings the homologous strands close enough to start recombination. The model also shows that initial pairing events additionally reduce fluctuations and promote further recombination. Importantly in our modeling we can describe both the steady state regime during one period of oscillations and the relaxation dynamics towards the steady state. Model results on the parameters describing the nucleus pulling and relaxation dynamics are in good agreement with experimentally measured values. With this level of quantitative understanding of chromosome alignment we can now try to answer the questions about the optimality of oscillatory dynamics and the corresponding progression of recombination during nuclear oscillations.

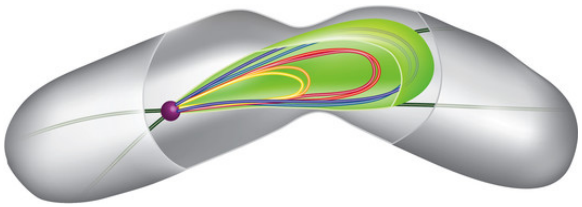

Figure 1. This figure shows a sketch of nuclear oscillations. Three chromosome pairs are attached by both ends to the spindle body inside of the nucleus. The nucleus is pulled by microtubules and dynein motors from one pole of the elongated cell to the other.

T39 A family of SUMO-E3 ligase-like proteins have distinct and essential functions in crossover formation in *C. elegans*

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Correct segregation of homologous chromosomes in meiosis depends on DNA crossovers (COs) that transform into cytologically visible physical linkages (chiasmata) between the homologs. Proteins homologous to SUMO (small ubiquitin-related modifier) E3 ligases have been implicated in synapsis and CO formation in a number of taxa including yeasts, plants, and mammals. We present evidence that a family of four SUMO-E3-like nematode proteins related to yeast Zip3 are required for CO and chiasma formation via distinct mechanisms in *C. elegans*. Isolation and characterization of genetic mutants and mutation of the RING finger domain of the putative E3 ligases has revealed that they form two distinct functional groups that are both essential for crossover formation. ZTP-2 localizes to the synaptonemal complex (SC) codependently with its paralog ZHP-3, followed by restriction to sites of COs; ZTP-1 and its paralog ZTP-3 show similar codependent SC localization, but are restricted to the short arm of the bivalent in the presence of COs. While recombination initiates in ztp mutants, early recombination intermediates fail to appropriately acquire late CO markers, and crossovers that do form correlate with the appearance of abnormal bivalents defective in the chromosome remodelling required for segregation. Our results suggest that ZTP-2/ZHP-3 and ZTP-1/ZTP-3 first function at an early step in the formation of CO intermediates; following CO designation, we propose they form a feedback loop linking this event with bivalent remodelling in preparation for segregation.

T40 Prdm9-controlled asynapsis in sterile hybrid mice

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Infertility of hybrids between closely related (sub)species belongs to early reproductive isolation mechanisms safeguarding speciation. Since the genetic control of hybrid sterility (HS) is complex only few HS genes have been identified so far including *Prdm9*, the vertebrate only known HS gene[1]. *Prdm9* controls infertility of male hybrids between PWD/Ph and C57BL/6J mouse strains derived from *Mus m. musculus* and *Mus m. domesticus* subspecies but within species the same gene determines the sites of programmed DNA double-strand breaks (DSBs) and meiotic recombination hotspots. In sterile hybrids PRDM9 allelic variants do not bind both homologs equally suggesting that the asymmetry or lack of symmetry of binding sites could explain failure of meiotic chromosomes to synapse[2,3]. To investigate the relation between DSB symmetry, asynapsis and meiotic arrest we prepared (PWD x B6)F1 hybrids with stretches of PWD/PWD homology on several pairs of homeologous autosomes and analyzed their ability to form full synaptonemal complex. We have found that 25 Mb of PWD/PWD homology was sufficient to rescue full synapsis in each of the eight examined autosomal homeologous pairs and estimated that minimum 2 symmetric DSBs per chromosome are needed for successful meiosis. Moreover, deliberate suppression of asynapsis of three smaller chromosomes enabled partial rescue of fertility of hybrid males. Thus our model links HS to meiotic recombination, chromosome synapsis and reproductive isolation. Further studies will show if such connection can be traced in other species, including those without *Prdm9* gene ortholog. Our hypothesis posits the *Prdm9*-directed HS as the special case of a more general mechanism of reproductive isolation between eukaryotic species via interaction of diverged genomic sequences with antirecombination mismatch repair machinery.

T41 The *Arabidopsis* Cdk1/Cdk2 homolog CDKA;1 controls the number and position of interference-sensitive cross-overs

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During meiosis homologous chromosomes recombine to generate new allele combinations in the offspring. Species typically show a relatively constant number of crossovers per meiosis, known as crossover homeostasis, while also the distribution of crossovers along the chromosome axis (the recombination landscape) remains stable. However, little is known of how such recombination patterns are established. Here, we analyze an allelic series of the *Arabidopsis* Cdk1/Cdk2 homolog CDKA;1 showing that plants with very little Cdk activity are sterile due to several severe meiotic defects, including a failure to pair and recombine homologues. Mutants with intermediate CDK activity are semi-sterile and homologues do pair and form crossovers, albeit about 30% less than wt plants. These lower recombination rates result from a partial loss of interference-sensitive crossovers, that seem to be preferentially lost from centromere-proximal regions and thus strongly altering the recombination landscape. Together with our observation that CDKA;1 can phosphorylate MLH1 that is essential for the formation of interference-sensitive crossovers, we identify CDKA;1 as a major regulator of meiotic progression in *Arabidopsis*. Many of our observations are not unlike the phenotypes described for mutants of the pairing regulator Ph1, that stabilizes recombination in hexaploid wheat and may indeed affect the activity of the wheat CDKA;1 homologue.

T42 Meiotic Defects And Quality Control In Oocytes From Genetically Diverse Mice.

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Most aneuploidies arise from errors in maternal meiosis. Chromosomes that fail to synapse or lack a crossover will be missegregated and result in aneuploid egg. Quality control mechanisms eliminate "at-risk" oocytes before they form fertilizable eggs. In human and mouse, female meiosis is more error prone than male, but it's unclear whether this is due to less efficient meiotic processes or weaker quality checkpoints or both. To identify mechanisms of quality control in oocytes we use a novel approach: genetically diverse mice from the Collaborative Cross (CC) and various inbred strains. The CC lines have been bred using a combinatorial funnel designed to produce a large number of multiparental recombinant lines from eight inbred founder strains. Each CC line is genetically different and represents unique mosaic of eight parental genomes; all mice within a given line are genetically identical. Importantly, CC lines and founder inbred strains show considerable variation in reproductive performance, which we show is due in part to female factors determining the quality and quantity of oocytes. Therefore, CC lines offer an excellent animal model for genetically heterogeneous human population. We find that the incidence of asynaptic chromosomes and persistent marks of DNA damage in late prophase I oocytes vary significantly among mouse inbred strains and CC lines. Results clearly reveal that genetic background regulates meiotic prophase I progression and efficiency and influences both quality and quantity of oocytes.

T43 Controlling DNA Breaks in Mammalian Meiosis

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Each chromosome is present in two distinct but homologous copies in diploid organisms. To generate haploid gametes suitable for fertilization, these homologous chromosomes must segregate during meiosis. To ensure correct chromosome segregation, homologous chromosomes must pair and form crossovers between each other during early meiosis in most taxa including mammals. Pairing of homologous chromosomes and crossover formation entail programmed generation of DNA double-strand breaks and the repair of DNA breaks by meiotic recombination. DNA double strand–breaks are potentially genotoxic, hence their formation must be under tight spatiotemporal control. We identified chromosome axis-associated proteins that play important roles in the control of DNA double-strand break formation. I will present our latest results on the regulation and function of these DNA break-promoting proteins, and discuss implications for the feedback control of DNA double-strand breaks.

T44 Regional control of meiotic DSB formation by the synaptonemal complex

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Meiotic double-strand break (DSB) formation is regulated by feedback mechanisms that limit DSBs on chromosomes that have successfully engaged in repair with the homologous chromosome. In *S. cerevisiae* and mice, this feedback regulation is linked to the formation of the synaptonemal complex (SC) and is disrupted in mutants that fail to synapse. Here, we identify large telomere-proximal domains that frequently escape this feedback regulation in *S. cerevisiae*. These privileged domains span roughly 100 kb and continue to form DSBs in pachynema when chromosomes have assembled a contiguous SC. Cytological analyses and chromatin immunoprecipitation of the major SC component Zip1 indicate that these regions are less likely to form an SC and instead retain binding of the DSB-promoting chromosome axis protein Hop1. This biased enrichment of Hop1 leads to a proportional increase in Hop1 levels and DSB repair signals on small chromosomes in pachynema. Deletion of the disassemblase Pch2 indicates that the biased enrichment of Hop1 is a result of differential removal of Hop1 from the chromosome center. In the absence of Pch2, DSB formation is proportionally decreased near chromosome ends whereas we observe strong enrichment of Hop1 and DSB formation near centromeres. Importantly, loss of Pch2 also eliminates the biased enrichment of Hop1 on small chromosomes suggesting that the telomere-proximal domains contribute to increasing the chance of recombination initiation on small chromosomes.

T45 Functional correlation between Spindle Assembly Checkpoint and Anaphase Promoting Complex activity during mammalian meiosis I.

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Mammalian oocytes and embryos are frequently affected by aneuploidy arising during chromosome segregation. The reason why chromosome segregation errors are more frequent in female germ cells and embryos in comparison to somatic cells is still not completely understood. We believe that the problem is partially caused by differences in function of chromosome segregation and anaphase control mechanisms operating in these cells. In our study we focused on relationship between activities of surveillance checkpoint mechanism called Spindle Assembly Checkpoint (SAC) and ubiquitin ligase controlling anaphase entry called Anaphase Promoting Complex (APC) in individual oocytes. Using mainly micromanipulation and whole cell volume confocal imaging we monitored the activity of SAC on individual kinetochores and global APC activity and correlated these with chromosome positions, spindle formation and polar body extrusion simultaneously in individual cells progressing through the first meiotic division and approaching anaphase I. Our results show similarities between oocytes and somatic cells in relationship between SAC and APC activity as well as important and unexpected differences. Apart from other valuable insights into function of these two mechanisms in oocyte meiosis this approach allowed us to analyze quantitatively the dependence of APC activation on the number of kinetochores producing SAC signal or consequences of SAC reactivation in time of APC activity. Our results revealed that the checkpoint mechanisms involved in monitoring chromosome segregation and the pathways controlling anaphase entry in oocytes show remarkable differences compared to somatic cells, which might contribute to the high incidence of aneuploidy in these cells.

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T46 Polo-like Kinase 4 Is Required for Homologous Recombination during Mouse Meiosis

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Production of viable gametes requires faithful haploidization of the genome during meiosis, a specialized program whereby one round of DNA replication is followed by two sequential rounds of cell division (MI, MII) in order to segregate homologs and sister chromatids, respectively. Crossover (CO) formation during prophase I is an essential process of the meiotic program that both increases genetic diversity and creates a physical link between homologs to ensure their accurate segregation during MI. Upon satisfaction of the prophase checkpoint for CO formation in males, the centriole is licensed to undergo duplication, followed by centrosome maturation and migration, resulting in the formation of bipolar spindles. Oppositely, centrioles are lost in oocytes during prophase, and remain absent until fertilization.

Polo-like kinase 4 (PLK4) has previously been shown to drive centriole duplication and centrosome maturation in somatic cells. Recently, we discovered that in addition to the centrosome, PLK4 localizes to sites of persistent DNA damage in the nucleus of spermatocytes and oocytes during prophase I. **This localization pattern suggests that PLK4 plays a novel, direct role in coordinating meiotic DNA repair and homologous recombination (HR).** We have shown that male mice harboring a mutant *Plk4* allele ($Plk4^{I242N/+}$) undergo delayed prophase progression, exhibiting persistent markers of DNA damage (DMC1/RAD51) and an increased number of CO's (MLH1/MLH3). Interestingly, the effect of depleting a structural component of the centrosome, CEP63, led to similar meiotic HR defects in male but not female mice.

In order to delineate the cause of these meiotic defects outside of the context of centriole duplication, we have recently begun to characterize meiotic progression in $Plk4^{I242N/+}$ oocytes. Our preliminary data suggest that $Plk4^{I242N/+}$ oocytes recapitulate the HR and CO defects observed in males, culminating in decreased follicles at birth. Notably, some abnormal $Plk4^{I242N/+}$ oocytes are not eliminated from fetal ovaries and frequently exhibit abnormal chiasmata morphology apparent at metaphase I. Accurate CO processing depends on recruitment of MLH1-MLH3 heterodimers, therefore, we hypothesize that suppression of the MLH1-MLH3 CO pathway in $Plk4^{I242N/+}$, will rescue the late meiotic defects observed in $Plk4^{I242N/+}$ oocytes.

Additional ongoing investigations are geared towards probing centriole loss as well as determining the precise timing of apoptosis in mutant spermatocytes and oocytes.

T47 The control of meiotic DSB formation by Prdm9

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Meiotic recombination is initiated by the programmed induction of DNA double strand breaks (DSBs) which are not randomly distributed along chromosomes. Two major pathways control the distribution of meiotic DSBs along chromosomes. In one pathway, likely ancestral, DSBs are formed in regions of accessible chromatin which can be promoters, enhancers or other regulatory regions such as CpG islands and where specific histone modifications are detected, in particular H3K4me3. This pathway has been characterized in details in yeast, and is also active in plants and some animals (canidae, birds, some fish). The other pathway depends on Prdm9 which is found in several vertebrate lineages. PRDM9 has a sequence specific DNA binding domain and a methyltransferase domain. Nucleosomes adjacent to PRDM9 sites are enriched for H3K4me3 and DSBs are formed at or next to PRDM9 binding sites.
We have tested the role of the methyltransferase activity of PRDM9. We show that both H3K4me3 and H3K36me3 detected at PRDM9 binding sites require PRDM9 methyltransferase activity. In addition, DSB activity is abolished at PRDM9 binding sites in strain expressing a catalytically inactive PRDM9. DSBs are however formed at regulatory regions in this context. This excludes the possibility that PRDM9 is only a recruitment factor for DSB formation. It also shows that PRDM9 binding is not sufficient for directing DSB away from regulatory regions and demonstrates the primary role of the histone modifications detected for DSB activity. I will also present further insight into the regulation of DSB activity based on analysis of mice expressing two different PRDM9 variants. Analysis of proteins interacting with PRDM9 allows us to propose a model linking the various steps required for DSB formation, from PRDM9 binding to SPO11 catalytic activity.

T48 Srs2 regulates Rad51 localisation during meiosis and protects from abnormal events.

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We have been analysing the function of the DNA helicase and translocase Srs2 in meiosis. It has been known for some time that Srs2 is required for wild-type levels of sporulation and spore viability. We find the first meiotic division is delayed so that after 6 h of meiosis approximately twice the proportion of *srs2* mutant cells have a single nucleus compared to wild-type. This delay of nuclear division is not due to meiotic arrest, as at 6 h over 30% of *srs2* cells have 4 spindle pole bodies with a single nucleus, a 6-fold increase compared to wild-type cells. Using integrated TetO repeated sequences and TetR-GFP, we have found that neither homologue nor sister chromatid separation at the TetO locus appear to be perturbed in these cells, suggesting that failure to divide the nucleus does not reflect a global failure of chromosome/chromatid separation. As Srs2 is believed to be a negative regulator of Rad51 binding to DNA, we examined Rad51 association to chromosomes by cytology. Over 30% of *srs2* cells contain bright foci of Rad51 that remain through late time points and can be inherited into spores. The phenotypes described require the activity of Spo11, and the abnormal Rad51 foci are dependent on Ndt80 function; raising the possibility that Srs2 is required for resolution of recombination intermediates in the crossover pathway.

Using an artificial hotspot to score recombinants and joint molecule intermediates on Southern blots, we found levels of crossing over and gene conversion similar to those seen in wild-type. Thus, a defect in 'normal crossover resolution' seems unlikely. However, unlike in wild-type, recovery of joint molecule intermediates at normal levels requires psoralen cross-linking. Also, *mek1 srs2* strains, which are expected to repair most Spo11-DSBs using the sister chromatid as donor, also have the nuclear division defects and persistent Rad51 foci. This implies that, while Spo11 dependent, the events causing these phenotypes may not be on the normal Mek1 dependent interhomolgoue recombination pathway. The surprising finding that bright long-lasting Rad51 foci still form in *sae2 srs2* cells supports this view. Potential underlying mechanisms will be discussed.

We would like to acknowledge that Prof. Akira Shinohara and colleagues have concurrently undertaken similar experiments with similar results, and we are grateful to them and for sharing resources and data.

T49 Meiotic recombination initiation in *A. thaliana*

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Meiosis is a specialized cell division at the origin of the haploid cells that eventually develop into the gametes. Recombination is one of the key events of meiosis. It gives rise to crossovers (COs), which are critical for the correct segregation of homologous chromosomes.
Meiotic recombination is triggered by the formation of large amount (several hundreds) of DNA double strand breaks (DSBs). The characterisation of mutants affected in DSB formation in *Arabidopsi thaliana* identified a group of proteins absolutely required for this step of recombination and that show a variable level of conservation among species. Among these, the MTOPVIB protein is required to promote the interaction between SPO11-1 and SPO11-2, the two *Arabidopsis* Spo11 paralogs required for meiotic DSB formation. Recent advances in the study of MTOPVIB dynamics during meiosis as well as a global overview of the interaction network among *A. thaliana* DSB proteins will be presented.

T50 Cell-type specific genomics and in silico modelling of the crosstalk between meiotic replication and recombination in mammals.

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Genetic recombination in most mammals occurs at specific hotspots defined by the PRDM9 protein. However, PRDM9 binding cannot fully explain the variation in frequency of DSB targeting to its recognition sites. This is particularly true in humans, where increased DSB formation at sub-telomeric regions cannot be explained by increase in binding of PRDM9 alone. DSB formation is preceded by DNA replication in meiosis, and in simple eukaryotes these two processes are intimately linked. We sought to investigate this link in humans and mice where technical challenges have precluded such studies to date. Previous work in our lab has generated genome-wide maps of meiotic DSB formation in mice, however, the landscape of meiotic replication has not been studied. We coupled nascent leading strand isolation to a method that directly sequences single stranded DNA to map origins of replication in mouse testis. Our strand-specific signal allows for highly accurate, model-based origin detection, eliminating the problem of false positive calls. We also, for the first time, describe the meiotic replication landscape by computationally inferring replication timing from whole genome sequencing of meiotic S-phase cells. Origin density is highest in early replicating regions and intriguingly, early replicating regions are also enriched for PRDM9 binding, for meiotic DSB formation and for genetic crossovers. This strongly implicates meiotic replication as a key determinant of recombination patterning in mammals. To more precisely dissect the mechanisms that link replication and recombination, we developed an *in silico* model of meiotic replication. Using only the locations of replication origins from testis this model accurately recapitulates experimentally determined replication timing. Intriguingly, just 1 origin every 5 Mb is optimal to complete meiotic replication in a biologically relevant time frame. Model-derived hypotheses can now guide experiments designed to identify the factors and mechanisms that mediate the apparent coupling of replication and recombination during mammalian meiosis.

T51 Spo11: A "broken" topoisomerase

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During meiosis, homologous maternal and paternal chromosomes exchange genetic information through the process of homologous recombination, which initiates with DNA double-strand breaks (DSBs) made by the Spo11 protein. Meiotic recombination has a profound effect on heredity and genome evolution, but is also a potential source of mutation if Spo11-generated DSBs are improperly repaired. Cells therefore exert exquisite control over Spo11 activity to ensure that it only acts at the right time and place. To better understand this control, we have purified recombinant yeast Spo11 in complex with several protein partners and are characterizing its structure, protein-protein interactions, and protein-DNA interactions. Current studies will be presented.

T52 Elucidating the Role of Cyclin N-terminal domain containing-1 (CNTD1) in Crossover Designation During Mammalian Meiosis

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Cyclin N-terminal domain containing-1 (CNTD1) is the mammalian ortholog of Crossover site-associated-1 (COSA-1), a *C. elegans* protein that has been shown to orchestrate crossover designation in nematodes. Studies of a mouse mutant for Cntd1 revealed an essential requirement for normal CNTD1 function during prophase I. Specifically, our previous studies demonstrated that loss of CNTD1 results in persistent localization of the MutS complex, consisting of the MutS homologs, MSH4 and MSH5, on chromosome cores during pachynema of prophase I. Foci of the RING finger protein (and putative SUMO E3 ligase), RNF212, also persisted on chromosome cores throughout pachynema. By contrast, the RING finger protein (and putative Ubiquitin E3 ligase), HEI10/CCNB1IP1, and the MutL complex, consisting of the MutL homologs, MLH1 and MLH3, fail to be loaded onto chromosome cores in mid-pachynema. To identify the functions of CNTD1 with respect to MutS and MutL loading and unloading, we have explored the interacting partners of CNTD1 by co-immunoprecipitation, Mass spectrometry, and yeast two-hybrid analyses. For the first two approaches, we made use of a tandem tagged Cntd1-FLAG-HA allele generated by CRISPR-Cas9 technology. Localization of the HA tag on chromosome spreads through prophase I revealed that CNTD1 accumulates on chromosomes in pachynema, at a frequency and distribution that is highly reminiscent of MutL loading. Moreover, the HA tag co-localizes with MLH1 for the most part, although individual HA-only foci are observed occasionally. Yeast two-hybrid analysis and co-immunoprecipitation studies revealed unexpected associations involving CNTD1 and other regulators of cell cycle progression and/or protein modification. Importantly, CNTD1 does not appear to interact functionally with any of the obvious cyclin-dependent kinases that are operating through prophase I. Taken together, our studies reveal unexpected functions of CNTD1 that were not initially predicted for this distant cyclin family member.

T53 PRDM9 binding symmetry impacts crossover versus non-crossover recombination event resolution in mice

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In humans and mice, binding by PRDM9 positions meiotic recombination hotspots. The double strand breaks (DSBs) that initiate recombination events resolve either as crossovers (COs) or non-crossovers (NCOs), but how this decision is made remains unknown. To study this question, and unknown properties of NCOs, we mapped over 400 NCO and CO event sites in both sexes genome-wide, by sequencing F2 hybrid mice from strains representing the *M.m. domesticus* (B6) and *M. m. castaneus* (CAST) subspecies, with the B6 mouse carrying a humanized version of the PRDM9 binding domain. We obtained the highest NCO event resolution yet obtained in a mammal, revealing that NCO tracts are very short (mean 26-35bp). NCO tracts profoundly differ from those found in humans, with >90% occurring within recombination hotspots in both sexes, an absence of complex NCO events, and an overall NCO rate >4-fold lower than in humans. By ChIP-seq-based mapping of H3K4me3 and DMC1-marked DSB hotspots, we found, surprisingly, that although humanization reprograms recombination sites, the CAST PRDM9 allele is dominant.

Previous work indicated that in males, "asymmetric" hotspots, where PRDM9 mainly binds only one homologue, show anomalous DMC1 signals compared to symmetric hotspots. Here, we show that this reflects strongly delayed DMC1 processing at asymmetric hotspots. Delayed repair may help explain the reduced fertility observed in hybrids in which asymmetric hotspots predominate. Moreover, symmetry influences CO/NCO pathway choice, with COs but not NCOs strongly avoiding asymmetric PRDM9 binding sites. These distinct phenomena might reflect a mechanism whereby early-repairing DSBs are more likely to become COs, for example through interference mechanisms.

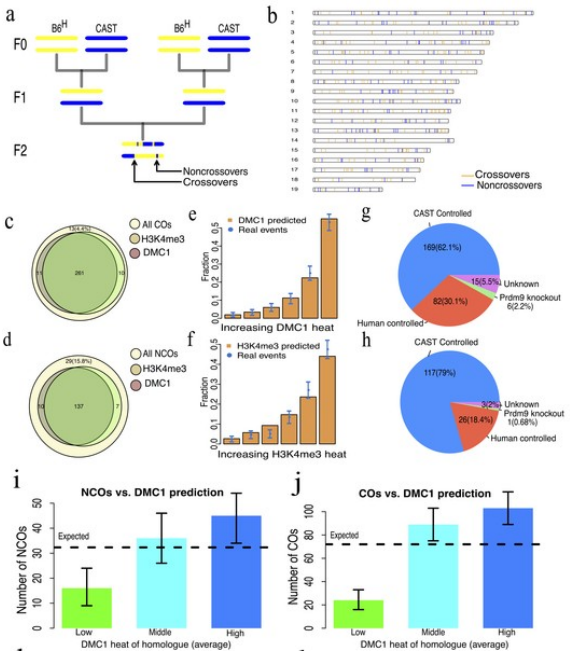

Figure 1

T54 Extensive sex differences at the initiation of genetic recombination

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Homologous recombination is essential for chromosome segregation during meiosis and understanding the differences between male and female recombination may help elucidating the reasons for higher aneuploidy rate in female germline. We provide the first genome-wide view of recombination initiation hotspots in female meiosis and find that in both male and female mice most hotspots exhibit sex-biased usage. DNA methylation mediates sex-biased hotspot activity consistent with the fact that recombination in females occurs in the context of a globally de-methylated genome. We find that sex-biased hotspots are clustered, and female-biased clusters are particularly prevalent in sub-telomeric regions. Intriguingly, recombination pathway leading to crossovers seems to be suppressed near telomeres in females, but favored in males. This work provides the basis for further studies of the factors that mediate sex differences in recombination.

T55 The mouse Shu complex SWS1-SWSAP1 is essential for meiotic recombination

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Homology recognition and DNA-strand invasion are steps in meiotic recombination that ensure faithful homolog pairing and segregation during meiosis to avoid the formation of aneuploid gametes. These central meiotic steps are catalyzed by two highly conserved recombinases, RAD51 and DMC1, with help from mediator proteins such as BRCA2. The five established RAD51 paralogs have also been implicated in regulating RAD51 activity, but their role in mammalian meiosis is poorly characterized, in large part due to the embryonic lethality associated with mouse knockouts. Furthermore, how these RAD51 paralogs collaborate with BRCA2 is not understood.

Recently, the Shu complex, composed of SWS1 and SWSAP1 subunits, was identified in human cells as a novel RAD51 paralog complex that promotes somatic homologous recombination. We generated mouse knockouts for both proteins and found that mice are viable but that both males and females are infertile. Adult females show an absence of oocytes, whereas adult males show a stage IV arrest. Few spermatocytes reach pachynema with normal homolog synapsis, and cells with abnormal chromosome structures dominate at late zygonema, although the defects are not as profound as those in a DMC1 mutant. RAD51 and DMC1 foci numbers are greatly reduced (~60%) in leptonema and zygonema. By contrast, foci of the meiotic-specific single-stranded DNA binding protein MEIOB are greatly elevated, consistent with normal DSB formation and end resection, but a deficiency in loading RAD51/DMC1. Foci that mark later recombination intermediates (MSH4) are greatly reduced. Not surprisingly, then, only a few cells progress sufficiently to have MLH1 foci and those that do show a large increase in bivalents without MLH1 foci (nearly 20%). Thus, the mouse Shu complex is critical for meiotic recombination, ensuring faithful homology search by promoting the formation of RAD51/DMC1- DNA intermediates.

We have also been characterizing a BRCA2 hypomorph in which the C terminus, known to be important for RAD51 filament stabilization in somatic HR, is deleted. These mice are viable and fertile and show only a minor defect in RAD51/DMC1 focus formation early in meiosis. However, loss of the BRCA2 C terminus in Shu-deficient spermatocytes greatly aggravates the defects in RAD51/DMC1 focus formation and homolog synapsis found in these spermatocytes, such that the chromosome structures resemble those found in a DMC1 mutant. Thus, these results demonstrate the requirement for the BRCA2 C terminus when the Shu complex is absent for the stabilization of the residual RAD51/DMC1 filaments observed early in meiosis.

T56 RNF212 Impedes DNA Break Repair to Enable Oocyte Quality Control

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Oocyte quality and number are important determinants of reproductive success (*1*). These attributes are influenced by the selective elimination of oocytes that experience problems during the early stages of meiosis. In mouse, epigenetic reprogramming during meiosis derepresses LINE-1 transposons triggering perinatal loss of more than two thirds of all fetal oocytes in a conserved process called fetal oocyte attrition (*2*). Defects in the chromosomal events of meiotic prophase also trigger oocyte loss, typically later than fetal oocyte attrition, during the early postnatal period before oocytes arrest and become quiescent (*3*). This second wave of oocyte death is mediated by interrelated pathways that signal defects is DSB repair and homolog synapsis (*3-7*). Together, these perinatal and postnatal processes balance the quality and size of the ovarian follicle reserve to maximize reproductive success. In this study, we implicate a new factor, RNF212, in postnatal oocyte apoptosis and show that it functions in a counterintuitive process that helps oocytes to gauge whether meiotic prophase was defective.

1. *J. B. Kerr, M. Myers, R. A. Anderson, The dynamics of the primordial follicle reserve. Reproduction **146**, R205-215 (2013).*
2. *S. Malki, G. W. van der Heijden, K. A. O'Donnell, S. L. Martin, A. Bortvin, A role for retrotransposon LINE-1 in fetal oocyte attrition in mice. Developmental cell **29**, 521-533 (2014).*
3. *M. Di Giacomo et al., Distinct DNA-damage-dependent and -independent responses drive the loss of oocytes in recombination-defective mouse mutants. Proceedings of the National Academy of Sciences of the United States of America **102**, 737-742 (2005).*
4. *E. Bolcun-Filas, V. D. Rinaldi, M. E. White, J. C. Schimenti, Reversal of female infertility by Chk2 ablation reveals the oocyte DNA damage checkpoint pathway. Science **343**, 533-536 (2014).*
5. *J. M. Cloutier et al., Histone H2AFX Links Meiotic Chromosome Asynapsis to Prophase I Oocyte Loss in Mammals. PLoS genetics **11**, e1005462 (2015).*
6. *L. Wojtasz et al., Meiotic DNA double-strand breaks and chromosome asynapsis in mice are monitored by distinct HORMAD2-independent and -dependent mechanisms. Genes & development **26**, 958-973 (2012).*
7. *H. Kogo et al., HORMAD2 is essential for synapsis surveillance during meiotic prophase via the recruitment of ATR activity. Genes to cells : devoted to molecular & cellular mechanisms **17**, 897-912 (2012).*

T57 Interplay between chromosome structure and meiotic recombination biochemistry

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I will address two interrelated topics, both focusing on meiotic recombination intermediate metabolism. One focuses on the recombination pathway, partner choice, and recombination intermediate resolution, with emphasis on functions of the conserved Sgs1-Top3-Rmi1 helicase-decatenase complex. The second examines the role that chromosome location, and by implication meiotic chromosome structure, plays in determining the biochemical mechanism of meiotic recombination.

T58 DNA Helicase Mph1FANCM Mediates Interhomolog Repair of Meiotic DSBs by Disrupting D-Loops between Sister Chromatids

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During meiosis, programmed DSBs preferentially undergo recombination with the homologous chromosome (homolog) instead of the equally available sister chromatid. This so called homolog bias ensures formation of crossovers as required for homolog segregation during meiosis I. DSB first end strand exchange represents a particularly critical transition during recombination. It determines whether the sister chromatid or the homolog is used as recombination partner, and whether a given interhomolog event is processed into a crossover or a noncrossover. Here, we have identified a role in homolog bias of DNA helicase Mph1. Mph1 is the presumed budding yeast ortholog of the universally conserved DNA repair factor FANCM which resides at the core of a larger protein complex defective in Fanconi anemia patients. Mph1FANCM plays demonstrated roles during DSB repair in vegetative cells. During plant and fission yeast meiosis, mutations in FANCM orthologs increase crossover formation. A lack of corresponding defects in the budding yeast mph1 mutant has raised questions about the evolutionary conservation of meiotic DNA helicase functions. We now provide evidence that Mph1 prevents recombination between sister chromatids by dismantling nascent D-loops *in vivo*, with downstream effects on crossover versus noncrossover differentiation. Mph1 is particularly critical for suppressing intersister repair during early meiosis when homolog pairing is not fully established yet. We will discuss mechanisms that direct Mph1FANCM towards strand exchange between sister chromatids, whereas strand exchange between homologs is protected from its dissolution activity. We propose that Mph1 is primarily involved in directing meiotic DSB processing towards the homolog. Among decreased interhomolog events in the mph1Δ mutant, noncrossovers are disproportionally reduced due to homeostatic crossover enhancement in response to weakened homolog bias.

T59 Dynamic suppression of Holliday junction resolution enables meiotic crossover patterning

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Meiotic crossing-over derives from the resolution of double-Holliday junctions (dHJs) by the MutLγ complex, Mlh1-Mlh3, and by three structure-selective endonucleases (SSEs): Mus81-Mms4(EME1), Yen1(GEN1) and Slx1-Slx4(BTB12). While SSEs contribute to dHJ processing, MutLγ has the unique ability to resolve dHJs at crossover (CO)-designated sites, which are spatially patterned along chromosomes. How and why cells implement pathway usage is unknown, but it has been proposed that chromosomal context plays an important role in specifying MutLγ function. Here, we show that CDK-mediated phosphorylation of Yen1 inhibits its nuclease activity and precludes its recruitment to dHJs during prophase I. Yen1 mutants refractory to inhibitory phosphorylation resolve dHJs at CO-designated sites and, moreover, restore CO formation in cells lacking MutLγ. Unexpectedly, unrestrained Yen1 function leads to a defective spatial distribution of CO events, suggesting that temporal control of dHJ processing is necessary for appropriate spacing of CO precursors along chromosomes. We propose that active suppression of Yen1 function, and by inference also Mus81-Mms4 and Slx1-Slx4, is a fundamental determinant in avoiding pathway competition and an important requirement for appropriate patterning of meiotic COs.

T60 Temporally and spatially distinct meiotic recombination pathways in mouse spermatocytes

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Meiotic recombination is not a single pathway, but is made up of multiple mechanisms to repair DNA double-stranded breaks. These repair pathways generate crossovers, which exchange chromosome arms between homologs, and noncrossovers, which are short patch-like repairs. Crossover and noncrossover recombination are important during Meiosis I to promote homolog pairing, synaptonemal complex formation, and the obligate crossover, which is required for accurate chromosome segregation. In mouse spermatocytes, most crossovers are MutLgamma-dependent and most noncrossovers are formed via synthesis-dependent strand annealing. In order to determine when and where different recombination pathways act during meiosis, we highly synchronized mouse spermatogenesis and monitored recombination in depth at hotspots. We find that half of noncrossover recombination is distributed evenly across the hotspot and fully completed during zygonema. The remaining noncrossovers are located in the central 200bp of hotspots and form coincidentally with MutLgamma-dependent crossovers in pachynema. We also find that MutLgamma-dependent crossovers are generated from the center of hotspots. We propose that early noncrossovers are important to mediate pairing, while the later noncrossovers are reserved for the crossover/noncrossover decision. By analyzing recombination in the absence of MutL amma, we observe a new class of long, co-converted noncrossovers that suggest double Holliday junction dissolution. We also document residual double Holliday junction resolution by structure-selective endonucleases. Both of these mechanisms are thought to be back up pathways that repair any remaining lesions prior to chromosome segregation. Consistent with this model, the dissolution and residual resolution signatures are found in diplonema. Finally, we analyze recombination in the absence of RNF212 and HEI10, SUMO and ubiquitin ligases, respectively. Cytological analysis of spermatocytes lacking RNF212 suggested it plays a role in crossover designation and/or MutLgamma activity. We find that in the absence of RNF212, all breaks are repaired as noncrossovers by synthesis-dependent strand annealing, providing strong support for the model that RNF212 acts early to designate crossover sites. In contrast, in the absence of HEI10 we observe an increase in double Holliday junction dissolution signature, suggesting an increase in crossover intermediates and supporting the model that HEI10 acts antagonistically with RNF212 for crossover designation.

T61 Repair delay is a key factor in the crossover/non-crossover decision in mouse meiosis

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While it has long been known that only a minority (~10%) of the double-strand breaks (DSBs) that initiate meiotic recombination in mice are resolved as crossovers (with the remainder as non-crossovers), the factors involved in this decision are not understood. We have developed a novel and reliable approach to whole-genome amplification from ultra-low DNA inputs which improves on currently available methods. This approach was used to allow whole-genome DNA sequencing from 217 individual mouse sperm, providing a new and powerful high-throughput approach to directly study meiotic recombination events across the genome. We detected more than 2500 crossovers, with the majority of these localised to within 1 kb. Nearly 300 crossovers were resolved to within 200bp, providing an unprecedented fine-scale view of crossover resolution in a mammal. Over 96% of all crossover events overlap known recombination hotspots. Comparison of stage-specific recombination markers H3K4Me3, Spo11, and DMC1 with crossovers in the same inbred mouse strain provides a powerful tool with which to unpick various aspects of meiosis. Our data establishes that DSBs which are first to pair with their homologous chromosome are more likely to be resolved as crossovers. In turn we identify a number of factors affecting repair times for DSBs, and hence the crossover/non-crossover decision. These include how likely it is that PRDM9 has bound at the same position on the homologous chromosome, distance from the (proximal) telomere, and chromatin features within 50kb of the break. We show that repositioning of nucleosomes by PRDM9 on the non-initiating, template, chromosome affects the lengths of repair tracts. Our results also shed novel light on the nature and functional definition of the pseudo-autosomal region (PAR) and the constraints on recombination within it.

P1 Identification of a new meiotic player that ensures correct germline development and progression

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Faithful transfer of genetic material is critical for the success of all cell divisions. As organisms age, this process becomes more error-prone and the mis-segregation of chromosomes leads to aneuploidy. This is the major cause of reduced fertility and inherited birth defects in the aging women. *C. elegans* offers a great opportunity to study meiosis progression, since in the adult gonad the nuclei are arranged in a perfect spatio-temporal order. Using RNAi screen for genes involved in late meiotic chromosome remodelling, we found that depletion of *ogr-2* expression leads to changes in correct chromosomal structure during diakinesis. The novel gene *ogr-2* is expressed in the gonads and has a putative SPK domain that is found in several chromatin modifiers proteins. To completely knockout its expression, we engineered a worm strain in which we deleted the entire *ogr-2* sequence by CRISPR/Cas9. DAPI staining of gonads in this strain revealed a significantly longer mitotic zone and a shorter leptotene/zygotene region pointing towards a delay in the entry into meiosis. In contrast to WT bivalents which always have only one crossover, in *ogr-2* we found bivalents with two chiasmata, suggesting reduced crossover interference. We also detected several changes in chromosomal structure in the oocytes of *ogr-2* indicating a multi-stage effect. We detected a mild shift in the dynamics of the double strand breaks repair (DSBR) as detected by RAD-51 foci, but no change in synapsis. When these two processes are not executed properly apoptosis is induced. Nevertheless, Acridine Orange staining indicated a 3-fold increase in apoptosis in *ogr-2* gonads, even though DSBR and synapsis seemed to progress normally or close to the WT dynamics. There was also an added apoptosis increase in *ogr-2;syp-1* vs *syp-1* gonads, a mutant in which both synapsis and DSBR are defective. This proves that the mutation in *ogr-2* increase apoptosis not through the canonical checkpoints. In agreement with previous reports indicating that aberrant ERK/MPK-1 activation elevates apoptosis, in *ogr-2;mpk-1*double mutant there was no increase in apoptosis. To test if OGR-2 controls apoptosis levels through ERK/MPK-1 activation, we stained the gonads for dpMPK-1, and indeed in *ogr-2* mutants it stained regions where in the WT it is silent. These results suggest that *ogr-2* has a role in controlling meiotic progression and correct chromosomal structure. We suggest that OGR-2 mediates oogenesis control, at least in part, through the MAPK pathway.

P2 Site-specific phosphorylation of SYCP3 correlates with synaptonemal complex disassembly in late meiotic prophase I

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SYCP3 is the major structural protein component of the lateral elements (LEs) of mammalian synaptonemal complexes (SCs). SYCP3 has the intrinsic capability to form highly ordered filamentous network systems and, thus, is assumed to represent the primary determinant of LE assembly. Although important for understanding of general SC function, as yet it remained largely unknown, how the assembly and disassembly of SCs and, in particular, of the LEs is regulated. In our current study, we analyzed site-specific phosphorylation of SYCP3 during meiotic prophase I and its impact on SYCP3 polymer assembly. Within the N-terminal non-helical domain we could identify an evolutionarily conserved serine residue, which was proven to be specific target of PKA. As prophase I progresses this site becomes phosphorylated and timing of its phosphorylation closely correlates with SC/LE disassembly in late meiotic prophase I. Biochemical and electron microscopical approaches suggest that phosphorylation of this serine residue is an important determinant for SYCP3 polymer formation and stability. Together, our results strongly support the hypothesis that phosphorylation of SYCP3 within its N-terminal region is involved in regulating SYCP3 disassembly during late meiotic prophase I.

P3 Disorders in Meiotic division leading to Aneusomies

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Aneusomies including trisomies are the most common chromosomal anomalies seen in live born. The most common of which is Trisomy 21 (Down syndrome), also including trisomy 18, 13. Monosomies also are aneusomies with missing chromosome, with the famous Turner syndrome, Monosomy X is the most common. Most aneusomies are the result of parental chromosomal non dysjunction in meiotic division, either in first or second meiotic division. Hence this highlights the role of abnormal meiosis in human malformations.

P4 The RNA-binding protein PUF-8 facilitates homologous chromosome pairing during meiosis by promoting dynein mediated chromosome movements in *C. elegans*

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Proper pairing of homologous chromosomes during meiosis is crucial for gametogenesis. While the roles of several components of the pairing machinery are known, regulation of the pairing process is still poorly understood. Here, we show that the *C. elegans* PUF-8 — a conserved PUF family translational regulator — controls the pairing event, in coordination with the proteasome. In a genetic screen, we isolated a hypomorphic allele of *pas-1*, as an enhancer of the *puf-8* mutant phenotype. *pas-1* encodes a subunit of the 20S proteasome complex, While the *puf-8* and *pas-1* single mutants are fertile, the *puf-8; pas-1* double mutant worms are sterile; they make sperm but do not produce any oocytes. Staining with DAPI, a DNA-binding dye, showed morphological abnormalities of meiotic chromosomes. This was confirmed firstly by the observation that SYP-1, a component of the central element of the synaptonemal complex is mislocalized in the *puf-8; pas-1* double mutant. SYP-1 normally localizes along the length of the paired chromosomes, however, in *puf-8; pas-1* germlines; SYP-1 forms poly-complexes at specific foci. Secondly, immunostaining against ZIM proteins showed that homologous chromosomes fail to pair in *puf-8; pas-1* double mutant. Consistently, recombination intermediates accumulate and number of cross-overs are decreased in the *puf-8; pas-1* double mutant. All these meiotic defects lead to germ cell death, hence the absence of oocytes. To identify the cause of chromosome-pairing defects in *puf-8; pas-1* germlines, we investigated the distribution patterns of proteins such as SUN-1, ZYG-12, and DHC-1 which concentrate in to specific "patches" on the nuclear envelope (NE). These patches connect dynein in the cytoplasm to chromosomes in the nucleus and mediate movements that help in pairing. While SUN-1 and ZYG-12 formed patches on NE in the *puf-8* and *pas-1* single mutants, they were uniformly distributed on NE in the double mutant germline. Consistently, chromosomal movements are reduced or absent in *puf-8; pas-1* worms. We propose that translational control by PUF-8 and proteasome-mediated protein degradation act in concert to promote the dynein mediated chromosomal movements, possibly by regulating the levels of factor(s) yet unknown.

P5 Effects of parental aging in *Nothobranchius furzeri* on embryo development and growth

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Studies on parental aging are a very attractive field although it is poorly understood how parental age affects embryonic development and the effects on adult traits of offspring.
For years, researches have tried to uncover the underlying biological processes responsible for aging wondering whether aging is the primary result of an evolutionary process that confers some benefit or it is the result of the age-dependent decline in the force of selection and of antagonistic pleiotropism between developmental an repair processes, as stated by the classical theories of aging.
It has recently been argued the progression of the aging process by developmental events 1.
In this study, we used the killifish Nothobranchius furzeri, an interesting aging vertebrate model with the shortest lifespan 2,3 .
The embryos from N. furzeri are able to either enter into or skip diapause, giving rise to different developmental pathways. Thus, this embryo plasticity allows this model to be used to study different factors that could affect their embryonic development including parental age.
The first goal of the present study was to highlight if parental aging could affect the embryo development. To do this, we collected F1 embryos from two breeder groups (old parents and young parents). We monitored the duration of embryonic development and analyzed genes involved in dorsalization process. The second goal was to investigate if embryos developmental plasticity could be modulated by an epigenetic process. Regarding this, the expression of dnmts genes was examined. Our data supported the hypothesis that diapause, mostly occurring in embryos from old parents, is associated with the increase of dnmt3a and dnmt3b expression suggesting an epigenetic control.
Finally, we analyzed whether parental age could affect metabolism and growth during adult life. Morphometric results and qPCR analysis of genes from IGF system showed a slower growth in adults from old breeders. Moreover, a gender-specificity growth emerged.
In conclusion, these results may contribute to the better understand the complex mechanism of aging.

1. *de Magalhães, J.P. FASEB (2012), 26: 4821–6.*
2. *Terzibasi, E. et al. Exp. Gerontol (2007) 42: 81–9.*
3. *Cellerino, A. et al. Biol Rev Camb Philos Soc. (2016) 91:511–533.*
4. *Furness, A. I. et al. Evolution (N. Y) (2015) 69:1461–1475.*

P6 Influence of temperature on recombination in barley

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Breeding work relies fundamentally on recombination but the control of this process is not fully understood especially in crop plants. In barley the distribution of meiotic crossover events is highly skewed, meaning that substantial proportions of the chromosomes are inherited together as large linkage blocks, preventing the generation of novel gene combinations and useful variation that could be exploited in breeding programmes. An ability to modify the pattern of recombination in these species could therefore have profound impact on the breeding of the crops. As part of a recently funded Marie Curie ITN entitled Control of Meiotic Recombination: *Arabidopsi* to Crops (COMREC) co-ordinated by Prof FCH Franklin (University of Birmingham) we are investigating the effect of temperature on recombination in barley at the James Hutton Institute. This could provide the means to manipulate recombination within a breeding programme as abiotic stress such as high temperature can affect the distribution of chiasmata. The initial experimentation has concentrated on the genetic mapping of progeny from F_1 plants that have been subjected to a different periods of temperature stress. This work has confirmed the reported effect of temperature in the distribution of crossovers in barley showing increases in recombination in peri-centromeric regions in the genetic maps derived from F_2 progeny of heat stressed F_1 plants. Having developed an experimental higher throughput working platform to reduce the developmental and temporal variation between plants, research is now focused on the dissection of the critical time interval for the effect on crossovers and recombination. This platform has also enabled transcriptomic studies to be carried out using pre-meiotic and early meiotic anthers under control and heat stress conditions and a wider range of barley lines to be studied to enable studies into the correlation of temperature response and eco-geographical variables. The results of these and other experiments will be presented.

P7 Enhanced occurrence of mutations during yeast meiosis

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One of the central roles of meiosis is to create new combinations of genetic material, which are uniquely transmitted to the next generation. While recombination, independent assortment and random meeting of gametes shuffle pre-existing sequences to form new haplotypes, only mutations can produce new genetic information, i.e. novel alleles. Old genetic experiments found higher mutation rates in meiosis compared to mitotic cells, and these meiosis-induced mutations were associated with recombination events[1]. In accordance, we show that mutation rates during meiosis are 3-10 fold higher than in mitotic cell divisions, in two independent loci. We find a firm dependence of meiotic mutations on spo11-induced DNA double-strand breaks (DSBs), as has previously been shown[2]. We present new data indicating that most mutations do not occur during the pre-meiotic S-phase, but during prophase I, at the time of recombination. Our data tighten the connection between meiotic mutations, DSB formation and recombination.

DSB-repair, including Homologous Recombination (HR), involves new DNA synthesis, offering opportunities for new mutations. Whereas meiotic DBS repair is achieved by canonical DNA polymerases such as Pol Delta & Epsilon[3], Trans-Lesion DNA polymerases (TLSPs) may also operate. TLSPs have a well established function in synthesizing across DNA lesions during DNA replication, but they are also involved in DNA synthesis associated with DSBs repair by HR[4]. We suggest that TLSPs might be involved in the processing of meiotic DSBs and thus lead to the enhanced mutations observed in meiosis.

We have recently shown that the three TLSPs of budding yeast, Rev1, PolZeta (Rev3 & Rev7) and Rad30, are induced during meiosis, at a time when DSBs are formed and homologous chromosomes recombine[5]. The absence of all three TLSPs leads to a reduction in both allelic and ectopic recombination. Yeast-Two-Hybrid (Y2H) tests in meiosis-arrested cells further support association between TLSPs and DSB-proteins - Rev1-Spo11, Rev1-Mei4, Rev7-Rec114, as well as between Rev1 and Rad30[5]. To reveal and characterize new mutations in meiosis we apply whole-genome sequencing of meiotic products to strains that have undergone 40 consecutive meioses (MMA lines), which were accumulating mutations during this process. We find that TLSPs contribute to mutation accumulation around DSB Hot spots.

Our results highlights *de novo* mutagenesis as an additional layer contributing to genetic diversification in meiosis. We suggest that the involvement of TLSPs in processing of meiotic DSBs could be responsible for the considerably higher frequency of mutations observed during meiosis and therefore may contribute to faster evolutionary divergence than previously assumed.

P8 Hybrid breeding: From *Arabidopsis* to crops

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The combination of different genomes often results in strongly enhanced plant vigor. This heterotic effect is often used in breeding programs and the it is the base for hybrid breeding. However, a major obstacle in hybrid breeding is that heterosis cannot be easily predicted; hence hybrid production often requires an empirical approach by selecting random parental lines and testing their offspring for enhanced performance. Here, we present a novel tool for hybrid breeding, i.e. the generation of partial hybrids and the reconstitution of a preselected best performing hybrid. This approach is based on the fact that genetic interactions in heterozygous hybrid plants are not always beneficial. Thus, focusing on only a few, positive interaction has the great potential to boost hybrid performance even further. In addition, partial hybrids allow the utilization of new genetic interactions that are not present in conventional hybrids. Our technique is based on the decrease of recombination frequencies during meiosis by *knocking down* the crossover regulator *MSH5* by Virus-Induced-Gene-Silencing (VIGS).

P9 The role of cohesin regulators in budding yeast meiosis

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Cohesin is a ring-shaped complex that holds sister chromatids together during mitosis and meiosis. During mitotic prophase in higher eukaryotes, cohesin is removed non-proteolytically from replicated sister chromatid arms through the action of Wapl. However, a subset of cohesin is protected from the destabilizing effects of Wapl because acetylation of the Smc3 subunit of cohesin allows binding of sororin. Phosphorylation events prevent sororin associating with chromosome arms, making cohesin susceptible to removal by Wapl. In contrast, at centromeres, shugoshin, with its binding partner the protein phosphatase, PP2A, counteracts these phosphorylation events to allow sororin binding, thereby protecting centromeric cohesin from Wapl. During meiosis, cohesin is proteolytically cleaved on chromosome arms during meiosis I, but protected from cleavage at centromeres until meiosis II. It is unclear, however, if the destabilizing activity of Wapl contributes to cohesin removal on chromosome arms during meiosis I or whether mechanisms are required to counteract Wapl activity near centromeres. I aim to address this question using budding yeast. Previous work showed that Wapl (Rad61) is expressed during meiosis, and that deletion of *RAD61* results in an increase in cohesin levels on chromosomes during prophase. Consistently, I found that Eco1 acetyltransferase is expressed during S phase of meiosis, and this expression coincides with Smc3 acetylation. Further findings suggest a critical role for Eco1-dependent acetylation in chromosome segregation during meiosis. I am currently investigating the relationship between Wapl-dependent cohesin loss and the mechanisms that protect cohesin at centromeres. My latest findings will be presented.

P10 PRDM9 methyltransferase activity is required for specifying meiotic recombination initiation sites in mouse

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An important feature of the prophase of first meiotic division is the programmed formation of several hundred DNA double-strand-breaks (DSBs), which are repaired by homologous recombination with the homologous chromosome. The recombination events play essential roles for ensuring accurate segregation of homologous chromosomes from maternal and paternal origins by participating in the process of recognition and pairing between them, and by generating crossing-over, which provide a physical link between homologous chromosomes that is essential to their segregation. We are interested in the controls of the frequency and distribution of meiotic DSBs.
The Prdm9 gene, which is expressed specifically during meiotic prophase, is the major local scale determinant of meiotic recombination sites in several mammals including humans and mice. PRDM9 is a PR/SET domain histone methyltransferase and contains a KRAB domain, most likely involved in interacting with other proteins, and a tandem array of C2H2 zinc fingers that recognize specific DNA motifs in the genome, where it is thought to promote histone H3 K4 and K36 trimethylations.
At sites bound by PRDM9, SPO11 is recruited by an unknown process and generates DSBs. In the absence of PRDM9 protein (Prdm9 KO mice), DSBs are formed at alternative sites characterized by PRDM9-independent H3K4me3 enrichment, such as transcription promoters. This raises the questions of the roles of PRDM9 interactions with other proteins and of its methyltransferase catalytic activity.
By using transgenic mice producing a catalytically inactive PRDM9 protein, we sought to understand the specific role of PRDM9 methyltransferase activity in specifying recombination sites, and more generally in supporting the progression throughout meiotic prophase in order to generate gametes. By characterizing this model, we showed that PRDM9 catalytic activity is required for H3K4me3 and H3K36me3 enrichments at recombination sites, and that this activity is required for DSB activity at these sites.
Strikingly, PRDM9 zinc finger array is extremely variable, and a large number of PRDM9 zinc finger variants, specifying each a set of recombination sites coexist in the same species. The functional relationship between PRDM9 zinc finger variants in heterozygous individuals has been the subject of debate. Our mouse model expressing two different PRDM9 zinc finger variants, one being functional and the other catalytically inactive, allowed us to gain insight into the functional interaction between PRDM9 variants and more broadly into PRDM9 molecular function.

P11 Super-resolution microscopy and quantitative EM tomography of mouse meiotic chromosomes

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Meiotic chromosome synapsis depends on the formation of meiosis-specific axial structures: synaptonemal complexes and cohesin axes. Furthermore, meiotic chromosomes are highly dynamic during the first meiotic prophase. Chromosome movement depends on telomere attachment to the nuclear envelope and forces generated in the cytoplasm by the cytoskeleton. The correct progression of directed chromosome movement and synapsis are interdependent processes that are essential for the faithful segregation of homologous chromosomes. Failure in any of these processes leads to massive meiotic defects and often to infertility. Thus, defining the molecular organization of the meiotic chromosome axial structures (i.e. synaptonemal complexes and cohesin axes) and their interaction with the nuclear envelope (i.e. attachment plates) are key aspects for the understanding of genome stability in the germ line. To this end, we are applying one- and two-color super-resolution imaging (i.e. *d*STORM) in combination with average position determination with nanometer precision. In parallel, these structures are analyzed by electron microscope tomography. New quantitative data obtained with the aid of these techniques will be presented and discussed.

P12 PCH-2 coordinates meiotic prophase events to ensure their fidelity

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During meiotic prophase, the events of homolog pairing, synapsis and recombination are coordinated to ensure that they result in recombinant chromosomes that can undergo proper meiotic chromosome segregation. We have hypothesized that the AAA-ATPase PCH-2 contributes to this coordination by disassembling intermediates that support pairing, synapsis and recombination to ensure their fidelity. This model, called kinetic proofreading, has also been used to explain the involvement of ATPases in the precision of splicing and vesicle fusion events. However, one weakness of our model was the inability to visualize inappropriate pairing, synapsis or recombination intermediates in the absence of PCH-2. We now report that pch-2 mutants that bind but fail to hydrolyze ATP (pch-2E253Q mutants), potentially preventing the release of PCH-2 substrates, exhibit non-homologous synapsis, in support of our model. We hypothesize that meiotic HORMA domain containing proteins (HORMADs), required for pairing, synapsis and recombination, are PCH-2's relevant substrates. We will present data demonstrating a genetic interaction between PCH-2 and HTP-3, a meiotic HORMAD in *C. elegans*, implicating HTP-3's HORMA domain as a key regulator of synapsis.

P13 Chromosome axis promotes recombination during mammalian meiosis

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Meiotic recombination is a defining event of mammalian meiosis that is required for proper chromosome segregation to next generation. It is initiated by SPO11-induced DNA double-strand breaks (DSBs) at recombination hotspots. The hotspots are activated by histone H3K4 trimethylation marks placed by the DNA-binding protein, PRDM9. During leptonema, when recombination events are initiated by these proteins, chromosomes also initiate the formation of axial elements (AEs) that incorporate meiosis-specific cohesin proteins. Most recombination repair-associated proteins, and the developing recombination nodules, are found on the AEs. Moreover, mutation of any of the meiotic cohesin proteins perturbs AE formation as well as recombination and/or DSB repair. These spatial, temporal and functional correlations of meiotic recombination initiation with AE formation prompted us to determine how the processes are related mechanistically. Both ChIP-seq and cytological data from multiple cohesin mutant models provide evidence that AE during mammalian meiosis has a scaffold-like function that promotes meiotic recombination initiation. Further, PRDM9 and meiotic cohesin protein STAG3 interact and synergistically promote meiotic DSB formation. Resolving these molecular relationships among recombination sites, DSBs and the chromosomal axis elucidates an underlying mechanism of chromosome dynamics essential for meiosis. Supported by the NIH, P01 GM99640 to KP and MAH, and JAX Scholar award to TB.

P14 Dissecting the role of cohesin complexes in murine spermatocytes

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Formation of proper chromosome axis and synapsis of homologous chromosomes are essential for meiosis I and orchestrate programmed double strand break repair and recombination events that are critical for genome haploidization in sexually reproducing organism. Chromosome dynamics during meiosis is regulated by the cohesin complex. In cohesin, SMC3 forms a heterodimer with either SMC1a or the meiosis specific SMC1b. SMC1a complexes are present but decline during prophase I. It is unclear why meiocytes feature two SMC1 variants. To address this question we expressed SMC1a driven by the *Smc1*b promoter in *Smc1b*$^{-/-}$ mice and asked if SMC1a can rescue some or all of the *Smc1b*$^{-/-}$ phenotypes. We found that SMC1a over-expression largely rescues deficiencies in axis formation, autosomal asynapsis, double strand break processing and sex chromosome synapsis in *Smc1b*$^{-/-}$ spermatocytes. The pachytene checkpoint arrest seen in *Smc1b*$^{-/-}$ spermatocytes is partially relieved, since 40% of SMC1a over-expressing *Smc1b*$^{-/-}$ spermatocytes are positive for H1t (a meiosis specific histone variant) compared to 1% in *Smc1b*$^{-/-}$, and these spermatocytes proceed up to late pachytene. However, they are unable to desynapse their chromosomes. We speculate that either the SMC1b cohesin complex is specifically involved in desynapsis, or that SMC1a over-expression prevents desynapsis, but more indirect mechanism may also stop development at that stage. SMC1b plays a specific role in telomere protection as SMC1a over-expression cannot rescue telomere deficiency phenotypes seen in *Smc1b*$^{-/-}$ spermatocytes. In absence of SMC1b, dysfunctional telomeres trigger an aberrant DNA damage response, indicated by mis-localisation of several recombinational proteins, particularly at the telomeric ends in both early and late prophase of meiosis I. Taken together, these results show certain overlapping functions of SMC1a and SMC1b, as well as specific functions of SMC1b cohesin in meiosis I.

P15 Alteration of local PRDM9 binding symmetry without PRDM9 allele changes completely restores successful synapsis on individual chromosomes, and fertility, in inter-subspecific mouse hybrids

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In mice and humans, the histone methyltransferase PRDM9 controls recombination genome-wide by positioning double strand breaks (DSBs) at hotspots during meiosis. Incompatibility between distinct Prdm9 alleles causes a range of fertility phenotypes in male hybrids of specific mouse subspecies.
In hybrids, PRDM9 can bind "asymmetrically", whereby only one of the two homologues is strongly bound, or symmetrically. Previously, we compared male hybrid mice with different PRDM9 alleles. Increasing levels of genome-wide asymmetric binding by PRDM9 predicted increasing rates of asynapsis, and reduced fertility including ultimately sterility [1]. Here, we investigate how symmetry/asymmetry impacts proper synapsis on individual chromosomes, and how this is driven by particular genomic regions. We use an orthogonal approach, fixing PRDM9 alleles but perturbing the genetic background and hence symmetry. We generated (PWDx(B6/CAST))F1-3 hybrid males, which differ from sterile (PWDxB6)F1 mice only by carrying introgressed segments of CAST DNA in place of B6. In mice carrying PWD and B6 PRDM9 alleles, a lack of evolutionary hotspot erosion in CAST means that PRDM9 binding symmetry is dramatically increased in CAST segments.
We observed a spectrum of asynapsis rates, and fertility from sterility to normal fertility, across 60 mice. Fluorescence in situ hybridization (FISH) analysis showed that symmetric hotspot-carrying CAST segments, even if only ~20Mb in size, consistently and completely restore synapsis of individual chromosomes. Because CAST, PWD and B6 are all approximately equally evolutionarily diverged genome-wide, this cannot be explained by the level of sequence differences outside PRDM9 binding sites. Moreover a model assuming independent synapsis of each autosome, and based only on CAST introgression levels, showed >90% correlation with observed synapsis rates genome-wide across 24 mice ($p<10^{-15}$). Statistical analysis estimated that each DSB within a CAST segment is associated with a >50% chance of proper chromosome-wide synapsis. Finally, DMC1 ChIP-Seq data we generated indicated reduced DMC1 level on CAST-containing chromosomes even outside the introgressed regions themselves, and suggesting increased DSB repair rate relative to non CAST-containing chromosomes. This strongly supports a mechanism whereby DSBs occurring at even a small number of symmetric PRDM9 binding sites show rapid repair, can "seed" synapsis chromosome-wide, and aid repair of DSBs at distant, asymmetric, sites.

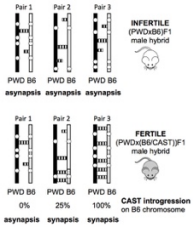

Figure 1 Homologues in (PWDxB6)F1 and (PWDx(B6/CAST))F1 male hybrid mice differ by a similar number of SNPs (circles). Upon CAST introgression (grey), synapsis is rescued by increased symmetric PRDM9 binding (striped rectangles), and fertility is restored.

P16 Spo11-independent recombination in the *Dictyostelium* sexual cycle

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Social amoebae are protists with an unusual and poorly understood sexual cycle. These amoeba proliferate as haploid unicells; zygotes do not proliferate, instead growing cannibalistically to form large, walled cysts. These macrocysts are believed to undergo meiosis before germinating to release haploid progeny. Although their genomes encode a number of genes involved in meiosis, social amoebae are unusual in having lost Spo11 and also ATM, which are critical for the controlled formation of double strand breaks during meiosis. By characterising for the first time genome-wide patterns of recombination in progeny from crosses between all three *Dictyostelium discoideum* mating-types, I show here that crossovers occur at high frequency in the sexual cycle of these organisms. I will discuss possible mechanisms underlying this apparently Spo11-independent mode of recombination and speculate further on the selective pressures that may have driven its evolution.

P17 Robust DSB repair and crossover formation require Mes19 during meiosis in mice

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Accurate segregation of homologous chromosomes (homologs) during the first meiotic division depends on inter-homolog crossovers that are formed by homologous recombination during the preceding prophase. Formation of crossovers is initiated by the introduction of DNA double-strand breaks (DSBs) into the genome. Intriguingly, whereas numerous DSBs form on each chromosome, only few of them, typically one per homolog pair, are turned into crossovers in mice; most DSBs are repaired as non-crossovers. Timely repair of recombination intermediates and the designation of a subset of them as crossovers are thought to require that MSH4/MSH5 (MutSγ)-associated recombination complexes are destabilised at non-crossover sites and selectively stabilised at crossover sites. MutSγ complex stabilisation depends on the SUMO-ligase RNF212 (Reynolds A. *et al., Nat Genet* 45, 269-278, 2013) and is opposed by the activity of the ubiquitin-ligase HEI10 (Qiao H. *et al., Nat Genet* 46, 194-199, 2014) and the cyclin-like protein CNTD1 (Holloway J. *et al., J. Cell Biol* 205, 633-641, 2014). Thus, a poorly understood interplay of these proteins was suggested to drive crossover designation, which enables future crossover sites to recruit MLH1/MLH3 (MutLγ) complexes that likely represent crossover-specific resolvases. Recently we identified a meiosis-specific protein MES19 (for MEiosis-Specific 19) which forms foci that co-localise with, and depend on MutLγ. This suggested a role for MES19 in late stages of crossover formation. Indeed, crossover numbers are dramatically reduced in *Mes19*-deficient cells. Interestingly, MES19 is also required for timely repair of non-crossover recombination intermediates. We found that both RNF212 foci and markers of incomplete DSB repair, including γH2AX flares and foci of RPA and MSH4, abnormally persist beyond mid-pachytene in *Mes19*$^{-/-}$ spermatocytes. A similar delay in DSB repair has been observed in *Cntd1*- and *Hei10*-deficient mice. Thus, we propose that MES19 is a new critical member of the crossover formation-promoting protein network, and it likely functions in the crossover-designation process alongside HEI10 and CNTD1.

P18 Genetic screen to identify new regulators of meiotic progression in *Arabidopsis*

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Meiosis is a conserved cell division that produces haploid cells from diploid parental cells, which is prerequisite for sexual reproduction and gamete formation. Transition from the mitotic to meiotic mode of chromosome partitioning requires extensive remodeling of the cell cycle machinery. In plants, mitotic cell division occur immediately after completion of meiosis to form mature gametophytes (embryo sac and pollen). In our previous work we showed that non-sense mediated RNA decay factor SMG7 and plant specific protein TDM1 are required for completion of meiosis and transition to subsequent mitotic divisions in *Arabidopsis*. The *smg7-1* null mutant shows arrest in anaphase II that is associated with delayed chromosome decondensation and aberrant rearrangement of the spindle. In contrast, phenotype of a weak *smg7-6* allele resembles TDM1 inactivation: haploid nuclei formed after second meiotic division re-condense, form bipolar spindles and attempt to undergo a third meiotic division without chromatin re-replication. This leads to the formation of multiple nuclei with unevenly distributed genetic material and to a reduced number of viable pollen. We proposed that activity of TDM1 and SMG7 enables remodeling of the cell cycle machinery to exit meiosis and enter subsequent mitotic divisions. To shed a light at molecular pathways controlling meiotic exit, we performed a genetic suppressor screen to identify mutations that increase fertility of *smg7-6* plants. We have obtained 90 mutant lines with increased fertility and candidate causative mutations were identified in selected lines by next generation sequencing. One of these suppressor lines presents a novel mutation in the putative phosphosites of TDM1. These findings reinforces the notion that posttranslational modifications constitute the main regulatory mechanism in the context of meiosis. Functional analysis of this mutation is in progress.

P19 The *C. elegans* Argonaute CSR-1 is Required for P granule Integrity and Meiotic Progression.

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Several aspects of the mechanisms that control cell fate decisions remain unknown. In metazoans, germ cells undergo proliferation and regulated meiotic entry. These regulatory decisions are thought to depend on germ granules, non-membrane bound structures found at the nuclear periphery of germ cells. Similar to other RNA-protein assemblies, germ granules form by phase separation, and are hubs for RNA and RNA regulators, including Argonaute (AGO) proteins, the critical effectors in small RNA pathways. Although AGO proteins are well known to function in translational control, RNA regulation, and epigenetic inheritance, their role in germ granule formation and function, and germline development remains unclear. We have been investigating the role of CSR-1, the only essential AGO in *C. elegans*, in germ granule assembly and germline development. We find that CSR-1 activity is required for maintenance of germ granule's biophysical properties, the robustness of the mitosis-to-meiosis switch, and meiotic progression. Our results reveal a function for AGO pathways as promoters of germ granule integrity and function, and suggest that germ granules may be critical for the robustness of cell fate decisions. Results from this work will help uncover mechanisms that may operate similarly in somatic cells under stress conditions, when rapid switches are required, as well as the unique biology of stem cells.

P20 Asymmetric phosphorylation of the synaptonemal complex promotes meiotic chromosome segregation

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Accurate chromosome segregation in meiosis requires the regulated two-step loss of cohesion between the first and second meiotic divisions. While centromeres serve as the locus of cohesion retention in most organisms, the nematode *C. elegans* which lacks single centromeres has innovated special mechanisms to determine this locus *de novo* for each chromosome at each meiosis. In late prophase of meiosis, several synaptonemal complex proteins localize asymmetrically into one of two domains defined by the site of the single off-center crossover. The domain from the crossover to the nearest telomere, termed the *short arm*, eventually becomes the first site of cohesion loss in meiosis I; while the domain from the crossover to the farthest telomere, termed the *long arm*, is where cohesion is retained until meiosis II. How an event at the molecular scale (DNA break repair resulting in crossing-over) is able to cause the robust establishment of chromosome-wide functional domains, in a manner sensitive to the distance to the chromosome end, remains unknown. The current work shows that phosphorylation of the synaptonemal complex component SYP-1 in early meiotic prophase is required for early steps in establishing this short and long arm distinction. Once COs are made, phosphorylated SYP-1 and its binding partner PLK-2 become asymmetrically localized to short arms and removed from long arms. This asymmetric distribution is required for the establishment of functionally distinct short and long arms in diplotene and diakinesis. When phosphorylation of SYP-1 is prevented, asymmetric chromosome domains are not formed. This leads to aberrant localization of phosphorylation of the axis component HIM-3, as well as phosphorylated histone H3 and the Aurora B kinase (AIR-2), resulting in chromosome segregation errors and progeny inviability. Our results show that PLK-2 bound to SYP-1 acts upstream of a mechanism that ensures confinement of the chromosome segregation machinery to the short arm subdomain and promotes correct segregation of chromosomes in meiosis I.

P21 A role for CENP-V in mammalian meiosis

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Kinetochores are composed of several functional units. The inner kinetochore mediates the direct contact to the centromere; the outer kinetochore ensures the attachment of spindle microtubules. Our project aims at elucidating the role of CENP-V in mammalian meiosis, a little-described centromere component in mitotic cells (1). CENP-V is conserved among vertebrates and in HeLa cells localizes to the mitotic kinetochores from prometaphase to metaphase. CENP-V then moves to the spindle mid-zone in anaphase, similar to the chromosomal passenger complex (CPC) proteins. Knock-down of CENP-V in HeLa cells leads to decompaction of chromosomes, decondensation of the heterochromatin and to mislocalization of CPC proteins. Metaphase alignment of chromosomes in CENP-V depleted HeLa cells is impaired and lagging chromosomes appear in anaphase. Cells that overexpress or are deleted for CENP-V eventually die as they cannot perform proper chromosome bi-orientation and cytokinesis (1). Nothing is known about a role of CENP-V in meiosis. We found CENP-V present in spermatocytes at pachynema, diplonema and metaphase I on all chromosomes except the sex chromosomes. CENP-V is enriched in the pericentromeric heterochromatin. We have recently generated CENP-V deficiency mouse models to study the role of CENP-V throughout meiosis. Spermatocytes lacking CENP-V show a few chromosomes that appear to be fused at their centromeres. In oocytes, preliminary data suggest reduced GV oocytes, a delay in MI, decreased GVBD efficiency leading to impaired polar body extrusion. Together this suggests an important role of CENP-V in meiotic chromosome structure and behavior.

1. *Tadeu AM, et al. EMBO J. 2008 Oct 8;27(19):2510-22.*

P22 Mi2 paralogs CHD-3 and LET-418 promote homologous recombination and ensure gamete quality in *Caenorhabditis elegans*.

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Meiotic progression requires the initiation of programmed, genome-wide double-stranded breaks (DSBs). Unrepaired DSBs result in defective gametes, which manifest as spontaneous abortions, infertility and developmental disorders. Accordingly, faithful repair of DSBs is accomplished by a series of conserved mechanisms which must take place within the constraints of a specialized chromatin architecture. Here, we demonstrate a role for the nucleosome remodeling and deacetylase (NuRD) complex in DSB repair, wherein the conserved Mi2 homologs Chromodomain helicase DNA binding protein (CHD-3) and its paralog LET-418 promote normal meiotic progression by attenuating meiotic checkpoints and ensuring faithful repair of DSBs through homologous recombination (HR). In loss-of-function *let-418* mutants, persisting recombination intermediates are detected, which is coincident with elevated checkpoint-dependent germline apoptosis. These data are corroborated by the presence of phosphorylated CHK-1 protein as well as elevated expression of the pro-apoptotic gene *egl-1*. Additionally, we uncovered a previously unreported role for CHD-3, whose loss alone results in the activation of meiotic checkpoints, a reduced brood size, and mild defects in the repair of DSBs, all of which are exacerbated when combined with *let-418* hypomorphs. Interestingly, we also discovered that when non-homologous end joining (NHEJ) is compromised, *let-418* germ lines are highly disorganized and repair of meiotic DSBs is severely disrupted. We are currently generating several strains which will enable us to further understand the molecular nature of these defects. Taken together, these data support a model wherein LET-418/CHD-3 maintain genomic stability through reinforcement of a chromatin landscape suitable for HR-driven repair mechanisms.

P23 Defining recombination hotspots in tomato

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During meiosis, homologous chromosomes (homologs) recognize each other and perform reciprocal exchanges (crossovers or COs) at a high frequency. In most eukaryotes, both homolog recognition and CO formation require inter-homolog recombinational interactions, which the meiotic prophase cell initiates by formation of double-strand DNA breaks (DSBs). The formation of COs is essential for proper meiotic chromosome segregation and contributes to the genetic diversity of gametes. In food crops, the genetic variation is limited and will soon become insufficient to meet the demands of an increasing world population. Sources of genetic variation are provided by related wild species. However, due to sequence divergence, recombination between species (homeologous recombination) is limited as compared to homologous recombination. To improve the transfer of genes that convey e.g. tolerance to changing climate conditions or pathogen resistance from wild to cultivated species, a better understanding and control of homeologous recombination is crucial. In this study we aim to analyze the genome-wide distribution of recombination events in tomato (*S. lycopersicum*), its wild relative S. pimpinellifolium and their F1 hybrids by ChIP, using antibodies against proteins involved in meiotic recombination. A pilot experiment has shown that the MLH1 protein binds mostly in the telomeric region and overlap with the genes. Development of a tomato meiotic nuclei ChIP procedure will be presented and results will be discussed.

P24 Biochemical studies of the *S. cerevisiae* meiotic DNA double-strand-break machinery

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Meiotic recombination is crucial to sexual reproduction as it allows the accurate segregation of homologous chromosomes necessary to form haploid gametes. Recombination is initiated by DNA double strand breaks (DSBs) made by Spo11 protein. Spo11 evolved from the DNA cleaving subunit of an ancestral type IIB topoisomerase (Topo VI). DNA strand breakage happens through a catalytic tyrosine on the protein carrying out a nucleophilic attack on the phosphodiester backbone, which generates a 5′ phosphotyrosyl protein-DNA covalent complex. Two Spo11 monomers work in concert to cleave both strands of the DNA duplex. Spo11 is then released by endonucleolytic cleavage and the DNA breaks are further processed and repaired by homologous recombination. So far, biochemical investigations of the initiation of meiotic recombination have been lacking, largely due to the insolubility of Spo11. We have recently solved this problem by co-expressing *S. cerevisiae* Spo11 together with some of its interacting partners and have purified soluble complexes. We will present our latest progress in characterizing their structure, protein-protein interactions and protein-DNA interactions.

P25 Meiosis and Recombination in Large Genome Crop

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All breeding work relies fundamentally on recombination but the control of this process is not fully understood especially in crop plants. A greater understanding of the control of recombination in crop plants would enable manipulation of this process to improve the speed and accuracy of plant breeding. This would be particularly useful for crop species such as barley (and wheat) where a highly skewed distribution of meiotic crossover events means that up to half of the genes rarely, if ever, recombine. Barley (*Hordeum vulgare* L.) is an inbreeding, diploid species with a 5.1 Gb genome organized into seven pairs of chromosomes. The presence of ring bivalents at metaphase I reflects the distal distribution of chiasmata. Barley is fortunate in having a considerable collection of characterised morphological mutants that include a number of 14 non-allelic desynaptic (*des)* mutants that exhibit perturbed meiosis and semi-sterility compared to wild type. A number of these mutants are currently being genetically mapped using the semi-sterility phenotype and cytologically characterized using 3D-SIM.

Taking advantage of the large barley genome and 3D-SIM and the use of non-disruptive fixation, here we describe the synapsis progression of a number of our mutants. We also revealed a new structure during the transition pachytene to diplotene. These structures, which we have named *tinsel* chromosomes, have not been previously described though they bear some structural similarities to the lamp-brush chromosomes of amphibian and avian oocytes. They are however a manifestation of the synaptonemal complex lateral elements remodelling that may not dependent on full synapsis.

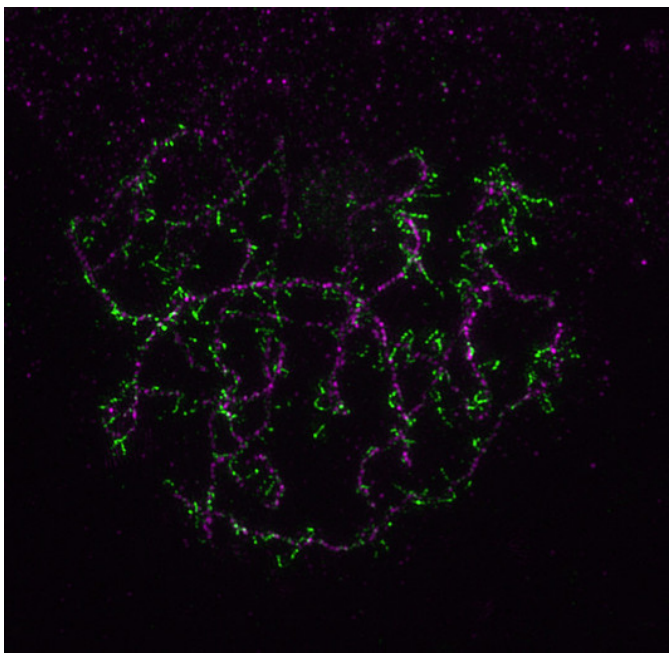

Figure 1 Tinsel chromosome in barley

P26 The molecular architecture of the bipolar spindle is remodelled during long oocyte arrest

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Oocytes of most species assemble and maintain a functional bipolar spindle in the absence of centrosomes. Strikingly, after bipolar spindle formation, oocytes arrest in metaphase for several hours before fertilization. How the dynamic spindle maintains its bipolarity during this long arrest is poorly understood. We hypothesise that the bipolar spindle is stably maintained by changes in the distribution of microtubule-associated proteins (MAPs) on the spindle during the long oocyte arrest. To test this, we generated transgenic flies expressing GFP-tagged microtubule-associated proteins (MAPs), and found that 12 out of 25 proteins change localisation after bipolarity is established in oocytes. In order to identify the molecular mechanisms triggering MAP relocalisation, we manipulated the kinase activity of the cell cycle regulator Cdk1. Expressing non-degradable cyclin A or B, the major activators of Cdk1, prevented re-localisation of distinct groups of MAPs and disrupted spindle bipolarity and accurate chromosome segregation in oocytes. Among them, we found that Pavarotti/MKLP1 localises the spindle equator only later during oocyte arrest. Removal of Pavarotti from the metaphase spindle by RNAi induced spindle defects in oocytes. Moreover, non-phosphorylatable Pavarotti/MKLP1 prematurely localises to the meiotic spindle and disrupts spindle bipolarity. It is likely that the microtubule cross-linking activity Pavarotti enhances the stability of the metaphase spindle during the long arrest. This supports our hypothesis that remodelling the molecular architecture of the spindle during the long oocyte arrest is important to stabilise the bipolar spindle without centrosomes.

P27 Evolutionary history of the mammalian meiotic telomere complex

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During meiotic prophase I, chromosome movements are crucial to facilitate chromosome synapsis dependent on telomere anchoring to the nuclear envelope (NE). Studies from yeast to mammals have provided clear evidence that LINC complexes of the NE are involved in force transduction from the cytoplasm into the nucleus. In addition, adaptor proteins of the so-called meiotic telomere complex are necessary to connect telomeres to LINC complexes. In fission yeast, the meiosis-specific proteins Bqt1 and Bqt2 mediate telomere anchoring at the NE. In mice, telomere anchoring involves the meiosis-specific proteins TERB1, TERB2 and the inner NE protein MAJIN. Despite their function in telomere anchoring, meiotic telomere complex proteins from fission yeast (Bqts) and mouse (TERB1/2-MAJIN) show no sequence homologies. Therefore, the lack of sequence homology suggests that the meiotic telomere complex might have different evolutionary origins in the different lineages. To shed light on this subject, we are analyzing the evolutionary history of mouse meiosis-specific telomere proteins through a phylogenetic approach. Additionally, we are investigating the corresponding expression patterns by RT-PCR, in situ hybridization, immunocytochemistry and immunoblotting. According to our ongoing bioinformatic and expression analysis, we conclude that across metazoans the components of the meiotic telomere complex would form monophyletic groups of orthologous proteins down to organisms as basal as hydra.

P28 Increasing the level of symmetric binding by PRDM9 rescues hybrid sterility between distinct species of mice

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The binding characteristics of PRDM9, as defined by its zinc finger arrays, determine the positioning of the double-strand breaks that initiate meiotic recombination in many species. We have previously shown that it is also these zinc finger motifs which underlie hybrid sterility phenotypes in certain hybrid male mice. In hybrids with two different PRDM9 alleles, binding of each PRDM9 to the two chromosome homologues tends to be asymmetric due to preferential binding of each parental PRDM9 to the chromosome of the other parent - a phenomenon resulting from the evolutionary erosion of PRDM9 binding sites on the chromosomes in which it resides. In certain hybrids, e.g. PWDB6F1, this asymmetric binding is extreme and results in failure of chromosome synapsis and sterility. We have shown that introduction of a genetically modified *Prdm9* allele harbouring the zinc fingers of the human B allele, the binding sites of which have clearly not been eroded on mouse chromosomes, restores symmetric binding in PWDB6F1 mice, resulting in normal chromosome synapsis and fertility.
Here we report a continuation of the above work and show that our humanized *Prdm9* allele is also capable of fully reversing the sterility that is seen in a male hybrid mice resulting from matings of C57BL/6 and the *Mus musculus musculus* strain, STUS, in both directions. Again, the reversal of the asynapsis that underlies this sterility phenotype correlates with a restoration of more symmetric PRDM9 activity, as demonstrated by DMC1 ChIP single-stranded DNA sequencing.
Male offspring of crosses between two separate mouse species, namely the laboratory mouse, *Mus musculus* and the Algerian mouse, *Mus spretus*, also fail to produce spermatozoa and are thus infertile. These species diverged over a million years ago, at least twice the divergence time of the subspecies between which we had previously rescued fertility. Remarkably, introducing the humanized *Prdm9* allele into hybrids generated from *M. musculus* and *M. spretus* restored sperm production. Failure of XY pairing was still prevalent, presumably due to critical differences in their pseudoautosomal regions, but a proportion of cells were now able to synapse correctly and complete meiosis. Although sperm counts and morphology were incompatible with fertility by natural mating, live offspring were obtained by in vitro fertilization of the humanized spermatozoa.
These studies provide further strong support for the model that when a double strand break occurs, binding of PRDM9 at the same position on the homologous chromosome facilitates homology search, and is thus central to successful synapsis and fertility.

P29 The interplay between *Caenorhabditis elegans* topoisomerase 3 and the other RTR complex members

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Crossovers (COs) between homologous chromosomes contribute to their faithful segregation during meiosis I. Only one of the *spo-11*-induced double strand breaks (DSBs) normally matures into a crossover in *C. elegans*. All the other DSBs are repaired as non-crossover (NCO) to restore genome integrity.
The determining events for CO and NCO formation are intensely studied topics.
The RTR complex, which works in the dissolution pathway during homologous recombination, provides a major NCO activity in different model organism.
The mammals RTR complex contains a type IA topoisomerase (TOP-3 in worms), Bloom's helicase (HIM-6) and RMI1/2 scaffolding proteins (RMH-1) and it was shown to mediate decatenation of joint molecules *in vitro*. Bloom is a cancer predisposition gene and patient chromosomes show elevated frequencies of COs. It is the major complex to antagonize COs in mitosis.
Using different alleles of *rmh-1* we have recently shown that RMH-1 functions in distinct complexes to promote both CO and NCO homologous recombination in *C. elegans* meiosis. It promotes CO designation and enforces the CO outcome at a late stage of recombination. Strikingly the protein is found in doublet structures at the CO sites, suggesting that it flanks the two junctions of the dHJ.
We isolated several alleles of *top-3* that are completely sterile and display chromosome aberrations at diakinesis reminiscent of unresolved joint molecules. I am currently working out the genetic dependencies of these joint molecules. This phenotype differs from the phenotype of the other member of the complex. Both *him-6* and *rmh-1* display an accumulation of univalents, which is more prevalent than the joint structures at diakinesis stage. I am currently working out the differences and similarities between *top-3* mutants and the other members of the complex. I will also report my progress on my studies specifically on the role of *top-3* in the *C. elegans* germline.

P30 Developing prediction models for meiotic crossovers in plant species

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Plant breeding uses meiotic recombination to introduce genes from wild species into crop genomes. Understanding which factors are associated with meiotic crossovers is important for developing successful breeding programs. We attempt to investigate these factors and develop predictive models for high resolution crossover (CO) sites in plants. For this purpose, we analyzed the genome sequences of published meiotic CO sites for tomato, rice, maize and *Arabidopsis* . Our aim is to determine the most informative features that distinguish between CO sites and randomly chosen genomic sites for the analyzed species. To this end, we applied a machine learning (classification) approach. We extracted 4kb long sequences surrounding CO sites from the reference genome of the corresponding species. We randomly selected the same number of similar sized regions from the euchromatin (for tomato) or from gene-rich regions (for the other species) as a negative set. Next, we determined features based on sequence (dinucleotide frequencies, predicted DNA shape features such as roll, propeller twist, helical twist and minor groove width), on genome annotation (distance to nearest transcription start site, proportions of gene elements covered, proportions of repeat elements covered) and on parental source information (nucleotide differences between two source parents). Using these features, we constructed species-specific models based on logistic regression, decision trees and random forest algorithms. Among the three classifiers, the random forest algorithm worked best as measured by the area under the receiver-operating characteristic (ROC) curve. This predictor correctly predicts 75% of all CO sites, while at the same time it mis-classifies only 30% of all randomly chosen genomic sites as CO sites. The feature importances as found by the random forest revealed that propeller & helical twist properties, AT/TA/AA frequencies, LTR repeat coverage and SNPs/INDELs between parental genomes collectively contribute to the prediction of CO sites in gene-rich regions of tomato genome. Similar features were found to be important for *Arabidopsis* and rice as for tomato. Conversely, the model for maize showed that vicinity of exons and 3' UTR were predictive, together with LTR repeat coverage. From all four species-specific models, it is noticeable that LTR repeat coverage is a common predictive feature, that may be found to be so in other plant species as well.

P31 A new member of the XPF-ERCC1 complex family is important for crossover formation in *Saccharomyces cerevisiae*

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How meiotic cells spatially and temporally choose to introduce a crossover (CO) among a large population of recombination intermediates is still not known.
The conserved Zip2 protein is a member of the pro-CO proteins, which are known to be important for stabilization of nascent recombination intermediates and promoting CO formation in *S. cerevisiae* (Börner *et al*., 2004). However, its role in stabilization of DNA intermediates is not well understood. *In silico* analyses indicate that Zip2 possesses an XPF domain, a motif known to recognize, and sometimes to cleave, branched DNA structures (Macaisne *et al*., 2008). This suggests that Zip2 may be involved in recognition and processing of branched-DNA junctions to promote CO formation.
Here we show that the XPF domain is important for Zip2 to perform its meiotic functions, such as binding to recombination hotspots, CO formation, meiotic progression and spore viability. We also tested the possibility that Zip2 works as an endonuclease even if it does not present any active site motif for DNA cleavage. Using structural prediction of the XPF domain, we targeted amino acids that might be important for endonucleolytic cleavage of Zip2. Point mutants did not show any meiotic defects, suggesting that Zip2 does not cleave branched DNA structures.
To work as functional nuclease, most XPF nucleases must form a complex with a non-catalytic ERCC1 domain subunit (Rad1-Rad10 and Mus81-Mms4 in *S. cerevisiae* and their mammalian homologs XPF-ERCC1 and MUS81-EME1). To determine factors that may collaborate with Zip2, we performed two converging approaches, TAP-tag purification from meiotic cells and yeast two hybrid assays. Our data indicate that Zip2 strongly interacts with two other pro-CO proteins, Spo16 and Zip4. Moreover, we found that Zip2, Zip4 and Spo16 colocalize genome-wide and are interdependent for their stability and for their binding to chromosomes, suggesting that they work as a single unit to promote CO. Strikingly, we found that Spo16 specifically interacts with the XPF domain of Zip2. Furthermore, we were able to identify, through structural bioinformatics, an ERCC1-like domain in the Spo16 protein. Taken together, our results suggest that the Zip2-Spo16 heterodimer form a meiosis specific XPF-ERCC1-like complex that recognizes specific DNA structures to promote CO formation. The *in vitro* binding activity of purified XPF(Zip2)-Spo16 complex to several DNA structures, including Holliday junction, will be shown.

P32 3'-terminal Overhangs Regulate DNA Double-Strand Break Processing in *Escherichia coli*

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Although double-strand breaks (DSBs) are lethal DNA lesions, they are essential intermediates during meiosis. DSBs are mostly repaired by a universal and evolutionary conserved process of homologous recombination, which involves degradation of the broken molecule, a process known as DNA-end resection. To study DSB processing, we induced DSBs into the *Escherichia coli* chromosome by gamma irradiation and measured chromosomal degradation. We show that DNA degradation is regulated by RecA protein concentration and its rate of association with ssDNA. RecA decreased DNA degradation in wild-type, *recB* and *recD* strains, indicating that it is a general phenomenon in *E. coli*. On the other hand, DNA degradation was greatly reduced and unaffected by RecA in the *recB1080* mutant (which produces long overhangs) and in a strain devoid of four exonucleases that degrade a 3' tail (ssExos). 3'-5' ssExos deficiency is epistatic to RecA deficiency concerning DNA degradation, suggesting that bound RecA is shielding 3' tail from degradation by 3'-5' ssExos. Since 3'-tail preservation is common to all these situations, we infer that RecA polymerization constitutes a subset of mechanisms for preserving the integrity of 3' tails emanating from DSBs, along with 3' tail's massive length, or prevention of their degradation by inactivation of 3'-5' ssExos. Thus, we conclude that 3' overhangs are crucial in controlling the extent of DSB processing in *E. coli*. This study suggests a regulatory mechanism for DSB processing in *E. coli*, wherein 3' tails impose a negative feedback loop on DSB processing reactions, specifically on helicase reloading onto dsDNA ends.

P33 Loss of spindle assembly checkpoint (SAC) proteins in oocytes from aged mares impairs the ability to correctly align chromosomes; the horse as an alternative animal model

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Embryonic aneuploidy of meiotic origin increases markedly in older women and is the leading cause of implantation failure and miscarriage. Correct spindle formation and attachment of chromosomes to the microtubules are prerequisites for the generation of oocytes of the correct ploidy. The SAC is a signalling system able to detect errors in kinetochore-microtubule attachment, and in the case of missing or erroneous attachments, it delays anaphase onset through inhibition of the APC/C so that these faulty attachments can be corrected. Although it has been previously proposed that a defective SAC could explain the maternal age-related increase in aneuploidy, the exact mechanisms underlying meiotic segregation errors are still obscure. While the mouse is a highly tractable model, the infrequency of chromosomal abnormalities in mouse oocytes and embryos renders it suboptimal for studying maternal age-related aneuploidy. Other species, such as the horse, with a time-interval to reproductive senescence more comparable to women, and a comparable increase in the incidence of aneuploidy and early pregnancy loss with maternal age could be more appropriate animal models.
The aim of this study was to evaluate the effect of advanced maternal age on the expression and function of key SAC components in horse oocytes.
Gene expression for MPS1, AURKC and SPC25, but not for other SAC components, was significantly reduced in in-vitro matured oocytes from old mares (≥15y) compared to young mares (<15y)(n=8 pools of 10 oocytes/group; $p<0.05$). Oocytes from old mares (n=20/group) displayed a higher rate of mild (1-5 misaligned chromosomes) (36.8 vs 4.6%) and severe (>5) (10.5 vs 0%) chromosome misalignments and spindle abnormalities (tri-, tetra-polar, severely misshapen) (5.3 vs 0%) than those from young mares. A similar difference in spindle and chromosomal abnormalities between old and young oocytes was also seen after spindle reconstitution (2h) following Nocodazole induced microtubules depolymerization (20µM for 10min). Conversely, after treatment with MPS1 inhibitor cpd5 (2h, following Nocodazole washout) oocytes from old mares showed little change in the incidence of abnormalities compared to Nocodazole exposure alone (spindle abnormalities 9.5 and 22.7%; mild chromosome misalignments 42.9 and 36.4 %; severe 9.5 and 45.5%; for 200nM and 500nM cpd5, respectively), whereas oocytes from young mares showed an increased incidence of both spindle abnormalities (5% and 16.7%) and chromosome misalignment (mild, 50 and 11.1%; severe, 5 and 16.7%).
In conclusion, MII oocytes from aged mares show an increased incidence of spindle abnormalities and chromosome misalignment. Since advanced maternal age is associated with a downregulation of MPS1 mRNA and reduced sensitivity to the MPS1 inhibitor, cpd5, it is proposed that compromised MPS1 activity in aged oocytes plays an important role in maternal age-related aneuploidy in horse embryos.

P34 Reverse evolutionary genetics of meiosis – what can genes under selection tell us about meiotic thermotolerance in *Arabidopsis arenosa*

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Meiotic recombination is a highly regulated process essential for maintenance of genome integrity and fertility in the vast majority of sexually reproducing organisms. From an evolutionary perspective, the importance of meiotic recombination lies in its ability to create new combinations of genes upon which selection can act. Thus, recombination can increase the efficiency of selection and the rate of adaptation. However, it is known that meiosis is itself sensitive to environmental conditions, especially temperature, but it is not yet clear if and how it can adapt as organisms evolve to adapt to challenging environmental conditions. In recent years, *Arabidopsis arenosa* has emerged as a model for studying meiotic adaptations to whole genome duplication. Interestingly, a subset of the axis genes that are suggested to have evolved to stabilize meiosis in the tetraploid lineage by modifying recombination rates and patterns, are also found to be under selection in a group of diploid plants that have adapted to a warmer habitat. Meiotic genes, especially the axis protein ASY3 (a homolog of the yeast protein Red1) and the cohesin subunit REC8, show strong differentiation and signatures suggestive of selection in diploid populations from the warm dry Pannonian basin relative to the temperate Carpathian Mountain populations. These proteins are involved in homologue pairing, axis and synaptonemal complex formation; meiotic processes that are known to be particularly thermo sensitive. Temperature is also known to affect recombination rates, which we hypothesize also depends on these processes. Considering that populations under study originate from two different thermal environments, with Pannonian Basin being a significantly warmer habitat than Carpathian Mountains, we hypothesise that the signatures of selection in Pannonian populations might be driven by meiotic adaptation to elevated temperatures. So far, we know that Pannoninan and Carpathian populations differ in frequency and positioning of crossovers at 20 ˚C and further efforts are underway to characterize meiotic differences under different temperatures in greater detail by using genomic and cytological approaches. In addition, we will use transgenic lines of *A. thaliana* to assay functional differences between Pannoninan and Carpathian alleles and to test if the genes showing signatures of selection indeed confer meiotic thermotolerance. Hence, applying the reverse evolutionary genetics approach suggested here, we are aiming to understand the mechanisms by which meiosis evolves to tolerate environmental change. We hypothesize that these changes have pleiotropic effects on recombination rate. Hence, meiotic evolution may not only influence fertility in the face of environmental challenges, but may also influence recombination rates and thus overall adaptive potential.

P35 Meiotic phasiRNAs – (more than) the plant version of piRNAs?

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Small RNA classes comprise the well-known miRNAs and siRNAs which regulate gene expression post-transcriptionally and by silencing through DNA methylation, respectively. However, there are additional classes of small RNAs that are not yet as well characterized, for example plant phasiRNAs. PhasiRNAs (phased small interfering RNAs) occur during male reproduction in monocot plants in high abundance and appear to be essential for fertility. PhasiRNAs occur in two waves (as 21nt premeiotic phasiRNAs and 24nt meiotic phasiRNAs), reminiscent of piRNAs from the animal kingdom. Plant phasiRNAs are however distinct from animal piRNAs since they are triggered by specific miRNAs and are generally strongly phased, i.e. originating from aligned and exactly sized intervals. Mammalian meiotic piRNAs have been shown to have functions in gene and transposable element regulation, while plant meiotic phasiRNA functions have not been elucidated yet. We used a special technique to isolate male meiocytes from maize plants during zygotene and subjected their DNA and RNA to Illumina sequencing to obtain a genome-wide DNA methylation profile together with sRNA and mRNA transcriptomes. Our analysis revealed peculiar AT-rich isochore-like islands around genomic phasi loci as well as specific DNA methylation of these ~600 phasi loci in maize meiocytes. We hypothesize a revolutionary role of meiotic plant phasiRNAs in chromosome dynamics and are currently testing first possibilities including subnuclear localization and initiation of pairing or synapsis.

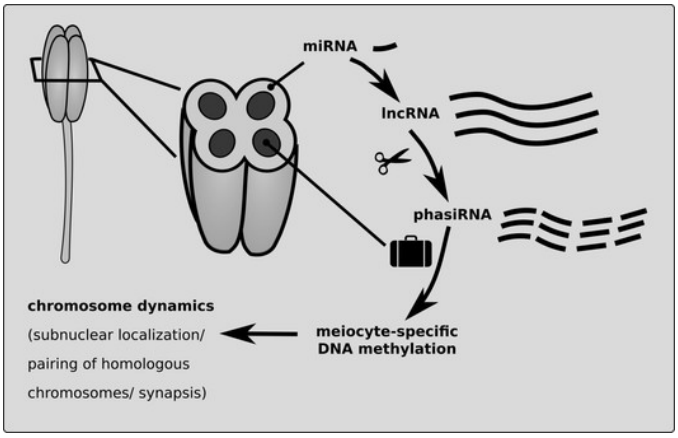

Figure 1 Overview of phasiRNA biosynthesis and functions

P36 Dynamic self-assembly of SYCP1 underpins meiotic chromosome synapsis

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Meiotic cell division is characterised by the formation of the synaptonemal complex (SC), a proteinaceous megastructure which "zips" homologous chromosomes along their entire length in tight synapsis. It induces a specific chromosome architecture essential for the repair of double-strand break intermediates via crossing over resulting in genetic exchange. In the human SC, the 976 amino acid elongated coiled-coil protein SYCP1 forms the "teeth" of this molecular zipper – self-associating via its N-termini at the midline central element (CE) whilst interacting with the chromosome axis via its C-terminus. SYCP1 contains a central structured domain sufficient in length to bridge the 50 nm distance between the CE and chromosome.

Here, we report the structural characterisation of the central structured domain of SYCP1 achieved through combined biophysical techniques. We utilised small angle X-ray scattering experiments for the determination of distance distributions and generation of *ab* initio molecular envelopes whilst light scattering experiments were used to determine the molecular weight and oligomeric state of key domains. We find that this central region is formed of two distinct domains – a compact parallel N-terminal tetrameric bundle which splays at its C-terminus into two elongated dimeric coiled-coils which together provide sufficient length to span the 50 nm separation between the CE and chromosome.

Further, crystallographic study of the N- and C-terminal self-assembly sites of human SYCP1 reveals two distinct mechanisms of dynamic assembly of dimeric coiled-coils at both termini of its central structured region. The N-terminal site mediates a dynamic and cooperative head-to-head association facilitating the formation and remodelling of SYCP1 assemblies. In contrast, under mildly acidic conditions, the C-terminus undergoes tail-to-tail assembly allowing for subsequent association with DNA. Further DNA-binding studies show that the unstructured C-terminus of SYCP1 can also interact with DNA at a physiological pH, providing a mechanism by which SYCP1 is initially bound to the chromosome.

Taking this data together, we propose a model in which tetrameric SYCP1 is recruited to the chromosome axis by its unstructured C-terminus followed by the recursive pH-dependent assembly of the C-termini which coat the chromosome axes. Synapsis of homologues is then initiated by local N-terminal associations – the cooperative and dynamic nature of these nascent assemblies allowing for their active remodelling, ensuring accurate alignment of tethered homologues. Subsequent recruitment of CE components SYCE1, SYCE3 and SYCE2-TEX12 promote stabilisation and elongation of these short regions of synapsis. It is only after the SC mediates this exquisite alignment and perfect synapsis of homologous chromosomes that repair intermediates may be processed and chromosome segregation may occur, underlining the essential role that SYCP1 plays in human fertility.

P37 Identification and characterization of alternative open reading frames in budding yeast meiosis

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Genomic regions that encode proteins are known as open reading frames (ORFs) and have been historically defined based on specific criteria including initiation at an AUG codon and the ability to encode one protein. These rules have been expanded through the characterization of alternative splicing in complex eukaryotes, but the use of alternative translation initiation sites has been observed less frequently and only in specific contexts. During certain stages of growth, development, and stress, alternative ORFs (altORFs) can modulate protein content and localization to meet the cell's changing needs, though this has not been widely reported in any eukaryote, including budding yeast (*Saccharomyces cerevisiae*). We profiled translation in budding yeast through meiosis, the developmental process by which haploid gametes are produced from diploid cells, and observed a significant increase in near-cognate start codon usage and translation occurring in regions upstream of annotated ORFs in comparison to vegetatively growing cells. In a few specific cases investigated thus far, we and others have observed that translation resulting in an N-terminal extension could append a targeting signal, causing a change in protein localization. We hypothesize that this type of mechanism may be broadly used in budding yeast meiosis to diversify the meiotic proteome.

P38 Essential roles for Puma and Noxa in the mammalian prophase I DNA damage checkpoint

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A key stage in the development of germ cells is meiosis, during which homologous chromosomes synapse and recombine, in order to form crossovers that are essential for accurate segregation. Errors in synapsis or recombination give rise to chromosome mis-segregation and aneuploid offspring. Hence, surveillance mechanisms operate during prophase I that arrest germ cells in which such errors occur. A recent study has found that induction of exogenous DNA damage in oocytes leads to up-regulation of the pro-apoptotic genes *Puma* and *Noxa*. Mice lacking these genes are protected from irradiation-induced oocyte apoptosis and produce healthy offspring. We hypothesised that the oocyte prophase I DNA damage checkpoint is a modified form of the exogenous DNA double-strand break (DSB) response; and therefore, might involve *Puma* and *Noxa*. To examine this possibility, we generated mice deficient for *Puma*, *Noxa* and *Dmc1*. *Dmc1* null is a mouse model exhibiting DNA damage characterised by persistent DSBs and defective recombination, resulting in germ cells loss at birth. Deleting *Puma* and *Noxa* in $Dmc1^{-/-}$ mouse rescued the oocyte loss. Indeed, three weeks old triple knockout ovaries contained a large pool of primordial follicles. Next, we wondered whether the rescue of germ cells loss in $Dmc1^{-/-}\ Noxa^{-/-}\ Puma^{-/-}$ females is attributable to activation of an alternative DSB repair pathway during pachetene. Immunostaining for a marker of DNA double-strand breaks (RPA) showed that all dictyate $Puma^{-/-}\ Noxa^{-/-}\ Dmc1^{-/-}$ oocytes have persistent unrepaired DSBs. A remaining question concerns whether the rescued oocytes are functional and can be fertilised. Six-week-old $Puma^{-/-}\ Noxa^{-/-}\ Dmc1^{-/-}$ females were hormone primed and mated to wild type males. Triple null oocytes were fertilised and were able to extrude two polar bodies, indicating that they completed meiosis. Zygotes with two pronuclei were obtained and were able to develop into embryos that arrested at two cells stage. We are now assessing whether overcoming the meiotic DSB-dependent checkpoint in triple-null females leads to increased aneuploidy resulting in the embryonic arrest. Our results suggest, that *Puma* and *Noxa* are responsible of the elimination of the primordial follicles pool with persistent DNA damage.

P39 BRCA1/BARD1 regulates repair choice, RAD-51 filament stability and crossover control differently during male and female meiosis

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During meiosis, homologous chromosomes pair, synapse and undergo crossover recombination initiated by SPO11-dependent DNA double strand breaks (DSBs). Homologous recombination (HR) is the preferred pathway to repair DSBs during meiotic prophase, and RAD51 assembly and disassembly serves as a readout of ongoing repair by HR. In mammalian cells, the breast cancer 1 gene (*BRCA1*) is required for HR in somatic cells. In mouse spermatogenesis, *BRCA1* is required for successful meiotic sex chromosome inactivation (MSCI), the failure of which leads to pachytene arrest and apoptosis of germ cells. Consequently, the role of BRCA1 in meiotic recombination is unclear. The *C. elegans* ortholog, *brc-1*, has been reported to promote HR at a subset of DSBs in meiotic cells as visualized by altered pattern of RAD-51 foci in *brc-1* oocytes (Adamo et al., 2008). We discovered that in *C. elegans* male germ cells, BRC-1/BRD-1 does not participate in MSCI, which allowed us to investigate the role of BRC-1/BRD-1 in meiotic recombination during spermatogenesis. In contrast to what was observed during female meiosis, *brc-1* and *brd-1* mutant male germ cells had a reduction in the number of RAD51 foci, which returned to wild-type levels when non-homologous end joining (NHEJ) was inhibited. Inactivation of NHEJ in *brc-1* or *brd-1* mutant hermaphrodites, in contrast, led to a further increase in the number of RAD51 foci, suggesting that BRC-1/BRD-1 regulates DSB repair choice differentially in the sexes. We also discovered that BRC-1/BRD-1 is important for stabilizing the RAD51 filament when the formation of a crossover-intermediate was disrupted in oogenesis but not spermatogenesis. Furthermore, crossover designation was differentially regulated in oocytes versus spermatocytes. We found that BRC-1/BRD-1 plays a critical in monitoring crossover numbers under situations where crossovers are unable to form on a subset of chromosomes. Consistent with a role in DNA repair choice, RAD51 filament stability and crossover designation, GFP::BRC-1 localized to foci in early prophase, and then was observed between homologs in mid pachytene before concentrating to the short arms of bivalents, co-localizing with central region components of the synaptonemal complex. Together, our studies indicate that BRCA1 and BARD1 play multiple roles during meiotic recombination, and these roles are modulated for production of oocytes versus sperm.

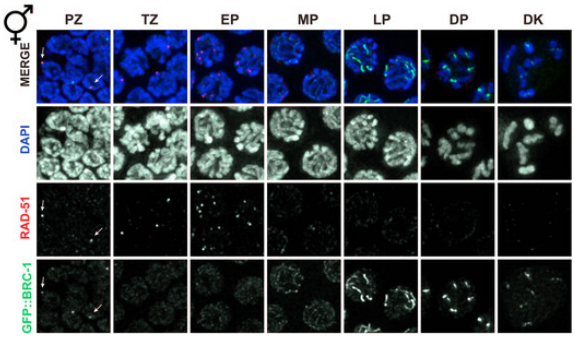

Figure 1 GFP::BRC-1 localization during meiosis

P40 Transmission of a human chromosome through mouse male meiosis

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Mammalian gametogenesis exhibits a distinct sexual dimorphism in the stringency of meiotic checkpoints, with oogenesis being more lenient than spermatogenesis. This is exemplified in many mouse models in which mutations or chromosomal abnormalities lead to complete male sterility, whereas females often display only a minor, or no, impact on fertility [1]. We investigated this sexual dimorphism in the Tc1 mouse, which is an elegant model for human Down Syndrome, carrying an aneuploid copy of human chromosome 21 [2]. We observed that while females display no obvious impact on fertility, and transmitted the human chromosome to their offspring with a high frequency (35 – 40% transmission rate), males showed a sub-fertility phenotype with histological abnormalities and a much lower transmission rate of chromosome 21 to their offspring (~10%) [3]. Careful dissection of the meiotic process in Tc1 males revealed that spermatocytes in these animals proceed normally through meiotic prophase but encounter an arrest at the first meiotic division. In addition to an overall increase of metaphase I cells in Tc1 males we observed a higher number of cells with congression defects, which showed misaligned chromosomes on the metaphase plate (Figure 1). In accordance with an activation of the spindle checkpoint we observed increased apoptosis in seminiferous tubules undergoing meiotic cell divisions but not during other stages of spermatogenesis. Genotyping the presence of human chromosome 21 in primary spermatocytes and round spermatids – before and after meiosis – revealed only a modest loss of human chromosome 21 during the meiotic division that cannot fully account for the low transmission rate via the male germline. This showed that Tc1 males produce an unexpectedly high number of aneuploid gametes (~35%) and show exceptionally high tolerance of aneuploidy in the male germline.

1. *Davisson et al. Hum Reprod, 2007*
2. *O'Doherty et al. Science, 2005*
3. *Ernst et al. eLife, 2016*

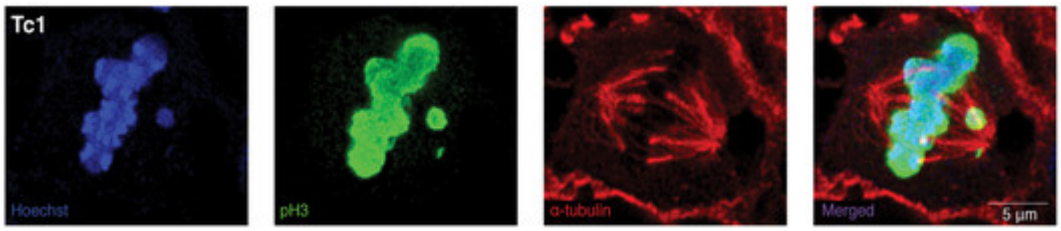

Figure 1 Representative confocal image of a Tc1 metaphase cell with a congression defect.

P41 The role of CDC25B phosphatase in meiotic maturation of mouse oocytes

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The development of mammal oocytes is physiologically arrested in the prophase of the first meiotic division. CDC25B phosphatase has been considered for a long time to be an essential regulator of meiotic resumption in mouse oocytes. Oocytes from CDC25B germ line knock-out (KO) mice are unable to resume meiosis, because of their inability to activate CDK1 (Lincoln et al., 2002). However, our data showed that CDC25B is not absolutely required for meiotic resumption in mouse oocytes, but it seems that it has additional roles during meiotic maturation and early embryonic development. In our research we used two mouse models: 1) CDC25B KO mice on the inbred C57BL/6 genetic background and 2) CDC25B KO on the outbred CD1 genetic background. We confirmed previous observation that CDC25B KO C57BL/6 oocytes are unable to resume meiosis. However, almost 47% of CDC25B KO CD1 oocytes resume meiosis but many of them arrest in metaphase I. Exogenously expressed CDC25A is capable to replace CDC25B function during meiotic resumption but not in later phases of meiotic maturation in CDC25B KO C57BL/6 oocytes. Additionally, metaphase II CDC25B KO CD1 oocytes are unable to start embryonic development. These data suggest that CDC25B is involved not only in the resumption of meiosis, but also in the metaphase I – metaphase II transition and initiation of the early embryonic development.

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P42 Vinculin in mouse meiosis

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The focal adhesion protein vinculin (VCL) acts as a molecular clutch between the actin cytoskeleton and the extracellular matrix via talins and integrins. However, the nuclear function of VCL has not been described yet. We find that VCL localizes in nuclei of mouse meiocytes undergoing the meiotic prophase I. In particular, VCL localizes in the nuclear interior along the meiosis-specific structure synaptonemal complex and in the centromeric regions of homologous chromosomes (homologs). To understand the role VCL plays in the early meiotic division, we prepared a VCL conditional knock-out of mouse meiocytes (VCL cKO), as the whole body VCL knock-out has been reported as embryonically lethal. In this model, VCL deletion occurs explicitly during the spermatocytes and oocytes development. In comparison with the wild-type control, our mouse model shows a decreased fertility, although the relative testis weight and the sperm count are increased. A FACS analysis performed on the VCL cKO testicular cell suspension reveals an enrichment of the prophase I spermatocytes and a reduction in the ratio of apoptotic cells. Homologs pairing and the crossing-over formation appear intact. However, homologs disassociation is impaired. The incidence of prematurely separated homologs on the centromeric sites increases in the VCL cKO. These data indicate defective splitting of homologs in late prophase I which might result in an improper chromosome segregation with a higher incidence of chromosomal aberrations. Therefore, a detailed study of these processes is required. Our findings indicate that VCL plays an essential role in the meiotic progression.
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P43 Uncovering the recombination landscape in *Lolium*

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With the ever-expanding human population and predicted climate change, it is important that plant breeders continue to produce new varieties that can meet increased demand for food. Meiotic recombination generates genetic variation, but in many crops such as wheat, barley, rye and ryegrass many genes for desired traits are embedded in 'cold' regions of the genome which rarely, if ever, recombine. *Lolium perenne* (ryegrass) is an agronomically important forage grass that provides sustenance for ruminating animals supplying the meat and milk industries worldwide. Using CRISPR/Cas9 gene editing technology, this project aims to redistribute crossovers to produce novel genetic variants, from which plant breeders can select new phenotypes. Since *L. perenne* is an outbreeding perennial and rather intractable experimentally, we are using as a model the closely related inbreeding annual species *Lolium temulentum*, as bioinformatic analysis shows that it shares high sequence homology. Meiosis in *Lolium* is not characterised as compressively as it is in other organisms. Many eukaryotes, including *Arabidopsis*, contain two CO pathways; class I exhibits interference, which is a phenomenon where one CO prevents others from forming nearby, and class II does not. The presence of these two CO pathways in *Lolium* will be established by targeting the ZMM gene Hei10 using CRISPR/Cas9 technology to produce a SNP and the resulting frameshift should cause a knockout. As Hei10 is essential for class I COs, in rice and *Arabidopsis*, this will leave the interference independent class II COs to facilitate chromosome segregation. Confirmation of a second CO pathway in *Lolium* will lead to the targeting of the anti-CO factor Recq4 gene in a similar manner to produce a knockout resulting in an increase in class II COs. A second Anti-CO factor, FANCM, will be targeted through homology independent target integration (HITI). Using CRISPR/Cas 9, a single intron will be targeted and a donor DNA template containing the same sgRNA will be co-transformed. This donor DNA template will contain the desired edit and all subsequent exons followed by the 3' UTR to create an AA substitution that, in *Arabidopsis*, results in an increase in Class II COs.

P44 Detection and Characterization of Non-crossovers in Mice

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At leptotene stage of meiosis I SPO11 induces approximately 250 DNA double-strand breaks (DSBs) per cell. From them, no more than 10% are resolved by homologous reciprocal recombination (crossovers, COs). It is supposed that the remaining 90% of DSBs are repaired by homologous nonreciprocal recombination (noncrossovers, NCOs), although the possibility of some recombination between sister chromatid cannot be excluded. The nonreciprocal recombination begins at DSBs by digestion of single strand 5'end, leaving the 3' overhang strand to invade the homologous chromosome, which is used as a template to extend the 3'strand. This process, also designated as gene conversion, is supposed to be essential for starting the homologous chromosomes synapsis, however very little is known about the extent and location of the sequence copied from the homologous chromosome during these events.

We used our C57BL/6J-Chr $\#^{PWD}$ chromosome substitution strains (abbreviated B6.PWD-Chr#) to directly identify and characterize the products of nonreciprocal recombination during the first meiotic prophase. Because in the consomic strains each PWD chromosome was introgressed into C57BL/6J (B6) background by 10 consecutive backcrosses (Gregorova *et al*. Genome Res. 2008), the number of NCOs was amplified ten-times (on average 10 x 12.5 expected DSBs per chromosome).

We detected NCOs in the six longest consomic chromosomes using mouse strains B6.PWD-Chr1 to B6.PWD-Chr6 by NGS sequencing. We found NCOs of variable length based on the SNPs present in the area. NCO lengths less than 100bp as well as greater than 200bp were found. Detected NCOs overlap DMC1 (markers of DSBs) and H3K4me3 (markers of PRDM9 binding sites) ChIP-seq peaks from B6, (B6xPWD)F1 and (PWDxB6)F1 males (Smagulova *et al*. Genes Dev. 2016, Davies *et al*. Nature 2016). The overlap can be used to monitor the gradual deposition of converted sites in the process of formation of consomic strains.

P45 The *Caenorhabditis elegans* RAD -51 isoform A is required for the induction of DNA damage-dependent apoptosis

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In some organisms, such as in *Sordariomycetes*, *Caenorhabditis elegans*, and *Drosophila melanogaster*, the key actor in the meiotic step of strand invasion, Dmc1, is missing and Rad51 alone seems to perform strand-invasion in meiosis. The rad-51 gene in *C. elegans* is transcribed into alternative mRNAs potentially coding three alternative protein isoforms. We have genetically modified this gene in order to investigate the potential roles of the longest isoform, namely isoform A, in genome stability. The RAD-51 isoform A appears to contribute to genome stability in late development, but is not implicated in meiosis or DNA repair in the germline. However, the RAD-51 isoform A has a pivotal role in DNA damage induced apoptosis, but not in DNA damage checkpoint activation or physiological cell death. This is a relevant new finding that improves our understanding of how DNA damage apoptosis is restricted to late pachytene stage preventing the inappropriate loss of nuclei undergoing the earlier stages of meiotic recombination, during which a large number of physiologically induced DSBs are present.

P46 Comparative behaviour of sex chromosomes during meiosis in two african pigmy mouse species: *Mus Minutoides* and *Mus Matheyi*

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Sex determination in mammals is provided by a XX couple of chromosomes in females and XY in males. The X and Y chromosomes are highly differentiated but usually share a small region of homology called PAR. In some species the PAR has completely disappeared, while in others it has been greatly expanded. An example of this two situations is the African pygmy mouse *Mus Matheyi*, in which there is not PAR, and *Mus minutoides*, in which a large autosome has been translocated to both the X and Y chromosomes, giving rise to a neo-XY chromosome system. To compare sex chromosomes behavior between this two species, we have studied the localization pattern of proteins related to synapsis (SYCP3), recombination (Rad51) and transcriptional inactivation (RNA polymerase-II, ATR, γH2AX, H3K9me3) throughout the first meiotic division. We found that in *Mus Minutoides* the neo-XY chromosomes displays a behavior comparable to other mouse species with standard XY chromosomes. More relevantly, we have observed that the characteristic inactivation of sex chromosomes is not extended to the neo-PAR. It seems that the centromere, which separates the autosomal and the sex specific part of the neo-XY chromosomes, acts like a physical barrier avoiding the extension of synapsis from the autosomal to the sex specific region and in turn preventing the expansion of sex chromosome inactivation to the PAR. On the other hand, we have observed that sex chromosomes in *Mus Matheyi* are completely asynaptic. Nevertheless, the X and Y chromosomes appear associated during metaphase I and properly segregate during anaphase I. We could not find any specific structure mediating this association, which contrasts to the behavior described for achiasmate sex chromosomes in other mammalian species. Overall, our results offer new clues about the interplay between meiosis and sex chromosome evolution.

P47 Investigating mechanisms that promote and limit meiotic CO formation in *C. elegans*

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Crossover (CO) formation at meiosis relies on the formation and repair of numerous double-strand DNA breaks (DSBs). DSBs are among the most dangerous DNA lesions for cell survival. Unrepaired DSBs can results in cell death or senescence whereas inaccurate repair of DSBs can cause genomic instability and carcinogenesis. Mechanisms that ensure timely error-free DSB repair are therefor crucial so that by the end of meiosis all DSBs are accurately repaired through HR to (i) ensure CO formation and proper chromosome segregation and (ii) guarantee genome integrity. Most species make very few COs per chromosome pair despite a substantial excess of DSBs, and *C. elegans* stands at one hand of this spectrum with one, and only one, CO formed per chromosome pair. We are using direct genetic screening approaches to elucidate and decipher the mechanisms underlying meiotic CO formation and its regulation. We will first report on our investigation of the mechanisms limiting meiotic CO formation in *C. elegans*. Our goal is to identify factors that normally function in antagonizing CO formation; as part of our strategy, we are conducting a genetic screen for suppressors of a temperature-sensitive mutation affecting the conserved CO-promoting complex MSH-4/MSH-5. The *msh-4(ts)* mutant is characterized by a decrease in CO formation at the restrictive temperature of 24°C, associated with a small brood size. We will report on the first 4 suppressor lines identified with a clear rescue of the progeny viability and number of CO per meiosis. We also conducted a screen for altered numbers of GFP::COSA-1 foci, which mark the sites of COs in late meiotic prophase in *C. elegans*. This screen identified CeNBS-1, the previously elusive *Caenorhabditis elegans* homolog of the NBS1 subunit of the conserved MRE11-RAD50-NBS1/Xrs2 (MRN/X) complex, as a key mediator of DSB repair via homologous recombination (HR) during meiosis. Loss of *nbs-1* leads to severely reduced loading of ssDNA binding protein RPA-1, recombinase RAD-51, and pro-CO factor COSA-1 during meiotic prophase progression, as well as aggregated and fragmented chromosomes (meiotic catastrophe) in oocytes at the end of meiotic prophase. This reflects a role for NBS-1 in processing of meiotic DSBs for HR that is common with its interacting partners (MRE-11, RAD-50 and COM-1/Sae2/CtiP). However, in contrast to MRE-11 and RAD-50, NBS-1 is not required for meiotic DSB formation, indicating that MRE-11 and RAD-50 function independently of NBS-1 to promote meiotic DSBs. Meiotic catastrophe and loss of CO formation of an *nbs-1* mutant are partially suppressed when the non-homologous end-joining (NHEJ) repair pathway is abrogated by loss of CKU-80. These and other data support a model in which NBS-1 promotes DSB resection functions both to antagonize NHEJ and to enable timely engagement of HR, thereby ensuring CO formation and restoration of genome integrity prior to the meiotic divisions.

P48 *In vivo* binding of PRDM9 reveals interactions with noncanonical genomic sites

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In mouse and human meiosis, DNA double-strand breaks (DSBs) initiate homologous recombination and occur at specific sites called hotspots. The localization of these sites is determined by the sequence-specific DNA binding domain of the PRDM9 histone methyl transferase. Here, we performed an extensive analysis of PRDM9 binding in mouse spermatocytes. Unexpectedly, we identified a noncanonical recruitment of PRDM9 to sites that lack recombination activity and the PRDM9 binding consensus motif. These sites include transcription promoters, where PRDM9 is recruited in a DSB-dependent manner. Another subset reveals DSB-independent interactions between PRDM9 and genomic sites, such as the binding sites for the insulator protein CTCF. We propose that these DSB-independent sites result from interactions between hotspot-bound PRDM9 and genomic sequences located on the chromosome axis.

P49 Determinants of interhomolog bias during meiotic DNA repair in *Arabidopsis thaliana*

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In meiosis, one round of DNA replication is followed by two rounds of chromosome segregation. In prophase I, homologous chromosomes are aligned along the chromosomal axis. In this structure, the respective sister chromatids are oriented in parallel, with chromatin loops protruding from the axis. To ensure proper segregation, the homologous chromatids need to be physically linked by crossovers. Crossovers are created by the repair of programmed DNA double strand breaks (DSBs) via the homologous partner chromosome. DSBs are created by the protein SPO11 which are subsequently processed by the MRN/X complex to yield 3‘-overhangs. The ssDNA stretches are bound by the RecA-like recombinases RAD51 and DMC1, giving rise to potentially asymmetrically decorated nucleoprotein filaments. In *Arabidopsis*, it is anticipated that the RAD51 covered filaments remain at the chromosomal axis, while the DMC1-ssDNA-filaments are free to search for repair templates. This filament invades duplex DNA in order to sample for a homologous sequence to repair the DSB. The closest repair template would be the sister chromatid, but proper chromosome disjunction in anaphase I requires at least one DSB per homologous chromosome pair to be repaired via the homolog. One of several models how DSB repair via the homolog is promoted proposes a requirement for the heterodimeric HOP2-MND1 complex: It binds to dsDNA and is thought to cluster DNA in the chromatin loops. Upon introduction of a DSB, a phosphorylation cascade requiring ATM and ATR could ultimately result in local depletion of HOP2-MND1. Because specific interaction of DMC1 with the C-termini of both HOP2-MND1 is a prerequisite for strand invasion, lack of HOP2-MND1 on the spatially close sister chromatids may disfavor DNA repair in the proximity of a DSB via the sister chromatid and bias DSB repair towards the homolog. Recent structural models of HOP2-MND1 from various organisms stimulated the idea that HOP2-MND1 stimulates DMC1 mediated strand invasion by localized DNA duplex melting.

We are using a combinational approach of biochemistry, structural biology and cytogenetics to gain an in-depth understanding of the interaction of HOP2-MND1 with the DMC1-ssDNA filament and how this interaction may promote interhomolog bias during plant meiosis. Since previous crystallization events were not successful, we generated and purified C-terminal truncated HOP2-MND1 complex proteins and are currently attempting co-crystallization with dsDNA to investigate DNA duplex melting. Utilizing various hypomorphic versions of HOP2, MND1 and DMC1 in *in vitro* and *in vivo* studies, we are aiming at more information about DMC1 catalyzed D-loop formation.

P50 Massive expression of germ cell specific genes is a hallmark of cancer and a potential target for novel treatment development

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Cancer cells have been found to frequently express genes that are normally restricted to the testis, often referred to as cancer/testis (CT) antigens or genes. When their expression is germ cell specific, the side effects of targeting CT-genes during cancer therapy would be limited to infertility. Moreover, male germ cell specific antigens are not recognized as "self" by the innate immune system, making them ideal targets for immunotherapy. However, thus far, CT-genes have been identified using whole testis, including the testicular somatic cells, and their germ cell specificity most often has not been demonstrated. By comparing the transcriptomes of micro-dissected germ cell types of the main developmental stages of human spermatogenesis with the publicly accessible transcriptomes of 2.617 samples from 49 different healthy somatic tissues and 9.232 samples from 32 tumor types, we discovered hundreds of true germ cell specific cancer expressed genes, or germ cell cancer genes (GC-genes). Moreover, we found these GC-genes to be widely expressed in all analyzed tumors. Many GC-genes appeared to be involved in processes that are likely to actively promote tumor viability, proliferation and metastasis. Targeting these GC-genes thus has the potential to inhibit tumor growth with infertility being the only side effect. Moreover, we identified a subset of GC-genes that are not expressed in spermatogonial stem cells. Because these stem cells can recover spermatogenesis after a gonadotoxic treatment, targeting of this GC-gene subset is predicted to only lead to temporary infertility. Our GC-gene dataset now enables more tumor types to be analyzed on the expression and function of true germ cell specific genes. We anticipate that this will contribute to a better understanding of tumor biology and improved treatment options.

P51 Adaption of meiosis in polyploids – Is diploidization accomplished by the downsizing of meiotic protein scaffolds?

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Whole genome duplication, a phenomenon that is common in the evolutionary history of eukaryotes. Genome duplication has contributed to variable environmental adaptations and the colonization of (new) ecological niches. But genome duplication poses serious challenges to meiosis: In newly-synthesized polyploid plants, chromosome-pairing, synapsis, and segregation are often impaired, leading to lower fertility. In contrast, meiosis in established polyploids is comparable to meiosis in diploids showing that these processes can evolve to overcome the initial problems they face. We use *Arabidopsis arenosa* as a model system to understand the adaptive evolution of meiosis in polyploids, as it occurs naturally as both diploids and stable tetraploids. In tetraploid *A. arenosa,* the average number of cross overs per chromosome is reduced to close to one, which is likely an important adaptation to prevent the formation of multivalent associations. Genome scans in *A. arenosa* revealed 44 genes showing strong evidence of selection and strong differentiation by ploidy, among which are eight meiosis-related genes. The majority of the identified meiotic genes encode for structural proteins of the axes and synaptonemal complex. Since the assembly and function of these multiprotein complexes may be limited by the availability of particular subunits, we are interested in whether or not meiotic adaptions in tetraploid *A. arenosa* include transcriptional changes of genes of the axes and synaptonemal complex. Initial qRT-PCR measurements showed that some of the identified ploidy-specific genes are expressed at low levels in tetraploids relative to diploids, especially the kleisin cohesion subunit REC8. Cohesin depletion can lead to shorter axes which in turn can affect the number of crossing-over events. Thus we hypothesize that after whole genome duplication, an important aspect of meiotic stabilization may be the downsizing of meiotic protein scaffolds by transcriptional adaptions.

P52 A mutation in Mcm5 disrupts cohesion, centromere clustering, and crossing over in *Drosophila*

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Proper segregation of chromosomes at the end of meiosis is dependent upon multiple meiosis I events, including the formation of crossovers between homologs. Prior to the onset of meiosis in multi-cellular animals, cells arising from the germline stem cell undergo multiple rounds of replication and modified division, preparing for meiotic onset. During these mitotic cycles, chromosomes pairing, clustering, and protein interactions occur. Although these pre-meiotic chromosomal events have been previous described, the importance of chromosomal dynamics and protein localization during these pre-meiotic mitotic divisions on meiotic crossing over and chromosomal disjunction is unknown. Interestingly, flies homozygous for the $Mcm5^{A7}$ mutation, which causes an amino acid substitution (D694V) in the replicative helicase Mcm5 protein, exhibit a 260-fold increase in meiotic nondisjunction as compared to wildtype flies[1]. Although the observed increase in nondisjunction is accompanied by a severe decrease in CO formation, DSB kinetics, formation of SC along the arms, and replication in mitotic cells appear normal in $Mcm5^{A7}$ mutants[1]. In this study, we aim to determine the role of Mcm5 in *Drosophila* meiosis using $Mcm5^{A7}$ mutants as a genetic tool. To initially evaluate the role that Mcm5 may be playing in meiosis, we examined whether the nondisjunction seen in $Mcm5^{A7}$ mutants were of the homolog or sister class. We found that the majority of nondisjunction events in $Mcm5^{A7}$ mutants are due to homolog missegregation. Through recombinational analysis, we conclude that the homologs in $Mcm5^{A7}$ mutants nondisjoin due to lack of crossing over. Interestingly, a minority of nondisjunction events in $Mcm5^{A7}$ mutants are that of the sister class. Through IF and FISH techniques, we found that sister chromatids are separating precociously in early meiosis I, possibility due to the mislocalization of the cohesion protein Smc1 at the meiotic centromere. Further, $Mcm5^{A7}$ mutants exhibit defective meiotic centromere clustering, an event that is established during pre-meiotic mitotic divisions. Through examining the pre-meiotic mitotic cells in $Mcm5^{A7}$ mutants, we show that centromere clustering dynamics, as well as Smc1 enrichment at the centromere, are indeed defective prior to the onset of meiosis. Collectively, our experiments strongly suggest that defects in pre-meiotic mitotic cells contribute to aberrations in meiosis I, resulting in chromosomal nondisjunction at the end of meiosis. These results highlight the importance of accurate pre-meiotic mitotic divisions on overall meiotic fidelity. Currently, we are investigating whether the pre-meiotic mitotic defects seen in $Mcm5^{A7}$ mutants directly cause the observed decrease in crossing over and chromosomal nondisjunction at the end of meiosis.

1. *Lake CM, Teeter K, Page SL, Nielsen R, Hawley RS. A genetic analysis of the Drosophila mcm5 gene defines a domain specifically required for meiotic recombination. Genetics. 2007;176:2151–2163. doi:10.1534/genetics.107.073551.*

P53 Short tandem repeats and their manifold influence on human meiotic recombination

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Not much is known about short tandem repeats (STRs) and their behavior during meiotic recombination in humans. Yet, recombination hotspots are enriched with polymorphic STRs and longer repeats of consecutive As strongly correlate with recombination. Moreover, in *Drosophila* insertions are significantly over transmitted compared to deletions in repeats with a difference of <5 bp. To analyze the effect of STRs on hotspot sequence evolution and crossover activity, we characterized using pooled sperm typing the crossover breakpoints and allelic transmission patterns of a human hotspot in chromosome 16 containing two STRs, A6/A7 and A9/A19, located 500bp and 200bp upstream of the mean hotspot center, respectively. We observed several different patterns linked to these STR sites: First, by comparing both reciprocal crossover products, our results show that the longer repeat is transmitted preferentially over the shorter one at the A6/A7 STR. When pooling all the donors, this insertion biased gene conversion (iBGC) is significant, with donors heterozygote at the central A9/A19 STR contributing to most of the data given their slightly different distribution of crossover breakpoints. We also observed an overall GC-biased gene conversion (gBGC) at strong (GC) vs weak (AT) SNPs. However, in heterozygous A9/A19 donors, the central SNPs flanking the hotspot center were transmitted preferentially with the longer allele (A19) regardless of being strong or weak, suggesting a potential meiotic initiation bias, a common phenomenon, if homologues are targeted differentially for double–strand breaks. The observed meiotic drive in our donors included SNPs over a stretch of 500-850bp. Second, we observed that the long heterozygous tract of polyAs (A9/A19) can reduce the overall crossover frequency, and heterozygous donors had a significant overall reduced crossover activity compared to homozygous donors. Moreover, in these heterozygous donors we observed an unexpected absence of crossover breakpoints at the A9/A19 STR. Finally, some donors showed an unusual high rate of complex crossovers. The molecular mechanisms inducing the observed patterns are not fully understood yet, but it is likely that different processes are involved. While the short repeat (A6/A7) outside the hotspot center could underlie the same base excision repair mechanism as gBGC leading to an insertion-biased gene conversion (iBGC), longer repeats (A9/A19) behave completely different. The A9/A19 polymorphic site possibly attracts the mismatch-repair machinery (MMR) active in larger mismatches. Poly-A runs are known to form stable loop structures and it is possible that this loop destabilizes the double strand break repair machinery that leads to crossovers. The complete repair mechanisms underlying our observations remain unclear, but our findings underscore the importance of recombination on STR evolution or *vice versa*.

P54 Natural variation and dosage of the HEI10 meiotic E3 ligase control *Arabidopsis* crossover recombination

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During meiosis homologous chromosomes undergo crossover recombination, which creates genetic diversity and balances homolog segregation. Despite these critical functions, crossover frequency varies extensively within and between species. Although natural crossover recombination modifier loci have been detected in plants, causal genes have remained elusive. Using natural *Arabidopsis thaliana* accessions we have identified two major recombination quantitative trait loci (*rQTL*) that explain 56.9% of crossover variation in Col×Ler F_2 populations. We mapped *rQTL1* to semi-dominant polymorphisms in *HEI10*, which encodes a conserved ubiquitin E3 ligase that regulates crossovers. Null *hei10* mutants are haploinsufficient, and using genome-wide mapping and immunocytology we show that transformation of additional *HEI10* copies is sufficient to more than double euchromatic crossovers. However, heterochromatic centromeres remained recombination-suppressed. Strongest *HEI10*-mediated crossover increases occur in sub-telomeric euchromatin, which is reminiscent of sex-differences in *Arabidopsis* recombination. Our work reveals that HEI10 naturally limits *Arabidopsis* crossovers, and has the potential to influence the response to selection. Our more recent work is further exploring natural modifiers of crossover frequency in *Arabidopsis thaliana*, where we have identified at least two further *rQTLs* and are characterizing their identity and function within meiotic recombination. In addition we have performed new experiments where we have combined additional *HEI10* copies with mutations in other recombination pathways in order to produce massive increases in crossovers throughout the genome. These studies reveal how natural variation in meiotic recombination can shed new light onto control of crossover numbers and distributions.

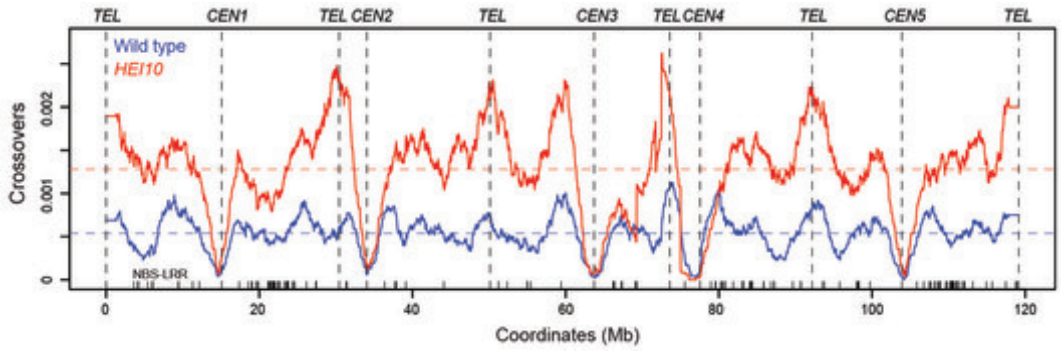

Figure 1: Increased HEI10 dosage elevates euchromatic crossover frequency genome-wide. Crossover frequency along the 5 chromosomes in wild type (blue) and HEI10Col (red) populations. Mean values are shown by the dotted horizontal lines and telomere (TEL) and centromere (CEN) positions indicated by vertical dotted lines and labels.

P55 Adaptation to Whole Genome Duplication: Biophysical Characterisation of the Diploid & Tetraploid Variants of ASY1 and ASY3 from *A. arenosa*

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In most eukaryote, the formation of crossovers (COs) between homologous chromosomes is essential for the successful segregation of an organism's genome. In diploids the pairing partner for a given chromosome is restricted to its single homolog, which results in the formation of bivalents that are easily separated by the spindle. In contrast, in polyploid organisms, more than one homolog of each chromosome is available as a potential partner. Hence a single chromosome can potentially form a CO with multiple homologs, resulting in the formation of multivalents. Such structures are difficult for the spindle to resolve and multivalents are associated with aneuploidy and reduced fertility.

However, in contrast to chemically induced polyploids, multivalent formation is infrequent in established polyploids. Instead, the chromosomes in these organisms show bivalent-like pairing. In allopolyploids, this behaviour is thought to be driven by differences in the composition of each genome, with homologs from within each genome pairing preferentially. However, in autopolyploids there is no genetic basis for pairing preferences as all the homologs are equally homologous. Instead it has been suggested that a reduction in CO number, to ~ one per chromosome, is responsible for the observed bivalent-like pairing in the absence of pairing preferences.

Genome-wide scans on autotetraploid and diploid populations of *Arabidopsis arenosa* have shown that selection has acted upon the proteinaceous components of the axial element (AE) and synaptonemal complex (SC): SMC1, SYN1*, ASY1, ASY3, PDS5,* ZYP1A and ZYP1B. Since both the AE and SC contribute to CO formation, it is plausible that the non-synonymous mutations in these proteins are driving the decrease in CO number. The mechanisms by which these changes could alter meiosis are currently unknown; however it is possible that they result from the alteration of each protein's function, and that the change in CO number may be due to increased interference strength.

Due to its proposed role in the transmission of interference, the characteristics of the proteinaceous components of the AE, ASY1 and ASY3, are of particular interest. However, very little is currently known about the function of these proteins e.g. how they interact with themselves/other proteins to form the higher order structure of the AE. Therefore, the functional characterisation of these proteins using biophysical methods is necessary to determine whether and how the novel mutations fixed in the tetraploids affect the structure and/or functions of the proteins. Also, in addition to providing insights into adaptation mechanisms after whole genome duplication, the proposed biophysical studies will also clarify basic mechanisms of AE formation in plants. In this poster I will show how the diploid and tetraploid variants of these proteins, that are required for *in vitro* studies, can be obtained using heterologous expression in *E. coli*. If possible, I will also discuss the results from any ongoing biophysical experiments.

P56 Meiotic cohesin directs DSB sites by regulating axial elements

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In meiosis, chromosomes exist as ordered, linear arrays of chromatin loops that protrude away from a proteinaceous axis, the axial element. This architecture provides the context, in which initiation of meiotic recombination via double strand break (DSB) formation occurs. Our previous results indicated that DSB hotspots are transiently tethered to cohesin sites on the axis for cleavage, despite residing at a considerable distance from them. Axis tethering of the DSB machinery requires axial element proteins Red1 and Hop1.

Our recent results show that meiotic cohesin Rec8, but not mitotic cohesin Scc1, recruits Red1, Hop1 to convergent transcription sites. In the absence of Rec8, chromosomal binding patterns of Red1 and Hop1 change dramatically. We found that DSB formation correlates closely with the altered Red1 and Hop1 pattern. DSBs are largely decreased in regions that lose Red1 signal when Rec8 is depleted. This result suggests that Rec8 regulates DSB formation (solely) by recruiting Red1. In addition, Rec8 focuses Red1 ChIP-seq signal sharply to the center of convergent transcription sites, while it spreads out by several kb in the absence of Rec8.

In contrast to cohesin, depleting condensin from meiotic nuclei resulted in no obvious defects for chromosome synapsis, which is DSB formation and repair, in our hands.

P57 The DEB-1 and CeLMN participate at the pairing of homologous chromosomes in prophase I

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Lamins are intermediate filaments (type V) which are main components of nuclear lamina and therefore they are a crucial part of the nucleus. They play important role in many nuclear functions like maintenance of nuclear shape, regulation of gene expression, transcription, DNA replication, segregation of chromosome, meiosis and apoptosis. It is known that mutations in lamin genes have negative effects on these functions, and can cause various diseases (e. g. Emery-Dreifuss Muscular Dystrophy or Hutchinson-Gilford Progeria Syndrome). In *Caenorhabditis elegans*, there is only one evolutionary conserved lamin (CeLMN). Interestingly, it was shown that point mutations introduced in CeLMN can mimic the human disease phenotype (Gruenbaum *et al*.).Our project focuses on the molecular mechanisms and regulation of chromosomal dynamics during gametogenesis. We showed that after depletion of DEB-1 (product of *deb-1* gene in *C. elegans*) in the gonad, there appears meiotic delay, improper chromosome pairing, synapsis defect and aneuploidy. Unexpectedly, we identified the protein DEB-1, a mammalian vinculin ortholog, in germ cell nucleus. We showed that this protein is involved in meiosis. Moreover, CeLMN can interact with DEB-1 as we observed that lamina was dislocated, fragmented and internalized after depletion of DEB-1 (using RNAi interference). Therefore, we localized DEB-1 in laminopathic strain of *C. elegans* which has point mutations in conserved residues of the rod and tail domains. Interestingly, we observed that levels of DEB-1 were reduced or lost from the germ cell nuclei. Therefore, we propose that DEB-1 and CeLMN participate in the dynamic processes during meiosis, especially during paring of homologous chromosomes in prophase I. (Acknowledgement: Supported by the project „BIOCEV" (CZ.1.05/1.1.00/02.0109) funded by the European Regional Development Fund, and by GACR (16-03403S), UMG/RVO: 68378050.)

P58 RNF212B, a novel SUMO E3-ligase, regulates meiotic crossing-over in mouse

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Meiotic crossing-over is essential for proper chromosome segregation at the first meiotic division. Defects in crossing-over cause aneuploidy or apoptosis, which can lead to congenital disease or reduced fertility. Alleles of human *Rnf212* cause heritable variation in crossover rate. We previously showed that RNF212 is a SUMO E3-ligase that functions in a dosage-dependent manner to promote crossing-over in mouse (*Reynolds et al., 2013*). Here we report initial characterization of an *Rnf212* paralog, *Rnf212b*. Immunostaining of surface-spread spermatocyte chromosomes reveals a punctate pattern of RNF212B foci that specifically localize to regions of synapsis during the zygotene stage. Numbers of RNF212B foci are maximal as cells enter early pachytene when chromosomes are fully synapsed. Subsequently, as most of RNF212B foci begin to disappear, one or two large/bright foci emerge along each chromosome pair, which are retained throughout pachytene and specifically mark sites where crossing-over will occur. Thus, the dynamics of RNF212B are similar to those of RNF212. Both male and female *Rnf212b* null mutant are sterile. While homolog pairing and synapsis appear to be normal, crossing-over is diminished. Crossover-specific markers, including MLH1, CDK2 and HEI10, are not detected and focus numbers of intermediate recombination markers (RPA, ZIP4/TEX11, MSH4, MER3) are reduced, as seen for *Rnf212* mutants. RNF212 foci are diminished in *Rnf212b* mutants and *vice versa*, indicating a co-dependent relationship between the two paralogs. RNF212B shows SUMO E3-ligase activity *in vitro*, and mutation of a conserved cysteine on the RING domain of *Rnf212b* causes sterility, indicating an essential role for RNF212B SUMO ligase activity in crossing-over. Finally, *Rnf212b* heterozygotes make significantly fewer crossovers than wild-type, indicating that RNF212B also regulates crossing-over in a dosage-dependent manner. Together these data indicate that RNF212B functions interdependently with RNF212 to stabilize intermediate recombination factors at designated crossover sites to enable crossover maturation. *Rnf212b* variants were recently linked to heritable variation in crossover rate in ancient and domesticated species of deer, sheep and cow, indicating a conserved function in mammalian meiosis.

P59 Tudor domain protein TDRD6 controls RNA splicing and nonsense-mediated decay in spermatocytes and spermatids

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Tudor containing protein 6 (TDRD6) is a male germ line-specific protein essential for chromatoid body (ChB) structure and male fertility (1). New data show that TDRD6 plays a major role in spermatocyte and spermatid RNA metabolism (2,3), of which quite little is known for meiotic and postmeiotic cells. In meiotic prophase I spermatocytes TDRD6 interacts with the protein arginine methyl transferase PRMT5, which supports spliceosome assembly, and with spliceosomal core protein SmB. In *Tdrd6*$^{-/-}$ diplotene spermatocytes PRMT5 association with SmB and arginine dimethylation of SmB are much reduced, and the assembly of spliceosomes is impaired. In the nucleus, SMN-positive bodies and Cajal bodies involved in nuclear snRNP maturation are reduced. Transcriptome analysis of TDRD6-deficient diplotene spermatocytes revealed a high incidence of splicing defects. In addition to its role in prophase I, TDRD6 regulates nonsense-mediated RNA degradation (NMD) in late spermatocytes and round spermatids. TDRD6 is essential for UPF1 localization and NMD-related protein interactions in ChBs. Upon removal of TDRD6, the long 3' UTR-stimulated but not the down-stream exon-exon junction triggered pathway of NMD is impaired. Reduced association of the long 3' UTR mRNAs with UPFs correlates with increased stability and enhanced translational activity. Thus, we identified TDRD6 as a major regulator of RNA processing machineries in spermatocytes and spermatids and will discuss the respective processes and mechanism.

1. *Tdrd6 is required for spermiogenesis, chromatoid body architecture, and regulation of miRNA expression. Vasileva A, Tiedau D, Firooznia A, Müller-Reichert T, Jessberger R. Curr Biol. 2009 Apr 28;19(8):630-9*
2. *Chromatoid Body Protein TDRD6 Supports Long 3′ UTR Triggered Nonsense Mediated mRNA Decay. Fanourgakis G, Lesche M, Akpinar M, Dahl A, Jessberger R. PLoS Genet. 2016 May 5;12(5):e1005857*
3. *TDRD6 mediates early steps of spliceosome maturation in primary spermatocytes. Akpınar M, Lesche M, Fanourgakis G, Fu J, Anasstasiadis K, Dahl A, Jessberger R. PLoS Genet. 2017 Mar 6;13(3):e1006660*

P60 Roles of conserved meiotic chromosomal protein Hop1 in DSB formation and axis-loop interaction in fission yeast

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Meiotic chromosome architecture is composed of distinct parts called “axis” and “loop”. The axis is a proteinaceous structure from which chromatin loops emanate. Spo11-mediated programed DNA double strand break (DSB) occurs at defined sites called DSB hotspots, preferentially found in chromatin loop regions; nonetheless the machinery of meiotic DSB formation and subsequent recombination processes are mainly enriched in the axis.
Mechanisms of linking the axis and DSB hotspots have been studied in budding yeast and fission yeast. We previously demonstrated in fission yeast that interaction between axis-associated proteins Rec10 and SFT (Rec7-Rec15-Rec24) complex (RMM complex in budding yeast) is involved in the connection between axes and loops (Miyoshi *et al*., *Mol. Cell*, 2012). Rec10 on axis can recruit SFT complex on hotspot via its interaction with Rec15. Simultaneously, SFT complex binds to DSB formation complex via Mde2, Rec15 interactor. Thus, Mde2 is likely functioning as a bridge protein (liaisonin) between axes and loops.
Here we found that conserved meiotic HORMA domain containing protein Hop1 interacts both Rec15 and Rec10 in fission yeast. ChIP-sequence analysis revealed that Hop1 serves as a tethering-base in axes to DSB hotspots, in collaboration with Rec15 and Rec10.
In some eukaryotes, HORMA domain containing proteins are found in meiotic chromosomal axes, possibly playing pivotal roles in axis/synaptonemal complex formation, DSB formation, and regulation of meiotic cross-over. Conserved interaction between meiotic HORMA domain containing proteins and Spo11-partner proteins led us to speculate the molecular function of Hop1 in the regulation of various meiotic recombination events.

P61 Transcriptome, methylome and metabolic analyses reveal a dramatic phenotypic switch during male meiosis

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Even after decades of meiosis research the regulatory networks governing mammalian meiosis remain an enigma. The adult germ cell lineage presents an example where spermatogenic and spermiogenic meiotic cells from all steps of differentiation coexist in the testicular seminiferous tubules, enabling isolation and characterization of the molecular repertoire of each cell population, belonging to different phases of the meiotic cycle. Previous methodologies employed to isolate cells of different stages relied on cell size, density and DNA content based on vital dyes. We have developed a novel method for the isolation of cells at different stages of spermatogenesis using transgenic mice expressing different levels of tomato fluorescent protein exclusively in germ cells; the level of tomato expression decreases along spermatogenesis, thus allowing to separate different cell populations both from pre pubertal and sexually mature mice using FACS sorting. The main advantages of this method are less contamination by somatic cells and the absence of DNA dyes which affect cell phenotype. Our rapid protocol allows harvesting >100,000 cells of each of the following populations: undifferentiated spermatogonia, differentiating spermatogonia, late spermatogonia, leptotene/zygotene spermatocytes, pachytene spermatocytes, secondary spermatocytes and round spermatids, all in highly pure populations. RNAseq and methylome analyses allowed us to follow transcriptional and metabolic changes throughout the process. We find a dramatic transcriptome switch occurring between leptotene/zygotene and pachytene phase cells with altered expression of ~7000 genes. Notably, the transcriptional network of meiosis in budding and fission yeasts is also divided into two main stages. We hypothesize that this division is a conserved feature of meiosis, enabling the division of meiosis into uncommitted and committed stages. We also identified multiple transcription factors which could drive this transition, and are conducting functional experiments to analyze these factors. Metabolic profiling of cells from premeiotic and meiotic populations revealed differential transcription of key metabolic enzymes; together with metabolic studies we show that substrate requirements and key metabolic pathways change drastically during the process of differentiation. Finally, preliminary results show a transient drop in DNA methylation during leptotene/zygotene-pachytene stages, partially overlapping with double strand break hotspot locations. Together, these analyses reveal an unprecedented level of regulation of meiosis at the level of transcription, DNA methylation and metabolism.

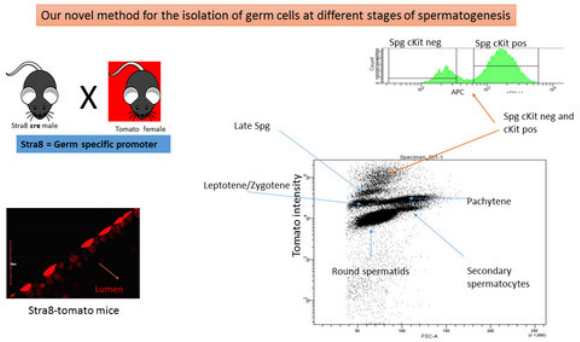

Figure 1. Our novel method for the isolation of germ cells at different stages of spermatogenesis

P62 Effect of polyploidisation on SYN1 (REC8 plant homolog) in *Arabidopsis arenosa*

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Arabidopsis arenosa is a close relative to the model plant *Arabidopsis thaliana* and exists in nature both as a diploid and as a tetraploid form. Yant *et al.* (2013) carried out a whole genome scan for selection of tetraploid and diploid individuals from six *A. arenosa* populations and found eight meiosis genes to have been strongly under selection after polyploidisation, presumably to stabilise meiosis. The Bomblies group is currently studying the evolution of each of these genes both at molecular and biochemical levels. My project focuses on SYN1 (*Arabidopsis* homolog of the kleisin subunit of meiotic cohesion, REC8). Diploid alleles of SYN1 can occasionally be found in tetraploid individuals. I generated F_2 populations of tetraploids segregating for diploid and tetraploid alleles of SYN1 and identified homozygotes for both (referred to hereafter as "DDDD" for homozygotes of the diploid allele and "TTTT" for homozygotes of the tetraploid allele). Five SYN1 DDDD plants were phenotypically compared to two SYN1 TTTT individuals. DAPI stained metaphase I chromosome spreads revealed that SYN1 TTTT meiocytes have fewer multivalents and more clearly separable bivalents than the SYN1 DDDD. This trend is consistent with results obtained when comparing DDDD and TTTT plants for the axis genes ASY1 or ASY3 (Chris Morgan and Huakun Zhang, unpublished data). However, SYN1 TTTT meiocytes contain fewer rod bivalents and more cross-shaped bivalents than SYN1 DDDD meiocytes, which goes the opposite direction from the axis proteins, and opposes the evolutionary trend of the tetraploids. This suggests that the T allele of SYN1 might make crossovers more central along chromosomes. The opposite effect was found for ASY1 and ASY3, where the T allele was shown to make crossovers more terminal. These results are preliminary and will need to be repeated on further SYN1 DDDD and TTTT individuals. If they are confirmed, we will investigate this further, as the effect of the T allele of SYN1 might be dependent on the genotypes of ASY1 and ASY3. Cross-pollinations are currently being carried out with the long-term plan of producing tetraploid individuals which will genotype as DDDD for one, two or all three genes (ASY1, ASY3 and SYN1) in order to study the effect of the T alleles in each context, and their interactions with each other. Future work will also involve cloning an *A. arenosa* SYN1 gene (TTTT) into *A. thaliana* syn1 mutants in order to determine whether the *A. arenosa* alleles rescue the mutant phenotype. If that is the case, then various regions of the SYN1 gene will be cloned individually in order to narrow down the part of the gene responsible for the rescued phenotype.

1. *Yant L, Hollister JD, Wright KM, Arnold BJ, Higgins JD, Franklin CFH, Bomblies K. Meiotic Adaptation to Genome Duplication in Arabidopsis arenosa.* Current Biology. *2013, 23(2151-2156).*

P63 The DNA damage checkpoint prevents polyploidy upon mitotic re-entry from prometaphase I

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Double-stranded breaks (DSBs) and repair of those breaks through homologous recombination can be beneficial or detrimental to a cell depending on whether they occur in meiosis or mitosis. In meiosis programmed DSBs are needed for proper chromosome segregation; however, in mitosis DSBs can lead to loss of heterozygosity and possible oncogenesis. *S. cerevisiae* offers a great tool for studying how DSB repair pathways differ between meiosis and mitosis. In a process historically called "return-to-growth", a cell that initiated meiosis in the presence of nutrient-poor medium can return to mitosis if nutrient-rich medium is provided on or before prometaphase I of meiosis. Intriguingly, cells that re-enter mitosis during prometaphase I have already undergone meiosis-specific DSBs and are completing the resolution of joint molecules. Whether the breaks are fully repaired and DNA damage is being monitored upon entrance into prometaphase I is unknown.

We used a microfluidics approach to determine if cells that are re-entering mitosis from prometaphase I are monitored by the DNA damage checkpoint. Cells in microfluidics chambers are exposed to nutrient-poor medium to enter meiosis, and then exchanged with nutrient-rich medium. Surprisingly, we found several interesting phenotypes in cells lacking DNA damage checkpoint proteins Mec1 or Rad53. These phenotypes include a premature nuclear segregation in the mother. Some of these cells become polyploid and others are able to bud and then move one of the nuclei into the daughter cell to correct the aberrant phenotype. These phenotypes were not seen with the loss of the meiosis-specific checkpoint kinase Mek1, suggesting that the Rad53-dependent DNA damage pathway monitors the re-entry of cells from prometaphase I into mitosis. By pinpointing the time of requirement for Mec1 and Rad53 function, we find that the checkpoint is needed as the cells are re-entering mitosis. In conclusion, our results suggest that the DNA damage checkpoint maintains genome stability by preventing polyploidy in cells that are re-entering the mitotic cell cycle from prometaphase I of meiosis.

P64 Cohesin-REC8 associates with nucleosomes and heterochromatin during *Arabidopsis* meiosis

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In *Arabidopsis* meiosis, an estimated number of 200 DNA double stranded breaks (DSBs) are formed and only ~10 of these recombination sites mature to form a crossover. We are investigating the factors controlling crossover formation. For this we have developed Chromatin Immunoprecipitation (ChIP) of meiotic recombination proteins and have identified their genomic binding sites. We observed that the cohesin subunit REC8, a component of the meiotic chromosome axis, is strongly enriched in centromeric heterochromatin, together with DNA methylation and histone H3K9me2. Within the chromosome arms REC8 shows varying abundance, which correlates positively with nucleosome occupancy. Sequencing of SPO11-oligonucleotides shows that REC8 occupies distinct genomic sites to those where DSBs form, supporting the chromatin loop-base array model. Here DSBs are formed in the chromatin loops away from the cohesin axis sites. In addition, we have observed polarised localisation of REC8 towards the transcription termination sites of highly transcribed genes, suggesting that transcription may displace REC8 towards the end of the genes, consistent with data from yeast. We are currently functionally investigating whether nucleosomes and H3K9^{me2} regulate REC8 binding sites and thereby influence crossover formation. I will also present immunocytological analysis of *rec8* mutants that reveal that both chromosome axis and DSB formation are defective. Interestingly, the few DSBs formed are all associated with the short axial structures, which indicates a link between chromosome axis and DSB formation. Together our data are consistent with REC8-cohesin performing a key organising role in meiotic chromosome recombination.

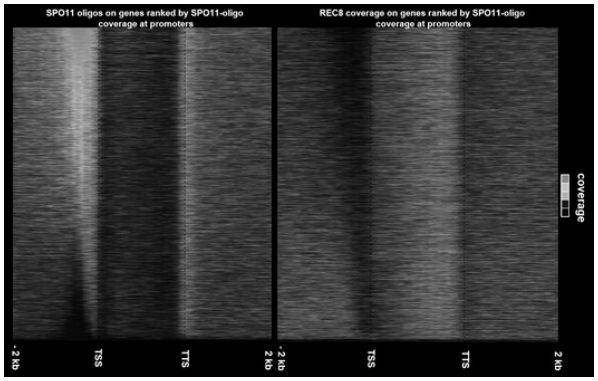

Figure 1: SPO11 is a protein that forms DNA double stranded breaks (DSBs), remaining covalently attached with the DNA molecules after a break is formed. We purified SPO11-oligo molecules and identified the binding sites using Illumina sequencing. The figure represents Arabidopsis genes ranked by SPO11-oligo coverage at the promoters (left), with the genes located on the top of the heatmap representing those with the highest SPO11-oligo coverage. We used the same ranking of genes to plot REC8 coverage (right). We observed that the genes with the highest SPO11-oligos were depleted of REC8 at the promoters indicating that REC8 and DSBs occupy distinct sites.

P65 Identifying natural modifiers of crossover frequency in *Arabidopsis thaliana*

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During meiosis, homologous chromosomes pair and undergo a process of reciprocal genetic exchange known as recombination, producing crossovers. This generates genetic diversity and is required for balanced homolog segregation. Despite the critical functions of crossovers, their frequency and distribution varies extensively within and between species. We are using natural accessions of *Arabidopsis thaliana* to identify genes underlying this crossover variation within the species, using a fluorescent-based reporter system to measure recombination frequency in specific genomic intervals. Previously, quantitative trait loci (QTL) mapping in a Col x Ler F_2 population identified the conserved ubiquitin ligase HEI10 as a major regulator of crossovers in this population. Extension of this approach in a Col x Bur F_2 population has revealed three major recombination QTLs (rQTLs). Additional mapping of a novel recessive rQTL on chromosome 1 has refined this interval to several genes, and confirmation of the underlying causal gene is ongoing. Our work shows that extensive natural variation exists that modifies crossover frequency in *Arabidopsis*, which has implications for interactions between selection and recombination.

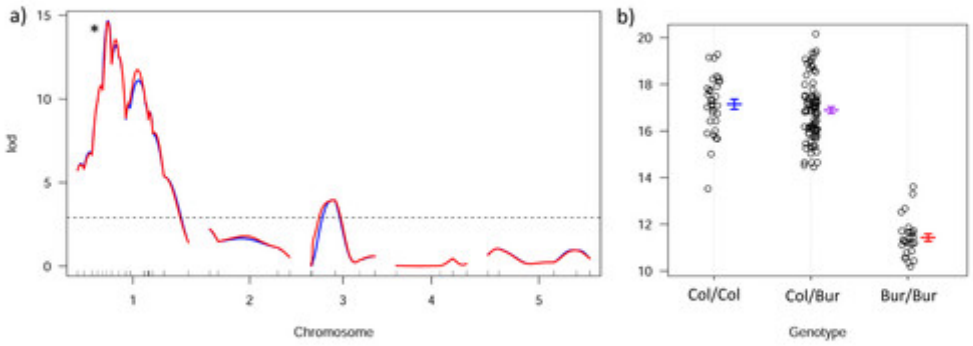

Figure 1.: QTL mapping within a genomic interval on chromosome 3 in a Col x Bur F2 population revealed 3 significant rQTLs affecting crossover frequency (a). Further mapping in a population segregating only in the region of a novel rQTL on chromosome 1 (asterisk) showed the Bur-0 allele to be acting recessively to reduce crossover frequency (b).

P66 Temperature increases cause transposon-associated DNA damage specifically during spermatogenesis

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Sexually-reproducing organisms use meiosis to generate haploid gametes, such as sperm and eggs, to transmit their genome to the next generation. All tissues are susceptible to dramatic increases in temperature; however, developing sperm in the testes are unusually sensitive to small temperature fluctuations. In contrast to oogenesis, spermatogenesis requires a narrow isotherm of 2-7°C below core body temperature. Failure to thermoregulate spermatogenetic tissue and prolonged exposure to elevated temperatures are linked to male infertility. Temperature increases are known to cause DNA damage in spermatocytes, but the molecular mechanisms underlying this damage are unclear. Here we show that upon a brief heat shock, the spermatocytes (but not oocytes) of *Caenorhabditis elegans* exhibit an increase in double strand DNA breaks (DSBs), and that these temperature-induced DSBs occur via a SPO-11 independent pathway and are dependent on the presence of transposons. Using an antibody against the recombinase RAD-51 (which marks sites of DSBs), we found that heat shock of the spermatocytes of both males and L4 hermaphrodites produces a dramatic increase in RAD-51 foci at all phases of meiosis I, and causes a 30-40 fold increase in RAD-51 foci in a *spo-11* mutant background where no endogenous meiotic DSBs are formed. Moreover, using a marker for a crossover repair outcomes (COSA-1), we found that heat shock can restore COSA-1-marked crossovers in *spo-11* males, indicating that at least a subset of the heat shock-induced RAD-51 foci represent DSBs with the capacity to be repaired as crossovers. Furthermore, we observed disorganization of chromosome structures specifically in late pachytene spermatocytes upon heat shock, suggesting that increased temperature alters chromosome structure. Interestingly, we found that the heat-shock induced RAD-51 foci are *Tc1* transposon dependent as males carrying a low *Tc1* copy number do not display increased RAD-51 foci following heat-shock. Previous studies have demonstrated that certain small RNA molecules such as PIWI-interacting RNAs (piRNAs) regulate transposon activity in the germline and some small RNA pathways promote male fertility at elevated temperatures. Our preliminary data indicate that mutations in specific small RNA pathways affect the efficiency of temperature-induced DNA damage in spermatocytes. Taken together, our data suggest that the increased DNA damage from heat-shock observed during spermatogenesis may be due to transposon mobilization, and may contribute to impaired fertility in heat-exposed males.

P67 Reorganisation of the nuclear lamina during meiotic chromosome movement in *Caenorhabditis elegans*

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Upon meiotic entry, chromosomes engage in a movement process to find and pair parental homologs, a prerequisite for their faithful segregation after crossover formation. This movement requires the transmission of cytoskeletal forces to the chromosomes as well as the reorganization of the nuclear envelope, including the nuclear lamina. In this work, we investigate the role of the nuclear lamina and its associated proteins in *C. elegans* meiosis and how the lack of reorganization affects meiotic progression.

C. elegans expresses only one lamin isoform in somatic and meiotic cells, encoded by a single gene (*lmn-1*). The disorganized gonads and sterility of hermaphrodite *lmn-1* deletion mutants indicate a meiotic role for LMN-1 especially during oocyte formation. Using mass spectrometry, we have found LMN-1 phospho modifications on multiple residues clustered within three distinct and conserved domains of the protein. Our work shows that at least some of these sites are modified during early stages of meiosis where chromosome movement occurs, they depend on the meiotic master regulator kinases *chk-2/plk-2* and regulate the distinct physico/chemical properties of the meiotic lamina network. Intriguingly, the lamina in early meiosis is subjected to modifications, which render it "more soluble", thus less cross-linked. We find that these alterations in the lamina influence chromatin distribution and the diffusion of membrane associated components, however, surprisingly, not SUN/KASH driven chromosome movement. Few resulting aberrant oocytes in the unphosphorylatable lamin mutant are efficiently culled by apoptosis. Previously, we showed that an N-terminal phospho cluster on the SUN-1 protein is required to sustain prophase movement when problems occur. An unphosporylatable SUN-1 mutant displays a synthetic defect with the unphosphorylatable lamin mutant resulting in chromosomal aberrations and defective diakinesis.

Overall our current work demonstrates the significance of the meiotic nuclear envelope and its specific modifications for chromatin organization and dynamics.

P68 Single cell meiotic heteroduplex DNA encyclopedia reveals biased resolution of double Holliday Junctions promoted by Mlh1-3

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Meiotic homologous recombination is essential for allelic shuffling and chromosome segregation during gametogenesis. Decades of studies focusing on a few loci only in different organisms led to models that explain most of but not all the complexity of the DNA gymnastic leading to crossover (CO) and non-crossover (NCO) recombinants. Here we analyzed meiotic heteroduplex DNA (hDNA) tracts genome wide in a mismatch repair (MMR) defective background and in combination with the genome wide map of meiotic DNA double strand breaks (DSBs) to test current recombination pathways. We systematically found DSB regions within NCO-associated trans hDNA tracts, which supports that they originate from single DSBs either by two-ended synthesis dependent strand annealing (SDSA) or double Holliday junction (dHJ) processing. These trans hDNA tracts allowed us to unambiguously orientate the corresponding DNA strands and we found that DSBs occur 5' of hDNA tracts compatible with SDSA, bringing further support to this pathway in meiotic cells. In addition, 15% of SDSA events occur in discontinuity with respect to the expected initiating DSBs supporting putative template switching between sister and non-sister chromatid. Remarkably, the structure of the CO-associated hDNA tracts shows that resolution of double Holliday junctions (dHJs) is biased toward the cleavage of the pair of strands that contain newly synthesized DNA near the junction, raising the possibility that nicks present in HJs are responsible for this bias. This resolution bias depends of the putative dHJ resolvase Mlh1-3. In addition, we found that Mlh1-3, Exo1 and Sgs1 promote the asymmetry of hDNA tracts positioning with respect to the initiating DSB of CO intermediates as well as their complexity. Finally, the recombination signatures we observed confirm the biased resolution of dHJs into COs when the ZMM pathway is functional. Altogether, this study brings new light on the processing of meiotic recombination intermediates and on the functions of meiotic proteins.

P69 Repair of SPO11-induced double-strand breaks by non-homologous end joining in ATM-deficient mouse spermatocytes

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DNA double-strand break (DSB) formation by the SPO11 protein initiates homologous recombination, the essential process required for accurate chromosome segregation in meiosis. DSBs activate the ATM kinase, which is best known for its function in the DNA damage-response in somatic cells. In contrast to mitosis, in meiosis ATM is essential, playing a critical role in controlling DSB numbers via a negative feedback loop (Lange *et al*, Nature 2011, Barchi et al, PLoS Genet 2008). Moreover, we have recently discovered that ATM plays a dominant role in shaping DSB distributions across the genome (Lange *et al*, Cell 2016).

In our current studies we hypothesize that deregulated DSB formation in ATM-deficient spermatocytes fosters use of aberrant DSB repair pathways. Here we investigated whether closely spaced DSBs occur and are repaired by non-homologous end joining (NHEJ), an error-prone DSB repair pathway thought to be largely suppressed in meiosis. To this end, we analyzed nucleotide resolution DSB hotspot maps to locate pairs of strong DSB hotspots that fit our criteria: they are separated by less than 2 kb and are hotspots in both wild-type and *Atm*$^{-/-}$ mice. A PCR-based strategy was designed to detect NHEJ between such DSB hotspots. Testing a DSB hotspot pair on chromosome 1, we found deletion events in *Atm*$^{-/-}$ testis but not in liver or wild-type testis DNA. Similarly, we detected NHEJ events in juvenile *Atm*$^{-/-}$ but not juvenile wild-type testis. For the tested DSB hotspot pair, the frequency of NHEJ events per meiotic cell is 0.0058% in adult (29 events per ~500000 meiotic genomes tested) and 0.0023% in juvenile testis (11 events per ~400000 meiotic genomes tested).

We sequenced PCR products to analyze breakpoints junctions. This revealed that breakpoints were located at or near hotspot centers. In addition, we found frequent 2 to 8-bp microhomologies (38%) at deletion junctions, which suggests that two DSBs are repaired via alternative NHEJ pathways in some cases. Interestingly, we also identified deletions accompanied with insertions (7 of 69 sequenced deletions). The inserted DNA sequences mapped to one of the hotspots of the pair (2 insertions) as well as to other genomic locations (5 insertions). Surprisingly, 4 of the latter insertions mapped to other DSB hotspots found in wild-type and *Atm*$^{-/-}$ hotspot maps, or within DSB clusters found only in *Atm*$^{-/-}$ maps. One potential mechanism for these latter insertions is that they originate from DNA fragments generated by double SPO11 cuts within narrow (sub-kb) genomic regions. Because the ATM homolog in yeast suppresses the formation of multiple, nearby DSBs (Garcia *et al*, Nature 2015), our observations suggest that ATM might play a similar role in mouse meiosis.

Taken together, our experiments demonstrate that in the absence of ATM, some DSB repair events are channeled into the NHEJ pathway and provide evidence for lack of spatial control of DSBs.

P70 Characterisation of Large-Scale Chromatin Organisation within Pachytene Mouse Spermatocytes

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Chromatin is a plastic and dynamic polymer whose structural organisation has the capacity to significantly impact upon key meiotic events. Electron microscopy has demonstrated that pachytene chromatin folds into sequential loop arrays, the base of which associates with the synaptonemal complex (SC) axis. The functional importance of such structures has been highlighted in budding yeast by chromatin immunoprecipitation experiments which demonstrate that DNA double strand breaks form within these chromatin loops and are subsequently recruited to the SC axis for recombinogenic repair. However, the structural organisation of meiotic chromatin remains elusive in mammals.

We have investigated large-scale chromatin organisation in mouse meiosis by combining SYCP3 (SC marker) immunofluorescence and fluorescent in situ hybridization (FISH). Using bacterial artificial chromosomes (BAC) as FISH probes we have shown that specific genomic regions tether to the pachytene SC axis in spermatocytes, while other genomic regions do not. This finding is consistent with mouse meiotic chromatin being organized into loops with their bases embedded in the pachytene SC axis in a manner which is conserved between individual mice. Furthermore, by employing successive adjacent BAC probes we have traced the length of an entire autosomal chromatin loop that extends for approximately 1.5 Mb.

P71 Synaptonemal Complex-Associated Proteins Protect Against Errors in Mismatch Repair During Meiosis in Budding Yeast

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Many of the events that process meiotic DNA recombination repair intermediates into interhomolog crossovers occur within the context of a meiosis-specific tripartite structure called the synaptonemal complex (SC), which assembles along lengthwise-aligned homologous chromosomes. We are interested in understanding the functional relationship between SC proteins, the mature SC structure, and interhomolog crossover recombination. In prior work we determined that although certain SC components such as the transverse filament protein Zip1 are required for generating a proper number of interhomolog crossovers, the tripartite SC structure itself is dispensable for meiotic recombination: SC central element components such as Ecm11, Gmc2 and the N terminus of Zip1 are dispensable for interhomolog recombination and instead are important for limiting the abundance of MutSγ crossovers. However, we observe that both transverse filament and central element components of the SC influence the processing of recombination intermediates. The resolution of heteroduplex during the processing of meiotic recombination intermediates is typically accompanied by mismatch repair, which results in either restoration of the original DNA sequence or conversion to the sequence associated with the homolog. A failure in heteroduplex repair, such as in the absence of the Msh2 and Pms1 mismatch repair enzymes, leads to mismatch-containing DNA within haploid spore nuclei. Such spores give rise to two distinct genotypes after their first mitotic division, resulting in sectored colonies. We found that mutants missing SC central element components, such as *ecm11* or *gmc2*, produce an abnormally large number of spore products that give rise to sectored colonies on SC-Thr media, indicating a role for SC central element proteins in protecting meiotic recombination intermediates against errors in mismatch correction. We have confirmed Ecm11's role in meiotic mismatch repair on a whole genome level by evaluating the genome sequences of octad meiotic products from wild-type, *ecm11*, *pms1*, and *msh2* hybrid strains carrying thousands of mismatches. We have also observed a similar elevation in mismatch repair errors at *THR1* in several non-null *zip1* alleles, in *zip3* mutants, and (surprisingly) in mutants missing MutSγ. Our observations suggest that SC proteins coordinate the processing of recombination intermediates with repair pathways that restore or convert heteroduplex DNA. We speculate that in the absence of Ecm11, Gmc2, Zip3, MutSγ, or in contexts where Zip1 is partially functional, Zip1-associated recombination intermediates are processed in a manner that is not properly associated with mismatch repair.

P72 Dna repair, chromatin morphogenesis and interlock resolution are controlled by topoisomerase II during meiosis

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During pairing and synapsis chromatin entanglements occur frequently, especially in organisms with long chromosomes like most plants. Chromatin cycles of expansion and contraction are postulated to be controlled by topoisomerase II (TOPII), among others, in mitosis and meiosis. Type II topoisomerases are highly conserved proteins. They resolve entangled structures between two dsDNA molecules by ATP-dependent induction of a transient double-strand break in one of the molecules to enable the other to pass through the broken double helix which is subsequently religated. This essential enzyme can decatenate sister chromatids, relax positive and negative supercoiling and solve DNA knots during any cell process involving DNA metabolism (replication, transcription, condensation...). Global chromatin topology is highly regulated during meiosis, towards achieving the pairing of homologous chromosomes and CO formation. In addition, almost 30 years ago, TOPII was proposed to be involved in one of the models for interlock resolution in meiosis, but very few studies since then have shed light on this matter. TOPII has been described to form part of the mitotic and meiotic chromosome axis and have a role in chromosome condensation and the spreading of the interference signal. However, a more global approach in meiosis assessing all the processes that underperform when TOPII is absent was still to be done. TOPII hypomorphic mutant and meiotic-specific RNAi line revealed a wide pleiotropic range of deffects in the development of *Arabidopsis thaliana*. DNA replication was severly affected during mitosis but only mild effects were seen in meiosis. DSBs repaired was delayed in prophase I leading to SPO11-dependent anaphase I bridges. CO distribution appears not different from the WT under normal circumstances, although HEI10 foci were increased in *topii-rnai* and higher chiasma frequency was present in *topii-1 mlh3-1* mutant. Finally, TOPII role in chromatin stress-release cycles must be responsible for the decondensed bivalents observed in metaphase I and the high frequency of interlocks. We show for the first time a role of TOPII in interlock resolution and proposed that both theoretic models proposed co-exists during prophase I: chromosome movement and breakage. Overall, we have investigated how sensing of chromatin global topology by TOPII affects several aspects during meiosis.

P73 Cdc14b prevents metaphase entry upon activation of the pachytene checkpoint in spermatocytes

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Pairing and recombination of meiotic chromosomes occurs during a prolonged specialized prophase. In the presence of defects in any of these processes, mammalian spermatocytes usually arrest in pachytene preventing entry into the first meiotic division. Despite its prominent role, how the pachytene checkpoint is regulated remains unknown. Our results demonstrate that this pathway is modulated by cyclin-dependent kinase-counteracting phosphatase Cdc14b. Cdc14b-deficient spermatocytes reach metaphase I in the presence of univalent sex chromosomes, suggesting defective surveillance mechanisms. Lack of this phosphatase overcomes the pachytene arrest observed in Cdk2- or H2AX-null spermatocytes, allowing these cells to progress to metaphase I in the presence of meiotic aberrations. Our study illustrates the relevance of Cdc14b phosphatase in the mammalian pachytene checkpoint, and reveals a role for Cdc14b in preserving male fertility and genomic stability in the germline.

P74 Metabolic studies reveal profound changes in energy sources during mouse male meiosis

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We have developed a novel method for the isolation of cells at different stages of spermatogenesis using transgenic mice expressing tomato fluorescent protein exclusively in germ cells. In this system the level of tomato expression decreases with the progression of spermatogenesis, thus allowing to separate different cell populations from pre pubertal as well as sexually mature mice using FACS sorting. This method does not require addition of DNA dyes, which cause significant metabolic perturbations. We performed RNAseq analysis on isolated populations of undifferentiated spermatogonia, differentiating spermatogonia, late spermatogonia, leptotene/zygotene spermatocytes, pachytene spermatocytes and round spermatids and analyzed data concerning different metabolic pathways. This analysis revealed profound changes in expression of metabolic genes along the course of meiosis progression with the most significant changes occurring between leptotene/zygotene and pachytene stages. Next we measured metabolic responses of the different cell populations to specific metabolic substrates using Seahorse XF Agilent technology. This analysis confirmed profound changes in energy source utilization particularly between leptotene/zygotene and pachytene stages. We hypothesize that these metablic changes are linked to the dramatic change in the transcriptome between these two steps and are studying potential mechanisms.

P75 Aurora B/phosphatase antagonism underlies maintenance of meiotic chromosome and spindle organization in *Drosophila* oocytes

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Aurora B is required for assembly of the spindle and kinetochores in acentrosomal *Drosophila* oocytes. Using drug inhibition, we have found that sustained Aurora B activity is required during meiosis I to maintain the spindle and the kinetochores. The requirement of a kinase for spindle maintenance suggests spindle dynamics could be regulated by phosphatases. Using tissue-specific RNAi, a division of labor was observed between two phosphatases, PP1 and PP2A, in regulating kinase activity in oocytes. The spindle microtubules were stabilized when Aurora B was inhibited in Pp2A RNAi oocytes, demonstrating that PP2A antagonizes the Aurora B spindle maintenance function. PP2A was also found to be required for maintaining sister chromatid cohesion during metaphase I, a role that appears to be independent of MEI-S332/SGO. PP1-87B was found to antagonize the Aurora B function during metaphase I that maintains chromosome organization and regulates kinetochore assembly. During these studies, we also found that PP1-87B is required for sister centromere co-orientation, the meiosis-specific function that promotes fusion of sister kinetochores in meiosis I to promote attachment to microtubules from the same pole. A similar phenotype had been previously observed in oocytes lacking the kinetochore protein SPC10R. However, the mechanism of PP1-87B and SPC105R function in co-orientation appears to be different. For example, the precocious separation of centromeres in Spc105R RNAi oocytes, and probably Pp2A RNAi oocytes, depends on separase, while in PP1-87B RNAi oocytes it does not. The precocious separation of sister centromeres in meiosis I of Pp1-87B RNAi oocytes does depend on the presence of microtubules (unlike Spc105R RNAi oocytes), Polo kinase and the SC protein C(3)G. These results show that multiple phosphatases maintain and regulate meiosis-specific spindle and chromosome structures in oocytes. In addition, we have found evidence of a pathway for co-orientation that begins with cohesion and SPC105R but ends with PP1-87B and spindle forces but not cohesion.

P76 Analysis of the crossover landscape in tetrads of hyperrecombinant mutants of *Arabidopsis thaliana*

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During meiosis, homologous recombination produces crossovers (CO) that are essential to promote the accurate segregation of chromosomes at the first division. Recent studies have shown that, in *Arabidopsis thaliana* as in many species, several pathways limit the number of COs within a narrow range despite a large excess of molecular precursors. To better understand the mechanism involved in the control of the COs number during meiosis we produced tetrads of plants issued from F1 plants exhibiting various combinations of mutations within the known anticrossover factors: RECQ4A, RECQ4B (the two *Arabidopsis* SGS1 homologs), FANCM, FIGL1 (the single representative of the AAA-ATPase FIDGETIN family). In total, 20 tetrads were sequenced and analyzed. We show that the number of COs per meiosis can be increased up to 5 times depending when a mutations in both RECQ4genes is associated to a mutations in FIGL1. Moreover the pattern of gene conversion associated to COs reveals frequent complex events possibly due to multiple invasions involving often 3 chromatids. Other features of the meiotic recombination landscape in *Arabidopsis* will be discussed.

P77 Manipulating crossover frequency in crops

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One of the limitations of breeding programs is the restricted number of meiotic crossovers (CO) between homologs (typically 1-3 per chromosome pair). Identification of genes that control CO formation in a large range of species has been very successful over the past 30 years leading to a tremendous increase in our understanding of this process. It has been notably shown that *FANCM*, *FIGL1* and *RECQ4* limits CO formation shown in *Arabidopsis thaliana* (W. Crismani Science 2012; M. Séguéla-Arnaud PNAS 2015; PNAS 2015). Disrupting one or a combination of two of this anti-CO factors provokes large increase in CO frequency without affecting meiotic progression in *Arabidopsis thaliana*. RECQ is a small but highly conserved helicase family involved in genome stability. Unlike members of the *brassicacae* family (and recent duplication events), most plants species have a single *RECQl4* gene. Here we demonstrate in rice that *Osrecq4l -/-* exhibit an average 3.1-fold increase in CO rate compared to wild type, without detrimental effect on meiosis and fertility. The genome wide distribution of COs will be presented. These results pave the way to manipulate recombination rates in in plant breeding programs.

P78 BAC transgene containing incomplete Prdm9 reduces the fecundity of semifertile mouse hybrids

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PRDM9 is germ-cell-specific histone-3-methyltransferase determining the sites of meiotic recombination hotspots in most mammals. *Prdm9* is essential for meiosis of both sexes; germ cells of *Prdm9*-deficient mice arrest at pachytene stage of meiotic prophase I in the laboratory mice, being predominantly of *Mus m. domesticus* (Mmd) subspecific origin. *Prdm9* was also identified as the Hybrid sterility 1 gene (*Hst1*) that causes meiotic arrest in F_1 male offspring from intersubspecific crosses of *M. m. musculus* females (e.g., the PWD strain) and some Mmd males (e.g., the C57BL/6 (B6) strain). The (PWD x B6)F1 (abbreviated PBF1) males are sterile because of complete pachytene block similar to that of B6 with removal of *Prdm9*. Males from the reciprocal (B6 x PWD)F1 cross (abbreviated BPF1) show partial meiotic arrest and contain sperm in their epididymides. BAC transgenes carrying the *Hst1* region with intact *Prdm9* gene from the C3H/HeJ mouse strain rescue the complete meiotic arrest of PBF1 and the partial one of BPF1, respectively. We found that another C3H/HeJ transgene (called BAC21) containing incomplete *Prdm9* did not rescue the fertility of PBF1 and, surprisingly, reduced the fertility of BPF1 hybrids. BAC21-carrying BPF1 males showed significantly decreased testes weight and epididymidal sperm count compared to BPF1 littermate controls. Fertility of B6-BAC21 heterozygous animals was similar to B6-BAC21 homozygotes and B6 controls. To reveal if BAC21 affects meiotic synapsis, we used fluorescence in situ hybridization probes specific for chromosomes 17 and 13 (where *Prdm9* and the integration site of BAC21 reside) along with immunofluorescent staining of synaptonemal complex proteins SYCP3 and HORMAD2 on spread nuclei. We identified no difference in synapsis of BAC21-carrying and control BPF1 pachytene spermatocytes. The progress of BAC21-carrying BPF1 testes is thus affected after prophase I of the first meiotic division. To specify the first affected stage, we analyzed apoptosis using TUNEL, metaphase I and II spermatocytes, and sections of seminiferous tubules from prepubertal and adult testes of BAC21-carrying BPF1 and controls. Moreover, the BPF1 mice carrying another transgene with similar gene content were also utilized to check the influence of BAC integration site. These results will be discussed to clarify the impact of BAC transgenes on mouse fertility.

P79 A derived ASY1 allele shifts crossover position in autotetraploid *Arabidopsis arenosa*

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Autopolyploid species face additional challenges during meiosis due to the presence of multiple homologous chromosomes, each of which are equally capable of pairing and recombining with one another. In newly formed autopolyploids this can cause problems such as homologue missegregation during anaphase I, however many established autopolyploid species have evolved molecular mechanisms to overcome these obstacles. The existence of extant diploid ($2n$ = 2x = 16) and autotetraploid ($2n$ = 4x = 32) populations of *Arabidopsis arenosa* provides a valuable tool for examining these mechanisms that underlie autopolyploid meiotic stabilisation. Previous studies comparing the diploid and autotetraploid genomes of *A. arenosa* have shown that ASY1, a functional homologue of the axis-associated protein Hop1 in yeast, has undergone strong ploidy specific differentiation (Hollister *et al.,* 2012, Yant *et al.,* 2013). Using a cytological approach we have examined meiotic behaviour in autotetraploid *A. arenosa* and transgenic *A. thaliana* plants expressing either the ancestral (diploid) or derived (autotetraploid) ASY1 alleles. We find that crossovers are shifted to a more distal position, towards the chromosome ends, in plants expressing the derived ASY1 allele and propose that this aids autotetraploid meiotic stabilisation by promoting the balanced segregation of homologues linked as multivalents during metaphase I.

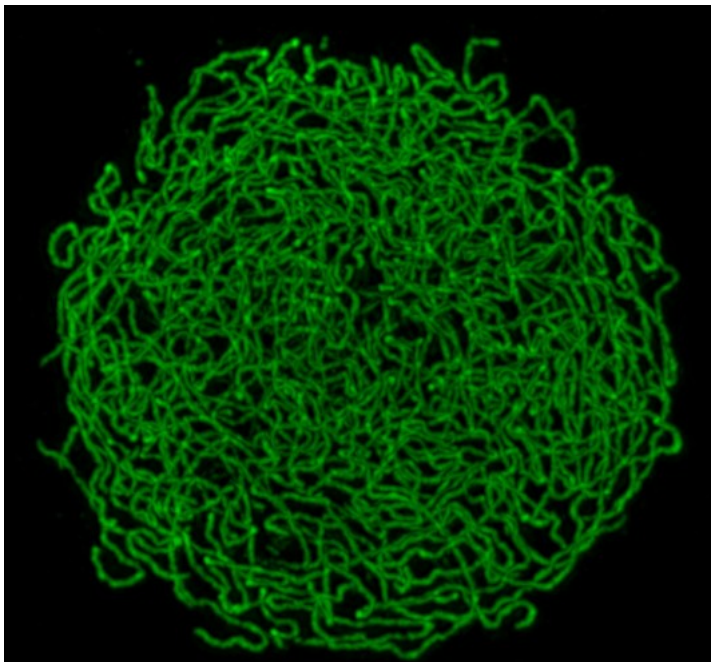

Figure 1 A leptotene cell from autotetraploid Arabidopsis arenosa *stained for ASY1 and imaged using structured illumination microscopy*

P80 Boosting meiotic recombination on short chromosomes

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Gametogenesis (meiosis) involves a stage of massive genome fragmentation, yet fragments are reassembled by homologous recombination, in most of cases without mishap. Meiotic recombination is initiated at hundreds of locations by programmed formation of DNA double-strand breaks (DSBs), yielding not only genetic diversity but also physical connections between homologous chromosomes, which are essential for proper allocation of chromosomes into sperms and eggs. Failures in meiotic recombination lead to chromosome abnormalities that can cause developmental disabilities or spontaneous abortion, therefore its initiation step (DSB formation) must be tightly regulated. For example, the control of DSB number per chromosome is vital: too few DSBs compromise chromosome disjunction, too many can cause genomic instability. If DSBs were to be distributed evenly, short chromosomes would receive fewer DSBs and would be at higher risk of chromosome nondisjunction.

Findings in budding yeast suggest the existence of mechanisms to attenuate this problem: short chromosomes exhibit higher densities of recombination, DSBs, and ChIP signal of proteins required for DSB formation than long chromosomes. We propose that preferential protein association to short chromosomes promotes higher DSB and recombination density. To explore underlying mechanisms, we measured the association and dissociation timing of Rec114, an essential component of the DSB protein complex, and found that Rec114 associates earlier and dissociates later on short chromosomes than on long chromosomes. The resulting elongation of the Rec114 loading duration would potentially ensure the overrepresentation of DSBs and recombination on short chromosomes. We are testing further mechanisms and results will be discussed.

P81 Interplay between DSB-signalling and progression of synapsis via phosphatase complex (PP^4

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In many eukaryotes Double stranded breaks (DSB) formation and DSB repair are known to affect pairing and synapsis of homologous chromosomes. The interplay between DSB-signaling and progression of synapsis is not well understood. The coordination between DSB formation and synapsis and meiotic progression could occur via wave of DNA damage signaling generated due to DSBs, or it could also require recombination intermediates, such as SEIs (single end invasions) or DHJs (double-Holliday junctions). Considering the events of meiotic prophase for DSB repair via homologous chromosome and to govern the faithful segregation of chromosomes, it is necessary to have synchronous coordination between DSB signaling and chromosomal structural changes including chromatin modification.

Our preliminary results show that a partially redundant network of proteins regulates phosphorylation level, with significant impact on synapsis. Deletion of *PPH3,* the yeast PP4 phosphatase causes delayed and incomplete synapsis. It is not known, which substrates of Pph3 prevent synapsis, if unphosphorylated. Analysis of different PP4 subunits reveal a major role for the catalytic subunit Pph3, but a minor one for the regulatory subunit Psy4 in controlling synapsis.

Deletion of *PPH3,* also delays meiotic progression compared to other PP4 phosphatase subunits. Our results show that PP4 phosphatase complex has different composing subunits, which might assemble to form PP4 phosphatase complexes to work temporally during meiotic prophase.

We found several modifiers of the effect of *pph3*Δ, suppressing or enhancing effects on synapsis. Suppressors distinguish between synapsis and cell cycle progression, suggesting that these are regulated by separable dephosphorylation events.

1. *Falk JE and Hochwagen A (2010) Dev Cell 19:599-611*
2. *Panizza S and Klein F. (2011) Cell 146:372-383*
3. *PhD Thesis, Jean Mbogning (Klein Group, MFPL)*
4. *Carballo JA, Klein F and Cha RS (2013) Plos Genet.*

P82 Recombination of Yeast Hybrids Through Meiotic Reversion

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S. cerevisiae strains can enter the meiotic developmental program, induce Spo11-dependent double-strand breaks genome-wide and return to mitotic growth before the reductional division, a process known as Return To Growth (RTG). Whole genome sequencing of RTG strains derived from the hybrid S288c/SK1 diploid strain demonstrates that the RTGs are bona fide diploids with mosaic recombined genome, derived from either parental origin (Laureau *et al.*, 2016). Individual and mother-daughter RTG genotypes are recombined, comprising homozygous regions due to the loss of heterozygous (LOH) events of various lengths, varying between a few nucleotides up to several hundred kilobases. To determine the biological relevance of the RTG process and its mechanistic features to create genetic diversity in sterile and hybrid yeasts, we examined the capacity of synthetic and natural wild type and *ndt80Δ S. cerevisiae*, *S. paradoxus*, *S. pastorianus*, *S. kudriazevii* and yeast industrial polyploids to perform the RTG process (they do!) and recombine (to what extent?) between polymorphic and highly heterologous chromosomes. Phenotype/genotype analysis of the RTG strains for various traits validate the use of this procedure of genome diversification to rapidly map complex traits loci (QTLs) without changing the parental ploidy.

P83 Regulation of meiosis-specific cohesin cleavage in mouse oocytes

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Female meiosis is prone to chromosome missegregation, which leads to formation of aneuploid oocytes and is a cause of severe genetic disorders in the resulting progeny. Cohesin, a protein complex that holds sister chromatids together, is essential in this process and its untimely removal from chromosomes is one of the primary causes of chromosome missegregation. Cohesin is removed in a step-wise manner through cleavage of its meiosis-specific subunit Rec8 by Separase. Most of cohesin is removed from chromosome arms during the first meiotic division in anaphase I, but a portion that connects sister chromatids remains untouched until anaphase II. This is achieved by protection of Rec8 from cleavage at the centromeric region of chromosomes. In yeast, only the phosphorylated form of Rec8 can be cleaved, however the state of mammalian Rec8 during cleavage, as well as precise sites of mammalian Rec8 phosphorylation and the required kinases are not known. This knowledge is essential for understanding the precise mechanism of cohesin protection at centromeres. In this project, we aim to determine how Rec8 phosphorylation affects its cleavage and what kinases may play a role in this process. We developed a system for *in vivo* detection of Rec8 cleavage in mouse oocytes in time-lapse images. It utilizes a fusion protein sensor H2B-mCherry-Rec8-YFP. This sensor is loaded through its H2B tag on the chromosomes independently of endogenous cohesin, and is successfully cleaved during anaphase. The change in ratio between the mCherry and YFP fluorescence brightness allows detection of sensor cleavage. By utilizing this sensor we can rapidly investigate cleavage of various phospho-mutant and phospho-mimetic modifications of Rec8 without the need for transgenic mice. We established that exogenously expressed mammalian Rec8 can be cleaved in phosphorylated form only, determined the region of Rec8 most likely to be phosphorylated, and confirmed the previously suggested cleavage sites preferred by Separase. The effect of inhibition of certain kinases on Rec8 cleavage can also be assessed, with Cdc7/Dbf4, CK1 (as determined to have a role in Rec8 phosphorylation in yeast) and other kinases, such as Plk1, Aurora B, CDK1 being the primary candidates. After confirming the precise location of Rec8 phosphorylation sites, we will verify the phosphorylation status of endogenous Rec8 in mouse oocytes by phospho-specific antibodies and generation of transgenic mice with a non-phosphorylatable Rec8 allele. The reported method of this Rec8 cleavage assay provides a significant advantage over *in vitro* biochemical methods. Information obtained in this project will improve the understanding of cohesin protection and deprotection, and the meiotic chromosome segregation process.

P84 Dynamic organization of chromatin domains revealed by super-resolution live-cell imaging

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The eukaryotic genome is organized within cells as chromatin. For proper information output, higher-order chromatin structures can be regulated dynamically. How such structures form and behave in various cellular processes remains unclear. Here, by combining super-resolution imaging photoactivated localization microscopy (PALM) and single nucleosome tracking, we developed a nuclear imaging system to visualize the higher-order chromatin structures along with their dynamics in live mammalian cells. We demonstrated that a certain number of nucleosomes form compact domains with a peak diameter of ~160 nm in live cells and move coherently. The heterochromatin-rich regions showed more domains and less movement. With cell differentiation, the domains became more apparent, with reduced dynamics. Furthermore, various perturbation experiments indicated that they are organized by a combination of factors, including cohesin and nucleosome–nucleosome interactions. Notably, we observed the domains during mitosis, suggesting that they may act as building blocks of chromosomes and serve as information units throughout the cell cycle.

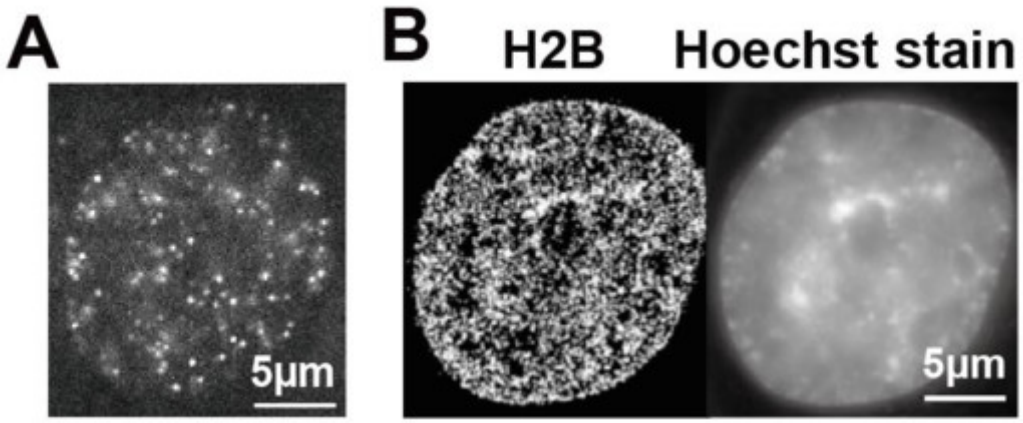

Figure 1 (A) Single-nucleosome (H2B-PA-mCherry) image of the nucleus of a live HeLa cell. (B) Live-cell photoactivated localization microscopy (PALM) image of histone H2B (left) and correlative Hoechst 33342 DNA staining of the same live cell (right).

P85 wtf genes are prolific dual poison-antidote meiotic drivers

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Meiotic drivers are selfish genes that bias their transmission into gametes, defying Mendelian inheritance. Despite the significant impact of these genomic parasites on evolution and infertility, few meiotic drive loci have been identified or mechanistically characterized. Here, we demonstrate a complex landscape of meiotic drive genes on chromosome 3 of the fission yeasts *Schizosaccharomyces kambucha* and *S. pombe*. We identify *S. kambucha wtf4* as one of these genes that acts to kill gametes (known as spores in yeast) that do not inherit the gene from heterozygotes. *wtf4* utilizes dual, overlapping transcripts to encode both a gamete-killing poison and an antidote to the poison. To enact drive, all gametes are poisoned, whereas only those that inherit *wtf4* are rescued by the antidote. Our work suggests that the *wtf* multigene family proliferated due to meiotic drive and highlights the power of selfish genes to shape genomes, even while imposing tremendous costs to fertility.

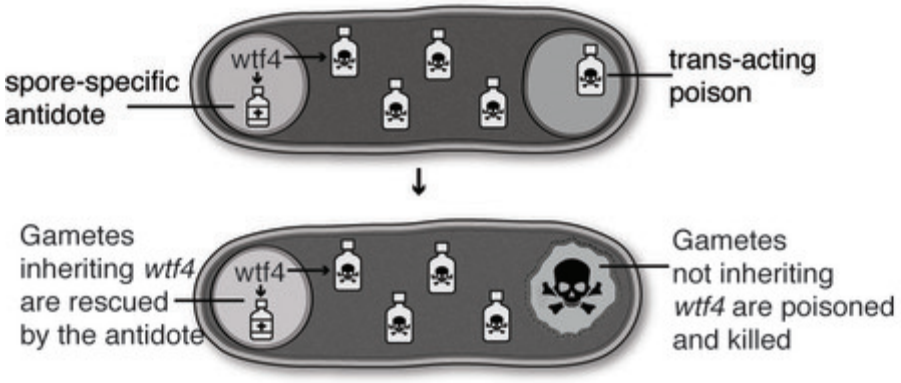

Figure 1: In fission yeast, the four meiotic products (spores) are encased in a spore sac called an ascus. Here, two of the four spores are shown for simplicity. wtf4 creates two proteins: a trans-acting poison and spore-specific antidote. The poison will kill any cells without the antidote; thus, only spores inheriting wtf4 survive. Therefore, wtf4 is a dual poison-antidote meiotic driver.

P86 Fine-scale dissection of a Prdm9-independent Canid meiotic recombination hotspot

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Meiosis requires homologous chromosomes to find each other, synapse fully and recombine correctly. In humans and most other mammals, meiotic recombination events are clustered in 1-2 kb wide recombination hotspots whose locations are determined in trans by the protein PR-domain containing 9 (PRDM9). Mice lacking PRDM9 direct recombination to promoters and functional elements, resulting in meiotic arrest and infertility. Canis familiaris (dog) lack a functional copy of PRDM9, yet linkage data show that historical recombination events cluster in functional elements, suggesting that there may be a mechanism enabling controlled recombination at these locations, and in the absence of PRDM9. However nothing is known about the de-novo activity of dog recombination hotspots and the patterns of recombination resolution in this PRDM9 deficient species. We investigated a dog recombination hotspot for de-novo recombination events using pooled sperm typing, and uncovered high crossover frequencies affecting up to 2% of sperm, with frequency differences of two orders of magnitude between individual dogs. Our crossover resolution data provides further insights into the morphology and dynamics of this Prdm9-independent canid recombination hotspot.

P87 Structural and Functional Remodeling of the Endoplasmic Reticulum during Budding Yeast Meiosis

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During meiosis, cells undergo a full remodeling in parallel with chromosome segregation in order to differentiate into viable gametes. In essentially all cases, little is known about how cells regulate the drastic changes in organelle structure and function that can be observed microscopically. The endoplasmic reticulum (ER) is one case in which an organelle is drastically remodeled in budding yeast cells undergoing meiosis, yet we don't understand the molecular basis for this remodeling or its impact on organelle function. Coincident with the second meiotic division, the cortical ER detaches from the plasma membrane and is reformed in mature spores. Here, we show that key features of ER remodeling are dependent on the meiotic transcription factor Ndt80, but occur independently of meiotic nuclear divisions. Deletion of genes regulating ER tubule formation and dynamics disrupts meiotic ER remodeling and reduces the viability of resulting gametes. Ongoing work seeks to define the signaling events controlling meiotic ER remodeling and the link between ER morphology defects and reduced spore fitness.

P88 Differential localization pattern of cohesin and synaptonemal complex proteins during male meiosis in the monotrene mammals *Ornithorhynchus anatinus* and *Tachyglossus aculeatus*

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Monotreme mammals present a striking complex of sex chromosomes. In platypus (*Ornithorhynchus anatinus*) males are $X_{1-5}Y_{1-5}$ and females are X_{1-5} X_{1-5}, while in the echidna (*Tachyglossus aculeatus*) males are X_{1-5} Y_{1-4} and females are X_{1-5} X_{1-5}. This amazing sex chromosome system presents an extraordinary behavior during male meiosis since the X and Y chromosomes form a chain, owing to the occurrence of reciprocal translocations between them. It has been previously reported that sex chromosomes in platypus accumulate the cohesin proteins SMC3 and STAG3 during the pachytene stage of first meiotic prophase. However, efforts to localize the proteins of the lateral element of the synaptonemal complex were unfruitful. Here we have extended the analysis of cohesin proteins by studying the localization patterns of Rec8 and Rad21 on meiotic chromosomes. The results indicate that in platypus REC8 might not follow the same pattern of SMC3 and STAG3, since it appears much less accumulated on the sex chromosomes. This mimics the localization of this cohesin in other mammals, where it is less abundant or even absent from the unsynapsed regions of the X and Y chromosomes. Then, using antibodies against the mouse SYCP3 protein we have been able for the first time to putatively localize the components of the synaptonemal complex lateral elements in monotremes. Its localization pattern differs from cohesin proteins in that SYCP3 appears only weakly during early prophase-I (letotene and zygotene) but distributes all along the chromosomes during pachytene, including the unsynapsed regions of sex chromosomes. On the other hand, the localization of cohesins and SYCP3 in the echidna greatly differs from the pattern found in platypus. This reveals the singularity of these two species in relation to sex chromosome behavior during meiosis.

P89 Efficient crossover formation between sex chromosomes requires a new pre-DSB recombinosome component in mouse spermatocytes.

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DNA double strand breaks (DSBs) are actively introduced into the genome by the SPO11 enzyme at the beginning of meiosis to initiate homologous recombination. DSBs are needed for the generation of crossovers, and thus for the correct segregation of chromosomes during meiosis. Successful meiosis depends on the tight control of DSB formation, which involves chromosome axis-associated complexes of SPO11 auxiliary proteins. These complexes are called pre-DSB recombinosomes. We identified a previously uncharacterised meiosis-specific protein as a component of these recombinosomes, hence we named this protein pre-DSB recombinosome component 4 (PRDR4). We found that PRDR4 interacted with REC114 in yeast two hybrid assays, and co-localized with axis-associated foci of MEI4 and REC114, which are two previously identified components of pre-DSB recombinosomes. We disrupted Prdr4 in mice using CRISPR/Cas9 genome editing. Despite normal body size and weight, adult Prdr4-deficient mice displayed reduced testis size and infertility in males. DSBs formed in high numbers and autosomes synapsed efficiently in Prdr4-deficient meiocytes. Nonetheless, we observed a partial defect in DSB formation and, in concordance with this defect, a nearly complete loss of crossovers between the X and Y sex chromosomes of spermatocytes. The late recombination nodule/crossover marker MLH1 did not form foci at the pseudoautosomal regions (PAR) of sex chromosomes, and PAR regions did not synapse in the majority of Prdr4-deficient spermatocytes. This defect seemed to cause a metaphase arrest in the first meiotic division leading to an almost complete absence of post meiotic cells in testes of Prdr4-deficient mice. Our findings raise the possibility that distinct components of pre-DSB recombinosomes are primarily dedicated to DSB formation on sex chromosome PAR regions. Alternatively, minor disturbances in DSB numbers, and/or alteration in the spatiotemporal control of DSB formation may impact on crossover assurance. Further analysis of *Prdr4*-deficient mice will be discussed in relation to these possibilities.

P90 Biophysical studies of the meiotic protein SYCP3

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The synaptonemal complex (SC) has a crucial but poorly-understood role in homologous chromosome pairing and genetic recombination. Its tripartite structure, comprising of lateral elements coating each homologue and linked in the midline by transverse fibres to the central element, is suggestive of a role as a molecular zipper. However, mechanistic insight at atomic level into the molecular determinants of SC architecture are largely missing.

We aim to understand the structural basis of SC assembly and the biochemical and biophysical properties that underpin SC function. We have focused initially on the lateral element protein SYCP3, due to its central role in SC architecture and in meiosis, and the ease of production in a recombinant system. During leptotene of meiotic prophase I, SYCP3 is deposited uniformly along the entire length of the chromosome molecule, in a process that contributes to the specific 3D architecture of the chromosomal DNA. Two biochemical properties of SYCP3, its ability to bind DNA and to self-assemble into fibres, are likely to be responsible for the observed cellular behaviour of SYCP3 (Syrjänen, Pellegrini, & Davies, 2014).

Here we describe our recent progress on the characterisation of SYCP3's biochemical properties and the implications for SYCP3 function in meiosis. Using an experimental setup that allows manipulation of DNA molecules at single-molecule level, we have shown that DNA-bound SYCP3 can accumulate in multimeric clusters that are compatible with its known role in DNA compaction (Syrjänen *et al*., 2017). We have also started to investigate the SYCP3 fibres by electron cryo-tomography. We have collected several datasets of SYCP3 fibres under native, cryogenic conditions, and we are currently attempting sub-tomographic averaging, to obtain a high-resolution view of the SYCP3 fibre.

1. *Syrjänen, J. L., Heller, I., Candelli, A., Davies, O. R., Peterman, E. J. G., Wuite, G. J. L., & Pellegrini, L. (2017). Single-molecule observation of DNA compaction by meiotic protein SYCP3. eLife, 6, 595. http://doi.org/10.7554/eLife.22582*
2. *Syrjänen, J. L., PellegriniL., & Davies, O. R. (2014). A molecular model for the role of SYCP3 in meiotic chromosome organisation. eLife, 3, 213. http://doi.org/10.7554/eLife.02963*

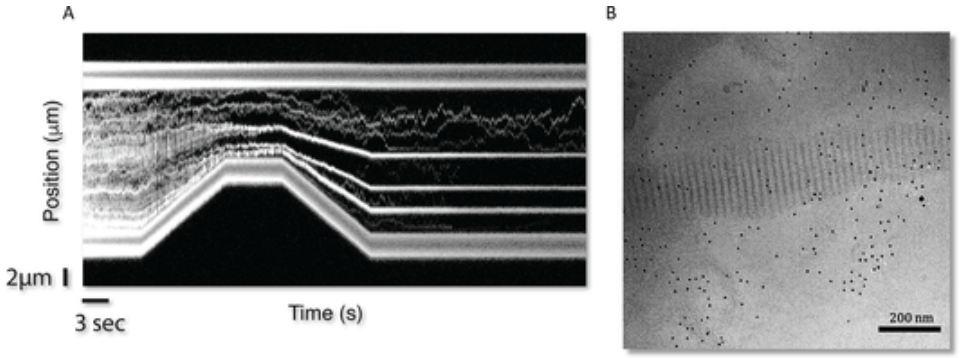

Figure 1. Biophysical studies of the meiotic protein SYCP3. A Single-molecule kymograph of SYCP3 bound to DNA (see Syrjanen et al, eLife, 2014). B Image of an SYCP3 fibre under native cryogenic conditions.

P91 EWSR1 regulates recombination hotspot determination and links hotspots to the chromosomal axis

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In mice and humans, meiotic recombination occurs at 1-2 kb long recombination hotspots, whose position and activity are determined by PRDM9, a meiosis-specific DNA-binding protein that trimethylates histone-3 at lysines 4 and 36. In male germ cells of mice, PRDM9 trimethylates histone 3 at about 5000 sites per cell; of these, ~300 acquire DNA double-strand breaks (DSB), and these in turn are repaired to yield only ~24 crossovers. Using yeast two-hybrid assay, we identified EWSR1 as one of the proteins that interact most strongly with PRDM9. EWSR1 is a single strand RNA/DNA binding protein that in somatic cells is involved in RNA splicing and transcriptional regulation. However, the function of EWSR1 in meiotic initiation and progression was previously unknown. To explore this question, we used an *Ewsr1* germ cell specific conditional knockout (CKO, *Ewsr1*$^{loxP/\Delta;Stra8\text{-}Cre}$) mouse model to determine the steps in the recombination process at which the EWSR1 function is essential. Male CKO mice were sterile, with a histological phenotype consistent with prophase I arrest. The CKO mice showed dramatic reduction of the number and activity of H3K4me3 marks at meiotic hotspots and asymmetric trimethylation at the weakest hotspots. Promoter and enhancer H3K4me3 sites were not affected. Although the total number of DSB per cell did not change, their positions were shifted towards closed chromatin, resulting in a decrease of crossover number from ~24 to ~18, less than one per bivalent, a number insufficient for proper chromosomal pairing and subsequent segregation. We also found that EWSR1, in addition to binding PRDM9, also binds to the meiotic-specific cohesin REC8. Since REC8 and PRDM9 do not bind directly to each other, EWSR1 provides a physical link between hotspot-bound PRDM9 and the chromosomal axis, thereby ensuring proper positioning of activated hotspots on the chromosomal axis for DSB initiation. Physically, our results support a model in which binding of EWSR1 both stabilizes PRDM9 dimer binding at hotspots, ensuring proper H3K4/K36 trimethylation, and incorporates activated hotspots into the forming chromosomal axis. In the absence of EWSR1, PRDM9-dependent trimethylation only occurs at the strongest hotspots, which failure affects all subsequent recombination processes.

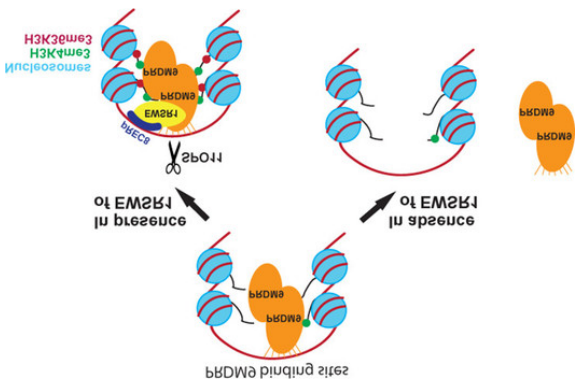

Figure 1 Model of EWSR1 interaction with PRDM9 and REC8 for proper hotspot activation

P92 Hormone signaling and meiosis: insights from the study of mutants defective in auxin-response

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Auxin phytohormones are master regulators of plant growth. They are involved in shoot and root development and are important for many environmental responses. Although the influence of these hormones on cell division has been extensively described, their potential involvement on meiosis remains to be investigated. Since auxin flow is critical in anther development, we are interested in providing evidences on the meiotic function of auxins by analyzing mutants defective in the auxin signaling pathway.

Mutations in the *Arabidopsis* *AUXIN-RESISTANT1* gene *AXR1*, linked to the ubiquitin-related protein degradation pathway, alter normal auxin response and produce severe vegetative defects. The group of Mathilde Grelon has demonstrated that fertility problems in *axr1* mutants are due to a reduction in bivalent formation in pollen mother cells (PMCs) (Jahns *et al*., 2014). Here, we have characterized mutants defective for *AXR1-LIKE* (*AXL*), a paralog gene of *AXR1* that encodes a protein with 80% of aminoacid identity. Despite AXL can replace AXR1 in the ubiquitin-related pathway, our results reveal that the function of both proteins on meiotic recombination is not redundant. *axl* mutant plants do not display univalents at metaphase I, but chromosome-specific contributions to the mean cell chiasma frequency are different than those observed in wild-type plants.

We have also analyzed plants with mutations that suppress *axr1* phenotype. Plants defective for *SUPPRESSOR OF AUXIN RESISTANCE1* (*SAR1*) show pleiotropic growth and developmental defects, and exhibit reduced fertility. SAR1 is a component of the nuclear pore complexes (NPCs) and is named NUP160. The cytological examination of meiosis in PMCs from *sar1* has revealed alterations in chromatin condensation during prophase I and chromosome fragmentation during second meiotic division.

In summary, the presented results give rise to the new perspective that auxins could influence on meiosis. This opens a new field for meiotic research that might be useful in plant breeding.

1. *Jahns et al. (2014). PLoS Biol. 12(8): e1001930.*

P93 Linking meiotic replication and recombination initiation in mammals

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Studies in yeast indicate coordination between meiotic replication and double-strand break (DSB) formation, but technical challenges have precluded investigating this link in mammalian systems. The replication timing program is determined by the distribution and efficiency of replication origins. We developed a novel approach to map origins by directly sequencing the nascent leading strand isolated from active origins. Our method is highly sensitive and specific and allowed us to detect origins from 10 million cells; at least an order of magnitude less than what current methods require. We identified ~17,000 origins of replication in mouse testes. These origins associate with CpG islands and with a permissive chromatin environment. To address whether replication influences recombination in mammals, we measured replication timing in cells undergoing meiotic S-phase in mice. We isolated actively replicating preleptotene cells, and identified early and late replicating regions. Replication origin density is highest in early replicating regions and intriguingly, these early replicating regions harbor more DSBs than expected. Conversely, DSB formation is depleted in late replicating regions. These data strongly suggest that in mammals, meiotic replication is a critical determinant of DSB formation. By integrating existing DSB maps, origins of replication and timing information we can now investigate the consequences of disturbing meiotic replication and origin selection and identify the factors that mediate the coupling of replication and recombination in mammals.

P94 EXO1 plays a key role in promoting crossover formation in mouse spermatocytes

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Chromosomes have to segregate accurately during meiosis to prevent infertility and aneuploidy. In order to segregate homologs accurately, a crossover (CO) recombination product is introduced between homologs. EXO1, an exonuclease, plays a key role in promoting CO formation in mouse spermatocytes(1). In yeast meiosis, Exo1 is required for MutLγ−dependent COs(2). Indeed, the crossover defect in Exo1-/- phenocopies Mlh1-/-(2). Unlike yeast, Exo1-/- mouse spermatocytes lose ~70% of COs whereas Mlh3-/- spermatocytes lose 95% of COs(3). This phenotype suggests that EXO1's role may differ between yeast and mouse and allows epistatic analysis between Exo1 and Mlh3. To get a comprehensive understanding of EXO1's roles in mammalian meiosis, we utilize cytological assays to reveal global meiotic defects and molecular assays to interrogate recombination products at hotspots. In agreement with previous reports, Exo1-/- mice have smaller testes and spermatocytes apoptose in metaphase I. Despite proper pairing and synapsis, Exo1-/- spermatocytes have 10% fewer MLH1 foci, a marker for MutLγ−dependent COs. Further, spermatocytes have reduced COs and lose ~70% of the bivalents. In agreement with the global reduction of bivalents, CO frequency at the 59.5 hotspot is ~60% lower than controls. However, the distribution of CO breakpoints is shifted in Exo1-/- spermatocytes relative to controls and suggests that the associated gene conversion tracts are shorter. In marked contrast, noncrossovers (NCOs) isolated from Exo1-/- spermatocytes are 4-fold longer than NCOs from Mlh3-/- spermatocytes. Finally, consistent with the established role of EXO1 in mismatch repair, ~25% of COs isolated from Exo1-/- spermatocytes have discontinuous gene conversion tracts. We will present our ongoing analyses to improve our understanding of the many different roles of EXO1 in mammalian meiosis.

1. *Wei K, Clark AB, Wong E, Kane MF, Mazur D, Parris T, et al. Mutation of Exonuclease 1 results in DNA mismatch repair defects, male and female sterility and increased cancer susceptibility in older mice. Genes Dev. 2003;17:603–14.*
2. *Zakharyevich K, Ma Y, Tang S, Hwang PYH, Boiteux S, Hunter N. Temporally and Biochemically Distinct Activities of Exo1 during Meiosis: Double-Strand Break Resection and Resolution of Double Holliday Junctions. Mol Cell [Internet]. 2010;40(6):1001–15. Available from: http://dx.doi.org/10.1016/j.molcel.2010.11.032*
3. *Holloway JK, Booth J, Edelmann W, Mcgowan CH, Cohen PE. MUS81 Generates a Subset of MLH1-MLH3 – Independent Crossovers in Mammalian Meiosis. 2008;4(9).*

P95 Spo11 generates short dsDNA fragments flanked by two double-strand breaks during initiation of meiotic recombination

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Spo11, a topoisomerase-related transesterase, initiates meiotic recombination via double-strand break (DSB) formation. After the cleavage reaction, which creates 2nt 5'-staggered overhangs (Liu *et al*., 1995), Spo11 monomers remain covalently bound to the 5'-DNA termini and are subsequently released by MRX/Com1 nuclease together with a short ssDNA-oligo (Neale *et al*., 2005). In *rad50S* mutants, resection is blocked and Spo11 remains attached to the DNA. When omitting the – previously standard – sonication step, prominent Spo11 qChIP signals were observed in *rad50S* mutants, consistent with the simultaneous generation of two adjacent DSBs. We developed a novel protocol to purify and deep sequence the resulting dDSB (double DSB) fragments in order to create genome-wide dDSB maps at nucleotide resolution in various mutant strains and wild type. We found that dDSB fragment formation depends on the catalytic activity of Spo11 and occurs both in resection-deficient mutants and in wild type. Moreover, we could observe the Spo11-specific 2nt overhang pattern, indicating precise mapping of the break sites at nucleotide resolution. The majority of dDSBs overlap with breaks mapped in a previous study by Spo11 oligo (ssDNA) isolation (Mohibullah and Keeney, 2017) and also prefer nucleosome-depleted regions, although to a lesser extent. Both in wild type and *tel1*Δ (*ATM-/-*) strains, the dDSB fragment length distribution peaks between 35 and 100nt, whereas in (resection-deficient) *rad50S, mre11S* and *com1*Δ mutants, additional, longer fragments can be observed. Furthermore, dDSB fragment lengths show an approximately 10.5 nt periodicity, corresponding to one turn in the B-DNA helix. The release of dsDNA fragments by closely spaced DSBs leads to the prediction of the existence of short-tract gene conversion events, which are not created by heteroduplex mismatch correction. We have set out to validate this prediction.

1. *Liu, J., Wu, T.C., and Lichten, M. (1995). The location and structure of double-strand DNA breaks induced during yeast meiosis: evidence for a covalently linked DNA-protein intermediate. Embo J 14, 4599-4608.*
2. *Mohibullah, N., and Keeney, S. (2017). Numerical and spatial patterning of yeast meiotic DNA breaks by Tel1. Genome Res 27, 278-288.*
3. *Neale, M.J., Pan, J., and Keeney, S. (2005). Endonucleolytic processing of covalent protein-linked DNA double-strand breaks. Nature 436, 1053-1057.*

P96 Cohesin dynamics at mitotic and meiotic DNA DSBs in *Arabidopsis thaliana*

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DNA damage can arise as a result of both endogenous and exogenous insults, as well as being part of normal cellular processes. DNA double strand breaks (DSBs) are one of the most hazardous types of DNA damage, since when left unrepaired they lead to chromosome fragmentation and genomic rearrangements. Therefore, DNA breaks need to be efficiently repaired in order to ensure genome integrity and proper chromosome segregation. Non-homologous end-joining (NHEJ) is the major DSB re-joining process and occurs in all cell cycle stages, yet it is error-prone. Homologous recombination (HR), in contrast, is an error-free homology template-dependent repair process, restricted to post-replicative cells. In order to detect and repair the DNA breaks, two master kinases, ATM (Ataxia telangiectasia-mutated) and ATR (Ataxia telangiectasia-mutated and Rad3-related), initiate a signalling cascade that attracts DNA repair proteins. In a previous phosphor-proteomic screen, three proteins related to sister chromatid cohesion (WAPL1, PDS5A and RAD21.3/SYN4) were identified as direct ATM/ATR downstream phosphorylation targets in *Arabidopsis thaliana*. Cohesins, are multiprotein ring-shaped complexes, holding sister chromatids together (among other functions) after DNA replication. In anaphase, the kleisin cohesin subunit (RAD21 family proteins) is targeted for cleavage and the entrapped sister chromatids can then be pulled to the opposite poles. The proteins PDS5 and WAPL have been shown to associate with the cohesin complexes, regulating their capacity to entrap the two sisters. It is anticipated that the two sister chromatids have to be in close proximity for HR in mitotic cells and indeed cohesion molecules have been found enriched around DSB sites in yeast cells (Ünal *et al*., 2007). In contrast, repair of programmed meiotic DSBs should preferentially be mediated via a chromatid of the homologous chromosome and not via the sister chromatid. In this sense, we anticipate that cohesion will be very differently regulated around mitotic and meiotic DSB sites. These aspects have not yet been studied in plants and we will present preliminary results and strategies how to investigate the differential regulation of cohesin at meiotic and mitotic DSB sites in plants. For now, we are focusing on the ATM/ATR mediated phosphorylation events, have generated specific antibodies and are currently generating plant lines expressing corresponding hypomorphic protein versions.

P97 Live imaging of meiosis in *Arabidopsis thaliana*

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Due to their size and accessibility, plant chromosomes are a powerful model to study meiosis. However, knowledge about the cytology of plant meiosis mainly results from the observation of fixed material. Although highly informative, this approach limits our understanding of the dynamics of meiosis. Here we present the development of a new method, based on classic confocal microscopy, to perform live imaging of anthers of *Arabidopsis thaliana*. Our method is based on the imaging of microtubules and the meiotic cohesion subunit RECOMBINATION 8 (REC8) that allowed us to concomitantly visualize six cellular/subcellular parameters and to describe the meiotic progression through a system of 16 clearly distinguishable hallmarks. This system delivers spatially and temporally quantifiable criteria for the analysis of meiosis, and will be applied on mutants to better investigate its entry and progression regulation.

P98 Human PRDM9 can bind and activate promoters, and other zinc-finger proteins associate with reduced recombination in cis

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Across mammals, PRDM9 binding localizes almost all meiotic recombination hotspots. However, most apparent PRDM9 motif sequence matches are not bound, and most PRDM9-bound loci do not become hotspots, but the reasons for this are currently unknown.

To explore factors that affect binding and subsequent recombination outcomes, we mapped PRDM9 binding sites for both human and chimpanzee PRDM9 alleles in a human cell line, and measured PRDM9-induced H3K4me3 and gene expression changes. These data revealed varied and previously unsuspected DNA-binding modalities of PRDM9. Binding involves all zinc-fingers to obtain very strong sequence specificity, but with different spacings and relative weightings between the fingers at different targeted sites, so the space of binding targets cannot be captured by a single motif. Particular histone modifications predict binding, but strong differences in even broad-scale patterns of binding exist between humans and chimps.

At sites where PRDM9 binds, we identified a collection of specific *cis* sequence motifs which predict whether recombination subsequently occurs. These motifs associate with TRIM28 recruitment, and histone modifications including H3K9me3. Follow-up analyses implicate the large family of KRAB-ZNF genes in consistent, localized meiotic recombination suppression in humans. Thus *cis* factors play a role in determining which PRDM9-bound sites become hotspots; we find that broad-scale factors further modify recombination rate, in both cases without impacting PRDM9's ability to bind or deposit H3K4me3.

The human *PRDM9* allele binds many promoters, but appears completely unable to localize DSBs to these sites. However, PRDM9 increases expression of a small number of genes including *CTCFL* and *VCX*, by binding nearby. Finally, we show that, surprisingly, PRDM9's DNA-binding zinc finger domain plays a key role in promoting the formation of multimers. Diverged alleles multimerize much less efficiently than identical alleles.

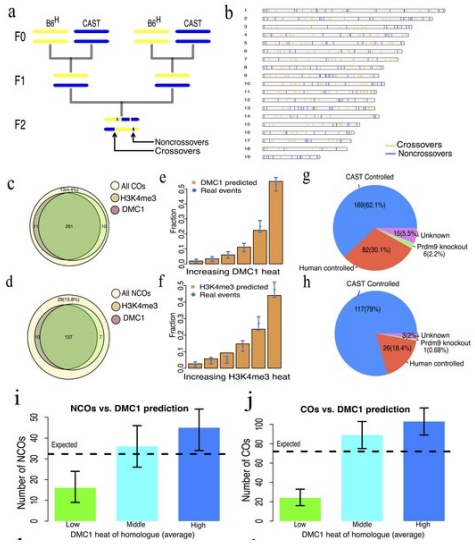

Figure 1 (a)Reduced recombination hotspot probability when KRAB-ZNF proteins bind near a PRDM9 binding site (b)Fold expression change of 3 genes in HEK293T cells after transfection with human PRDM9 (c)ChIP-seq and RNA-seq coverage around the VCX gene.

P99 Effective use of DNA double-strand break repair for the pairing of homologous chromosomes requires MCMDC2 in mammalian meiosis

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Orderly chromosome segregation during the first meiotic division requires meiotic recombination to form crossovers between homologous chromosomes. We discovered that MCMDC2, an atypical yet conserved member of the minichromosome maintenance (MCM) helicase family, plays crucial roles in meiotic recombination and is required for fertility in mice. Meiotic recombination begins with the introduction of DNA double-strand breaks (DSBs) into the genome. DNA ends at break sites are resected. The resultant 3-prime single-stranded DNA overhangs recruit RAD51 and DMC1 recombinases that promote the invasion of homologous duplex DNAs by the resected DNA ends. Multiple strand invasions on each chromosome promote the alignment of homologous chromosomes, which is a prerequisite for inter-homolog crossover formation during meiosis. We found that although DNA ends at break sites were evidently resected, and they recruited RAD51 and DMC1 recombinases, these recombinases were ineffective in promoting alignment of homologous chromosomes in the absence of MCMDC2. Consequently, RAD51 and DMC1 foci, which are thought to mark early recombination intermediates, were abnormally persistent in *Mcmdc2-/-* meiocytes until mid prophase. Importantly, the strand invasion stabilizing MSH4 protein, which marks more advanced recombination intermediates, did not efficiently form foci in *Mcmdc2-/-* spermatocytes. This indicated that MCMDC2 plays an important role in either the formation, or the stabilization, of DNA strand invasion events that ensure alignment and the formation of crossovers between homologous chromosomes during meiosis. Unexpectedly, we found that numbers of recombination foci which were marked by RPA and DMC1 dramatically dropped in late prophase *Mcmdc2* -/- oocytes. Thus, despite failed alignment of homologous chromosomes and defective SC formation, DSBs might be repaired in late prophase *Mcmdc2* -/- meiocytes. We tested if this putative repair of DSBs depended on the meiosis-specific DMC1 recombinase that is thought to be especially important for the repair of DSBs with the help of the homologous chromosomes. High numbers of RPA marked recombination foci persisted in late prophase *Mcmdc2 -/- Dmc1-/-* oocytes, which indicated persistence of unrepaired DSBs. Thus, a DMC1-dependent recombination pathway might repair DSBs in *Mcmdc2* -/- oocytes. We hypothesize that this pathway abnormally uses the sister chromatids as opposed to homologous chromosomes as repair templates, because homology search fails in the absence of MCMDC2.Thus, our observations suggest that MCMDC2 is specifically needed for inter-homolog repair of DSBs and not for DSB repair *per se*. This identifies MCMDC2 as a key putative factor that distinguishes meiotic recombination from mitotic recombination and enables efficient recombination between homologous chromosomes.

P100 Meiotic recombination defects induced by the lack of the MEIOB/SPATA22 complex

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Homologous recombination is a conserved process among eukaryotes. During meiosis, this mechanism is essential to the formation of crossovers and thus for the proper segregation of chromosomes. Meiotic recombination is ensured by the combined action of mitotic and meiotic factors. MEIOB has been recently identified and shown to be essential to the repair of meiotic DNA double-strand breaks. MEIOB is a paralog of RPA1, the large subunit of RPA, which is a ubiquitous ssDNA-binding trimeric composed of RPA1, RPA2 and RPA3. MEIOB has been shown to interact with SPATA22 and RPA2. This observation suggested that MEIOB, SPATA22 and RPA may work together. Based on the homology existing between structural domains of MEIOB, SPATA22 and the RPA subunits, we deciphered the modality and the role of their interactions. We show that MEIOB and SPATA22 interact through their C-terminal OB domains like RPA1 and RPA2 and cooperate to interact with the RPA complex. Using Transmission Electron Microscopy, we evidenced that the presence of MEIOB/SPATA22 induces a strong compaction of the RPA/ssDNA filament. Immunofluorescent microscopyperformed on murin meiotic chromosomes revealed that in the absence of MEIOB, the BLM helicase accumulates on chromosomes axis and correlates with the eviction of the DMC1 recombinase from unrepaired meiotic breaks. Finally, we show that the absence of MEIOB favors abnormal recombinase distribution observed by SIM microscopy. Together, our results evidence that MEIOB, SPATA22 and RPA act together to insure the integrity of recombination intermediates during strand invasion.

P101 Investigating meiotic mechanisms in the nematode *Pristionchus pacificus*

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The establishment of the satellite nematode model organism *P. pacificus* has enabled us to investigate how meiotic programs have evolved within the nematode lineage. Like *C. elegans, P. pacificus* is a free-living, self-fertilizing hermaphroditic species that is easily cultured in the lab. Although the lineages of *C. elegans* and *P. pacificus* diverged hundreds of millions of years ago, their body plan and germline organization appear very similar. Using CRISPR/Cas9 to disrupt or epitope-tag meiotic genes, we have discovered that meiosis in *P. pacificus* follows a more "canonical" program, in which recombination plays an essential role in homolog pairing and synapsis. Although the *P. pacificus* genome contains both *rad-51* and *dmc-1* genes, analysis of their localization and loss-of-function phenotypes indicates that DMC-1 is essential, while RAD-51 is dispensable, for pairing and synapsis. This is in contrast to the *C. elegans* lineage, which has lost *dmc-1* and developed recombination-independent mechanisms to accomplish these essential features of meiotic prophase. DMC-1 localizes densely along chromatin in early prophase, while RAD-51 appears as DMC-1 disappears, and has a much sparser, more punctate distribution. While RAD-51 does not seem to play an early role in pairing and synapsis, it is nonetheless essential for proper meiosis since *rad-51* mutants have significantly smaller broods and high embryonic inviability. Intriguingly, we observe additional differences from *C. elegans* in the timing and interdependence of other meiotic events. In particular, analysis of widely conserved factors required for crossover formation suggests that they play a much earlier role in *P. pacificus*. In mammals and *C. elegans*, CNTD1/COSA-1 acts fairly late in CO designation, and is used as a molecular marker for CO sites. We found that *cosa-1* is essential for synapsis in *P. pacificus*, suggesting that CO designation occurs much earlier and that the formation of the SC is tightly linked to this process. Indeed, COSA-1::3xFLAG localizes as discrete foci along the SC, beginning as early as zygotene and increasing in brightness through pachytene. While genetic mapping indicates that multiple crossovers occur per chromosome pair, most pachytene nuclei display only 6 COSA-1 foci (presumably marking designated CO sites), leading us to tentatively conclude that *P. pacificus* exhibits complete class I CO interference, as in *C. elegans*, and that the "extra" crossovers likely arise through the Class II pathway. Moreover, disruption of a homolog of *Rnf212/zhp-3* in *P. pacificus* results in a severe phenotype in which central features of the zygotene stage are completely abrogated: nuclei do not become polarized upon meiotic entry, DMC-1 does not localize to chromosomes, and synapsis fails. Further investigation of meiosis in these and other nematode species will likely reveal how regulatory networks that drive meiosis are rewired in response to niche-specific selective pressures.

P102 CHK2 roles in mammalian oogenesis

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Mammalian oogenesis begins with the proliferation of oogonia which complete mitosis but do not complete cytokinesis, leaving the daughter cells linked together forming a cysts. These oogonia cysts enter meiosis, turning into oocytes, and advance through prophase I. At this point, double strand breaks (DSBs) are formed throughout the genome. Their repair by meiotic recombination leads to the synapsis of the homologous chromosomes. Errors in any of these two processes trigger the activation of the pachytene checkpoint to either repair them or send the cells to a programmed death. Interestingly, and contrary to what is found in spermatocytes, most oocytes present a high number of unrepaired DSBs at pachynema. At the same time, around birth, there is a massive oocyte death accompanying cyst breakdown and the posterior follicle formation. Thus, we hypothesize that the apparently inefficient meiotic recombination occurring in the oocytes may be required to promote massive oocyte death by the pachytene checkpoint in order to regulate the oocyte number, promote cyst breakdown and follicle formation in mammalian females. To test this idea, we analyzed the presence of DSBs, the oocyte number in both perinatal and adult females, cyst breakdown, follicle formation and reproductive lifespan using control and mutant mice for the effector kinase of the recombination-dependent pachytene checkpoint, CHK2. We confirmed the involvement of CHK2 in the pachytene checkpoint and we detected the involvement of CHK2 in the regulation of the oocyte number in fetal ovaries prior to birth. Furthermore, our results show an increase in the number of oocytes in old *Chk2* mutant females suggesting a possible extension of the reproductive lifespan in mutant mice.

P103 Genome-wide analysis of meiotic prophase chromosome organization in mouse

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While eukaryotic genomes are highly organized in all cell-cycle stages, meiosis presents unique challenges that require highly specialized mechanisms for genome organization. In meiotic prophase, telomeres are tethered to the nuclear envelope to generate the distinctive "bouquet" arrangement first observed over 100 years ago, and chromosomes themselves are organized as a linear array of chromatin loops. These organizational features and the underlying protein machinery are required for successful meiotic recombination, homolog pairing, and chromosome segregation in meiosis I. To gain insight into the detailed spatial organization of the genome in meiotic prophase, we have applied chromosome conformation capture (Hi-C) to highly purified populations of mouse spermatocytes in two prophase stages, zygotene and pachytene. We observe strong evidence of the chromosome bouquet in the global pattern of chromosome-chromosome contacts, as well as spatial segregation of the X-chromosome into the sex body. On an individual-chromosome level, we find no evidence for the "chromosome compartments" commonly observed in interphase cells, but we do observe megabase-scale features that may represent local chromatin folding or looping. Finally, as these assays were performed in a high-SNP-density (Black-6/Castaneus) F1 hybrid, we have the ability to unambiguously detect inter-homolog interactions and correlate these interactions with the known pattern of DNA double-strand breaks in this hybrid strain.

P104 The molecular basis of synaptonemal complex elongation mediated through SYCE2-TEX12 self-assembly

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The assembly of the synaptonemal complex (SC) during meiotic prophase I is vital for homologous chromosome synapsis and the formation of crossovers that generate genetic variation between gametes. Double strand breaks are induced across the genome, prompting chromosomes to find their homologous partner. The tripartite SC forms a ladder-like structure between homologues to hold them within a tight 100 nm distance for the repair of these breaks by homologous recombination, with a subset of the breaks forming crossovers.

The SC has lateral elements (LEs) which assemble along the chromosome axis, a central element (CE) that forms mid-way between the chromosomes and transverse filaments that bridge the CE to the chromosome axes. In mammals, LEs are constituted by SYCP2 and SYCP3, the central element is formed from SYCE1, SYCE2, SYCE3, TEX12 and C14ORF39/SIX6OS1 and the transverse filaments are made by SYCP1. Each component is crucial for SC formation and failure to form an intact SC can cause aneuploidy and infertility due to meiotic arrest. Therefore understanding the molecular choreography that these proteins execute is vital.

The CE proteins SYCE2-TEX12 form a constitutive 4:4 complex with a proclivity for higher-order assembly. In the absence of SYCE2 or TEX12, short regions of synapsis and recruitment of the SC proteins are observed, but a mature SC fails to form. Therefore SYCE2-TEX12 are thought to govern SC extension along homologous chromosomes. We aim to elucidate the molecular structure of SYCE2-TEX12, the mechanistic basis of its self-assembly, and how it coordinates with the rest of the CE, using a combination of biophysical and structural biology methods.

Through the use of electron microscopy and size-exclusion chromatography multi-angle light scattering (SEC-MALS), a TEX12 mutant is shown to abolish SYCE2-TEX12 assembly and restrict the proteins to a 2:2 complex. We also identified that TEX12 forms a stable unit in the absence of SYCE2 and we present an X-ray crystal structure of TEX12. TEX12 forms an antiparallel four helical assembly in the crystal, highlighting how SYCE2-TEX12 may interact in the 2:2 complex. Small angle X-ray scattering (SAXS) experiments have provided low resolution structural information about SYCE2-TEX12 in solution. Analysis of the 4:4 and 2:2 complexes reveals that both complexes have the same length in solution, with the 4:4 complex having double the cross-sectional radius of the 2:2 complex. Combining the SAXS data with the TEX12 structure leads to a model whereby a four helical assembly is formed from the interaction between two SYCE2 and two TEX12 molecules. These units can interact to form the 4:4 complex and also undergo higher-order assembly through recursive interactions between these units. We have identified a TEX12 region essential for the interaction between the SYCE2-TEX12 2:2 complexes and therefore the region that may be fundamental for the elongation of the entire SC.

P105 Chromosome loop structure in *S. cerevisiae* meiosis

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The chromosomal axis-loop structures formed during meiotic prophase are crucial for a successful meiosis. The meiotic cohesin subunit Rec8 is required for such structures to form and, together with RMM (Rec114-Mer2-Mei4), has a periodic signature along chromosome axes. However, the mechanism of the formation, and the molecular architecture, of the axis-loop structure remain poorly understood.

We are combining genome-wide chromosome conformation capture (Hi-C) and genetics in *S. cerevisiae* to investigate the axis-loop structure in meiotic cells. We detect interactions along chromosome arms that are not observed in pre-meiotic cells. The location of such putative axial interactions agrees with previously published axis protein ChIP data, confirming that factors such as RMM and Red1 are bona fide markers of loop bases. Such loop structures are dependent on Rec8 but not dependent on homologous recombination and homolog pairing, indicating that they represent interactions formed in cis along sister chromatids. Furthermore, we can detect misalignments of homologs in mutants that disturb chromosome pairing.

Our assay of loop detection enables us to revisit previous predictions of how different factors affect the axis-loop structure and allows us to investigate the processes that regulate the formation and molecular structure of meiotic chromosome loops.

P106 Control of meiotic recombination at the rDNA

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Meiotic DSBs, a prerequisite for meiotic recombination, occur at specific regions of the genome known as “hot spots” and, conversely, are excluded from other regions named “cold spots”. Different studies have revealed *cis* and *trans* acting factors that regulate and mediate meiotic DSB formation. As shown before, the linear differentiation of “hotness/coldness” for DSB formation and recombination along a chromosome is defined hierarchically, including chromosomal features like kinetochores and telomeres, organisation of the chromatin in loops and axis associated cores, transcriptional activity and others. Post-translational modifications of histones, possibly in conjunction with the DNA methylation status, contribute to this differentiation. Yet, relatively little is known on how meiotic DSB formation and recombination is suppressed in some defined genomic regions, such as the rDNA. In order to clarify the underlying molecular mechanisms, we are studying the rDNA loci during meiosis in *Arabidopsis thaliana*.

We generated plants with ectopically integrated single rDNA repeat units and we tested whether they affect recombination by modifying the genomic regions adjacent to them. To measure recombination we used the FTL based marker system (developed by the Copenhaver lab). While recombination is significantly lower around the integrated rDNA units, it is elevated in intervals that are located more distantly to the ectopic repeat (1,5 Mb), compared to the wild-type.

We also performed immuno-FISH, monitoring the ectopic and endogenous rDNA loci during meiotic progression, probing for axis-related proteins, synaptonemal complex (SC) formation, recombination mediators and chromatin modifiers. We observe that at the rDNA the axis protein ASY1 is deposited very late, compared to the rest of the genome, and the SC protein ZYP1 not at all. Additionally, the rDNA loci have very few RAD51 foci during meiotic prophase, indicating that these regions have very limited meiotic DSB formation or do not form canonical recombination intermediates. In *Arabidopsis mre11-4* mutant plants, but not in *Atcom1-1* mutant plants, we observe DNA fragmentation of the rDNA during meiosis. We are currently investigating if this fragmentation is indeed SPO11 dependent or, alternatively, a by-product of pre-meiotic replication events. Additionally, LIG4 is needed for efficient DNA repair during meiosis in the rDNA region.

Our current understanding is, that while locally being a “cold spot” for DSB formation and recombination, the rDNA loci serve as “pairing” centers, promoting recombination in their vicinity. Furthermore, it appears that the few breaks observed in the rDNA during meiosis are presumably SPO11 independent, and repaired via micro-homology mediated end joining (MMEJ – a branch of the NHEJ pathway, depending on MR

P107 The ATM/ATR mediated DNA damage response in plants may be transmitted by CK2

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DNA is the carrier of genetic information in all living organisms and therefore its integrity is crucial for assuring survival. Here we focus on DNA double strand breaks (DSBs) that have dramatic consequences for a cell if left unrepaired. Key for successful repair of DSBs is an efficient detection mechanism, attracting all relevant DNA repair factors by means of a fast and functional signaling cascade. On top of this activation process, two closely related DNA damage kinases, highly conserved throughout the kingdoms of life, ATM (*Ataxia telangiectasia*-mutated) and ATR (Ataxia telangiectasia-mutated and Rad3-related) can be found. Most of the downstream targets of ATM and ATR have been systematically identified in animals and yeast, but for many of those components, including transducing kinases and transcription factors, no homologues have been found in higher plants (e.g. CHK1, CHK2, p53). Recently, we published a study that introduced a mass spectrometry based phospho-proteome wide screen and presented novel targets of ATM and ATR in *Arabidopsis thaliana* following genotoxic stress. Interestingly, significantly enriched phosphorylation motifs not only highlighted ATM/ATR consensus sequences but also the consensus motif for Casein Kinase 2 (CK2). This inspired the idea that in plants CK2 could be one of the long sought DNA damage transmitter kinases acting downstream of ATM/ATR. The presented study is building on these data and hypothesis and aims to establish one important node in the DNA damage signaling network in plants with emphasis on CK2. We will also present data concerning the DNA repair protein MRE11, previously identified in above mentioned phospho-proteomic screens as ATM/ATR and CK2 target following genotoxic stress. The respective phosphorylation site is located within a DNA binding domain. We hypothesize that this phosphorylation is implicated in regulating MRE11's function, by limiting it's binding capacity to DNA, during somatic and meiotic DSB processing. We will present strategies and novel data to corroborate these ideas.

P108 Characterization of the PRDM9 zinc-finger array binding and multimer formation

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PRDM9 (PR-domain containing protein 9) has been identified as a meiosis specific protein that plays a key role in determining the location of meiotic recombination hotspots by recognizing hotspot-specific DNA motifs. PRDM9 is an epigenetic modifier, which binds DNA via its long C-terminal zinc-finger (ZnF) array, consisting of several tandem zinc-fingers (e.g. 11 zinc-finger repeats for the murine CAST allele; *Mus musculus castaneus* origin), and directs in its close vicinity double strand breaks (DSBs) necessary for the initiation of recombination. Therefore, PRDM9 interacts with additional proteins via its N-terminal KRAB domain so that the hotspot DNA is directed to the chromosomal axis where the DSB machinery is located. The different players in this interaction are not known, but it was shown that PRDM9 acts as a multimer *in-vivo*. However, to date it is still unclear what factors drive the multimerization and the number of units in an active multimer. Using *in-vitro* binding studies, in this work we characterized the number of PRDM9 units forming an active multimeric complex with a high-affinity DNA target. Specifically, we first established that the molecular weight (MW) of a protein-DNA complex can be inferred from a native polyacrylamide gel in an electrophoretic mobility shift assay (EMSA). For this purpose, we used DNA sequences of increasing lengths containing one or two specific target sites for PRDM9. The relative increase in MW with the change in migration distance of two protein complexes bound to DNA compared to one was used to infer the MW of the protein in the complex. Next, we assessed which domains of PRDM9 drive the observed multimerization using different PRDM9 constructs missing distinct domains of the PRDM9 full-length protein, as well as, parts of the ZnF array. We observe that all tested PRDM9 constructs (full-length PRDM9, ZnF domain consisting of 11, 10, 7 or 5 zinc-fingers) form functional multimeric complexes of at least two or more monomer units that are mediated solely within the C-terminal ZnF domain. Interestingly, the binding affinity of the $PRDM9^{Cst}$ ZnF domain was reduced with decreasing numbers of zinc-fingers, as measured with protein titration experiments used to infer a dissociation constant, K_D. Our results suggest that the number of zinc-fingers within an array might play an important role in the overall affinity of PRDM9, and could explain the dominance of one *Prdm9* allele over the other in a heterozygous PRDM9 complex, as has been observed for human and mice crosses. Specifically, the stoichiometry of zinc-fingers with different affinities is affected in multimeric complexes and modifies the overall dosage of PRDM9.

P109 Proteinaceous structure of the meiotic sex chromosomes in the domestic cats

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The X and Y chromosomes behave in a special way during the first meiotic prophase in mammalian spermatocytes compared to autosomes (reviewed in Solari, 1994 and in Handel, 2004). The heteromorphic X and Y chromosomes, generally, synapse only partially at the pseudoautosomal region (PAR) leaving two unsynapsed (or unpaired) differential regions. Moreover, both chromosomes form a well-known subnuclear structure characterized by the special compaction of its chromatin fibers: the XY body (Solari, 1974). Testes from four European shorthair cats and one Siamese cat were surgically removed by veterinarians during a normal castration operation at the Instituto de Zoonosis "Luis Pasteur" (C.A.B.A., Argentina) following all institutional and national guidelines for the care of animals. For each animal, two pieces of tissue were processed for semithin (0.5 μm thick) and thin (0.08 μm thick) sections to analyze the seminiferous epithelium in detail by light microscopy and the ultrastructure of the XY body in spermatocyte nuclei by electron microscopy (EM), respectively. Another piece of tissue was used to perform spermatocyte spreads for synaptonemal complexes. The aim of the present work is to analyze the fine structural characteristics of the sex chromosomes and the immunolocalization of the meiotic proteins SYCP3, SYCP1, SYCP2, SYCE3, SMC3, γ-H2AX, BRCA1, 3meH3K27 and MLH1 in the primary spermatocyte nuclei of the domestic cat. The analysis of those primary spermatocytes shows that the X and Y chromosomes undergo sequential changes along pachytene stage: first bullous expansions, then subdivision into multiple fibrils, all involving axial thickening. The number of split fibers, as well as their narrowness, is very different from the substructure of the autosomal axes. That multistranded, split appearance of the X and Y axes is due to the appearance of fibrils containing SYCP3 protein, which develop into tangles and bridges in the late substages of pachytene. Some of those fibrils make a bridge across the space between the X and Y chromosomes at a few sites. In some short stretches of the multistranded X-axes, SYCP1 is located at the inner side. Unexpectedly, one of those cross-bridges in the PAR co-localize with the late recombination protein marker, MLH1. The chromatin of the XY body shows the immunolocalization of γ-H2AX and 3meH3K27. At mid-late pachytene stages, the XY pair undergoes a premature desynapsis as a consequence of the disassembly of the synaptonemal complex at the PAR. Those results show that even if the XY body of the domestic cat shows the usual patterns of meiotic sex chromosome inactivation (MSCI) in mammals, striking variations in the structure of unpaired axes and their behavior during meiotic prophase are observed. The present work will discuss the supramolecular structure of the synaptonemal complex under the light of those observations.

P110 A serine-rich duplicated region in the N-terminus of ASY3 confers meiotic stability in autotetraploid *Arabidopsis lyrata*

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Whole genome duplication (WGD) is often associated with increased ecological fitness and adaptation to new biological niches. However, the doubled set of chromosomes can lead to complex meiotic configurations at meiotic metaphase I, thus causing sterility. Our aim is to understand the mechanisms underlying the stabilization of bivalent formation during meiosis after WGD in naturally occurring stable polyploid plants. *Arabidopsis lyrata* subsp. *petraea* is an outbreeding relative of the model plant *A. thaliana* that has both extant diploid and stable autotetraploid populations. Whole genome resequencing of diploid and tetraploid populations of *A. lyrata* has showed strong evidence of selection on a number of synaptonemal complex (SC) genes. Sanger sequencing of the coding region of these genes revealed two major ASY3 (Red1 homolog) alleles in diploids and tetraploids. One has a 78bp (26aa) in-frame duplication in a serine rich region of exon 2 (SD allele), the other has a reference genome-like ASY3 allele (ND allele). PCR genotyping of these alleles and cytological analysis in a number of diploid and tetraploid populations showed that tetraploids homozygous for the SD allele were meiotically stable (bivalents with distal chiasmata), whereas tetraploids with both SD and ND alleles formed multivalents and univalents. Diploid populations of *A. lyrata* were almost always homozygous for the ND allele and displayed stable bivalents with a large proportion of proximal/interstitial chiasmata. Computer based predictions of both *in silico* translated ASY3 alleles indicated Ataxia telangiectasia mutated (ATM) sites in the duplicated region, with the SD allele harboring potentially more than double the sites than the ND allele in that region. We are currently crossing diploid *A. lyrata* heterozygotes to generate homozygous SD and ND plants to measure the effect on chiasma distribution and investigate whether the difference is due to a change in inter-homolog bias or strength of CO interference. We are also investigating meiotic stability in *A. arenosa*/*A. lyrata* natural hybrids where gene flow in tetraploids appears to have overcome the species barrier, thus enabling a much greater pool of allelic combinations to be selected upon for meiotic stability.

P111 The function of the meiotic protein Emi2

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Zinc fluxes characterize mammalian meiosis; zinc content increases by 50% between germinal vesicle stage and metaphase II, and then decreases by 20% after fertilization. The anaphase promoting complex/cyclosome (APC/C) is a ubiquitin ligase that governs cell cycle progression. Emi2 is a meiotic spindle-checkpoint protein that starts to be expressed during anaphase I. It causes metaphase II arrest through inhibition of the APC/C (preventing the degradation of cyclin B) and is itself degraded after fertilization. Emi2 has two zinc-binding regions which are necessary for its proper functioning. We hypothesize that the activity of Emi2 is regulated by zinc, as the binding regions have significantly different affinities for zinc. Here we present biochemical characterization of Emi2 to understand its mechanism during meiosis.

P112 Massive crossover elevation via combination of increased HEI10 dosage and RECQ4 helicase mutations during *Arabidopsis* meiosis

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During meiosis, homologous chromosomes undergo reciprocal crossovers, which generates genetic diversity and underpins classical crop improvement. Meiotic recombination initiates from DNA double strand breaks, which are processed into single-stranded DNA that can invade a homologous chromosome. The resulting joint molecules can ultimately be resolved as crossovers. In *Arabidopsis*, competing pathways balance the repair of ~100-200 meiotic DSBs into ~10 crossovers per meiosis, with the excess DSBs repaired as non-crossovers. In order to bias DSB repair towards a crossover fate, we simultaneously increased dosage of the pro-crossover E3 ligase gene HEI10 and introduced mutations in the anti-crossover helicase genes RECQ4A and RECQ4B. As HEI10 and recq4a recq4b increase interfering and non-interfering crossover pathways respectively, they combine additively to yield a massive meiotic recombination increase. Importantly, we show that patterns of interhomolog polymorphism and chromatin drive recombination increases within the chromosome arms in both HEI10 and recq4a recq4b, while the centromeres remain crossover-suppressed. These results provide a genetic framework for engineering meiotic recombination landscapes in plant genomes.

P113 Why is it difficult to introgress genes from *Brassica rapa* (AA) and *B. nigra* (BB) into the established polyploid *B. juncea* (AABB)

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Polyploidy is thought to be significant in the success of up to 70% of the angiosperms, and is likely to be important for crops that are used to feed both human and animals. Different genomes combining in a single nucleus provides an excellent model for studying behaviour of individual genomes for their survival and expression in polyploid cells. *Brassica juncea (AABB)* is an allopolyploid species which came into being by hybridization between *Brassica rapa (AA)* and *Brassica nigra (BB)*. This species is important in the sub-continent of India and Pakistan for both animal and human foods, and we need to be in a position to breed new varieties for the increased population and potential climate changes over the next years by introgression of novel genes from *B.nigra* or *B. rapa*. At this time it is difficult to succeed in achieving this objective, thus my study is to investigate the reasons for this barrier. In this species we know very little about role of its progenitors in its evolution and their individual behaviours in plant development. Meiosis plays an essential role in maintaining and restoring the ploidy level during life history of sexually reproducing eukaryotes. We have used a combination of cytogenetics, immunochemistry and FISH analysis to further our understanding of how meiosis works in these Brassica diploid and tetraploid species. Currently I have developed a technique for identifying the B genome in a tetraploid background *B. juncea* and these results will be reported here. I have also identified a shift in crossover distribution in the constituent genomes that make up *B. juncea* compared to the diploid genomes that may be significant in introgressing genes from the constituent species into this species. In artificially generated lines, there appears to be a problem with synapsis, leading to infertility. I will discuss these results in pinpointing the problems associated with introgressing genes from the A and B genome into these important crops.

P114 Meiosis specific modification of mammalian telomeres

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During meiotic prophase I, rapid chromosome movements facilitate pairing and recombination of homologous chromosomes, by facilitating physical juxtaposition of homologous partners and also resolving unwanted entanglements of non-homologous partners. Chromosome ends, telomeres, play a central role to drive the movements in most of eukaryotes. To this end, telomeres are physically fused to the Nuclear Envelop (NE) and connected to the cytoskeleton, such as microtubules (fission yeast, plants and mammals) or actin filaments (budding yeast), in a transmembrane manner. The transmembrane linkage transmits cytoskeletal movement forces to the NE-anchored telomeres and drives its movements. This functional and structural differentiation of meiotic telomeres requires specific molecular modifications. Here, we report a molecular mechanism, that converts meiotic telomeres into the specialized movement apparatus in mammalian meiosis.

P115 Prophase pathway for removal of a meiosis-specific cohesin from chromosomes during late prophase I of meiosis

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Sister chromatid cohesion (SCC) is essential for proper chromosome segregation in mitosis and meiosis. SCC is mediated by a protein complex called cohesin, which forms a ring structure embracing two sister chromatids. The mitotic cohesin consists of two Smc subunits, Smc1 and Smc3, and α-kleisin subunit, Scc1/Mcd1 as well as other regulatory subunits. Although the cohesin is critical chromosomal components for the interphase, it is removed for accurate chromosome segregation during M phase. In higher eukaryotes, cohesin removal occurs through two-step processes. In late G2 or early pro-metaphase, a bulk of cohesin complexes are dissociated from chromosome arms. This removal of the cohesin is so called "prophase pathway" which is controlled by Plk1 (Polo-like kinase) and Wapl (Rad61), a regulator subunit of the cohesin complex. At metaphase-to-anaphase transition, cleavage of the centromeric cohesin by the separase, a protease which degrades Scc1/Mcd1, triggers the separation of sister chromatids.

During meiosis, a meiosis-specific cohesin promotes not only the segregation of chromosomes at both meiosis I and II but also chromosome dynamics in prophase I. In budding yeast meiosis, it is known that the removal of cohesin from chromosome arms by the cleavage of Rec8, a meiosis-specific α-kleisin subunit, triggers the segregation of homologous chromosomes at metaphase-anaphase transition of meiosis I. However, during prophase I (G2) when drastic chromosome morphogenesis occurs, dynamics of the cohesin molecule, particularly in late prophase I, is poorly described. We found that large amounts of Rec8, thus meiosis-specific cohesion, is removed from chromosomes, particularly from chromosome arms in the late prophase I of the yeast. This removal of Rec8 is also seen in the depletion mutant of the *CDC20*, which encodes an activator of APC/C (Anaphase promoting complex/cyclosome) for the cleavage of Rec8 by the separase. Thus, like late G2 phase in mammalian mitosis, late prophase I shows cleavage-independent removal of the cohesin, thus prophase pathway. This meiosis-specific prophase pathway requires both Plk1 and Rad61/Wpl1(Wapl). We will discuss the biological significance for the removal of cohesin from chromosome arms in late prophase I.

P116 Synapsis-dependent meiotic CO control in long-sized chromosome

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CO formation is strictly controlled to produce chiasma structure properly along entire chromosomes. In budding yeast, meiotic recombination reaction is synchronized with synaptonemal complex (SC) formation. SC components ZMM (Zip-Mer-Msh) complex are essential for crossover (CO) control as well as SC formation. Because zmm mutants also show a defect in SC elongation, it has been believed that SC elongation itself has critical function for CO control. It was difficult to distinguish between CO formation and SC elongation in ZMM function. ZMM complex can be defined as two subgroups by cytological observation; one is the transverse element comprised of Zip1 polymerization, the other is the initiation point of Zip1 polymerization, named ZMM nodule, which is comprised of other ZMM components. To identify the function of SC elongation, we focused on Gmc2-Ecm11 complex which is essential for Zip1 polymerization.

We revealed that CO formation was affected in the gmc2*Δ* mutant in a short chromosome (chr. III) as reported previously. However, genetical analysis revealed that CO formation was more frequently than in the gmc2*Δ* mutant than that in wild type on a long-sized chromosome (chr. VII). The gmc2*Δ* mutant showed normal level of Zip3 and Msh5 focus formation on meiotic spread. However, reduced number of Zip3 foci and irregular distribution were observed specifically on the long-sized chromosomes in the gmc2*Δ* mutant. In addition, we observed increase of DSB formation in the gmc2*Δ* mutant. From these result, we hypothesized that increase of CO on long-sized chromosomes in the gmc2*Δ* mutant cell might be caused by ZMM-independent pathway originated by additional DSB formation.

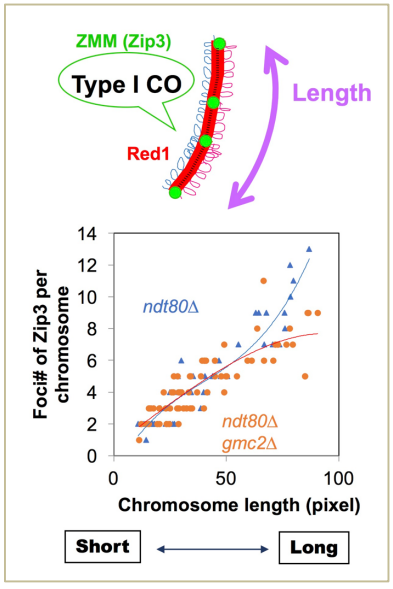

Figure 1 gmc2 mutation showed decrease of pro-CO localization in long-sized chromosomes

P117 Interplay between BRCA1 and catabolism of ADP-ribose ensures crossover homeostasis and maintenance of genome integrity

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Faithful transmission of the genetic information relies on meiosis, a specialised cell division mechanism that halves the genome and ensures its correct partitioning into the gametes. To properly segregate, parental chromosomes (homologs) must recognise each other (pairing), tightly associate (synapsis) and recombine. Meiotic recombination is triggered by the deliberate formation of DNA double strand breaks (DSBs) by SPO-11, which in turn undergo homology-dependent repair to yield crossovers (COs). This entails physical attachments between the homologous chromosomes that are crucial to promote their correct segregation at anaphase I. In most organisms, the number of DSBs greatly exceeds the final number of COs, suggesting that a large cohort of DNA breaks is repaired via non-crossover pathways.

ADP-ribosylation at sites of DNA lesions is crucial for DNA repair in higher eukaryotes. However, the determination of the roles of this post-translational modification in DNA repair during gametogenesis has been hampered by the embryonic lethality caused by depletion of both ADP-ribose polymerases PARP1/2, or PARG, the enzyme responsible for removing ADP-ribose moieties from substrates.

We provide data which highlight a pivotal and previously unknown role for ADP-ribosylation, in particular its removal, in DSB repair during meiosis. By using *Caenorhabditis elegans* as a model system (mutations in PARP1/2 or PARG do not cause embryonic lethality in worms) we show that although not essential for CO formation, knockout of the worm ortholog of PARG, *parg-1*, causes accumulation of SPO-11-dependent recombination intermediates which require *brc-1/BRCA1* function for repair.

In fact, *brc-1; parg-1* double mutants display synthetic embryonic lethality (indicating that BRC-1 and PARG-1 function in distinct pathways) coupled with a severe impairment of RAD-51 loading in pachytene cells and extensive chromosome abnormalities observed in diakinesis nuclei. Aberrant chromatin masses were largely dependent on non-homologous end joining and disappeared upon SPO-11 removal, suggesting an abnormal processing of meiotic DSBs. Furthermore, lack of the CO-specification site marker COSA-1 and premature loss of synapsis were observed on the X chromosome but not on the autosomes, suggesting that BRC-1 and PARG-1 are required to elicit CO formation on the X chromosome and that simultaneous depletion of PARG-1 and BRC-1 impairs multiple DNA repair pathways. Furthermore, cytological analysis revealed extensive co-localization of PARG-1 and BRC-1 with each other, and with CO-promoting factors, such as COSA-1 and ZHP-3 at the presumptive CO sites. Moreover, co-immunoprecipitation experiments show that PARG-1 and BRC-1 are found in a complex *in vivo*. Taken together, our data suggest a crosstalk between PARG-1 and BRC-1, which is essential for efficient and correct meiotic DSB repair to maintain genome stability.

P118 Wapl-mediated cohesin removal from bivalent chromosomes during mammalian meiosis

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Sister chromatid cohesion is essential for proper chromosome segregation during mitosis and meiosis. During mammalian meiosis, cohesion is established before birth during pre-meiotic S and must be maintained until meiosis resumption, which only occurs month or years later during ovulation. The increase of trisomic pregnancies, which lead to spontaneous abortions or developmental defects, with maternal age correlates with a gradual loss of chromosome cohesion. To gain new insights on the "maternal age effect" it is therefore crucial to understand how chromosome cohesion is regulated during mammalian meiosis and how chromosome cohesion is deteriorated with age. In mitotic cells, cohesin can be removed from chromosomes by the protein Wapl, but whether Wapl also releases cohesin complexes from bivalent chromosomes in meiosis is unknown. By using conditional mutagenesis in mice, we have analyzed the functions of Wapl in mouse oocytes. Our results indicate that Wapl has important roles in regulating cohesin, in the generation of normal bivalent chromosomes, and for meiotic chromosome segregation. The implications of these results for maternal age-related egg aneuploidy and infertility will be discussed.

P119 Meiotic crossing-over in the absence of RAD51 activity

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The process of eukaryotic sexual reproduction is based on the production of gametes of halved ploidy, the fusion of two of which regenerates the original ploidy in the subsequent generation. Homologous recombination during the first meiotic prophase ensures the balanced segregation of homologous chromosomes essential for the production of gametes and shuffles the genetic information, generating genetic variation.
Previously published data from our lab points to differing requirements for RAD51 and DMC1 in meiotic pairing of heterochromatic centromeric and 5S rDNA regions versus chromosome arms in *Arabidopsis* (Bleuyard and White, EMBO J. 2004. 23: 439-49; Da Ines *et al*, PLoS Genet. 2012. 8: e1002636). Taking advantage of the fact that DMC1 is the only active strand-exchange protein in plants expressing the dominant-negative RAD51-GFP fusion protein (Da Ines *et al*, PLoS Genet. 2012. 8: e1002636; Kobayashi *et al*. Genes Genet Syst 2014. 89**:** 169–179), we present here work further characterizing the meiotic roles of these two proteins, through an analysis of the effects of the absence of RAD51 strand-exchange activity in meiosis. Meiotic recombination in *rad51* mutants expressing this protein is thus catalysed only by DMC1 and we have exploited this to test for effects on crossing-over rates in different chromosomal regions, chromosomes and genome-wide. Chiasmata counting, crossing-over rate measurements in centromere-spanning and chromosome-arm genetic intervals and EdU (5-Ethynyl-2´-deoxyuridine) meiotic time course experiments show no detectable effect of the absence of RAD51 strand-exchange activity in *Arabidopsis* meiosis These data will be discussed in the context of the specificities of the roles of the DMC1 and RAD51 strand-exchange activities in *Arabidopsis* meiosis.

P120 3D model of meiotic telomere attachment sites at the nuclear envelope

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During prophase I of meiosis the tripartite synaptonemal complex forms as a scaffold for the homologous chromosomes to synapse, recombine and segregate. Early electron microscopic images of murine synaptonemal attachments at the nuclear envelope show characteristic filamentous structures that span the nuclear membrane. Available evidence indicates that these filaments correspond to LINC complexes. These complexes are thought to transduce the forces necessary for chromosome movements by connecting microtubules to meiotic telomeres. Despite past observations of the ultrastructure of meiotic telomeres by transmission electron microscopy, a structural map that would provide true insight into their functionality is still missing. To bridge this gap, we acquired dual-axis electron tomograms of the telomere attachment sites of wildtype mice. Within the tomograms we manually annotated the synaptonemal complex with its associated filaments to generate a 3D model of the structure. Based on this structural model, we quantified the amount and length of the cytoplasmic filaments, analyzed their distribution and elucidated their relation to microtubules. The quantitative and topological data based on the 3D model of synaptonemal attachment sites provide a deeper understanding of the molecular architecture of meiotic telomeres and their interaction with the cytoskeleton.

P121 Reduced level of centromeric histone increases fertility of SMG7 deficient *Arabidopsis*

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Meiotic division produces haploid cells from diploid precursors in two rounds of chromosomal divisions. While we have relatively detailed knowledge on evolutionary conserved genes governing meiotic recombination and chromosome segregation, regulation of meiotic progression is evolutionary less conserved and less studied. In our previous work we have discovered that SMG7, a conserved nonsense mediated RNA decay factor, is required for completion of meiosis in *Arabidopsis thaliana*. Partial loss of SMG7 function results in third meiotic division and formation of aberrant spores, which drastically reduces plant fertility.

To unveil the SMG7 function in meiosis, we performed an EMS suppressor screen to find mutations that can bypass meiotic defects and increase fertility in SMG7 deficient plants. The screen revealed two independent lines carried the same mutation in the CENH3 gene, coding for the centromere-specific histone H3 variant. The new mutant allele, *cenh3-2*, affects splicing of the first intron, substantially reducing the amounts of functional transcript and of the CENH3 protein. Despite the very low levels of CENH3, the mutant plants grow normally and are fertile, suggesting that centromeres and kinetochores are functional.

Detailed phenotypic and epistatic analysis indicated that reduced level of CENH3 does not prevent entry to the third meiotic division, but rather facilitates post-meiotic cytokinesis in *smg7-6 cenh3-2* double mutant plants. This may increase the formation of normal haploid microspores with full sets of chromosomes and explain the restored fertility. Live imaging of mitotic divisions revealed that the metaphase lasts longer in *cenh3-2* mutants in comparison to that in the wild type and that a reduced level of CENH3 delays chromosome biorientation. In sum, the results suggest that a proper level of CENH3 is important for coordinated progression through mitotic and meiotic cell divisions.

P122 Characterization of autosomal and X-Y pairing defects in mice with a mono-allelic expression of Spo11-beta splice isoform

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Meiotic Recombination starts with DSBs formation, a stochastic process catalysed by Spo11 protein. In mammals, Spo11 gene produces two main alternative transcripts: Spo11-beta and Spo11-alpha (Keeney S. *et al*., Genomics (61) 2, 1999). Recent findings suggest that in mice, the transgenic expression of the single Spo11-beta isoform under Xmr promoter, in C57BL/6 strain background (Xmr[Spo11-beta]-only mice), is sufficient to support meiotic recombination between the homologs, but the sex chromosomes (X-Y) (Kauppi L. *et al*., Science (331) 6019, 2011). As consequence, these mice are sterile. Conversely, when the Spo11-beta transgene is expressed in a mix-genetic background, X-Y pairing defect and fertility are rescued (Faisal I., Chromosome 125: 227, 2016). These findings indicate that in mice, with an intrinsic predisposition to X-Y asynapsis, the degree of failure of segregation of the sex chromosomes (thus, fertility) is modulated by the genetic background. However, whether it is the direct consequence of a change in Spo11-beta expression or activity is unknown.

We knocked-in Spo11-beta within the Spo11 locus and analysed the phenotypic consequences of its mono-allelic expression in a Spo11-/- background (Spo11[Spo11-beta-ki]-only mice). All mice, in a mix C57/129 background, were fertile. However, a detailed analysis showed that mouse progeny had two different phenotypes: some mice were characterized by an obvious reduction in testicular weight ("small-size testis", [ST]), while in others testis size was more similar to that of wild type ("mild-size testis" [MT]). To understand the origin of these differences, we kept the two phenotypes separated by inbred crossing, and analysed spermatocytes meiotic progression and recombination proficiency. In ST mice, we observed defects in pairing of the autosomes in about 40% of the nuclei and asynapsis of the sex chromosomes in 60% of pachytene stage nuclei. These defects were milder in MT mice (30% and 20%, respectively). This indicates that a) in a mix-genetic background, the mono-allelic expression of the only Spo11-beta isoform predisposes to a defective synapsis of the autosomes b) accordingly with previous findings (Faisal I., Chromosome 125: 227, 2016), the degree of autosomal and X-Y synapsis likely differs with genetic background. Importantly, the side-by-side analyses of Spo11-beta expression (real-time PCR) and quantification of DMC1 foci (a marker of DSBs) in ST and MT spermatocytes did not reveal significant differences between ST and MT mice. This suggests that the observed phenotypic differences are likely attributable to modifier genes, whose expression changes according to the genetic background. Our future goals will be to analyze the consequence of the expression of the only Spo11-beta-ki splice isoform in a pure C57BL/6 background and the identification of major modifier genes, involved in controlling autosomes and X-Y chromosomes synapsis in mice.

P123 Meiotic Nuclear Oscillations Are Necessary to Avoid Excessive Chromosome Associations

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Pairing of homologous chromosomes is a crucial step in meiosis, which in fission yeast depends on nuclear oscillations. Yet, how nuclear oscillations help pairing is unknown. Here we show that homologous loci typically pair when the spindle pole body is at the cell pole and the nucleus is elongated, whereas they un-pair when the spindle pole body is in the cell center and the nucleus is round. Inhibition of oscillations demonstrated that movement is required for initial pairing, and that prolonged association of loci leads to mis-segregation. The double-strand break marker Rec25 accumulated in elongated nuclei, indicating that prolonged chromosome stretching triggers recombinatory pathways leading to mis-segregation. Mis-segregation was rescued by overexpression of the Holliday junction resolvase Mus81, suggesting that prolonged pairing results in irresolvable recombination intermediates. We conclude that nuclear oscillations exhibit a dual role, promoting initial pairing and restricting the time of chromosome associations to ensure proper segregation.

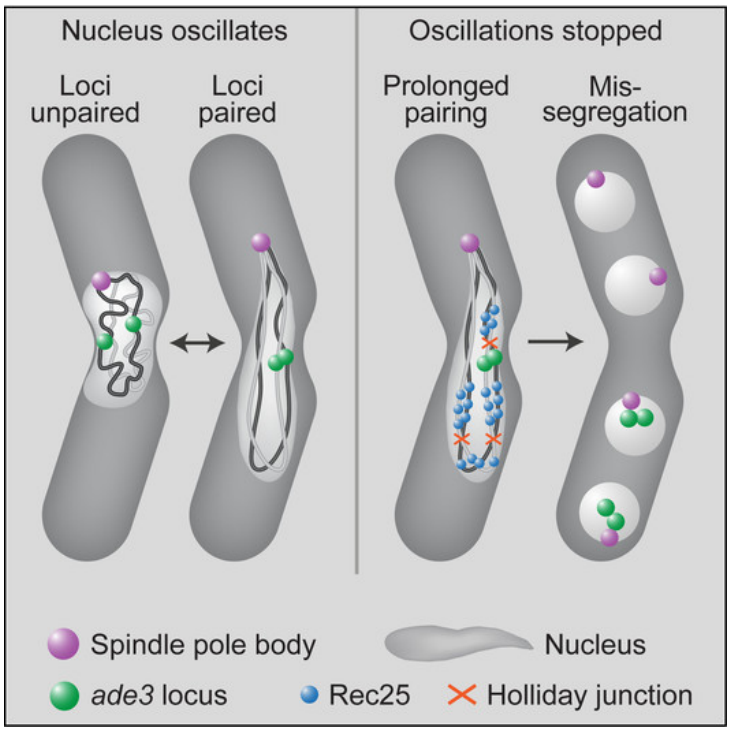

Figure 1. Meiotic nuclear oscillations have a dual role in chromosome dynamics.

P124 Chromosome synapsis and recombination in black tern (*Chlidonias niger*) and common tern (*Sterna hirundo*)

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Recombination rate variation in birds is undeservedly little studied. Conservative karyotype and high crossover frequency make them a good model for studying evolution of this trait. High number of crossovers on macrocromosomes make possible to access variation independent on obligatory chiasma and could help to reveal the adaptive component of recombination rate variation comparing species with similar karyotypes and different habitats. Differences in the degree of the sex chromosome differentiation between taxa make birds a good model for studying the stages of their evolution. We analysed chromosome synapsis and recombination in oocytes of two tern species (*Laridae*) - black tern (*Chlidonias niger*) and common tern (*Sterna hirundo)* using immunolocalization of key meiotic proteins (SYCP3, MLH1, centromere proteins). These species diverged about 8-9 MYA (Bridge et al. 2005) and differ for food sources, genetic and spatial population structure (Szczys et al., 2012, 2016). We first described the pachytene karyotype of the black tern (2n=74, FN=94) and reconstructed suggestive rearrangements fixed during its divergence from the common tern (2n=68, FN=90) comparing the morphology and relative sizes of macrobivalents. The mean number of MLH1 per autosomes significantly differed between species ($p<0.0001$, 53.0±4.2 in black and 44.1±5.0 in common tern). The lengths of their genetic maps were estimated as 2700 cM for black and 2254 cM for common tern. However, the recombination density per unit of SC length did not differ between species both for individual macrobivalents and whole set of autosomes. For individual macrochromosomes we plotted recombination maps and showed that difference in recombination patterns was higher between the rearranged homeologues than between the non-rearranged ones. This indicate that the difference in the crossover numbers per cell mostly depends on the difference in the SC length and chromosomal rearrangements. We analysed the pairing pattern of heteromorphic sex chromosome pair ZW and estimated the length of the pseudoautosomal regions by distribution of the MLH1 foci. Despite the black and common tern karyotypes are differed by atypically high number of rearrangements in autosomes, synaptic and recombination patterns of sex chromosomes did not differ between them. Supported by Russian Foundation for Basic Research (project #15-04-08389) and the Federal Agency of Scientific Organizations via the Institute of Cytology and Genetics (project #0324-2017-0003).

P125 Histone-methyltransferase PRDM9 is not essential for rat meiosis

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PRDM9 (PR/SET-domain 9) is a histone-3-lysine-4-trimethyl(H3K4m3)transferase that determines the double-stranded breaks (DSBs) initiating meiotic recombination in the mouse, human, and cattle. The rapidly evolving DNA-binding domain of the PRDM9-encoding gene can set off the meiotic paradox (the most often used recombination sites – hotspots - disappear most quickly from the population). Deletion of the *Prdm9* gene from the laboratory mouse induces a shift of the recombination initiation hotspots to other H3K4m3-marked sites including promoters and a complete arrest of meiotic prophase I. However, dogs, birds, and baker´s yeast that lack PRDM9 have recombination hotspots often located near promoters. Moreover, a human female that could produce offspring without PRDM9 function was identified. We therefore investigated if PRDM9 is essential for meiosis in another rodent species, *Rattus norvegicus*. Mutants carrying deletions in one of the exons encoding the PR/SET domain were produced by injecting mRNAs of programmed endonucleases into rat zygotes. Four resulting founders were bred to homozygosity. Compared to littermate controls, the *Prdm9*-deficient rats displayed decreased fertility parameters, but some produced offspring. Therefore, the rat can be utilized to model human germ cell development, as it is able to accomplish meiosis using both PRDM9-specified and other recombination sites.

P126 Human infertility alleles identified in mice: a Spo11 allele that may alter crossovers

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Infertility affects up to 15% of child-bearing aged couples. In half of these cases, the causes are unknown but it is believed that they derive from a genetic origin. Over the last several years, we have worked on an association- and linkage-free approach to identify segregating infertility alleles. With the use of CRISPR/Cas9 genome editing, we have modeled multiple putatively deleterious, nonsynonymous SNPs in mouse orthologs of fertility genes. I will present data on an interesting allele of the gene *Spo11*, which causes a subfertility phenotype in our edited mice. SPO11 is a critical protein that initiates recombination by creating double-stranded breaks (DSBs) at several hundred sites in the genome, a fraction of which are repaired by crossover recombination. These crossovers, when properly distributed across all chromosomes due to interference, are essential for proper disjunction at the first meiotic division. The phenotypes of our mutant mice include decreased testes size and lower sperm counts. Preliminary data suggests that crossover defects in Prophase I may be the cause for this subfertility phenotype, suggesting a potential alteration in the pathways used for DSB repair.

P127 An intact ER unfolded protein response is important for meiotic success

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Successful gamete formation depends on faithful execution of meiosis via a highly coordinated developmental program. Recent work has identified timed induction and silencing of the conserved endoplasmic reticulum (ER)-localized unfolded protein response (UPR) during late stages of budding yeast meiosis, suggesting a role in gamete formation for this conserved cellular stress response. Decreased sporulation efficiency and spore viability were observed as a result of meiotic depletion of the UPR transcription factor, Hac1. We are currently examining the precise meiotic stages during which an intact UPR is required, as well as using conditional UPR mutants to determine the transcriptional output of the meiotic UPR. Through use of a variety of genetic and cell biological techniques, we hope to gain insight into developmental UPR activation and its relevance to meiotic success.

P128 Regulation of SOD1 during budding yeast meiosis

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In the budding yeast *Saccharomyces cerevisiae*, meiosis is accompanied by dramatic changes in expression at the levels of transcription and translation. One interesting case of meiosis-specific gene regulation that we have observed is the alternative transcription of the *SOD1* locus, which encodes a conserved superoxide dismutase. The levels and proper folding of Sod1 protein are known to be essential to cellular fitness, as Sod1 is important for detoxification of the cellular products of oxidative stress. Sod1 is also unusually prone to aggregation, with aggregation status associated strongly with the human disease ALS. During vegetative growth, the *SOD1* mRNA transcript is approximately 600 nucleotides in length. Upon entering meiosis, cells selectively translate an extended *SOD1* transcript with an extremely long 5' leader region containing a translated upstream open reading frame (uORF). We are investigating the dynamics and function of this alternative *SOD1* transcription in meiosis.

P129 Microtubule sliding in the bridging fiber pushes kinetochore fibers apart to segregate chromosomes

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During cell division, microtubules of the mitotic spindle segregate chromosomes by exerting forces on kinetochores, protein complexes on the chromosomes. The central question is what forces drive chromosome segregation. The current model for anaphase in human cells includes shortening of kinetochore fibres and separation of spindle poles, processes that require kinetochore-pole connections. By combining laser ablation, photoactivation and theoretical modelling, we show that kinetochores can separate without the attachment to the pole. This separation requires the bridging fibre, which connects sister k-fibres. Bridging microtubules in intact spindles slide apart together with k-fibres, indicating strong crosslinks between them. Kinetochore segregation and pole separation is slower after depletion of MKLP1/KIF23 (kinesin-6), faster after depletion of KIF4A (kinesin-4), and unaffected by reduction of Eg5/KIF11 (kinesin-5) or KIF15/Hklp2 (kinesin-12). We conclude that motor-generated sliding in the bridging fibres drives pole separation and pushes k-fibres poleward, thereby working together with k-fibres shortening to segregate chromosomes.

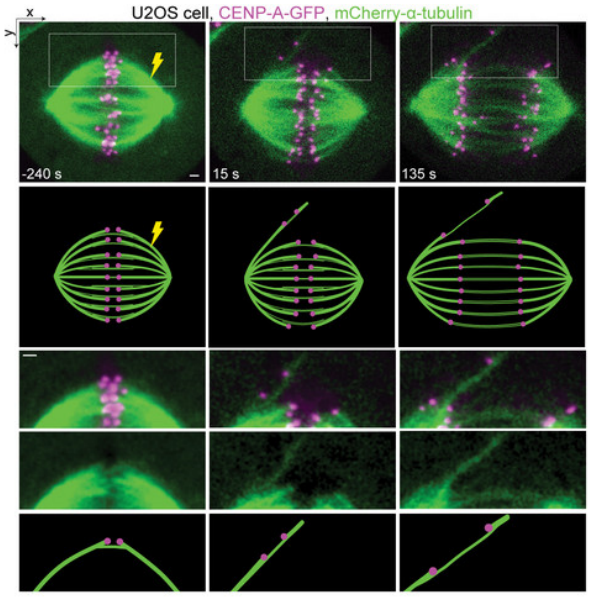

Figure 1: Time-lapse images of spindle (first row) in a U2OS cell expressing centromere protein CENP-A-GFP (magenta) and mCherry-α-tubulin (green), and smoothed enlargements (third row, forth row in green). Yellow lightning sign represents ablation. Time 0 is anaphase onset.

P130 Meiosis in the industrial workhorse fungus *Trichoderma reesei*

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Trichoderma reesei (syn. *Hypocrea jecorina*) is a widely used model organism for plant cell wall degradation, industrial enzyme production and plant-fungal interactions. *Trichoderma reesei* undergoes a heterothallic reproductive cycle and generates fruiting bodies (stromata) containing linear asci with 16 ascospores, which are are generated via meiosis and two rounds of postmeiotic mitosis. We have determined the complete (telomere-to-telomere) genomic sequences of two different wild-isolate haploid strains and their F1 progeny using both next- and third-generation sequencing platforms. Our results indicate that the genome of *Trichoderma reesei* encodes Rad51 Rad52, Rad54, Rad55 and Rad57 but not Dmc1, Hop2, Mnd1, Mei5 or Sae3. Deletion of the only *spo11* gene results into normal sexual development, including the formation of fruiting bodies, asci and ascospores. However, the interhomlog recombination products *in spo11* are different from those in the wild-type strain. We suggest that *Trichoderma reesei* is a unique model for studying *spo11*- and *dmc1*-independent meiotic recombination.

P131 CO sites set up axis/SC hyperabundance domains

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During meiosis, crossover (CO) recombination plays an evolutionary role in maintaining genomic diversity. In addition, COs create physical linkages between maternal and paternal homologs which ensure their regular segregation to opposite poles at the first meiotic division. COs occur at different positions in different meiotic nuclei but, nonetheless, along each given chromosome, tend to be evenly spaced. This spatial patterning is a reflection of the classical phenomenon of crossover interference. The final outcome of CO patterning can be observed cytologically at mid-prophase of meiosis via the positioning of corresponding recombination or recombination-related complexes (e.g. Zip3 foci) along the synaptonemal complex (SC), a structure that links the structural axes of the homologs along their lengths. Previous studies show that, at pachytene, an SC central region component (Zip1) and an important axis component (HORMAD protein Hop1) occur with alternating abundance. Careful quantification of Zip3, Hop1 and Zip1 patterns in wild type cells, together with other data, suggest that designation of a CO site (with accompanying CO interference) sets up divergent domains of hyperabundant Hop1 and Zip1 emanating to either side. Possible sources of Hop1/Zip1 asymmetry and possible roles for this feature during meiotic recombination are discussed.
Additional findings further show that AAA+-ATPase Pch2, whose primary role is to remove Hop1 from chromosomes is not required for CO-designation or CO interference.

P132 Investigating the Cdc5 dependent phosphoproteome during meiotic prophase I

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In meiosis, haploid gametes form from diploid germ cells through a pre-meiotic DNA replication phase which is followed by two consecutive nuclear divisions. Homologous chromosomes segregate in meiosis I, while sister chromatids disjoin in meiosis II. During this process, reciprocal recombination is required for the generation of crossovers, which, together with monopolar attachment of sister-kinetochores, enable the meiosis I spindle to pull maternal and paternal centromeres in opposite directions. In turn, crossover formation depends on the resolution of Holliday junctions (HJs), which arise during the repair of deliberately introduced DNA lesions. HJs are key intermediates in the process of crossing-over. However, due to their stability in connecting the recombining DNA duplexes, they are also dangerous DNA repair intermediates. If left unresolved, HJs impair chromosome segregation and lead to formation of aneuploid gametes. It is known that HJ processing is tightly coupled to cell cycle progression by the activity of Polo kinases. Polo kinase Cdc5 is considered the master-orchestrator of HJ resolution events and in budding yeast, is known to target the HJ resolvase Mus81. Yet, cells lacking Mus81 still resolve most HJs in a Cdc5-dependent manner, indicating that vital targets of Cdc5 remain elusive. We have acquired a mass-spectrometry data set, depicting the Cdc5 dependent phosphoproteome during meiotic prophase I. Ongoing biochemical and genetic approaches will help us to reveal and validate novel Cdc5 substrates involved in meiotic HJ resolution.

P133 Modifying meiotic recombination in tomato

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The mechanisms underlying the control of meiotic recombination have been extensively studied in plants over recent years, utilising the model plant *Arabidopsis thaliana*. With the global population predicted to exceed 9 billion by 2050 and with the estimated adverse effects likely to occur as a result of climate change, it is now of great importance to translate this knowledge to crop species. The reciprocal exchange of genetic information during meiotic recombination has been shown to be actively repressed in plants by certain proteins, such as the RTR complex, with only a fraction of double-strand breaks being processed as crossovers. These constraints limit the genetic variation available to plant breeders, thus restricting the ability to generate new crop varieties that are able to tolerate various abiotic and biotic factors. As a means to aid with the improvement of crop production, these crossover-limiting mechanisms should be investigated in crops to develop strategies to be able to modify recombination in such a way to either increase or suppress meiotic crossover formation. In this study, the plant-specific CRISPR/Cas9 genomic editing tool [1] was used for targeted mutagenesis of two of the RTR complex partners, the type IA topoisomerase TOP3alpha and the structural protein RMI1, in tomato. These proteins, in conjunction with the RecQ helicase RECQ4A and the second structural protein RMI2, have been shown to be involved in the dissolution of recombination intermediates to form non-crossover products in *Arabidopsis*. *rmi1* and *top3alpha* mutants are sterile with extensive chromosome fragmentation and the arrest of meiosis during telophase I, demonstrating the integral role of these proteins in meiotic recombination [2]. Such a severe meiotic phenotype was surprising, as it has not been demonstrated in other eukaryotic organisms. Generating and analysing such meiotic mutants in tomato would increase our understanding of the control of crossover formation and ascertain as to whether this severe meiotic phenotype is plant-specific and conserved in crop species.

1. *Fauser F., Schiml S. and Puchta H. (2014) Both CRISPR/Cas-based nucleases and nickases can be used efficiently for genome engineering in Arabidopsis thaliana. Plant J 79, 348-359.*
2. *Hartung F., Suer S., Knoll A., Wurz-Wildersinn R. and Puchta H. (2008) Topoisomerase 3A and RMI1 suppress somatic crossovers and are essential for resolution of meiotic recombination intermediates in Arabidopsis thaliana. PLoS Genetics 4 (12), 1-11.*

P134 Visualizing meiotic crossover patterning in *C. elegans*

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DNA crossovers (COs) are one possible product of homologous recombination. During meiosis, a subset of DNA double-strand breaks (DSBs) are designated to mature into COs; a fate that both generates genetic diversity and creates the physical connections between pairs of homologous chromosomes that are necessary for accurate chromosome segregation at Meiosis I. The mechanism by which a subset of DSB precursors are designated for CO formation is unknown, but it is coupled to a second mysterious process (known as CO interference) that reduces the probability of nearby precursors from also being designated for CO formation. As a result, there is a tendency for COs to be evenly spaced along chromosomes. In order to investigate the mechanism of this patterning process we have developed an assay for visualizing the process and allowing the response of chromosomes to perturbation to be measured in real-time. Specifically we have developed a microfluidic device that allows us to image oogenesis in the model organism *Caenorhabditis elegans* at high spatial and temporal resolution for over 50 hours, allowing us to follow all stages of Meiosis I. Additionally, we can flow in chemicals allowing for protein induction, depletion, and inhibition. Specifically, by combining the visualization of markers for homologous recombination and crossover designation with inducible depletion of proteins of interest, this assay should provide new insights into the mysterious processes of CO designation and interference.

P135 The dynamic architecture of DNA repair complexes at sites of meiotic recombination in context of the synaptonemal complex

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We re-assessed the dynamic localization of DNA double strand break (DSB) repair proteins in context of the Synaptonemal Complex Lateral Element (SC-LE) and Central Region (SC-CR), by Structural Illumination Microscopy on spread *C. elegans* germline nuclei. Throughout early prophase, RAD-51, the only recombinas utilized during *C. elegans* meiosis, initially decorates both DSB ends. RAD-51 marked DSBs convert into repair intermediates, decorated by two populations of both, BLM helicase and ssDNA-binding RPA-1. These foci orient parallel with and between SC-LEs. At inter-homologous repair sites, BLM and RPA-1 doublets flank a single population of MSH-5 and COSA-1, essential factors for crossover formation during *C. elegans* meiosis. DSB continue to be introduced, turned over and accumulate as inter-homologous repair sites, until each bivalent receives at least one crossover-eligible intermediate. Once that happened, MSH-5 vanishes from future non-crossover sites, while RPA-1 and BLM remain temporarily, but disappear shortly thereafter. In contrast, crossover-designated sites, lose RPA-1 and acquire a second MSH-5 population, which orientates perpendicular to the axes, in contrast to the BLM doublet at the same sites. These crossover-designated intermediates are found enveloped in a bubble of SC-CR proteins and endure pachynema. In diplonema one BLM focus is lost, before chiasmata, the cytological manifestations of crossing-over, become apparent in diakinesis, not marked by any recombination proteins anymore. In absences of the SC-CR, BLM is completely lost from inter-homologous repair sites marked by MSH-5/COSA-1 prematurely and crossover formation fails. From these and other data presented we concluded that the SC-CR protects crossover-designated sites from being dismantled inappropriately and promotes the stepwise maturation of crossover-designated sites into crossover.

P136 Adaptive evolution of meiosis in response to genome change

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I'm using *A. arenosa* as the model plant to detect how alleles of meiosis genes under selection in autotetraploid functionally differ from diploid alleles, and test whether they alter crossover number and interference. Autopolyploids have more than two copies of every chromosome, and all are potential partners that can recombine with one another. When this happens, chiasmatic multivalents can form, which frequently lead to chromosome mis-segregation and gametic aneuploidy, multivalents occur commonly in newly arisen autopolyploids. In theory, autopolyploids can overcome the meiotic challenge of multivalent formation by reducing crossover (CO) frequency to one per chromosome, which ensures bivalent formation rather than multivalents and thus improves chromosome segregation. Recently, through population genomic approaches, the Bomblies lab established a list of candidate genes that are likely responsible for stabilizing meiosis and reducing crossover (CO) frequencies in the plant *A. arenosa*. Strikingly, these genes are involved in coordinated structural processes in meiosis I, especially the formation of linear axes between replicated sister chromatids (ASY1, ASY3, SYN1), and the formation of a zipper-like synaptonemal complex (SC) between homologs (ZYP1). These processes are essential for recombination, as well as chromosome pairing and regular segregation. Capitalizing on the rare occurrence of diploid-like alleles in natural tetraploid populations along with the colchine treatment the Bomblies lab have bred tetraploid plants homozygous for diploid ASY1 or ASY3 or both alleles, and found that these plants showed altered crossover placement consistent with differences observed between the diploids and tetraploids. Thus, the major goal in my project is to study the functional consequences of the evolution of axial element (ASY1/ASY3). I have taken advantage of both cytological and immunocytological approaches to determine the critical differences of meiosis among these plants. I am quantifying the persistence of multivalents and unresolved interlocks at metaphase1 and detect mis-segregation (laggards across the division plate) at anaphase1 by assessing cytological DAPI cell spreads. I will study axis formation and structure by examining localization of ASY1 and ASY3 in different meiotic stages, crossover maturation by staining for the recombination protein MLH1, and synapsis by ZYP1. Furthermore, I will make use of super-resolution microscopy to examine chromosome axis and SC structure in high resolution. Through these studies, I will be able to generate a comprehensive and specific cytological feature during meiosis in response to whole genome duplication.

P137 The impact of chromosome heterozygosity pattern on crossover designation

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It has been recently shown that juxtaposition of homozygous and heterozygous regions in *Arabidopsis* favours COs in the heterozygous region, at the expense of the homozygous region. The mechanism responsible for the heterozygosity juxtaposition effect is unclear, but may occur via recruitment of 'late' double-strand breaks (DSBs), caused by delayed recombination progression in mismatched heterozygous regions (hypothesis #1). This would cause heterozygous regions to recruit higher DSB levels, thereby leading to elevated CO frequency, with interference then responsible for decreased COs in adjacent homozygous regions. Alternatively, it is possible that recombination proteins respond directly to mismatched strand invasion sites (hypothesis #2). For example, the MutS mismatch repair factors can directly recognize mismatched DNA substrates. The major goal of the project is to investigate the molecular mechanisms by which the juxtaposition effect affects meiotic recombination. In the course of the project both hypothesis, which are not mutually excluding, will be tested. The research will also provide an answer to the question, how mismatches arising during strand invasion in meiotic recombination influence the control of DSB formation and its feedback mechanism via ATM and ATR kinases. The project will also shed more light on the role of eukaryotic homologs of mismatch repair proteins MutS and MutL in the interhomolog crossover formation. The role of these complexes in meiosis has been investigated in the mechanistic context of crossover formation, however our knowledge on their role for template selection during meiotic DSB repair is elusive.

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Transport from Split Airport to Hvar (hotels Amfora & Pharos)

Regardless of what transportation means you are going to use you will undoubtedly experience an interesting sea journey to Hvar island. When you see the city of Hvar and start enjoying its benefits you will know that your journey has paid off.
The closest international airport is **Split Kaštela Airport**, from where you can take a bus or a taxi, or rent a car to **Split Main Bus Station**, which is within walking distance to **Split port** from where you need to travel by catamaran or ferry (if you are arriving by car) to **Hvar**.

BUS from Split Kaštela Airport to Split Main Bus Station (Pleso prijevoz)

Buses drive from **SPLIT Kaštela Airport** to **SPLIT Main Bus Station** (platform No.1). Split Main Bus Station is directly adjacent to the Split port, so it is easy to continue your journey to Hvar.
The departure of the bus from **Split Kaštela Airport** is in front of the airport main entrance. The final stop of this bus is the **Split Main Bus Station**. Split airport is very small, so you will not miss it. Tickets can be bought on the bus, inside the airport building, or in the Split Main Bus Station (for the journey back). You will need Croatian currency, the Croatian kuna (HRK), in order to buy the ticket. There is an exchange office in the airport building.
Time table of departures from Split Main bus station can be found HERE
Time table of arrivals to Split Main bus station can be found HERE
** For more information about departures of buses in Split, contact Pleso prijevoz at +385 (0) 21 2031190 or write an e-mail to split@plesoprijevoz.hr.
One-way ticket costs 30,00 HRK. Travel time is half an hour.

Taxi

Taxi is available during **Split Kaštela Airport** operating hours.
Telephone: +385 (0)21 895 237

Sea transport from Split port to Hvar

In order to travel from Split port to Hvar we recommend the **catamaran** (1 hour) or the **ferry** (2 hours – for those who arrive by car). Price of one-way catamaran ticket is 100 HRK, while the ferry price for one car is 318 HRK plus 47 HRK per person. The Catamaran stops in Hvar, very close to the venue, while the ferry stops in Stari Grad, approximately 19 km from the venue.
Regarding your arrival to Hvar by catamaran / ferry from Split port we would like to advise you to book your catamaran (or ferry, if you travel by car) ride as soon as possible via the link https://www1.jadrolinija.hr/Shop/en/ .
The conference is taking place during the high season and the catamaran / ferry line Split-Hvar is a very popular and busy line. In order to avoid long queues on a hot weather and to avoid any chance to miss your catamaran ride, please book it on time.
You can see the map of the Split port below, with the location of the bus station, the catamaran ticket office, and both the catamaran and the ferry ports, all within waking distance.
When you arrive to **Hvar**, whether by catamaran, or by ferry, there will be a trolley waiting to take your bags to the hotel, while you take a pleasant walk (it takes 15 min – see Map of the city of Hvar at the end of the document).

Contacts of the Amfora and Pharos hotels are:

Ana Štambuk, Head of concierge
Sunčani Hvar Hotels, Sunčani Hvar d.d.
Dolac b.b., 21450 Hvar, Croatia
tel : +385 (0) 21 750 308
fax : +385 (0) 21 750 301
mob: +385 (0)91 1741 500
astambuk@suncanihvar.com
www.suncanihvar.com

If you have any problems with the organization of your transport or if you run into any problems in Croatia, please contact me (Ana Vidoš, avidos@irb.hr , +385 (0)1 457 1333).

Catamaran timetable:

Split-Hvar (transfer time 1:05)

Thu, 8/24/2017	Fri, 8/25/2017	Sat, 8/26/2017	Sun, 8/27/2017	Mon, 8/28/2017	Tue, 8/29/2017
9:15	9:15	9:15		9:15	9:15
9:45	9:45	9:45	9:45	9:45	9:45
10:30	10:30	10:30	10:30	10:30	10:30
15:00	15:00	15:00	15:00	15:00	15:00
15:45*	15:45*	15:45*	15:45*	15:45*	15:45*
17:00	17:00	17:00	17:00	17:00	17:00

* arrives in Hvar at 17:30

Hvar - Split (transfer time 1:05)

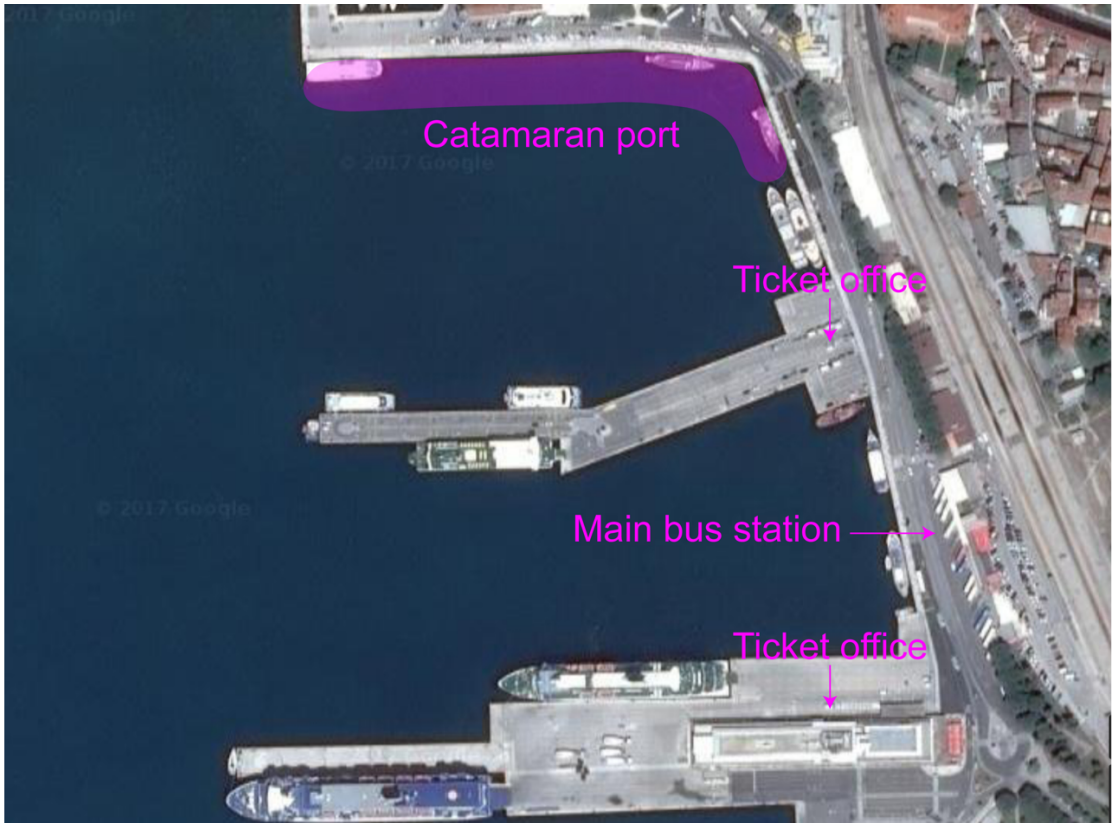
Tue, 8/29/2017	Wed, 8/30/2017	Thu, 8/31/2017	Fri, 9/1/2017	Sat, 9/2/2017	Sun, 9/3/2017
6:35	6:35	6:35	6:35	6:35	
8:00	8:00	8:00	8:00	8:00	8:00
10:45*	10:45*	10:45*	10:45*	10:45*	10:45*
11:30	11:30	11:30	11:30	11:30	11:30
13:00	13:00	13:00	13:00	13:00	13:00
14:35	14:35	14:35	14:35	14:35	14:35

* arrives in Split at 12:30

Ferry timetable:

Split- Hvar every day from 6/30 – 9/3	**Hvar-Split** every day from 6/30 – 9/3
1:30	5:30
5:00	7:45
8:30	11:30
11:00	14:00
14:30	17:30
17:00	20:00
20:30	23:00

Map of Split port



Map of the city of Hvar

Useful expressions:

ENGLISH	CROATIAN
Hello	Bok
Goodbye	Doviđenja
Good morning	Dobro jutro
Good afternoon	Dobar dan
Good evening	Dobar večer
Good night	Laku noć
I'm sorry	Oprostite
Excuse me…	Oprostite…
How are you?	Kako ste?
I'm fine thanks! And you?	Dobro, hvala! A vi?
What is your name?	Kako se zovete?
My name is	Moje ime je…
Do you speak English	Govorite li engleski
I don't understand	Ne razumijem
Please speak more slowly	Molim vas govorite sporije
Thank you	Hvala
Where is the toilet?	Gdje je toalet?
Please call me a Taxi	Možete li mi pozvati taxi?
How do I get to…?	Kako mogu doći do…
A beer/ two beers please	Pivo / dva piva molim
A glass of red/white wine please	Čašu crnog/bijelog vina molim
The menu, please	Jelovnik, molim
Is there a local specialty?	Koji je lokalni specijalitet?
I'm Vegetarian	Ja sam vegetarijanac
I'm allergic to…	Alergičan sam na…
It was delicious	Bilo je jako ukusno
The bill, please	Račun, molim
I have a headache	Boli me glava/Imam glavobolju
I have a sore throat	Boli me grlo
My stomach hurts	Boli me trbuh
I need a doctor who speaks English	Trebam doktora koji govori engleski