Regulation of meiotic recombination and prophase I progression in mammals

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Summary
Meiosis is the process by which diploid germ cells divide to produce haploid gametes for sexual reproduction. The process is highly conserved in eukaryotes, however the recent availability of mouse models for meiotic recombination has revealed surprising regulatory differences between simple unicellular organisms and those with increasingly complex genomes. Moreover, in these higher eukaryotes, the intervention of physiological and sex-specific factors may also influence how meiotic recombination and progression are monitored and regulated. This review will focus on the recent studies involving mouse mutants for meiosis, and will highlight important differences between traditional model systems for meiosis (such as yeast) and those involving more complex cellular, physiological and genetic criteria. ***BioEssays*** **23:996–1009, 2001.**

Introduction

Meiotic cell division differs from somatic cell mitotic division in that germ cells undergo two rounds of chromosomal division following a single round of chromosome replication. In prophase of meiosis I, chromosomes first replicate into sister chromatids, and then search for and align with their homologous partner. Recombination between homologous chromosomes is initiated during this stage of meiosis, and is mediated by a cohort of enzymes that accumulate at the site of recombination, the recombination (or meiotic) nodule. All of these events occur within the framework of a proteinaceous structure known as the synaptonemal complex (SC) that physically tethers the homologous chromosomes together through much of prophase I, and is the landmark feature of this stage of meiosis.

Until recently, the study of mammalian meiosis was hindered by the relative paucity of analytical techniques with which to study chromosomal events and outcomes in mammalian germ cells. In contrast, yeast geneticists have had at their disposal a wide variety of techniques with which to study these processes.[1,2] These include, analysis of recombination outcomes by tetrad analysis, rapid induction of different allelic mutations along with genetic screening for random mutations affecting meiosis, and the use of known recombination hotspots as target sites for proteins of interest. This review will take advantage of the significant advances made in the study of yeast meiosis to provide a framework for analogous processes and regulatory mechanisms in mammals and will be restricted only to those protein families that are known to be involved in homologous chromosome interactions, DNA modification and crossing over during mammalian meiosis.

Overview of prophase I

Prophase I can be divided into five distinct substages, as defined by the chromosomal events occurring during each particular stage, as well as by the status of the SC (Table 1; Fig. 1). Sister chromatids replicate and enter prophase I at leptonema. At this early stage, a proteinaceous backbone forms along each chromatid pair, known as the axial element. The axial element joins the two sister chromatids together in a linear array, with their chromatin forming loops that extend out from each protein backbone. The chromosomes at this stage are long, uncompressed structures, but as they progress through prophase I they become progressively shorter and more condensed. During the second stage of prophase I, zygonema, numerous proteinaceous foci, termed recombination nodules, become associated with the axial elements at intervals along their length.

The homologous chromosomes begin to align with one another and become closely apposed at several regions along their length. The recombination nodules that are present at multiple sites along the axes form the earliest interaction site and are thought to promote interactions between the homologous chromosomes. At this stage, many recombination nodules exist, and these are described as early recombination

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Table 1. Characteristics of different stages of Prophase I

Stage*	SC components	Recombination nodules	Synapsis	Chromosome length	Centromere
Leptonema (leptotene)	Axial elements form ($Cor1^+$)	Early	Single homologs comprised of sister chromatids	Long, uncompacted	Single centromeres
Zygonema (zygotene)	Axial elements become lateral elements ($Cor1^+$); central elements accumulate ($Syn1^+$)	Early	Regions of synapsis appear	Shorter, compacting	Closely paired
Pachytene (pachynema)	Lateral elements persist ($Cor1^+$); central element fully formed ($Syn1^+$)	Late	Full synapsis (except XY)	Compacted	Closely paired
Diplotene (diplonema)	All components disintegrate; central element persists around centromere ($Syn1^+$)	None	Chromosomes paired at chiasmata only	Dense and compacted	Centromeres move apart

*Each stage name is provided in noun (adjective) form.

nodules because not all of them will go on to represent true sites of crossover (and, hence, fully mature sites of homologous recombination). The paired axial element regions, now lateral elements, become tethered together by the third component of the SC, the central element, which functions as a zipper to bind the homologs together in a process termed synapsis. As cells progress through to pachynema, the central element is completed, with chromosomes being synapsed along their entire length. The chromosomes at this stage are well compressed, short structures whose individual homologs are indistinguishable from their homologous partner.

Towards the end of prophase I, during diplonema, when the SC disintegrates and the homologous chromosomes begin to move apart, the sites of recombination become apparent as individual chiasmata, which hold the homologs together until the chromosomes are appropriately aligned along the midplate of the cell. At the first meiotic division (MI), these chiasmata are released, allowing the homologs to move to opposite poles of the cell, producing two daughter cells that then enter meiosis II. The correct segregation of homologous chromosomes to opposite poles at the first meiotic division is essential for the continuation of meiosis, and is ensured, at least in part, by the recombination process itself.

In mammals, superimposed upon this basic system is the need for temporal regulation of meiotic events (especially in females), as well as the requirement for endocrine feedback regulation. Thus, in male mammals, the regulation of meiotic events is achieved by the co-ordinate interactions between germ cells and neighboring Sertoli cells together with endocrine regulatory cues provided by the Leydig cells of the testicular interstitium. In male mice, spermatogenesis is initiated at around day 7 pp and, at this stage, the testis contains only somatic cells and spermatogonia. Early prophase I spermatocytes appear by day 12 pp, with later stages being apparent at around day 17 pp. By day 23 pp, the first wave of meiosis II begins, with early spermatids being evident within the core of the seminiferous tubular lumen.

In female mice, the entire population of oocytes enters meiosis I more or less synchronously at around e13 of gestation. By term, the oocytes have progressed through pachynema and into early diplonema, and shortly thereafter they undergo meiotic arrest in a stage known as dictyate. During this dictyate stage, which lasts until after puberty, the chromosomes retain their pachytene/diplotene morphology and remain arrested until gonadotropins stimulate the resumption of meiosis I during the estrous cycle in a small cohort of oocytes.

Initiation of recombination during prophase I

Recombination in Saccharomyces cerevisiae

Recombination is an essential pre-requisite for meiosis in yeast, since mutations that affect recombination also disrupt meiosis. Moreover, in yeast, the formation of the SC is also dependent on recombination, unlike species such as *Drosophila melanogaster* in which SC formation appears to precede recombination events.[3] In contrast, meiosis in *Schizosaccharomyces pombe* occurs in the absence of the SC, a situation that is more analogous to the mitotic recombination process.[4]

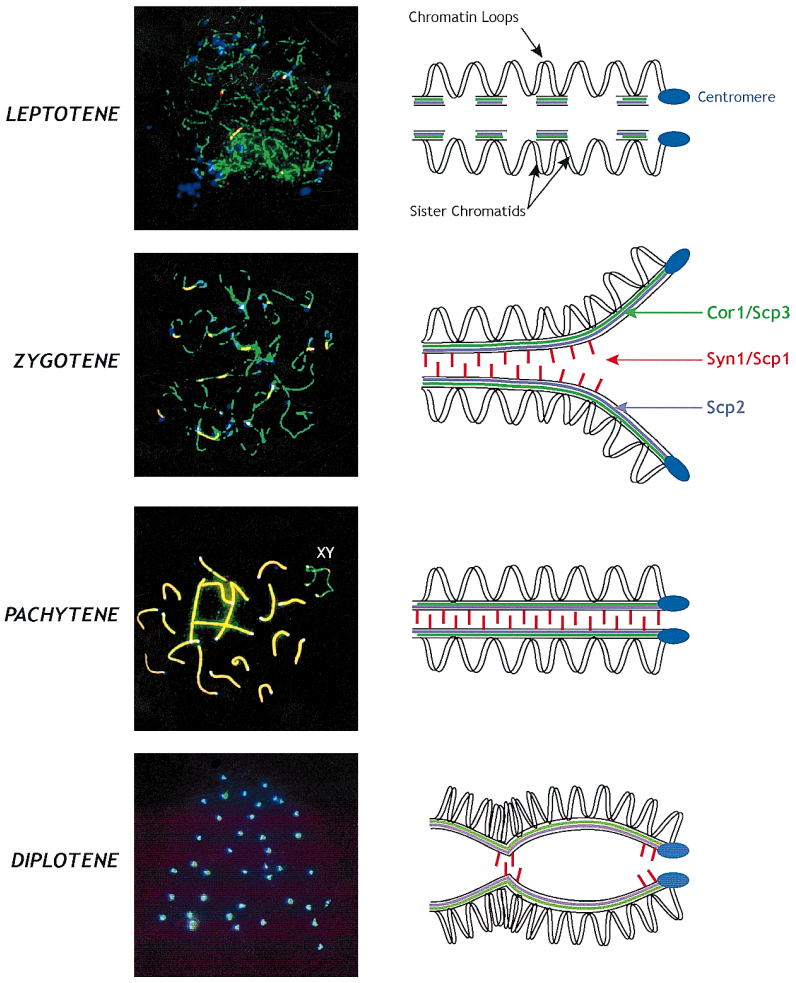

Figure 1. Overview of meiotic prophase I in mice. Synaptonemal complex (SC) formation in spermatocytes from male mice, as demonstrated by immunofluorescence localization of the SC proteins, Cor1 (green) and Syn1 (Red). Centromeres are stained in blue using the human Crest antiserum. As chromosomes synapse, the Cor1 and Syn1 signals merge to generate a yellow fluorescent signal. At the pachytene stage, the XY bivalent is the only chromosome pair that does not show complete synapsis. Instead, pairing occurs only at the pseudoautosomal region, as demonstrated by the limited colocalization of Syn1 and Cor1 (single region of yellow signal). Cartoon adapted from P. Moens, B. Spyropoulos and N. Kolas (University of York, Toronto, Canada).

In yeast, meiosis is initiated by the formation of a double-strand break in one of the homologs, a process that is mediated by the endonuclease product of the *SPO11* gene.[5] The resulting short 5′ overhang is then nucleolytically resected to yield variable-length 3′ single-stranded DNA tails, which are capable of invading the opposing homolog, thereby displacing one of the sister chromatids and producing double Holliday junction intermediates. The entire complex of intermingled DNA strands, termed a heteroduplex, is encased in the multi-proteinaceous recombination nodule (see below).

The enzymatic processes leading to the formation and processing of DSBs and the Holliday junction intermediates requires the products of at least eleven different genes, including *SPO11, MEI4*, *MER2, RAD50, MRE11, REC102, REC104* and *XRS2*. While SPO11 is required for formation of the DSBs, the other proteins appear to function downstream of the DSB formation event, either for the removal of SPO11 from the 5′ end of the break site or for the resection of DSB ends to produce the heteroduplex (for review see Ref. 1). Yeast MRE11, RAD50 and XRS2 have been implicated in a surprisingly wide range of processes, but during meiosis this complex appears to function at numerous stages in the recombination process, both by regulating the activity of SPO11, and by catalyzing the removal of SPO11 from the DSB and the subsequent resection of the break. Thus, null mutants in any of these genes are unable to initiate DSB formation, while some hypermorphic mutants will initiate DSB formation, but fail to remove SPO11 from the 5′ ends of the DSB or fail to resect the chromosomal ends.

Recombination in mammals

Evidence from studies in *C. elegans*, *D. melanogaster* and *S. pombe* has indicated that the mechanisms underlying homologous recombination have been well conserved amongst sexually reproducing organisms. Recently, the mouse and human homologs of *Spo11* were cloned based on sequence similarities to other *Spo11* family members.[6–8] Expression of the *Spo11* mRNA is limited almost entirely to gonadal tissues

in both species.[7] Moreover, in situ hybridization studies have revealed that the message is restricted to embryonic oocytes in females consistent with the restricted role for SPO11 in early prophase I.[6,8]

The recent disruption of the *Spo11* locus in mice has confirmed the conserved role of this protein in the initiation of recombination since the mice exhibit both male and female infertility.[9,10] Spermatocytes progress through early prophase I, but homologous chromosomes fail to pair and the cells enter apoptosis soon thereafter.[9,10] In light of the early requirement for SPO11 in the initiation of recombination, the RN markers, DMC1 and RAD51, are not found on meiotic chromosomes at zygonema[9,10] while Cisplatin induction of DSBs in *Spo11*$^{-/-}$ spermatocytes restores DMC1/RAD51 foci and synapsis to some degree.[10]

The Rad50–MRE11–XRS2 complex that is vital to yeast meiosis and recombination also appears to exist in some form in mammalian species. In human cells, XRS2 is replaced by p95 (also known as NBS1), the gene that is mutated in Nijmengen Breakage syndrome.[11] Patients suffering from NBS exhibit a variety of phenotypes including immunodeficiency, increased incidence of hematopoeitic malignancies and chromosomal instability.[12,13] Interestingly, mutations in yeast *XRS2* result in similar phenotypes to that observed in cells from NBS patients,[14] suggesting similar functions of these two gene products. Murine *Rad50* has also been cloned, and targeted mutations in this gene result in early embryonic lethality,[15] whereas mouse MRE11 has been studied at the mitotic level only where it exhibits similar enzymatic activity to that of its yeast counterpart.[16] Mutations in human *Mre11* result in an Ataxia-Telangiectasia-like disorder (ATLD) which has thus far been identified in two distinct families.[17] Interestingly, the genetic mutations that are responsible for ATLD cause spore inviability when reconstituted in the yeast *MRE11* gene.[17]

Processing of recombination intermediates

While the induction of DSBs in yeast and mice is mediated by SPO11, the processing and resolution of recombination structures arising from these break points appears to be differentially regulated. Certain players appear to be conserved between species, including the RecA homologs, DMC1 and RAD51, while additional proteins appear to be required in mice. In mice, the proteins involved in these processes are all localized to discrete recombination nodules that, during synapsis, far outnumber the frequency of recombination sites. By pachynema, however, the number of recombination nodules is pared down to reflect only true sites of homologous recombination. Interestingly, the composition of these recombination nodules changes as their number declines, suggesting a strict temporal order to the resolution of recombination. The overabundance of recombination nodules prior to synapsis is unique to higher eukaryotes, since the number of recombination sites in yeast appears to more closely approximate the number of DSBs generated by SPO11.

RecA homologs

RAD51, the best characterized of the yeast RecA homologs, was identified in a screen for yeast mutants that exhibit ionizing radiation sensitivity, and is a member of the *RAD52* epistasis group. Yeast *rad51* has been shown to be involved in the repair of DSBs during both mitosis and meiosis.[18] Thus, *rad51* mutant yeast strains that are defective in DSB repair accumulate chromosomal intermediates with 3′ single-stranded termini.[18] During meiosis, yeast RAD51 is a component of the early recombination nodule, an observation that has also been demonstrated in other species as diverse as lilies[19] and humans.[20]

Another RecA homolog, *DMC1* (disrupted meiotic cDNA), was identified in a screen for meiosis-specific, prophase-induced genes that caused a meiotic defect when disrupted.[21] Yeast mutants for this gene exhibit extensive meiotic defects, including defective synaptonemal complex formation, hyper-resected, unrepaired DSBs and meiotic arrest in late prophase I.[21] The meiotic arrest in these mutants can be overcome by upstream mutations that prevent DSB formation, such as those occurring in *spo11* mutants.[5] However, *spo11dmc1* double mutant spores are inviable as a result of a failure to undergo reductional segregation at each meiotic division.[5] DMC1 forms discrete foci upon chromosomes prior to synapsis, where it colocalizes with RAD51.[22] However, DMC1 complexes do not form in *rad51* mutants, while RAD51 foci persist indefinitely in *dmc1* mutants.[22]

In mice, the genetic analysis of the role of RAD51 in meiosis has been precluded by the embryonic lethal phenotype of mice lacking a functional *Rad51* gene.[23,24] However, as in yeast, multiple RAD51 foci are localized to meiotic chromosomes early in leptonema in mice, and persist until pairing occurs.[25] Initially, the frequency of these foci far outnumbers the estimated number of recombination sites found in mouse germ cells. As prophase I progresses, however, the number of foci declines, but are localized to corresponding sites on converging homologous chromosomes and disappear completely by mid-pachynema (Fig. 2; Ref. 26).

Dmc1 is localized exclusively in the testis and embryonic ovary in mice,[27] in the former being further restricted to leptotene and zygotene spermatocytes.[27] DMC1 localization exactly mirrors that of RAD51.[28] Disruption of the *Dmc1* gene in mice results in male and female sterility resulting from a zygotene failure of synapsis and chromosomal condensation.[27,29] In the testes of *Dmc1*$^{-/-}$ males, cells progress to the early spermatocyte stage and then are lost via the process of apoptosis. Chromosomal spreads from *Dmc1*$^{-/-}$ spermatocytes reveal that, while some homologous and non-homologous synapses are formed between discrete regions of chromosomes, most of the chromosomes fail to pair.[27]

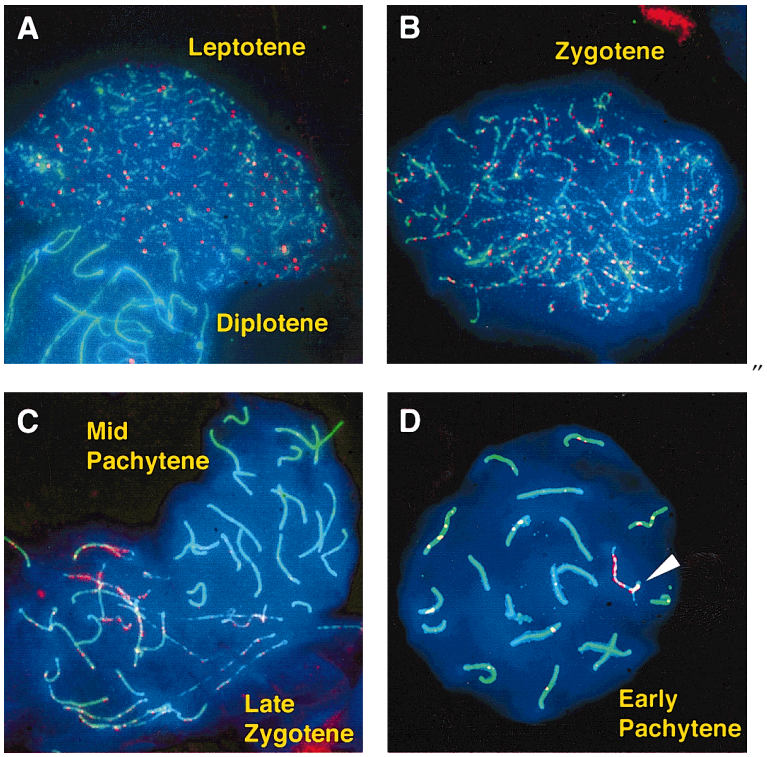

Figure 2. Localization of Rad51 on meiotic chromosomes from mouse spermatocytes. Immunofluorescent co-localization of Rad51 (red) with the SC protein Cor1 (green). Cells are counterstained with DAPI (blue) to highlight the nucleus. The XY pair is indicated by the white arrowhead in panel D.

In *Dmc1*$^{-/-}$ females, oocytes are lost as early as e17, when wild-type oocytes have already progressed through to pachynema.[29] By the neonatal stage of development, most oocytes are lost, and those that remain are already undergoing apoptosis.[27] Interestingly, while the ovary of adult *Dmc1*$^{-/-}$ females contains no oocytes and is significantly smaller than that of age-matched wild-type females, ovarian structures do nevertheless persist into adulthood, unlike that of *Msh4*$^{-/-}$ and *Msh5*$^{-/-}$ females[29] (see below).

Blooms syndrome helicase

Bloom's syndrome is a rare autosomal recessive disorder characterized by immunodeficiency, genomic instability and predisposition to a variety of cancers. Somatic cells from BS patients exhibit an elevated frequency of chromosomal breaks and rearrangements, coupled with an increased rate of sister chromatid exchange (reviewed by Ref. 30). The product of the gene mutated in Bloom's syndrome, *BLM*, belongs to a subfamily of helicases sharing homology with the RecQ helicase of *Escherichia coli* that also includes *Saccharomyces cerevisiae* Sgs1p, *Schizosaccharomyces pombe* rqh1p, and human WRN, the protein mutated in Werner's syndrome.[31–33] SGS1, BLM and WRN possess 3′–5′ DNA helicase activity,[34–36] suggesting similar modes of action. In yeast, Sgs1p is essential for ensuring chromosomal stability,[37] while *Sgs1* mutations result in elevated levels of homologous recombination during meiosis that can be suppressed by ectopic expression of either *BLM* or WRN.[38] Moreover, SGS1 and BLM interact physically and genetically with topoisomerase III, an enzyme that alleviates the torsional stress resulting from helicase activity.[39] Interestingly, topoisomerases are also thought to be essential for the resolution of recombination intermediates during meiosis.[40,41]

Given the role of SGS1 in yeast meiosis, several groups have turned their attention towards the function of BLM protein in mammalian meiosis. However, mice bearing homozygous mutations in mouse *Blm* are embryonic lethals, thus precluding their usefulness in studying the role of this gene product in meiosis.[42]

Localization of BLM on meiotic chromosomes in mice has been the subject of contention between scientific groups. Results have differed slightly with respect to the temporal localization of BLM in mouse spermatocytes. Walpita et al. demonstrated that BLM is present on meiotic chromosomes from late zygonema, but is lost gradually from early pachynema and is no longer present at mid pachynema.[43] By contrast, Moens et al. showed that BLM localization is evident as early as leptonema and steadily increases through to the mid pachytene stage.[44] BLM initially localizes with RAD51/DMC1-positive foci at chromosomal cores, and persists as the

RecA homologs disappear during synapsis.[44] By pachynema, the few RAD51/DMC1 foci that are present are all associated with BLM, but the total number of BLM foci far outnumbers that of RAD51/DMC1. The number of BLM/DMC1/RAD51-positive nodules throughout prophase I far outnumbers the presumed number of crossover sites, suggesting that only a subset of these foci result in true recombination events. This idea, coupled with the fact that *Sgs1* mutations in yeast result in hyper-recombination, suggests that BLM acts to stabilize the chromosomal structures and to limit the frequency of meiotic recombination. Moreover, it is possible that the dissociation of RecA homologs from the SCs is mediated, at least in part, by the persistent localization of BLM.

ATM and ATR

The ATM (ataxia telangiectasia-mutated) and ATR (AT- and Rad3-related) proteins are members of the phosphatidylinositol 3-kinase (PIK3)-like kinase group. These proteins have been implicated in the control of DNA damage-induced mitotic cell cycle checkpoints. They are homologous to products of the *MEC1* and *TEL1* genes of *Saccharomyces cerevisiae*, the *RAD3* gene of *Schizosaccharomyces pombe*, and also to the *mei-41* gene of Drosophila melanogaster.[45–48] MEC1, RAD3 and MEI-41 are all important regulators of checkpoint control during mitotic cell cycles and, more particularly, are responsible for S phase and G_2–M cell cycle arrest following ionization irradiation-induced DNA damage.[49–52] MEC1 and TEL1 have been categorized as sensors of DNA damage.[53] More importantly, all of these proteins have also been implicated in meiosis.[46,48]

Mutations of the human *ATM* gene are responsible for the autosomal recessive hereditary disorder, Ataxia Telangiectasia, which is characterized by increased cancer predisposition and immune deficiencies. Somatic cells from AT patients exhibit increased sensitivity to ionizing radiation, chromosomal instability, elevated numbers of unrepaired DSBs during mitosis and inefficient G_1/S phase checkpoint control. More relevant to this review, however, is the fact that these patients are sterile, suggesting a role for this gene product in meiosis.

Mutations in the gene encoding mouse ATM results in a disorder similar to that seen in human AT patients, and the mice are sterile,[54] while ATR-deficient mice die early on in embryogenesis (at e7.5). In $Atm^{-/-}$ male mice, spermatogenesis is arrested as early as the leptotene stage, resulting in a progressive decline in spermatocyte numbers from postnatal day 8 onwards.[55] Few, if any, spermatocytes from these males reach the pachytene stage, and the chromosomes exhibit high levels of abnormal and non-homologous pairing.[55] Furthermore, many SC fragments are observed in chromosomal spreads from ATM-deficient spermatocytes. In $Atm^{-/-}$ females, oocytes begin to undergo apoptosis from embryonic day 16.5, a stage at which the majority of oocytes from wild-type mice are in pachynema, suggesting a temporally similar phenotype.

ATM-deficient spermatocytes display hyperaccumulation of ATR, but this ATR does not associate with RAD51/DMC1-positive nodules.[55,56] Interestingly, in spermatocytes from wild-type males, ATR aggregates are also found on unpaired regions of the X and Y, as well as on autosomes whose pairing is slightly delayed compared to the rest of the autosomal contingent.[56] Such late-pairing chromosomes are rarely observed in spermatocytes from wild-type males and would be predicted to result in aneuploidy should meiosis proceed before their pairing is complete. These results suggest that ATR signals a delay and/or checkpoint in the absence of complete synapsis that prevents the progression through to pachynema.

Keegen et al. reported that ATR and ATM exhibit complimentary localization patterns along chromosomes, with ATR being localized exclusively to unpaired chromosomal axes and Atm being restricted to synapsed chromosomes.[57] However, Moens et al. found that ATR foci, but not ATM, can be associated with chromosomal cores and SCs during early and mid-zygonema.[56] In view of the limited localization of ATR and the failure to colocalize either ATR or ATM with DMC1/RAD51 nodules, they concluded that there is no relationship between the sites of recombination and the DNA damage detection/signaling machinery. This conclusion was further supported by the observation that RAD1, another ATR/ATM family member, is also not associated with DMC1/RAD51-positive meiotic nodules.[58] However, the absence of ATM in $Atm^{-/-}$ males results in the mislocalization of DMC1/RAD51 within the spermatocyte nuclei such that there is an increased localization of these RecA homologs to chromatin and a decrease in their localization to the developing SC.[55] This suggests that ATM is required for the appropriate localization of RecA homologs to the DSB.

Mismatch repair proteins in meiosis

The MutHLS DNA mismatch repair (MMR) system is responsible for the repair of DNA mismatches that can result from a number of different mechanisms including DNA replication, genetic recombination and chemical modification of DNA. The system was first described in *Escherichia coli* where the MutS protein first recognizes and binds to mismatched nucleotides and initiates a cascade of downstream events (reviewed by Ref. 59). In a subsequent step a second protein, MutL, interacts with MutS and activates a third protein, MutH, which is an endonuclease. MutH, taking advantage of the transiently unmethylated state of the newly synthesized DNA strand, nicks the unmethylated strand of hemimethylated DNA in the vicinity of the mismatch and thereby directs repair to the daughter strand.

While the essential components of this MMR system have been conserved in eukaryotes, the repair system is more complex than in *E. coli* and involves several MutS and MutL homologs. In *Saccharomyces cerevisiae* there are six homologs of the DNA-binding protein MutS designated MutS homolog (MSH) 1–6. There are also four known homologs of the MutL gene in yeast, designated MLH1, MLH2, PMS1 (for post meiotic segregation 1, known as PMS2 in mammals) and MLH3 (reviewed by Ref. 59).

Somatic cell MMR in eukaryotes requires one of two MSH heteroduplexes: MSH2–MSH3 or MSH2–MSH6 (Fig. 3). The two MSH complexes interact with the complexes of MLH1–PMS1 (PMS2 in humans) or MLH1–MLH3 for the repair of the different mismatches, although heterocomplexes of MLH1–MLH2 might also play a minor role in repair.[60] These various complexes differ in their affinities for different types of mismatches and are therefore able to repair each different mismatch with similar affinity.

MutS homologs in meiosis

MSH4 and *MSH5* were identified in yeast screens for meiosis-specific mutants specifically defective in homologous recombination.[61] Mutant alelles of *MSH4* exhibit an approximately 62% reduction in spore viability in the absence of any defects in gene conversion.[61] The reduction in spore viability is associated with a 50% reduction in crossing over and a resulting increase in homologous chromosome nondisjunction. Similarly, yeast mutants defective in the *MSH5* gene also display diminished spore viability, increased meiosis I nondisjunction and decreased levels of reciprocal exchange between, but not within, homologous chromosomes.[62] As with the *MSH4* mutants, the meiotic aberrations in *MSH5*-null yeast strains are not accompanied by alterations in gene conversion, nor are any MMR deficiencies observed.[62] Furthermore, neither MSH4 nor MSH5 are capable of MMR activity due to absence of the N-terminal domain that is essential for mismatch recognition.[63]

In yeast and humans, two-hybrid analyses revealed that MSH4 and MSH5 interact in a manner analogous to that seen for MSH2–MSH3 and MSH2–MSH6.[64,65] Levels of *Msh5* mRNA also increases steadily throughout meiosis I in the whole testis RNA from mice.[66] Similarly, in humans, high expression of *MSH5* mRNA is apparent in the testis, along with lower levels of expression in bone marrow, lymph node, thymus, spinal cord, and adult ovary.[64,67] *MSH4* transcripts are also present in other human tissues, including placenta and liver, although the transcript size differs from that found in testis.[68] In mice, MSH4 forms discrete foci along meiotic chromosomes during the zygotene and pachytene stages of meiosis, with the number of foci being maximal at zygonema and declining to approximately 47±4.5 foci per nucleus by mid-pachynema (Fig. 4; Ref. 69).

Disruption of the *Msh4* gene in mice results in male and female sterility due to meiotic failure.[69] In *Msh4*$^{-/-}$ males, spermatocytes fail to complete meiosis and instead undergo apoptotic cell death. Closer analysis of chromosomal spreads from these spermatocytes reveals that meiosis is initiated, as indicated by the chromosomal localization of RAD51 and SC components.[69] Thus, axial elements form along each homologous chromosome, indicating appropriate progression through zygonema and the initiation of recombination at the very least in these cells. At leptonema, however, when homologous chromosomes initiate pairing, the absence of MSH4 protein induces meiotic failure (Fig. 4). Instead, a high frequency of aberrant chromosomal morphologies become apparent. These include non-homologous pairing, partial pairing, and associations between more than two chromosomes. Of the total spermatocyte pool, 70% of spermatocytes from *Msh4*$^{-/-}$ males show some pairing (whether homologous or non-homologous), while 31% of cells show no chromosomal pairing.[69]

In *Msh4*$^{-/-}$ females, meiotic arrest occurs at a similar time in meiosis to that in *Msh4*$^{-/-}$ males. Due to the temporal

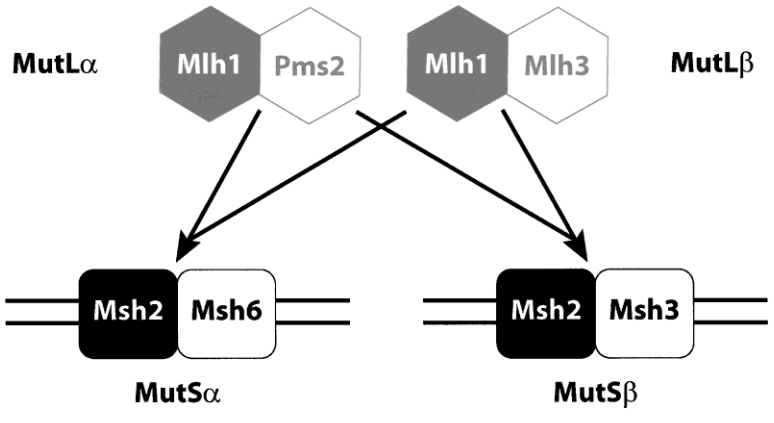

Figure 3. Mismatch repair complexes in somatic cells. At least two different MutS heterodimers exist (MutSα and MutSβ), along with at least two different MutL heterodimers (MutLα and MutLβ). MSH2 and MLH1 are obligate members of these respective heterodimers. Different combinations of MutS and MutL homologs exhibit varying affinities for different mismatch substrates (as reviewed in Ref. [59]).

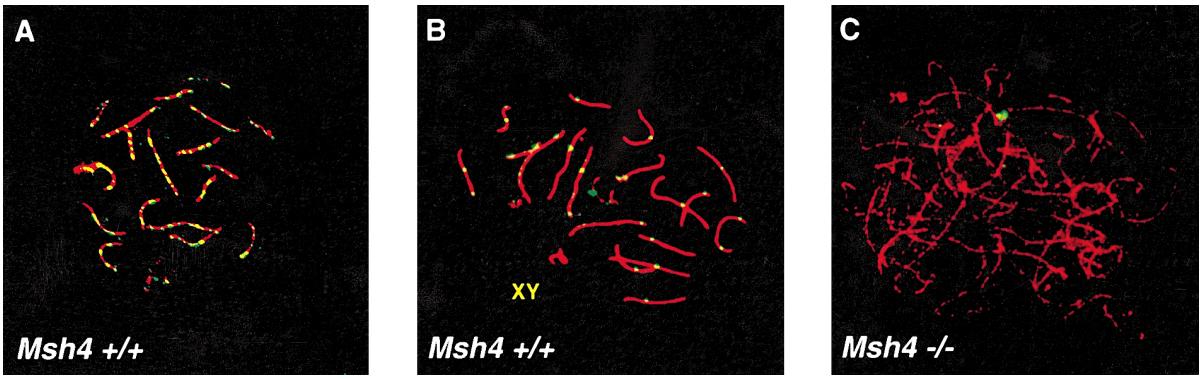

Figure 4. Localization of Mlh1 and MSH4 on meiotic chromosomes during meiotic prophase in male mice. **A:** Localization of MSH4 (green) on SCs (red) from early pachytene spermatocytes, (**B**) Localization of Mlh1 (green) on SCs (red) from mid pachytene spermatocytes; and (**C**) Localization of Mlh1 (green) on SCs (red) from mid pachytene spermatocytes from *Msh4*$^{-/-}$ males mice. Note the disorganized chromosomal configurations and absence of MLH1 foci. This is most likely due to the failure of these cells to progress beyond the zygotene stage of prophase I (MLH1 appears on the chromosomes from mid-pachynema).

differences in meiotic program between males and females, however, the phenotypic consequences are quite different, as also observed in DMC1-deficient mice.[29] Indeed, this is the case for *Msh4*$^{-/-}$ females; by day 4 pp almost all the oocytes have undergone apoptosis, while prior to e18, the oocyte numbers are comparable to that seen in the ovaries of wild-type females.[69] Thus the meiotic arrest that occurs in the absence of MSH4 is similar between males and females.

In *Msh5*$^{-/-}$ mice, meiosis is also arrested prior to synapsis at zygonema, and the degree of the arrest appears greater in these mice than in *Msh4*$^{-/-}$ mice.[66] For example, over 90% spermatocytes from *Msh5*$^{-/-}$ males have absolutely no pairing of homologous chromosomes, while this figure is only 30% for *Msh4*$^{-/-}$ males.[66,69] In both mutants, however, complete synapsis is never observed.

Slightly different phenotypes have been observed in another mouse line harboring a deletion in the *Msh5* gene.[70] In the case of this mouse mutant, meiosis was also arrested at the zygotene stage of prophase I, but the degree of chromosomal interactions were higher than that demonstrated by Edelmann et al.[66] 80% of spermatocytes from *Msh5*$^{-/-}$ mice were at a stage that resembled zygonema, with up to 25% SC formation across the genome.[70] De Vries et al. also report that the axial elements from Msh5-deficient spermatocytes were often broken and/or discontinuous, an observation supported by the often weak immunofluorescent staining with anti-SC antibodies (P. Cohen, unpublished observations). The reasons for the differences in meiotic phenotypes between the two mice is unclear, but might be a result of allelic variations caused by different gene targeting strategies.

Msh2 and Msh3 are also found in the mouse testis.[71] *Msh2* mRNA expression is limited to spermatogonia and to leptotene/zygotene and early pachytene spermatocytes, while expression of *Msh3* mRNA is coincident with the onset of meiosis I, and persists throughout meiosis and into the post-meiotic differentiation stage of spermatogenesis.[71] The functions of MSH2 and MSH3 in meiotic cells are unclear, however, since no meiotic phenotype is apparent in *Msh2*$^{-/-}$ or *Msh3*$^{-/-}$ mice.

MutL homologs in recombination

PMS1 was identified during screens for mutations that alter recombination rates in yeast and was found to be a homolog of the bacterial *MutL* gene. Mutations in yeast *PMS1* result in reduced recombination and gene conversion during meiosis, and reduced spore viability. In addition, these mutant strains exhibited a mutator phenotype, indicative of a role for this gene product in mitotic MMR. Similarly, *MLH1* also appears to function both in meiotic events and in post-replicative repair during mitosis. The consequences of a mutation in *MLH1* are much more severe than that of a *PMS1* mutation, however, since the numbers of resulting spores were considerably lower in yeast strains harboring *MLH1* mutations, and was accompanied by a significant reduction in crossing over.

In mice, MLH1 foci are found on SCs only at pachynema, at a frequency that resembles the estimated number of chiasmata (Fig. 4; Refs. 72–74). Mutations in the mouse gene encoding *Mlh1* results in male and female infertility. Closer examination of the seminferous epithelium revealed that germ cells beyond meiosis I were completely absent in *Mlh1*$^{-/-}$ males.[75] At the chromosomal level, homologous chromosomes that have already replicated into sister chromatids appear to be fully synapsed at pachynema, as indicated by silver staining of the SC in chromosome spreads,[75] as well as by immunofluorescent localization of SC proteins.[72,73]

Beyond pachynema, however, homologous chromosomes desynapse and are no longer maintained at their chiasmata attachment sites in the absence of MLH1.[73]

More recently, Woods et al. demonstrated that the chiasma frequency was diminished more than two-fold in both *Mlh1*$^{-/-}$ males and females compared to their wild-type littermates.[76] Moreover, 15% of meiotic germ cells from *Mlh1*$^{-/-}$ animals failed to show any chiasmata exchanges at all.[76] Associated with this reduction in chiasma frequency in males was an almost complete failure of observable recombination in the presence of a marker Y chromosome that enabled detection of recombination and partially resolved recombination intermediates.[76] Using Eppig's technique for the culture of oocytes in vitro, they showed that 65% oocytes extruded a first polar body after 15 hours in culture, while only 7% oocytes from null mutants were competent to extrude a polar body.[76] Moreover, only 18% cells from the mutant ovaries actually complete metaphase I. This failure to complete metaphase I was attributed to the failure of meiotic chromosomes to congress at the midplate of the cell, instead forming ''flower petal''-like radial arrays around the forming meiotic spindle. Chromosomes that were centrally located were bivalents, indicating that the aberrant chromosome behavior is limited to univalent chromosomes.

The human homolog of PMS1 has been termed, rather confusingly, PMS2. *Pms2* transcripts in whole mouse testis extracts decline steadily throughout the first wave of meiosis between day 12 pp and day 23 pp and are limited to spermatogonia, leptotene/zygotene and early pachytene spermatocytes, with no expression being apparent throughout later stages of pachynema and beyond.[71] In contrast to *Mlh1*$^{-/-}$ mutant females, however, *Pms2*$^{-/-}$ female mice are fully fertile.[77] Male *Pms2*$^{-/-}$ mice, on the contrary, are infertile, the epididymides of these mice containing less than 25% of the spermatozoa found in wild-type littermates.[77] Those spermatozoa that are present in *Pms2*$^{-/-}$ males are irregular in shape, being grossly malformed with truncated tails that render them, presumably, incapable of motion. In line with the reduction in spermatozoa numbers in the epididymides of *Pms2*$^{-/-}$ males, their seminferous tubules contain reduced numbers of round and elongating spermatids, while the numbers of spermatogonia and primary spermatocytes appears similar to that of wild-type males. Associated with these spermatogenic abnormalities, Baker et al reported an increased rate of germline mutations resulting from instability of simple dinucleotide repeat sequences.[77] At the chromosomal level, silver-staining analysis of meiotic chromosome spreads from *Pms2*$^{-/-}$ spermatocytes revealed that 80% of nuclei displayed severe prophase I abnormalities, including asynapsed portions of chromosomes, non-homologous synapsis, discontinuous synaptonemal complex formation, interlocking bivalents and interactions between XY bivalents and autosomes.[77]

The studies described above demonstrate that MLH1 is an essential component of the meiotic MMR complex and that, in males at least, PMS2 is also an important player. In line with the known heterodimeric MutL complexes in somatic cells, therefore, it seems reasonable that these two MutL homologs might form heterodimers that interact with the MSH4/5 recognition complex. In somatic cells, however, MLH1 forms heterodimers with at least one other MutL homolog, Mlh3, and perhaps also with Mlh2. Mlh2 (also known as Pms1 in mice) appears to have no role in meiosis, either in yeast or in mice, but yeast mutants for Mlh3 show a slight increase in postmeiotic segregation[78] and a 70% reduction in crossing over.[60,79] The recent cloning of human *MLH3* and its similarity to the yeast gene predicts a similar function in mammals,[80] although no mouse models have yet been reported. It seems likely, however, that Mlh3 would play a role in meiosis since the fertility of PMS2-deficient females suggests that MLH1 has no known binding partner in oocytes. Thus one hypothesis might be that MLH1 heterodimerizes with PMS2 in spermatocytes and with Mlh3 in oocytes (Fig. 5). Alternatively, MLH1 might recruit both PMS2 and Mlh3 during meiosis in both sexes, and there might be relative differences in function of the two MutL homolog complexes between the sexes. The fact that PMS2-deficient mice show abnormal chromosome synapsis during prophase I of meiosis, while MLH1-deficient mice do not, would certainly lend credence to such a suggestion.

Perspectives

The number of different regulatory systems involved in recombination is quite overwhelming, ranging from RecA homologs, RecQ helicases, MMR proteins, and classic tumor suppressors (BRCA1 and BRCA2). The functions of many of these proteins appear to be remarkably conserved between higher and lower eukaryotes, and yet distinct differences exist in the phenotypic consequences of loss of individual gene products. Thus, loss of MSH4/MSH5 function in mice results in meiotic arrest and germ cell apoptosis, while loss of function of these proteins in yeast only increases meiosis I nondisjunction and reduces spore viability. An explanation for these differences might lie in the checkpoint regulation that exists during meiosis in yeast and higher eukaryotes. In yeast, unresolved DSBs trigger a recombination checkpoint at pachynema[79] whereas in mammals, studies of meiotic mutant mice have demonstrated that an earlier checkpoint exists to ensure complete synapsis of homologous chromosomes prior to entry into pachynema. Such a checkpoint was first demonstrated by Odorisio et al. and was shown to be p53-independent.[81] Indeed, meiotic arrest in *Msh5*$^{-/-}$ males results in a p53-independent checkpoint that results in spermatocyte apoptosis prior to pachynema (P. Cohen and W. Edelmann, unpublished observations). It appears that a later checkpoint also exists in mice at metaphase I, however,

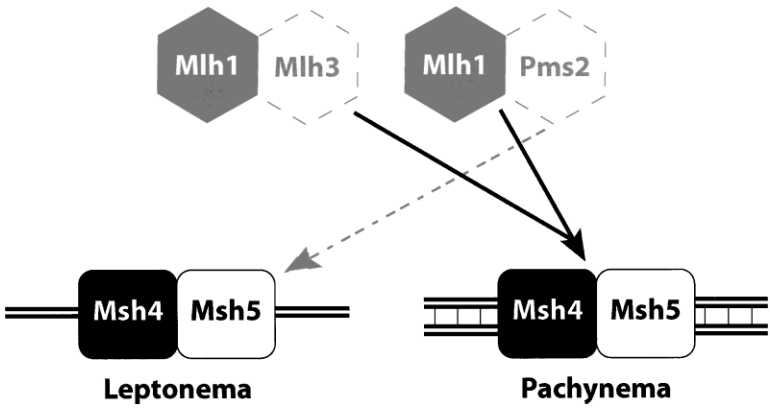

Figure 5. Mismatch repair complexes in mouse germ cells. Unlike somatic cells (Fig. 3), MSH4 and MSH5 heterodimers appear to be the only MutS complexes in meiotic cells. These proteins accumulate on the chromosomes at leptonema, but their associated MutL homologs do not appear to localize to SCs until pachynema. At the current time, MLH1 is the only confirmed MutL homolog to localize to SCs during prophase I, although biochemical evidence suggests that PMS2 is present at the same time. In yeast, Mlh3 also appears to play a role in meiosis, and interacts with MLH1 in two-hybrid screens.

since *Mlh1*$^{-/-}$ mice exhibit a later meiotic arrest. Spermatocytes from these males enter pachynema normally, but homologous chromosomes fall apart at diplonema, and are triggered to enter apoptosis by metaphase. Whether this later checkpoint is p53-dependent remains to be seen. Thus, while one meiosis I checkpoint exists in yeast, there appears to be at least two such checkpoints in mice. The reasons for this difference is unclear at present, but might be due to the increased complexity of meiotic regulation in higher eukaryotes resulting from the increased genome size. In addition, while the term checkpoint has been applied to functional arrest in a variety of specific developmental pathways, there are often quite different endpoints to such disruptions. The term checkpoint usually applies to those developmental arrests that result in apoptosis, but the mechanisms and regulators (such as p53) are often quite different. Furthermore, it is not clear whether apoptosis is always the direct consequence of such developmental arrests, or whether cellular death occurs further downstream. As further proof of this ambiguity, PMS2-deficient and ATM-deficient males exhibit meiotic defects leading to apoptosis, but this meiotic arrest occurs throughout the synapsis event and into metaphase. Thus, both *Pms2*$^{-/-}$ and *Atm*$^{-/-}$ males contain spermatocytes exhibiting a range of normal/abnormal chromosome configurations, both pre- and post-synapsis.

Another interesting feature of meiosis in both yeast and mice is the high number of recombination nodules relative to the actual number of crossovers observed in each species. One possible explanation for this lies in the theory that recombination nodules are involved in the initial homology searching and/or interactions between homologous chromosomes. Indeed, the recombination nodules represent the initial point of contact between homologs, either as a result of the underlying DSB-induced 5′ tails, and/or perhaps as a result of homology-seeking proteins such as the MSH4/MSH5 heterodimer.

The initiation of recombination and the processing of recombination intermediates occurs across a temporally broad period, stretching from leptonema to diplonema, when the final recombination events are evident as individual chiasmata. Mutation of different genes involved in these processes, together with the temporal localization of different proteins has begun to uncover stage-specific functions for these proteins (Fig. 6; Table 2). Thus MSH4, MSH5 and DMC1 appear to function prior to synapsis, in leptonema and zygonema, while PMS2 and MLH1 are involved in post-synaptic functions at pachynema and beyond. In the case of MSH4 and MSH5, however, it is unclear whether their true activity is initiated before of after synapsis, since their function

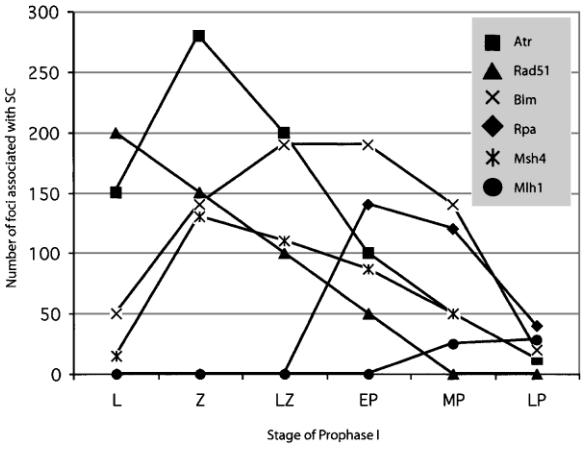

Figure 6. Overview of regulatory proteins localized to meiotic chromosomes during prophase I in mouse germ cells. Data taken from various sources, as described in the text. L, leptonema; Z, zygonema; LZ, late zygonema; EP, early pachynema; MP, mid pachynema; LP, late pachynema.

Table 2. Summary of phenotypes of mouse mutants exhibiting meosis I defects

			Phenotype of Homozygous Mutant Animals					
Gene Family	Gene	Protein Localization	Female	Males	Meiotic Disruption	Sperm Production (%)	Apoptosis	Meiosis-specific
RecA	*Rad51*	L-EP	Lethal	Lethal	?	?	?	No
Homologs	*Dmc1*	L-EP	Sterile	Sterile	Zygonema	0	yes	Yes
PI3K-like Kinases	*Atm*	L-Z	Sterile	Sterile	Leptonema to Pachynema	0	yes	No
	Atr	L-MP	Lethal	Lethal	?	?	?	no
Helicases	*Blm*	L-MP	Lethal	Lethal	?	?	?	no
Mismatch	*Msh4*	L-LP	Sterile	Sterile	Zygonema	0	yes	yes
Repair	*Msh5*	?	Sterile	Sterile	Zygonema	0	yes	yes
	Mlh1	EP-LP	Sterile	Sterile	Post-pachynema	0	yes	no
	Pms2	?	Fertile	Sterile	Leptonema to Pachynema	<20	yes	no

L, Leptonema; Z, Zygonema; EP, Early Pachynema; MP, Mid-Pachynema; LP, Late Pachynema; Lethal=embryonic lethal phenotype

is dependent on the formation of canonical MMR heterotetramers, involving MLH1 and other MutL homologs. Analysis of all known MutL homologs to date suggest that these proteins are only present from the pachytene stage onwards, and thus would imply that the biochemical activity of the meiotic MMR complex is not induced until this later stage. Why, therefore, are MSH4 and MSH5 required earlier than their MutL homolog partners? It could be that MSH4 and MSH5 act as homology recognition molecules, as suggested above, or that the loading of MSH4 and MSH5 act as apoptosis checkpoint monitors to ensure appropriate regulatory elements are present before the completion of synapsis. Such a function is as yet untested and would represent a novel meiosis-specific function for MMR proteins in mice.

Analysis of MMR-deficient mice exhibiting meiotic defects revealed interesting sex-specific roles for specific proteins. For example, *Pms2*$^{-/-}$ males are infertile as a result of prophase I defects while *Pms2*$^{-/-}$ females are fertile. Such sex-specific differences in meiotic regulation contradicts the long-standing assumption that meiotic progression is similarly regulated in males and females, and underscores the importance of studying meiosis in mammals. Similarly, studies in other meiotic mutants demonstrate that the consequences for meiotic disruption are also quite different in males and females. In *Msh4*$^{-/-}$ and *Msh5*$^{-/-}$ mice, for example, meiotic failure in the males results in spermatogenic failure, but male libido and testicular architecture are maintained. In *Msh4*$^{-/-}$ and *Msh5*$^{-/-}$ females, in contrast, the disruption of meiosis during embryogenesis results in the loss of the total germ cell population and loss of the entire ovarian structure, such that adult females contain only a pair of cyst-filled dysgenic ovaries. These different consequences for gonadal structure and function also reveal interesting questions concerning the consequences of meiotic failures in the human population and might, for example, partially explain the higher frequency of meiotic defects observed in women as opposed to men.

From the growing list of proteins now known to be involved in meiotic recombination, several interesting correlates arise. First, as described earlier, there is functional conservation of such proteins between higher and lower organisms. A second remarkable feature is the similarity between protein complexes during meiotic and mitotic recombination/repair processes. Thus, RAD51, BLM, ATM, and ATR are all required for recombination events in both somatic and germ cell lineages. By contrast, while the MMR system functions in both cellular contexts, it appears that different detection mechanisms exist, since different MutS homolog complexes are utilized in each system. The major MutL homologs are utilized both in somatic and meiotic cells, however, indicating that some functional/enzymatic similarities do exist between the two cell types. Alternatively, this conservation of MutL function in both cell types might also suggest that the activity of the MutL heterodimer is dictated by the species of MutS heterodimer to which it binds, such that binding to one or other MutS heterodimer subtype might result in a different biochemical activity of the MutL heterodimer (a repair function versus a heterodimer stabilization function, for example?).

Another important difference between the repair complexes existing in somatic versus germ cells is the requirement in the latter for an additional RecA homolog, namely DMC1. That this protein is essential for meiotic recombination is demonstrated by the infertility of DMC1-null mice, and yet the protein is always localized with RAD51 on meiotic chromosomes. Why should two such proteins exist in germ cells and

both be apparently essential for meiotic progression while only one is required for analogous processes in somatic cells? In somatic cells, RAD51 forms filaments along each pair of sister chromatids, and it is assumed that a similar structure is formed along sister chromatids in meiotic cells. In the case of meiotic cells, however, the relative distance between recombinant strands is considerably larger than in somatic cells (homologous chromosomes compared to sister chromatids), giving rise to the possibility that a more sophisticated RecA structure is necessary to bridge the chromosomal structures. Alternatively, or in addition, the emphasis on DSB repair by intrahomolog recombination, rather than sister chromatid conversion, might be conferred by the presence of DMC1.

What is clear from the above discussion is that, despite the overall conservation of players between mitotic repair/recombination and meiosis, and between meiosis in yeast and vertebrates, there are distinct functional differences for individual proteins in each of these scenarios. In addition, the generation of distinct sexes in higher eukaryotes has resulted in the need for more specific regulatory systems for males and females. Such regulation is necessarily linked to physiological and endocrinological events, such that meiosis becomes an event that is no longer solely autonomously regulated. Instead, the complexity of the vertebrate reproductive system is now imposed on a system that was essentially an extension of the mitotic cell cycle and which has evolved to create a new process with similar players but entirely different temporal and spatial regulation. Thus, meiosis in higher eukaryotes can be regarded as a finely tuned system in which environmental, sociological and physiological cues all play important roles, albeit poorly understood. Importantly, however, this complexity can often result in meiotic interference, as evidenced by the increased nondisjunction seen in older women and by the decreased meiotic competence in vertebrates exposed to environmental toxins. In light of the added complexity of meiotic regulation in mammals, therefore, the availability of mouse models for studying recombination events will allow for a greater understanding of these problems.

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