Meiosis is a double-division process that is preceded by only one DNA replication event to produce haploid gametes. The defining event in meiosis is prophase I, during which chromosome pairs locate each other, become physically connected, and exchange genetic information. Although many aspects of this process have been elucidated in lower organisms, there has been scant information available until now about the process in mammals. Recent advances in genetic analysis, especially in mice and humans, have revealed many genes that play essential roles in meiosis in mammals. These include cell cycle-regulatory proteins that couple the exit from the premeiotic DNA synthesis to the progression through prophase I, the chromosome structural proteins involved in synopsis, and the repair and recombination proteins that process the recombination events. Failure to adequately repair the DNA damage caused by recombination triggers meiotic checkpoints that result in ablation of the germ cells by apoptosis. These analyses have revealed surprising sexual dimorphism in the requirements of different gene products and a much less stringent checkpoint regulation in females. This may provide an explanation for the 10-fold increase in meiotic errors in females compared with males. This review provides a comprehensive analysis of the use of genetic manipulation, particularly in mice, but also of the analysis of mutations in humans, to elucidate the mechanisms that are required for traverse through prophase I. (Endocrine Reviews 27: 398–426, 2006)

I. Introduction

Genetic Analysis of Chromosome Pairing, Recombination, and Cell Cycle Control during First Meiotic Prophase in Mammals

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do not exhibit reciprocal recombination (reviewed in Refs. 2–4).

The regulation of SC formation and recombination in mammals has been the focus of much attention recently, and the advent of genetic and biological tools for mammalian systems has resulted in increased interest in these processes. Comparative studies have revealed increasing levels of complexity with regard to the meiotic regulation in mammals, as a result of the larger genome size and/or because of the extrinsic regulation imposed by the physiological and endocrinological environment of the gonads. This is well illustrated by the observation that meiotic error rates rise with increasing genome and organismal complexity. Thus, meiotic errors that give rise to unequal separation of homologous chromosomes at the first meiotic division (nondisjunction) occur at a rate of 1:10,000 in budding yeast, at 1–2% in mice and men, and as great as 20% in women (reviewed in Ref. 5). Understanding how meiosis is regulated in mammals, and extrapolating between mouse and human, will allow for a greater understanding of the mechanisms by which maternal meiotic errors arise in women. In addition, our understanding of these processes is significantly enhanced by examining the meiotic phenotypes of mutant mouse lines harboring targeted gene mutations and by examining the biology of patients with genetic syndromes that result in sterility or reduced fertility. Both of these approaches have yielded a large number of genes that are now known to have roles in meiosis, and the current review is aimed at providing a comprehensive examination of these genes and their function in meiotic recombination.

II. Formation of the SC and Its Role in Meiosis

The formation and structural status of the SC defines the five substages of prophase I. Replicated sister chromatids arrive in prophase I at leptotene (adjective: leptotene) and begin to condense their chromatin structures while at the same time initiating homolog searching and pairing. At this time, a protein filament structure, known as the axial element, begins to accumulate along each sister chromatid pair, such that by the end of the leptotene stage, it forms a continuous structural backbone along each chromosome. During zygonema (adjective: zygotene), homologous chromosomes align, and a protein structure known as the central element begins to form between the homologs and tethers them together in a process known as synopsis. The two axial elements, now termed lateral elements, together with the central element, constitute the mature SC structure (Fig. 1). Extension of the central element results in a zippering effect such that, as cells enter pachynema (adjective: pachytene), the longest stage of prophase I, the homologs are synapsed along their entire length. Chromosomes remain in this state for several days in mice, and up to weeks in human oocytes, and they continue to compact throughout this period. Toward the end of the pachytenic stage, the central element begins to disassemble. The homologous chromosomes repel each other, progressing into the next stage of prophase I, diplonema. They remain connected at chiasmata, the physical manifestation of reciprocal recombination (crossover) events, through to the final stage of prophase I, diakinesis, and it is these hybrid DNA structures that are responsible for ensuring that homologs remain paired through to metaphase. By diakinesis, much of the SC structure is lost, and only remnants of the lateral elements remain.

The formation of the SC occurs within the temporally distinct framework of prophase I in all organisms, and yet the proteins that constitute the SC vary widely between species. In mammals the SC is comprised primarily of three distinct proteins, termed SC protein (SYCP)1, SYCP2, and SYCP3, together with minor components and with members of the

![Fig. 1. SC dynamics through prophase I. A, Cartoon of SC structure, showing loops of chromatin (paired sister chromatids) extending out from the SC “Core”, made up of the two axial/lateral elements and the central element. B, An electron micrograph showing a stretch of SC in situ in a mouse spermatocyte. C, Immunofluorescence of SC components at zygonema, pachynema, and diplonema, showing the axial element protein, SYCP3, in red and the central element protein, SYCP1, in green. The centromere is labeled with CREST autoimmune serum and is blue. When synopsis has occurred, the overlap of the red and green colors produces a yellow signal. CEN, Centromere.](image)
cohesin complex. SYCP3 (also known as SCP3 or COR1 in hamsters) is the main constituent of the axial elements (Fig. 1C), the two structural elements that align along each pair of sister chromatids and which begin to assemble in early leptonema of prophase I (6–11). SYCP3 is present in meiotic nuclei in early leptonema and persists until diplonema when the SC begins to break down. The importance of the axial elements is underscored by the fact that Sycp3−/− male mice are sterile as a result of a mass apoptosis of the spermatocyte population before pachynema (12). Not surprisingly, axial elements fail to form during leptonema in Sycp3−/− spermatocytes, resulting in a failure to assemble meiotic nodules (MN) components and an absence of SYCP1 recruitment at zygonema. However, reverse filamentation, reminiscent of those that make up the central element of the SC, accumulate throughout the chromatin of spermatocytes from Sycp3−/− mice, suggesting that SYCP1 accumulation is not wholly dependent on axial element formation (13). Interestingly, Sycp3−/− females are fertile and generate viable offspring, albeit with drastically reduced litter sizes coupled with increased oocyte aneuploidy (14). Thus, although SYCP3 is a central feature of the axial element in both male and female mice, and is critical for appropriate synapsis in both sexes, SYCP3 is not replaced by another protein in Sycp3 nullizygous females; therefore, female meiosis appears not to be entirely dependent on proper SC formation.

During zygonema, the SYCP3 filament becomes continuous along each chromosome, and a third structure, the central element, begins to accumulate. The mammalian central element consists of SYCP1 (Fig. 1C) and is responsible for synapsis and bivalent formation through zygonema and into pachynema. Other components of the central element have recently been identified in mice, including FK506 binding protein-6 (FKBP6), which localizes to synapsed regions of chromosomes and physically associates with SYCP1 (15). Targeted inactivation of Fkbp6 in mice results in aspermic males and the absence of normal pachytene spermatocytes. Instead, loss of FKBP6 results in abnormal pairing and misalignments between homologous chromosomes, nonhomologous partner switches, and autosynapsis of X-chromosome cores in meiotic spermatocytes. Interestingly, however, fertility and meiosis are normal in Fkbp6 mutant females, indicating that FKB6 is not essential for meiotic progression in oocytes (15). Two other proteins, SYCE1 and CESC1, were identified by microarray profiling and appear to be novel components of the mammalian central element (16).

Cohesins are also intertwined with the SC structure, and meiosis-specific cohesin complexes are present in mammalian germ cells. Mitotic cohesin contains four subunits; two SMC (structural maintenance of chromosome) proteins, SMC1α and SMC3, and two non-SMC subunits, RAD21 and SCC3. Collectively, this complex is essential for ensuing sister chromatid cohesion through mitosis until metaphase when cohesion is lost between the chromosomes arms, followed by loss of centromere cohesion (reviewed in Ref. 17). In meiotic cells, SMC1α is replaced by SMC1β, whereas RAD21 and SCC3 are replaced by REC8 and STAG3, respectively. Recent evidence has indicated that an intact cohesin complex is essential for progression through prophase I of meiosis. Smc1β−/− male and female mice are sterile as a result of meiotic failure during pachynema and early metaphase, respectively. Spermatocytes from these mice exhibit impaired axial element formation, shortened axial element lengths, chromosome synopsis errors, and a failure to process recombination events, whereas oocytes progress through prophase I and accumulate a few crossovers that enable them to progress through metaphase I and beyond, but with increased rates of nondisjunction and premature sister chromatid separation (18). Similarly, two recently isolated mutations in Rec8 result in sterility in mice as a result of similar synopsis defects and recombination failure (19, 20). Interestingly, the absence of REC8 in one of these mouse lines results in SC formation between sister chromatids (20), indicating a role for the cohesin complex in directing appropriate SC assembly. Thus the SC, although essential for meiotic progression, is not sufficient to ensure sister chromatid cohesion without specific participation of the meiosis-specific cohesin complex.

An important feature of the developing SC is the meiotic (or recombination) nodule (MN), an electron-dense structure first described by Adelaide Carpenter in 1975 (21). The original term for the MN was the recombination nodule, but this is somewhat misleading because not all MN/recombination nodule structures will become true recombination events. Molecular and cytogenetic analysis has revealed that the components of the SC vary through prophase I, as does the frequency and placement of MNs (reviewed in Ref. 2). In mice, the number of MN structures is highest in leptonema and early zygonema and declines steadily thereafter to reach a stable plateau at midpachynema (reviewed in Ref. 23). The number of MNs at this time correlates well with the number of eventual crossovers observed at metaphase. Indeed, studies with the phosphatase inhibitor, okadaic acid, which accelerates prophase I progression through pachynema and induces precocious chiasmata formation, have demonstrated that two key MN components, the MutL homologs MLH1 and MLH3, are also found at nascent chiasmata (24). That the stabilization of MNs and ultimate crossover formation are intricately linked, and that both are dependent on a functional SC, is demonstrated by mouse gene knockout studies showing MN loss in mice lacking components of the SC. For example, the frequency of MNs is severely reduced in spermatocytes from Smc1β−/− mice, suggesting a role for the cohesin complex in mediating events at these sites (18). An important question arises, however, as to the function of the excess MNs observed in early prophase I. Because these sites presumably represent recombination initiation sites (see below), their function in promoting meiotic progression is unclear because only a fixed number of MNs persist through to late pachynema. The events that give rise to this fixed number of late MNs remains poorly understood, particularly in mammals, but will be discussed in the context of recombination events in the following sections.

The timing and length of prophase I vary widely between species and even between sexes. In male mice, for example, prophase I lasts over 10 d, with pachynema constituting 5–7 of those days (Fig. 2). The first cohort of spermatogonia enters prophase I at around d 13 postpartum and progresses through leptonema and zygonema, reaching pachynema by around d 17 (25). They remain in pachynema until d 22–
postpartum, and the first cohort of metaphase I cells is observed at this time. Mature spermatozoa are then observed from d 27 postpartum onward, with cohorts of new cells progressing through prophase I in waves from that time onward.

In female mice, the entire oogonial pool initiates meiosis in utero, with prophase I entry being observed at around embryonic day (e) 14 (Fig. 2). By e17, cells enter pachynema and progress through pachynema, reaching early diplonema by birth (26). Instead of progressing through to metaphase I, however, the cells enter a stage of meiotic arrest known as dictyate arrest in which all meiotic activity ceases and the cells undergo a protracted stage of diplonema. The nascent chiasmata are established and maintained through early postnatal development, and the meiotic events do not reinitiate until after puberty, when cohorts of cells are stimulated to resume meiosis by the action of the gonadotropins, principally FSH. These oocytes progress through metaphase I and into meiosis II, arresting once again soon after ovulation and resuming the second meiotic division upon fertilization. Thus, some oocytes will reinitiate meiosis soon after sexual maturity is achieved, whereas others may remain in dictyate arrest for much longer periods of time.

A similar temporal framework exists for human female meiosis. Oogonia begin to populate the primitive ovary as early as 7–9 wk gestation, with mitotic activity being evident by 10–12 wk (27). Meiosis is initiated soon thereafter, with prophase I cells being observed between 12 and 20 wk gestation (27), and maximal pachytene cell numbers being observed by 26 wk gestation (27–30). By 20 wk gestation, two thirds of the total germ cells are intrameiotic, and one third are still undergoing mitosis. By 7 months gestation, germ cell mitosis ceases, and the last of the germ cells enter meiosis (29). Others are either still in the process of meiosis, undergoing atresia, or are arrested at the dictyate stage (31). By term, many of the oocytes are at dictyate arrest, although a high proportion are still progressing through meiosis, highlighting the less synchronous manner of human oocyte meiosis (31–35).

III. Recombination Events during Mammalian Meiosis

In most sexually reproducing organisms, meiosis is marked by the formation and resolution of hybrid DNA molecules between maternal and paternal homologous chromosomes in the process of homologous recombination or reciprocal exchange. Similar to the DNA repair process, homologous recombination repair and homologous recombination during meiosis involve the activity of many different repair pathways and proteins that are conserved throughout meiotic species. In mammals, our understanding of the molecular events has been slower to emerge than in yeast and flies because of the inherent difficulties associated with studying recombination events in mammals. However, the past 10 yr or so have seen a surge of interest in meiotic prophase I studies, and our knowledge of these processes has been particularly helped by the extensive comparative study of yeast and mammalian meiosis.

Homologous recombination is initiated in almost all meiotic species by the formation of a double-strand break (DSB) in one sister chromatid of one homolog. The SPO11 topoisomerase is responsible for the formation of DSBs in a conserved manner throughout all meiotic species, including Saccharomyces cerevisiae, S. pombe, Coprinus cinereus, Caenorhabditis elegans, and mammals (reviewed in Ref. 36). Not surprisingly, therefore, Spo11−/− mutant mice are infertile as a result of a failure to induce recombination during leptotene of prophase I (37, 38). In male mice, the failure to induce DSBs is paired with a failure of chromosome condensation and synapsis in leptotene and zygonema, coupled with an absence of recombination markers (37, 38). Cisplatin treatment, which induces DSB formation, restores recombination events in spermatocytes from Spo11−/− males, resulting in resumption of prophase I and at least partial spermatogenesis in these animals (38). Interestingly, oocytes from Spo11−/− females reach the diplotene/dictyate stage of prophase in nearly normal numbers, but most are destroyed soon after birth as the meiotic defects become apparent to the checkpoint machinery (37, 39).

The events that proceed from SPO11-induced DSB induction are not yet fully elucidated in mammals but have been extensively characterized in other species such as yeast, flies, and worms. Our current understanding of these events is that DSB resolution can occur through several pathways (summarized in Fig. 3), but that recombination events are directed via DSB repair (DSBR) that involves double Holliday junction formation or via synthesis-dependent strand
FIG. 3. Summary of the current model for reciprocal recombination according the double Holliday junction model for DSBR. For simplicity, one (two-stranded) sister chromatid of each homologous chromosome is shown (one homolog is green; the other is orange). A DSB event in the orange chromatid results in a nick that is further processed to reveal 3'-overhangs that can then invade the opposing chromatid homolog, resulting in strand displacement. DNA synthesis at the 3'-end of the invading strand, followed by second-end capture, concurrent with DNA synthesis to restore the noninvading strand, results in the formation of a mature double Holliday junction as shown. This may then be resolved by the elusive meiotic “resolvase” in a number of permutations (A–D), resulting in the formation of crossovers (cutting at positions A or B) or noncrossovers (cutting at positions C and D). A second mechanism for resolving the DSB event, known as SDSA, involves the first three steps as in this cartoon, but the mature D loop never forms. Instead, the D loop is rejected, resected ends are resolved by limited DNA synthesis, and the structure results in noncrossover events. Gene names provided to the left of each cartoon are indicative of putative regulators of the corresponding events. Model is based primarily on extensive yeast studies, but also on data from mice.
annealing (SDSA) (40, 41). In both pathways, DSB ends are degraded, providing 3'-overhangs that are then capable of invading an opposing homolog. This event results in the displacement of one homolog, producing stretches of heteroduplex DNA, and the formation of a D-loop structure that then becomes stabilized by subsequent branch migration via DNA synthesis. In the DSBR pathway, the second end of the DSB becomes captured, resulting in a mature double Holliday junction structure that may be resolved as a gene conversion event with or without crossing over (to produce a recombinant molecule), whereas in SDSA the invading strand gets displaced and reanneals with the other end of the DSB (after DNA synthesis). Repair of mismatches then results in a gene conversion tract without crossover formation (42). Other mechanisms for crossover formation have been identified in yeast but have yet to be verified in mammals (43, 44).

An interesting feature of meiotic recombination is that crossover events (those involving reciprocal exchange of information between homologs) are nonrandom, following a defined set of rules. For example, at least one crossover event occurs per chromosome pair, and the placement of one crossover will decrease the potential for a nearby crossover event, in a process known as “crossover interference.” Underlying these principles is the observation that from a large set of initiating DSB events only a few (some 10% in mammals) are selected for subsequent reciprocal exchange (reviewed in Ref. 23). In yeast, and probably in mice, the remaining DSB events are resolved as noncrossover events via different pathways involving SEI or SDSA processes (41, 45). The process by which crossovers are selected from all possible DSB initiation sites is known as crossover control (40), and the mechanisms involved in this process remain poorly understood but are thought to involve members of the SC and DNA mismatch repair (MMR) machinery. Undoubtedly, this selection process is of prime importance to the success of the first meiotic division, as supported by the evidence of the strict conservation of crossover (CO) numbers in lower eukaryotes.

The recombination process in mammals is less clearly defined than that outlined above for yeast. However, as with yeast meiosis, the ultimate crossover number is extremely conserved in mammals, arising from a starting number of DSB events that outnumber the final chiasma count by more than 10-fold (46). Analysis of orthologs of yeast genes involved in the recombination process has revealed that many conserved gene products are involved in these selection events in mammals. These gene products include the RecA homologs, RAD51 and DMC1, and members of a number of DNA repair families including the breast cancer susceptibility genes, Brca1 and Brca2, the RecQ helicases, Blm (B5 muted) and Wnt (Werner syndrome-associated), the MMR family, and the Atm/Atr [ataxia telangiectasia (A-T)-mutated and A-T-related] genes (see below). Given the importance of these genes in DNA repair and modification in general, it is not surprising that targeted mutations within this group result in embryonic lethality, cancer predisposition, and/or premature aging of mice. Of those mutations that do allow for viable pups, it is interesting to note that the homozygous mutant animals also exhibit severely reduced fertility (or complete sterility) along with their cancer predisposition/genomic instability phenotypes, highlighting the similarities in function of these genes in DNA repair and recombination events. As discussed below, these mutant animals thus allow us to explore the function of their respective genes in the meiotic process and to expand our knowledge of recombination events in mammalian systems. The ultimate goal of such studies is to understand how recombination is regulated and what role the DNA repair mechanisms play in the decision between crossover and noncrossover (NCO) fates.

IV. Cell Cycle Regulation and Checkpoints in Prophase I

A. Cell cycle mediators in meiosis

Primary germ cells undergo mitotic divisions before they pass into the meiotic state, in which a replicative round of DNA synthesis, characterized by its long duration compared with somatic cells, is followed by the prolonged G2 phase of the cell cycle known as prophase I discussed above, before the first reductive division (MI). Although there have been some significant advances in understanding the control of the decision to leave mitotic cycles and enter into meiosis in both fission and budding yeast (47–51), the control of this transition is poorly understood in mammals but is thought to involve signals derived from surrounding support cells (52). In contrast, the regulation of mitotic cell cycles in somatic cells has been well studied, and it might be expected that some of the regulatory genes identified will also play an essential role in germ cells. Despite the considerable experimental data derived from studies in cultured cells, recent analysis of mice containing mutations in genes for apparently essential regulators of the G1 to S phase of the cell cycle has been somewhat perplexing and suggests considerable redundancy between members of different gene families. Indeed, in several cases, many of the phenotypes identified for mutants in mitotic cell cycle-regulatory genes only become apparent in prophase I and not, as would have been expected, during the premeiotic replicative DNA phase or its preceding G1 (53). These data call into question the essential roles for these molecules during mitosis and suggest novel nonredundant functions in the complex chromosomal dynamics that occur during meiosis. These mutants have also revealed striking sexual dimorphism in these processes (Table 1 and Ref. 35).

In mammalian somatic cells, progress through G1 is regulated by the sequential action of cyclins acting with their cyclin-dependent kinase (CDK) partners (54). The primary action of these heterodimeric kinases appears to be to phosphorylate members of the retinoblastoma (Rb) family of proteins. Phosphorylation of Rb results in release of E2F transcription factors from an inhibitory Rb complex and activation of genes required for cell cycle progression (55). The first acting cyclins belong to the D family, and these together with their catalytic partners, CDK4 and CDK6, act as intracellular sensors of extracellular signals (56). This phosphorylation of Rb by cyclin D-associated kinases is required for further phosphorylation by CDK2, acting together with cyclin E initially and then cyclin A, as cells progress
through S phase of the cell cycle. At the end of S phase, cyclin A binds to CDK1 for transit into G2, after which cyclin A is degraded to be replaced by B-type cyclins (B1, B2, and B3), which in turn bind to CDK1, the function of which is essential for transit into and passage through mitosis (53).

The actions of cyclins and their associated kinases are inhibited by CDK inhibitors that fall into two families, the INK4 (a–d) and the Cip/Kip (p21Cip/Waf1, p27Kip1, and p57Kip2), families of inhibitors (57). Cyclin D/CDK4 and CDK6 complexes are negatively controlled only by the INK4 and Cip/Kip families (57). Although this sequence of events has been largely validated in cell culture experiments, recent genetic analyses of cell cycle events in mice lacking these proteins have not been straightforward to interpret, and the results suggest considerable compensation within and between gene families (59). Indeed ablation of the supposedly essential cyclin E1 (Ccne1), or its partner CDK2 (Cdk2), did not result in embryonic lethality, and mice were fully viable (60, 61). Even ablation of both Ccne1 and e2 did not block embryogenesis until late in development (60). These data indicate that cell cycles can progress normally even in the absence of any CDK2 activity. However, deletion of cyclin A2 (Ccna2), a widely expressed cyclin, results in early embryonic development immediately after implantation, probably as a result of loss of cyclinA2/CDK2 whose activity is required for S phase transverse (62).

Disruption of the genes encoding the cell cycle-regulatory proteins has revealed important roles in meiosis. Mice with null mutations in Cdk2 show severe abnormalities in both male and female meiosis with complete loss of germ cells in both sexes (61). However, the timing of loss is a little different between sexes. CDK2 is required for full chromosome synapsis in males. Thus, Cdk2−/− males trigger a pachytene checkpoint and exhibit massive apoptosis of spermatocytes before diplonema even though mitotic divisions of spermatogonia seem unaffected. In contrast, Cdk2−/− females do not display defects at pachynema, but significant defects were found in oocytes as they reached dictyate with the aberrant distribution of SYC3 along chromosomes and a random dispersal of centromeres. Immediately after dictyate arrest oocyte loss occurs, and all oocytes are lost by postnatal d 14 (61).

Cyclin E2 (Ccne2)-deficient mice also display meiotic abnormalities in males but, in this case, females are fully viable and fertile. The block in spermatogenesis in cyclin E null males appears incomplete with the report of aberrant meiotic figures and low sperm count but is made more severe in mice that are also heterozygous for a mutation in the gene encoding cyclin E1 (Ccne1) (60). Mice completely lacking all E-type cyclins die during embryogenesis, and in these mice meiotic analysis in females was not performed (60). Male meiotic cells uniquely express cyclin A1 (Ccna1), and targeted ablation of this gene blocks spermatogenesis and, as expected from the expression data, not oogenesis (63–65). This block is not in prophase S phase, but instead manifests itself at the end of prophase before MI and is associated with desynapsis abnormalities followed by the propulsion of cells into apoptosis (65).

Mice lacking all D-type cyclins (66), as well as those mice lacking their partners CDK4 (Cdk4) and CDK6 (Cdk6), also

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<td>EL</td>
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<tr>
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F, Fertile; IF, infertile; EL, embryonic lethal; PL, perinatal lethal.

a Infertile due to failure of FSH-induced granulosa cell proliferation.
b Infertile due to an endocrine perturbation during implantation.
die during embryogenesis (67), whereas those lacking a single cyclin D are viable (68). Cyclin D2 (Ccnld2) null male mice are fertile but have reduced testicular size and lower sperm number, whereas mice harboring mutations in the other cyclin D subtypes (D1 and D3) do not have any apparent abnormalities in spermatogenesis or oogenesis (66). Cyclin D2, however, is required for FSH-regulated granulosa cell proliferation, and the females are infertile due to a failure in follicle maturation (69). Null mutant mice for Cdk4, the cyclin D partner, have hypoplastic testis with abnormal spermatogenesis consisting of reduced numbers of spermatagonia and spermatocytes but exhibiting normal oogenesis until CDK4 is required together with cyclin D2 for FSH-induced follicular development (69, 70). These data suggest that at least one cyclin D is required in partnership with CDK4 for normal spermatogenesis but that this is not involved in the generation of female germ cells. It is unclear exactly where the defect in spermatogenesis lies, although it does seem to involve meiotic phases.

It is unfortunate that mutations in Rbl result in embryonic lethality (71) because the absence of Rb during meiosis cannot be analyzed without the creation of a conditional Rbl allele. However, the recent rescue of this early embryonic lethality by tetraploid aggregation techniques (72) paves the way for analysis of female meiosis during later embryonic development. In the case of the E2F transcription factors that are downstream of Rb, there is remarkable redundancy, and all three E2F family members need to be removed before an impairment in cell cycle progression is observed (73). This has not yet been performed in mice. Thus, it is still unclear what the role for these nuclear transcription factors is in the regulation of meiosis.

The activity of the CDKs is regulated by the CDK inhibitors (74). P27kip1 is a ubiquitous regulator of the cyclins, and mice homozygous for a null mutation in the Cdkn1b gene show testicular hypertrophy but without apparent effect upon ovarian maturation; however, there are endocrine disruptions that make female mice infertile (75, 76). Homozygous null mutations for both Cdkn1c and Cdkn2d in mice result in male infertility due to a failure of spermatogenesis but without any effect on female fertility. However, the disruptions in spermatogenesis appear additive, with defects in the timing of mitotic exit and in progression through meiosis. This apparently triggers an apoptotic pathway(s) that results in a gradual depletion of germ cells with few spermatids and spermatooza being detected in the seminiferous tubules of these mice (77). These data suggest that these inhibitors act to extinguish the activity of a D-type cyclin that, together with the activity of P27kip1 during normal physiology, allows appropriate exit from mitosis without reactivation of DNA replication and normal progression through meiosis. However, the data also suggest that some of these cell cycle regulators may continue to have functions through the meiotic cell cycles maintaining proper timing in association with correct chromosome segregation (Table 1).

These studies of mice carrying mutations in cell cycle-regulatory genes are only preliminary and as yet do not explain the control over timing of the transitions from mitosis to meiosis in mammals, or their mode of action in regulating the chromosome dynamics after chromosome replication or the progression in M1. The dramatic loss of germ cells in Cdk2 null mutant mice evident in prophase I without apparent effects during mitosis strongly suggests that, in addition to known targets of proteins involved in DNA replication, elements of the SC are either direct or indirect targets for this kinase. Furthermore, these studies indicate that without CDK2 action, SC proteins fail to assemble on the homologous chromosome pair, resulting in desynapsis and nonhomologous pairing. Consistent with this is the unusual association of CDK2 with chromosomes during anaphase (78). Furthermore, lacking in the analysis of these mouse mutants is an understanding of why female meiosis is unaffected by mutations in some of these cell cycle-regulatory molecules despite apparently similar progression through mitotic and meiotic cycles in both sexes. Only the absence of CDK2 appears to profoundly affect both male and female meiosis, whereas the other mutants have a meiotic phenotype that is restricted to effects on spermatogenesis (Table 1).

In addition to the regulation of DNA synthesis by cyclin-dependent mechanisms, DNA replication also requires the organization of the prereplicative complex (pre-RC) at the origins of replication. This “licenses” the DNA to be copied, and the assembly of this complex is tightly controlled to ensure one, and only one, round of DNA replication per cell cycle (79, 80). Origin licensing begins with the recognition of the origin by the origin replication complex, a heterohexameric complex (81). The association of CDT1 and CDC6 with this complex facilitates the loading of a hexameric complex consisting of the minichromosome maintenance (MCM) proteins 2–7 in stoichiometric amounts (82). This, in turn, triggers a conformational change that allows firing of the pre-RC by the action of CDK and CDC7, resulting in recruitment of DNA polymerase, proliferating cell nuclear antigen, and CDC4 (82). The MCM complex melts the chromatin, allowing replication to be initiated by the primase activity of DNAse polymerase a (82). This licensing complex is then removed to block reinitiation of DNA synthesis. This removal includes down-regulation of origin replication complex 6, CDT1, and MCM proteins as well as geminin expression that binds to CDT1, thereby blocking the loading of MCM proteins onto the chromatin (81, 82).

Proteins of the pre-RC complex are required for premeiotic DNA replication in yeast (83, 84). Given the importance of these proteins for DNA synthesis, it has not been possible to analyze the meiotic phenotypes of mice carrying inactivating mutations in the pre-RC complex genes. Nevertheless, it would be expected that these genes would be down-regulated during early prophase I to prevent unscheduled DNA synthesis. Interestingly, this is not the case, and instead the down-regulation of CDC6 and CDT1 does not occur until the early spermatid stage, whereas MCM2 expression is not lost until late spermatid stage (85). Similarly in females, MCM2 and CDT1 are found in oocytes beyond dictyate arrest. Moreover, regulation may be different between males and females because geminin is expressed in primary spermatocytes but not in primary oocytes, whereas CDC6 expression is down-regulated in oocytes but is maintained in early spermatocytes (85). This suggests two different mechanisms for the suppression of replication firing in males and females. The continued expression of many components of the pre-RC com-
plex in prophase I of both sexes suggests additional roles in meiosis such as in recombination or DNA repair. However, identification of such roles will necessitate the existence of conditional or hypomorphic alleles.

The final phase of prophase I is the entry into the first meiotic division (MI). Like the mitotic cycle, meiosis is regulated by maturation-promoting factor (MPF), the complex that includes cyclin B and CDK1 (49, 86). Although the regulation of MI is beyond the scope of this review, it is clear that MPF is essential during mammalian meiosis because, despite the lack of conditional alleles to ablate these proteins specifically in germ cells and the very early embryonic lethality of CDK1 null mutants (53), genetic ablation of the CDK1-interacting protein, CKS2, results in normal progression of CDK1 null mutants (53), genetic ablation of the CDK1-specifically in germ cells and the very early embryonic lethality of CDK1 null mutants (53), genetic ablation of the CDK1-interacting protein, CKS2, results in normal progression through prophase I including normal chromosome synopsis, but then followed by a failure to undergo the first meiotic division in both male and female mice (87). Similarly, the inhibitor of CDK1, -2, and -5, roscovitine, inhibited MI in cocultures of rat spermatocytes and Sertoli cells in a germ cell-specific manner (88). However, there are no mutations that directly allow the analysis of MPF in mammalian systems. Nevertheless, the extensive biochemistry that has been performed in Xenopus and the genetic analysis in yeast (49, 86) should pave the way for a similar analysis in mammals using conditional alleles of the relevant genes.

B. Checkpoints in prophase I

The absence of cell cycle-regulatory proteins, particularly CDK2 and cyclin A1, triggers apoptotic pathways in developing germ cells. In somatic cells a major checkpoint is triggered through detection of DSBs in a process that involves ATM and AT/Rad3-related (ATR) gene products (89). These kinases propagate their signal through p53–p21 and CHK1 and 2 (CDC25A and CDC25C-mediated pathways), resulting in inhibitory phosphorylation of CDK2 and its subsequent degradation and consequent cell cycle arrest (89). This is coupled to DSBR that requires another substrate, the Nijmegen breakage syndrome (NBS1) protein (see Section V) that may also be involved in the checkpoint itself. However, if repair is not mediated, the cells are triggered to die via apoptosis. The proteins p53 and p21 are required for this response because their targeted ablation partially rescues ATM-deficient somatic and meiotic cells (90–92). During meiosis, as will be discussed in the next section, the absence of ATM or NBS1 triggers apoptosis in both male and female germ cells with few cells surviving beyond pachytene. These data indicate the importance of the ATM DNA damage-sensing pathway during meiosis, perhaps resulting in the blockage of passage toward MI, while the DSBs introduced as a necessary prelude to homologous recombination are repaired. Thus in the absence of ATM, cells are sent into apoptosis at what has been determined as the epithelial stage IV in the testis, approximately equivalent to midpachytene (92), to avoid the inevitable aneuploidy that would follow improper chromosome synopsis. Whether the mechanism is the same as found in somatic cells remains undetermined, although absence of NBS1 and CDK2 (downstream of CHK2-CDC25), both ATM direct or indirect targets, result in meiotic abnormalities during pachytene and apoptosis as discussed in Section IV.A. However, it has not been established, for example, whether CDK2 accumulates in the absence of ATM, resulting in the failure of cell cycle arrest, as would be predicted from experiments in somatic cells. Furthermore, this checkpoint, unlike those in somatic cells, is p53 independent (92). This suggests that the pathway through CHK1 and -2 proteins is emphasized in meiosis, consistent with the association of these proteins with meiotic chromosomes (93).

Analysis of other meiotic mutants in mammals suggests that there are at least two checkpoints during prophase I (94, 95). The first senses unpaired chromosome and/or unprotected double-strand breaks. Its presence is graphically demonstrated by the complete loss of germ cells through apoptosis in males and females in mutant mice lacking Msh4 and Msh5 (96, 97) as discussed in detail below. This checkpoint is p53 independent (Ref. 94 and our unpublished results). A similar checkpoint appears to be initiated after loss of DMC1, a RecA homolog (98) and may also be congruent with that used by ATM. However, the depletion of meiocytes in Atm−/− mice appears more rapid and synchronized, whereas in females lacking MutS homologs (MSH4 or -5), checkpoint activation is associated with disruption of ovarian structures that is not observed in ATM-deficient mice (96, 97). This may indicate the presence of more than one checkpoint or of other signaling pathways that modify a single process. Genetic epistasis experiments may help to sort out these alternatives.

A second meiotic checkpoint is revealed by the loss of MutL homolog 1 (Mlh1), and this appears to sense univalent chromosomes without appropriate chiasmata as the meiocytes exit from pachytene (99, 100). In this case, apoptosis is executed either at or before MI (101). This checkpoint is also p53 independent (our unpublished data). Mlh1 null females show no oocyte loss, and ovulation proceeds in an apparently normal manner. However, these oocytes fail to fertilize properly and rapidly lose viability, with few progressing past MI. Thus, although the chiasma loss is the cause of the meiocyte loss, the timing of cell death is quite different. This may reflect on the different processes with the female oocytes entering dictyate arrest before the checkpoint is activated. The oocytes instead appear to be eradicated as a result of improper chromosome segregation and subsequent developmental failure, rather than through apoptosis that is initiated by the actions of a stage-specific checkpoint per se. A similar sex difference is observed in mice nullizygous for the MLH1 partner, Pms2, where males are infertile due to a prophase I defect, whereas females are fertile (102). The loss of such a stringent checkpoint post-pachytene in females may be a partial explanation for the increased numbers of meiotic abnormalities in this sex.

V. Studies of Meiotic Mutants Involved in DNA Recombination in Mice and Man

The processes described in Sections II to IV prepare the chromosomes for homologous recombination and, therefore, the generation of genetic diversity within individuals. To effect this during prophase I, the recombination machinery creates profound DNA damage. This must be repaired ac-
curately without inducing apoptosis before the cells progress into MI; failure to repair this DNA damage triggers checkpoints that result in the loss of the germ cells, thus preventing propagation of the errors to subsequent generations. It is not surprising then that many proteins identified in somatic cell DNA repair, several of which were discovered as the result of genetic diseases in humans, play an important role in meiosis. This section will review the genetics of these proteins and their meiotic specific paralogs as well as the new information obtained from genetic manipulation of these genes in mice.

A. The RecA homologs, RAD51 and DMC1

Bacterial RecA protein is involved in homologous recombination and repair of DNA damage caused by ionizing radiation and/or chemical mutagens (103, 104). The eukaryotic homolog, RAD51, was identified by Shinohara and colleagues (105–108) and was shown to catalyze strand transfer between homologous DNA strands in yeast and humans. Human RAD51 maps to chromosome 15q15.1 and promotes homologous pairing and strand exchange (109). A second RecA homolog, DMC1, appears to be restricted to meiosis (110) and colocalizes with RAD51 at early MNs (111) in a variety of organisms, including Escherichia coli (112), mice (109, 111, 113, 114), and humans (109, 115). Interestingly, a number of RAD51-like paralogs have been identified, of which there are five in mammalian cells (RAD51B, RAD51C, RAD51D, XRCC2, and XRCC3; reviewed in Ref. 116).

RAD51 attaches to the 3' end of a DNA DSB site and mediates the transfer of the resulting nucleoprotein filament to a complementary homologous strand on the intact chromatid. This invasion displaces the noncomplementary intact strand, an essential step in D-loop formation (reviewed in Refs. 116 and 117). Yeast rad51 mutants are defective in meiotic DSBR, displaying an accumulation of recombination intermediates coupled with increased chromosomal nondisjunction and decreased spore viability (108, 118). The requirement for other factors in the Rad51-driven meiotic events is demonstrated by the observation that (replication protein A) RPA, Brca2 Rad52, and Rad55 all augment the recombinational activity of Rad51 in yeast (119–123) and in human cells (124).

In human spermatocytes and oocytes, RAD51 forms nuclear foci from early zygonema to late pachynema (109, 115), colocalizing with the lateral element SC protein, SYCP3, most strongly on unsynapsed segments. In contrast to the situation in mice, however, where RAD51 is rapidly lost from the chromosome core after synopsis (109, 111, 113), RAD51 immunoreactivity persists on synapsed human chromosomes until late pachynema, although the number of RAD51 foci declines rapidly through pachynema to reach a level that is comparable to the frequency of mature MNs that are thought to give rise to the final population of chiasmata (115). Thus, in both mouse and human meiocytes, RAD51 focus numbers start out at a frequency of approximately 10-fold higher than the final chiasma tally (>250 in the mouse and >350 in humans) and decline thereafter to undetectable in the mouse and less than 100 in humans.

The meiosis-specific RecA homolog, DMC1, is highly conserved among meiotic species, ranging from yeast to lilies to mice (98, 110, 125, 127). Yeast dmc1 mutants are meiotically inactive, yet have no demonstrable mitotic defects (110). Yeast Dmc1 and Rad51 proteins colocalize during meiotic prophase I and are localized to the SC before synopsis. Interestingly, although DMC1 fails to localize to these sites in rad51 mutants, Rad 51 is found in dmc1 mutants, indicating a temporal loading order for the RecA homologs (110, 112). Similar to Rad51, Dmc1 protein forms a nucleoprotein filament on single-stranded DNA in the presence of ATP (128–132).

Mice that are homozygous for a null mutation in Dmc1 are sterile, exhibiting meiotic disruption early in prophase I before pachynema. Spermatocytes from Dmc1<sup>−/−</sup> males show apparently normal progression through leptotena and early zygote, with no complete pachytene-stage cells being observed (98, 127). No attempts at synopsis are apparent, and chromosome cores remain in their uncompacted state, indicative of early prophase I. Interestingly, RAD51 continues to be present on the chromosome cores in numbers indicative of leptotene to zygote stages, but no diminution of RAD51 focus frequency (that would be indicative of a progression through to pachynema) is observed (98).

In Dmc1<sup>−/−</sup> females, the loss of DMC1 causes a similar meiotic disruption, but the consequences to the overall biology of the gonad are more severe. By e17, the number of oocytes within the fetal ovary is significantly diminished in Dmc1<sup>−/−</sup> females compared with wild type (98) and, by d 4 of life, fewer than 10% of oocytes remain (39). The consequence of this global loss of oocytes from the Dmc1<sup>−/−</sup> ovary is the subsequent degeneration of ovarian structures through life, with adult females displaying a “streak-like” ovarian morphology reminiscent of ovaries from Turner syndrome patients.

The loss of ovarian structures after mutation of a key early recombinogenic gene is now a common theme in murine meiosis. Several of the genes that will be discussed in this review, when mutated, give rise to a similar female phenotype. Interestingly, the earliest of the recombinogenic genes, Spo11, is quite different. As described earlier, Spo11 is thought to be the enzyme responsible for generating DSBs in mammalian meiocytes and, indeed, its activity is essential for DSB formation in mice (37, 38, 133). However, unlike the genes, the products of which process these DSB events, mutation of Spo11 in females results in at least some progression through prophase I, with oocytes progressing through to the diplotene/dictyate stages before succumbing, after birth, to apoptotic elimination (37). Interestingly, however, some oocytes from Spo11<sup>−/−</sup> neonates progress further than diplotene, with 15–20% of oocytes being capable of initiating primordial follicle formation indicative of entry into dictyate arrest (39). By contrast, no oocytes in Dmc1<sup>−/−</sup> females remain to this point; instead they die by diplote. These observations suggest that once DSBs are formed, they must be dealt with to ensure the health and survival of the oocyte. In line with this theory, oocytes from Spo11<sup>−/−</sup> Dmc1<sup>−/−</sup> double-mutant females are a phenocopy of oocytes from Spo11<sup>−/−</sup> single-mutant animals (39). Taken together, these results demonstrate that the failure to induce DSBs in Spo11 mutant animals results in meiotic disruption.
that is independent of a DNA damage checkpoint and that
is therefore distinguishable from the DNA damage check-
point to which oocytes from Dmc1 null females succumb.

By contrast with the situation in females, male meiocytes
are wholly dependent on DSB formation and recombina-
tion to progress through prophase I. Spermatocytes from Spo11−/−
and from Dmc1−/− males show similar meiotic pheno-
types, arresting their meiotic program at or before pachytene
(37, 38, 98, 127); in contrast, meiotic progression in Spo11
nullizygous males is partially restored by introducing cis-
platin-induced DSBs in spermatocytes, whereas Dmc1−/− males are unaffected by such treatment (37). Taken together,
these results indicate that RAD51 and DMC1 cooperate in the
downstream processing of SPO11-induced DSB events, and
that the checkpoint machinery monitoring their actions may
differ in stringency between male and female mammals.

B. The MMR machinery in mammalian meiosis

The MMR pathway, as its name implies, was first identi-
fied in its capacity as the primary mechanism through which
dNA mismatches are detected and corrected. The system
acts primarily to correct mismatches arising as a consequence
of errors during replication in S phase, but also participates
in other DNA stability functions associated with stabilization
of repeat structures, such as trinucleotide repeats (134–136),
cisplatin-induced cross-links, and damage from oxidation/
alkylation agents (reviewed in Ref. 137). This pathway also
prevents recombination between divergent sequences,
known as homologous recombination, which could result in
potentially lethal chromosomal imbalance. Components of
the MMR pathway are highly conserved throughout evolu-
tion and are closely linked with components of the cell cycle
and checkpoint machinery in higher eukaryotes (reviewed in
Refs. 138 and 139). That these events are essential for cell
viability and integrity is demonstrated by the observation
that defects in the mammalian MMR system result in cancer
predisposition (reviewed in Ref. 140). In humans, germline
mutations in certain MMR genes are responsible for heredi-
tary polyposis colorectal cancer, characterized by early-
onset colorectal cancer, along with cancers of the endome-
 trium, stomach, urinary tract, and ovary (reviewed in Ref.
141). Mutations in the MMR gene family are also responsible
for a high number of spontaneous colorectal cancer cases.

In bacteria, where the MMR system was first defined,
mismatch recognition is effected by the MutS protein, which
binds as a homodimer to the site of the mismatched bases. An
ATPase-containing protein, MutL, which binds to the MutS
homodimer is then recruited, and this MutS-MutL complex
then activates the MutH endonuclease (reviewed in Ref. 138).
MutH is a methylation-sensitive endonuclease that binds to
a methyl group on either side of the mismatch. Taking ad-
Vantage of the fact that, in prokaryotes, newly synthesized
DNA is transiently unmethylated, MutH preferentially nicks
the daughter strand, thereby directing the repair machinery
away from the template strand and instead marking the
erroneous strand for exonucleolytic resection and resyn-
thesis (142). Depending on the side of the MutH activity, one of
four exonucleases is then recruited, possessing either 5′-3′
exonuclease (exonuclease VII or RecJ) or a 3′-5′ exonuclease
(exonucleases I or X) activity, to degrade the mismatch-
containing nicked strand (reviewed in Ref. 138). These events
are assisted by the MutU (UvrD) helicase that unwinds the
DNA from the nick through to the mismatch.

In eukaryotes, the MMR system is complicated by the
presence of several homologs of MutS and several of MutL.
No MutH homolog exists in eukaryotes, possibly because of
the absence of methylation status-directed strand discrimi-
nation. Instead, strand discrimination is thought to come
about as a result of interactions of the MMR family with the
replication fork machinery, such as proliferating cell nuclear
antigen (143, 144). Seven MSHs have been identified, five of
which exist in mammals, MSH2–MSH6. These act as het-
erodimers, with MSH2 being the principal MutS homolog
involved in repair events, in conjunction with either MSH3
or MSH6. Together, the MSH2–MSH6 (known as MutSα) and
the MSH2–MSH3 (known as MutSβ) bind to and repair all
common mismatches, but each complex has its own substrate
specificity. Mammalian MutSα can bind to, and repair, DNA-
containing base-base mismatches and insertion-deletion
loops, whereas MutSβ can only bind to insertion-deletion
loops. In addition, evidence from yeast suggests that MutSβ
is capable of binding to unpaired loops as large as 5.6 kb that
could arise as a result of heteroduplex formation during
meiotic recombination (145).

Like the MutS homologs, MutL also has several homologs
in eukaryotes, and in mammals these are named MLH1,
MLH2, MLH3, and PMS2, the latter being named for its
postmeiotic segregation (PMS) phenotype in yeast (where it
is confusingly called PMS1). MLH1 is the principal MutL
homolog acting in MMR events, and it primarily functions in
this regard with PMS2 (MutLα), although interactions with
MLH2 (to form MutLβ) and MLH3 (to form MutLγ) may
provide repair mechanisms for specialized classes of muta-
tional intermediates, at least in yeast (146, 147). In mammals,
the most likely function for MutL heterodimers other than
MutLα is in meiosis, where MutLγ is essential for recombi-
nation and crossing over, as it is in yeast (see below). MLH3
appears to be the primary binding partner for MLH1 in these
meiotic processes, both in yeast and in mammals (42, 148–
151), whereas the role for MLH3 in MMR and colorectal
cancer is contentious at the present time (149, 152–158).

In addition to its role in DNA repair and cell cycle control,
the MMR pathway is also essential for meiosis in a variety
of species including S. cerevisiae, Arabidopsis thaliana, C. el-
egans, and Mus musculus (23, 42, 159–162). For these pur-
poses, however, other MutS homologs are recruited, and the
MSH4–MSH5 heterodimer (MutSγ) appears to function
solely in meiotic recombination; these proteins lack the N-
terminal MutS/MSH domain that is necessary for mismatch
binding (163). In mice, mutation of Msh4 or Msh5 results in
meiotic disruption early in prophase I and sterility in both
males and females (96, 97, 164). At the cytogenetic level,
chromosomes replicate in premeiotic S phase, apparently
normally, and then enter meiosis, accumulating SYCP3 pro-
tein and forming complete axial elements. The axial elements
appear somewhat elongated, however, and stain less inten-
sely for SYCP3 (P. E. Cohen, unpublished observations).
Most significantly, chromosome synopsis is severely reduced
in both Msh4−/− and Msh5−/− animals, with SYCP1 rarely
appearing between the axial elements (P. E. Cohen, unpublished observations), and with a high degree of mispairing, self-synapsis, and nonhomologous pairing being observed (Fig. 4). As a result, spermatocytes succumb to apoptosis early in prophase I, at or before zygonema, such that the testis of adult Msh4\(^{+/−}\) and Msh5\(^{−/−}\) males is largely devoid of spermatogenic cells except for one or two layers of spermatogonia and early spermatocytes.

Msh4\(^{−/−}\) and Msh5\(^{−/−}\) females undergo a meiotic disruption similar to that seen in the males. However, the timing of meiosis in females is such that the consequence of such meiotic disruption is much more severe. In females from both knockout strains, prophase I oocytes progress through leptotema and zygonema but by e20/21 (d 1 postpartum) most of the oocytes are in the process of being lost by apoptosis so that fewer than 10 oocytes remain per ovary by d 3 postpartum (39, 96, 97). Interestingly, the loss of MSH5 can be partially overcome by deletion of Spo11, and therefore prevention of DSB formation, indicating that oocyte loss in the Msh5\(^{−/−}\) females is primarily the result of failure to repair DSBs rather than failure of synopsis (39). This failure to resolve DSBs as crossover or noncrossover events, therefore, results by birth in the induction of apoptotic events resulting in almost complete removal of germ cells and consequent severe ovarian dysgenesis in the adults of Msh4\(^{+/−}\) and Msh5\(^{+/+}\) mice.

Given the stoichiometry of the MMR complexes in repair functions, it was not surprising to find that MSH4-MSH5 interacts with both MLH1 and MLH3 in yeast and in mice (165, 166). Mutations of either of these MutL homologs in mice also result in meiotic failure but at a distinctly later time point to that seen for Msh4 and Msh5 mutations (99, 100). Spermatocytes from Mlh1 and Mlh3 null animals progress through to pachynema apparently normally, with complete synopsis being observed (99, 148). Then at diplonema, when the central element of the SC disassembles and the chromosomes begin to move apart, instead of remaining together at chiasmata sites, the chromosomes in Mlh1\(^{−/−}\) and Mlh3\(^{−/−}\) males fall apart prematurely. This premature desynthesis is thought to occur as a result of a failure to stabilize these crossovers, and, as a consequence of this destabilization, fewer than 10% of chiasmata are observed at metaphase in Mlh1\(^{−/−}\) males, whereas none are seen in Mlh3\(^{−/−}\) males (99, 100, 148, 170).

More recently, studies of MLH3 and MLH1 function in meiotic cells revealed some interesting dynamics with respect to the accumulation of these proteins in SCs during prophase I. MLH3 is recruited first to the chromosome core in late zygonema or early pachynema, with MLH1 being recruited in midpachynema in mice (170). This observation is further supported by the persistence of a few MLH3-positive foci on chromosome cores from Mlh1\(^{−/−}\) males but the absence of MLH1 on chromosomes from Mlh3\(^{−/−}\) males (148, 170). Furthermore, this persistence of MLH3 in Mlh1\(^{−/−}\) is coupled with the retention of 10% chiasma numbers at metaphase (171). Thus MLH3 may function independently of MLH1 to select MN sites for further processing into crossover events. The phenotype of Mlh1 and Mlh3 null females reflects the later function of MLH1 and MLH3 in mammalian meiosis as compared with MSH4 and MSH5. Thus, chromosomes progress through to pachynema and into early diplonema (99, 149, 172). However, at birth, when the oocytes enter dictyate arrest, meiotic failure has not yet become apparent, and so the ovary assumes its normal dictyate developmental pattern, even though the oocytes are destined to fail in the resumption of the meiotic program. Thus, adult Mlh1\(^{+/−}\) or Mlh3\(^{+/−}\) females have normal ovaries possessing oocytes at all stages of follicular development. These oocytes are capable of resuming meiosis upon ovulation, but at metaphase, it becomes apparent that few, if any, intact chiasmata remain.

As a consequence, the chromosomes fail to establish stable bipolar spindle orientation, the spindle poles are destabilized and continue to elongate, and most oocytes fail to achieve an anaphase configuration (172). In vitro fertilization studies demonstrate that oocytes from Mlh1\(^{+/−}\) and Mlh3\(^{+/−}\) females progress poorly through early fertilization events (99, 148). Although first polar bodies are, in fact, extruded, it appears unlikely that these polar bodies contain the appropriate chromosome count because chromosomes appear to be arrayed randomly over the first meiotic spindle (Ref. 172, and N. Kolas and P. E. Cohen, unpublished observations).

In line with the phenotypes of the meiotic MMR genes, their gene products are intrinsic to the chromosome cores of prophase I meiocytes. MSH4 (and presumably MSH5) is localized to meiotic chromosome cores of mouse spermatocytes and oocytes as early as leptotena (97), at a frequency that is thought to reflect the status of DSB-processing events (Fig. 5). That MSH4 is localized to MNs at this time is shown by colocalization with RAD51 and DMC1 (173) and, in human oocytes at least, MSH5 (115). However, MSH4 localization on chromosome cores persists beyond these other early MN markers, through until at least late pachynema and, from

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**Fig. 4.** Examples of mispairing in spermatocytes from Msh5\(^{−/−}\) (A) and Msh4\(^{−/−}\) (B) males. Most of the chromosomes remain unsynapsed through to pachynema in both mutants, and any synapse that does occur is often between nonhomologous partners or involves many different chromosomes. Examples of mispairing are shown in the box in panel B(i) and are magnified in panel B(ii). In general, more synopsis is observed in spermatocytes from Msh4\(^{−/−}\) than from Msh5\(^{−/−}\) males. Chromosomes were prepared by sucrose spreading and staining with antibodies against SYCP3 (red) to highlight synapsis events.
early to midpachynema onward, interacts with components of the late MNs, including MLH1 and MLH3 (165, 166). Thus, MSH4 and MSH5 constitute core components of the MNs that are resident through most of the DSB-processing events, and the frequency of which diminishes gradually through prophase I, reflecting the sequential selection of recombination intermediates along a reciprocal recombination pathway (Figs. 5 and 6). Biochemical data indicate that the MSH4/5 heterodimer associates specifically with Holliday junction substrates and that this binding stimulates ATP hydrolysis (174). This, in turn, instigates a sliding clamp configuration for MSH4/5 that moves away from the Holliday junction and encircles the heteroduplex arms (174). It is postulated that MLH1–MLH3 stabilizes these clamp structures, allowing more to accumulate along the heteroduplex stretch, and thus ensuring the progression of this site through to later crossover stages (23, 174, 175). This suggestion is confounded, however, by studies in yeast, which demonstrate the existence of three distinct crossover pathways, only one of which involves MSH4–MSH5 (161, 176).

MLH1 and MLH3, as mentioned earlier, form the MutL heterodimer that predominates in mammalian meiocytes. In both male and female mice, they appear on meiotic chromosome cores only after synapsis has occurred (100, 155, 177) and at a frequency that is far reduced from the initiating DSB frequency. Studies by many groups have indicated that the MLH1–MLH3 heterodimer at late pachynema reflects the ultimate selection of crossover events and, indeed, MLH1 accumulates at the precocious chiasmata that arise after treatment of mouse spermatocytes with the phosphatase inhibitor, okadaic acid (24). The importance of MLH1 and MLH3 localization to these nascent crossover events is underscored by the observation that chiasmata are completely absent in spermatocytes from $Mlh3^{-/-}$ males (148).

**LEPTONEMA:** Processing of DSBs by recombinogenic machinery and labeling of these sites by RAD51 and then by MSH4/MSH5

**ZYGONEMA:** Systematic loss of MSH4/MSH5 positive foci either by positive selection (dark green foci) or by negative selection (light green foci)

**PACHYNYEMA:** Stabilization of MSH4/MSH5 event by MLH1/MLH3 at a subset of MNs results in the final number of mature meiotic nodules

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**Fig. 5.** Correlation of MSH4 and MLH1 focus frequency with DSB processing events. Graphical representation of immunoreactive foci for MSH4 (orange) and MLH1 (green) through prophase I in mouse spermatocytes. [Data adapted from Ref. 97 and P. E. Cohen, unpublished.] The estimated number of MNs, representing DSBs and/or processing DSBs (indicated by RAD51 localization in leptonema and zygonema), is shown in brown and is an estimate taken from a compilation of many studies (22, 36, 37, 113, 114).
C. The RecQ helicases, Bloom syndrome (BS) mutated (BLM) and Werner syndrome-associated (WRN)

BLM is an autosomal-recessive disorder characterized by growth deficiency, dermatological sun-sensitive pigmented lesions, immunodeficiency, predisposition to malignancies, and chromosomal instability, including increased frequency of sister chromatid exchanges (178). The BS gene product, BLM (also known as RecQ-like 3, RECQL3), is a member of the RecQ family of 3'-5' single-stranded DNA helicases, molecules that recognize and bind to and expand single-stranded gaps in duplex DNA (179), and include the human Werner’s syndrome-associated (WRN) and Rothmund-Thomson syndrome (RECQL4) gene products, as well as S. cerevisiae Sgs1. Men with BS are sterile due to azospermia; women, although sometimes fertile, have a shortened reproductive life span, displaying normal puberty, but a high incidence of premature ovarian failure (178, 180). Human male heterozygote carriers of BLM mutations display sperm with higher frequencies of structural chromosome abnormalities (181). Interestingly, the rare occurrences of pregnancy among women with BS are associated with higher rates of preterm labor (182, 183).

The human BLM gene spans 250 kb on chromosome 15q, constitutes a 4437-bp cDNA, and encodes a 1417-amino acid protein (179). Transfection of BLM coding sequence into cells from BS patients restores BLM activity and partially complements the phenotype of S. cerevisiae sgs1 top3 mutant strains (184). Similarly, murine Bim spans 4780 bp of chromosome, contains an open reading frame of approximately 1420 bp, and shares 81% identity with human BLM cDNA, whereas the proteins are 76% similar (185). Bim is expressed at high levels in the testis where the protein localizes to germ cells in prophase I.

BLM protein contains a C-terminal nuclear localization sequence, whereas the RecQ helicase domain is located in the central portion of the protein (179). BLM unwinds DNA in 3'- to 5'-direction, but can also recognize and disrupt other DNA structures such as Holliday junctions, triple helices, and complex duplex structures (186–189). Other RecQ-specific domains exist in the C-terminal portion of the protein, including a zinc-binding RecQ-Ct domain that appears to direct interactions with the DNA and to modulate the ATPase and helicase functions of BLM (190). The C terminus also regulates nucleolar localization and interactions with other proteins that function in DNA metabolism, including topoisomerase IIα (TOPOIIα), RAD51, and MLH1, whereas other regions directly bind of BRCA1, ATM, p53, FEN1, NBS1/MRE11, MSH2, and MSH6 (191–200), to name a few. The interaction with ATM is functionally important because it abolishes both meiotic recombination (207, 208). The sgs1 mutation leads to meiotic chromosome missegregation as a result of increased paternal associations between chromosomes and increased crossing over without a concomitant increase in gene conversion rates and subsequent arrest at pachynema (208–210).

In mouse spermatocytes, BLM protein becomes evident along unpaired chromosome cores, by immunofluorescence and electron microscopy immunogold techniques, starting in leptotena (211). The number of BLM foci is lower than the RAD51/DMC1 foci at this time, but they are somewhat coincident with these RecA homologs (211). BLM localization increases during zygonema (211) and is found as discrete foci along the SCs of synapsed chromosomes in late zygonema I (212), eventually localizing to sites at which RAD51 and DMC1 are no longer present (211). The BLM foci progressively decline through early pachynema I, until they are no longer seen at midpachynema (212). There is also a statistically significant excess of BLM signals in the synapsed pseudoautosomal region of the X-Y bivalent, a known recombinational hot spot (211), suggesting a role of BLM in meiotic recombination.

BLM also colocalizes with RPA during late zygonema and early pachynema, suggesting a role for BLM in the later establishment of early prophase interhomolog interactions (212). Later, in pachynema, BLM exhibits a more dispersed nucleoplasmic location over the chromatin, suggesting additional roles of BLM in resolution of sister chromatid interactions during anaphase I (212). Of note, the distribution of BLM foci in pachytene spermatocytes rarely corresponds with the pattern of MLH1 localization (100, 177), but occasional interactions between these two proteins are found in mouse spermatocytes at the immunoprecipitation-Western blot level (P. E. Cohen, unpublished data). Either way, this would lend credence to the finding that BS cell lines show mitotic hyperrecombination, not diminished homologous recombination (210).

BLM and TOPOIIα colocalize to human nuclei, can be coimmunoprecipitated from human cell extracts, and bind to each other in vitro (193). BLM and TOPOIIα also colocalize substantially with one another on human meiotic spermatocyte spreads (192). In meiotic human spermatocyte spreads, TOPOIIα is found on axial or lateral elements during zygonema, before and after synopsis, continues to be associated...
with lateral elements as zygonema proceeds, and is concentrated at sites of synopsis (192).

A second RecQ homolog prevalent in mammalian cells is WRN (also known as RecQ-like 2, RECQL2), the gene that is altered in the human condition known as Werner syndrome. Features of this progeroid disorder include scleroderma-like skin changes, especially in the extremities, cataract, calcification, premature arteriosclerosis, diabetes mellitus, and a wizened and prematurely aged face. Like BLM, the WRN gene encodes a protein that has been implicated in a variety of DNA maintenance functions, including repair, recombination, replication, telomere processing, and transcription (reviewed in Refs. 214–216). For example, the helicase activity of WRN facilitates long-patch base excision repair by unwinding stretches of DNA and exposing these regions to the actions of DNA polymerase polβ (216) and may also provide a proofreading component to polymerases (such as polβ) that do not themselves possess such ability (217). WRN also exhibits exonuclease activity and is able to remove 3’-mismatches (218); also, it can interact with other exonucleases, such as Exo1, to encourage their cleavage activity (219).

WRN has been implicated recently in recombination repair of telomeric ends, in a process known as alternative lengthening of telomeres (ALT). The molecular basis for ALT remains unclear, but it can be undertaken by other members of the RecQ family, such as Sgs1, and involves interactions with the RAD51, NBS1, and BRCA1 repair pathways that are active in mammalian germ cells (reviewed in Ref. 216). The role of WRN in meiotic recombination, therefore, is implied by all these observations, but requires further study.

D. BRCA1/BRCA2 cancer susceptibility syndromes

The breast cancer susceptibility gene, BRCA1, was cloned in 1994 by Skolnick and colleagues (220) after King and associates (221) had assigned it to chromosome 17 by linkage analysis of kindreds exhibiting early-onset breast cancer. In the same year, another breast cancer susceptibility locus was identified on chromosome 13 in families exhibiting a high incidence of male breast cancer, and this was named BRCA2 (222–224). Heterozygous mutations in BRCA1 or BRCA2 confer susceptibility to breast and ovarian cancer, with the majority of tumors showing loss of heterozygosity with retention of the mutant allele (225). Women carriers of these BRCA1 and BRCA2 mutations have breast cancer risks of 37% to more than 85% by age 70, and ovarian cancer risks of 63% for BRCA1 carriers and 27% for BRCA2 carriers, although a 16% lifetime ovarian cancer risk was found in a combined BRCA1/BRCA2 population (226–230). Although these cancer susceptibility phenotypes are similar, showing a propensity for diagnosis at younger ages, the genes are not related by sequence or function (231).

The protein product of the BRCA1 gene is a 1863-amino acid nuclear phosphoprotein containing a RING finger domain, two nuclear localization signals, a DNA-binding domain, an SCD domain, and two BRCT domains (reviewed in Ref. 231). That this latter domain is a feature of DNA repair and/or cell cycle checkpoint proteins, whereas the RING finger domain is commonly found in proteins possessing ubiquitinylation functions, indicates a role for BRCA1 in cell cycle checkpoint activation after DNA damage (reviewed in Ref. 232). Indeed, BRCA1-mediated ubiquitinylation occurs in response to replication stress in conjunction with its binding partner, BRCA1-associated ring domain (233).

BRCA1 is a core component of BRCA1-associated genome surveillance complex, consisting of the MMR proteins, MSH2, MSH6, and MLH1, together with ATM, BLM, replication factor C, and the RAD50-MRE11-NBS1 complex (234). Given the role of all these proteins in sensing abnormal DNA structures and in subsequent repair events, this implicates BRCA1 as a major coordinator of the cellular response to DNA damage to ensure genome integrity. In somatic cells, BRCA1 appears in S-phase nuclei as discrete foci throughout the nucleus and colocalizes to these sites with RAD51 (235), an association that is dependent on BRCA2 (235, 236). Breast-deficient murine embryonic stem cells have impaired homologous recombination activity (237, 238), whereas transgenic rescue with human BRCA1 restores the frequency of homologous recombination and corrects the radiation sensitivity (238).

Bra1 expression levels increase during late pachynema and diplonema of prophase I (239). In human spermatocytes, BRCA1 localizes with RAD51 on unsynapsed axial elements (235), along with BRCA2 (236, 240). As with RAD51, both BRCA1 and BRCA2 are first observed at early zygonema on all chromosomes and persist through pachynema on the unpaired X- and Y-chromosomes (235, 236).

Mice homozygous for mutations in the Brca1 gene exhibit lethality before d 10 of embryogenesis, precluding further analysis of BRCA1 deficiency. It is also known that mutations in p53 (Trp53) confer a growth advantage, with extended survival of BRCA1-deficient embryos in the absence of p53 expression (241, 242). Cressman et al. (243) identified three mice, two males and one female, that had homozygous mutations for both the p53 and Brca1 genes. They found, in the males, smaller testis size and a failure to impregnate female mice despite normal copulation. In addition, although numbers of spermatagonia appeared normal (compared with p53 null mice), the meiotic spermatids and postmeiotic spermatocytes were absent. Only two pachytene spermatocytes were identified. Interestingly, staining for early prophase I markers, such as the heat shock protein HSP70–2, was present in some cells. Together, these data indicate that mouse spermatocytes lacking both p53 and BRCA1 show meiotic failure during prophase I, implicating possible roles for BRCA1 at this stage of meiosis. These findings were expanded upon by Xu et al. (244), who found that Brca1 plays essential roles in DNA-damage repair and crossing over during murine spermatogenesis, by studying male Brca1/−/− mice, which carry a homozygous deletion in exon 11 of the Brca1 gene and which can survive to term as a result of a single mutant allele of p53. In this mouse model, meiosis was disrupted in the spermatocytes, whereas oocytes progressed normally through meiosis. In spermatocytes from Brca1/−/−/− males, chromosome synapsis occurs normally, and the cells progress through pachynema (244). However, MLH1 fails to accumulate at late MNs in spermatocytes from these mice, suggesting a failure to stabilize and process DSBs appropriately. In line with this suggestion, DSBs fail to become repaired in the correct
temporal framework, as demonstrated by persistent γH2AX immunolocalization.

BRCA2-deficient murine embryonic stem cells and human CAPAN-1 (a BRCA2-deficient pancreatic adenocarcinoma cell line) cells also display diminished repair of double-strand breaks by homologous recombination (245). The need for DNA DSBR in meiotic cells implies that these proteins may play similar roles in meiosis, but this has yet to be demonstrated. Connor et al. (246) generated mice homozygous for a Brca2 truncation mutation in exon 11 and found that, in contrast to the situation for Brca1, some homozygous animals were viable, but at much lower rates than Mendelian ratios would predict. Interestingly, other Brca2 mutant mouse lines exhibited complete embryonic lethality (240, 247, 248), possibly indicating that the Connor mice are hypomorphic. Nevertheless, the homozygous mutant males harboring the exon 11 disruption were found to be completely devoid of germ cells, whereas females exhibited very much smaller ovaries, but with signs of some persistent oocytes (246).

In another mutant mouse line in which the embryonic lethality had been overcome by a BAC transgene encoding human BRCA2, but which failed to be expressed in germ cells (249), spermatocytes progress through early prophase I, but with only partial synopsis being observed. In view of the known association between BRCA2 and RAD51, the localization of RAD51 is very much reduced in spermatocytes, whereas RPA accumulation appears to be normal. However, no cells progress through to pachynema, suggesting a requirement for BRCA2 in early prophase I. Interestingly, the female Brca2 mutant animals that are rescued by the BRCA2 transgene appear to progress further through meiosis than the males. In 3-wk-old animals, the ovaries were indistinguishable from wild-type controls, but with an 8- to 10-fold reduction in the number of primary and primordial follicles. However, normal antral follicles were observed and oocytes could be obtained by superovulation of the transgene-rescued females (249), albeit at significantly reduced numbers. These oocytes could be fertilized and matured in vitro but displayed increased abnormalities such as failed chromosome condensation, abnormal DNA distribution between the egg and the polar body, and oversized polar bodies. Some fertilized eggs from Brca2−/− transgene rescued females could undergo implantation, but at very much reduced frequency to that of wild-type littermates. Thus BRCA2 is essential for both male and female meiosis, but the dynamics of this requirement appear to differ between the sexes, because male spermatocytes arrested their meiotic process before pachynema, whereas female germ cells appeared to progress through to the end of prophase I.

**E. Nijmegen breakage syndrome (NBS) and ataxia-telangiectasia (A-T)-like disorder (ATLD): components of the MRN complex**

NBS is an autosomal-recessive chromosomal instability syndrome characterized by microcephaly, distinctive facioskeletal abnormalities (“bird-like” faces), growth retardation, immunodeficiency, predisposition to cancer, reduced fertility, and gonadal dysfunction (250–252). In adult female patients, there is a high incidence of primary ovarian failure, lack of secondary sexual development, and primary amenorrhea (250). The gene defective in NBS is NBS1, which encodes a protein known as nibrin or p95 (250, 253, 254). In NBS cells, despite the absence of NBS1, its binding partners, MRE11 and RAD50, still form complexes, although they remain in the cytoplasm (254), suggesting a role for NBS1 in DNA damage recognition and targeting.

A related disorder, ATLD, has recently been shown to be the result of a functional hypomorphic allele of the MRE11 gene, the gene product of which forms part of a tripartite complex together with NBS1 and RAD50 (255, 256). MRE11 possesses multiple activities, including 3′-5′ double-strand and single-strand exonuclease activities along with single-strand DNA endonuclease activity (reviewed in Refs. 257 and 258). These activities are responsible for processing of DNA secondary structures and removal of covalent linkages between proteins and DNA (259, 260).

Structural analysis of the RAD50 coiled-coil region reveals a unique apical dimer interface consisting of interlocking Zn2+-binding hooks. The result of dimerization at this position is the formation of a flexible bridge of up to 1200 Å that is capable of bridging a DSB gap or of linking sister chromatids together during homologous recombination (257, 261). In addition, Walker A and B motifs confer ATPase activity to the protein and are brought into close alignment upon folding of the intervening heptad repeats that make up the coiled coil domain (Fig. 7). The current model for MRN function, therefore, involves two molecules of RAD50 linked at their coiled-coil Zn2+-bridge, which extends away from the Walker A/B globular domain “head” by virtue of the coiled coil “tail”. An MRE11 dimer associates with the head region and tethers the entire complex to the DNA through its two DNA-binding domains (257, 261–268). NBS1 presumably associates with MRE11, also as a dimer (257, 258).

The MRE11-RAD50-NBS1 (MRN) complex localizes to sites of DNA damage (250) where it plays diverse roles in genome maintenance by participating in DNA recombination and checkpoint activation after DNA damage (reviewed in Ref. 269). In somatic cells, the major function of this complex in homologous recombination events is the promotion of recombination between sister chromatids (270), by binding to both ends of a DSB, unwinding the ends, acting as a bridge between the two, and by recruiting ATM (269). ATM phosphorylates NBS1 (271), whereas NBS1 is essential for recruitment of ATM to sites of DNA damage through interactions that involve the C terminus of NBS1 (269, 272, 273). In this way, the MRN complex facilitates the actions of ATM, including phosphorylation of downstream targets, such as CHK2, p53, and histone H2AX (274), and the recruitment of other recombinogenic proteins, including WRN (275).

The MRN complex has also been implicated in DSB resolution during meiosis in a number of eukaryotic organisms. In *S. cerevisiae*, the MRE11-RAD50-XRS2 (the functionality, but not structurally, related protein to NBS1) complex (MRX) is essential for Spo11-mediated DSB formation (276, 277). Separation-of-function mutants of MRE11 indicate that the MRX complex is required for removal of Spo11 from the 5′-ends of the DSB and for subsequent processing of the DSB (277, 278). More recently, studies have indicated that MRE11
and XRS2, in the absence of RAD50, bind to sites along the chromosome in the absence of SPO11 and, in so doing, may establish a network of "pre-DSB" sites that ultimately become the target for SPO11-mediated events (279–281). Further studies suggest that MRX may facilitate chromatin configurations that are favorable for the induction of DSBs (282). The MRX complex also functions in meiotic events in *S. pombe* (283), *C. elegans* (284), *A. thaliana* (285), and in mice (reviewed in Ref. 286). In this latter case, however, the embryonic lethality of mice bearing mutations in genes encoding the MRN components has precluded extensive functional genetic analysis of the mammalian MRE11 complex in meiosis (287).

Rad50, Mre11 and Nbs1 are all highly expressed in the mouse testis (288). In both mouse and human spermatocyte meiotic spreads, NBS1 and MRE11 localize to the distal ends of chromosomes during late leptonema, zygonema, and early pachynema (253). NBS1 also colocalizes with TRF1 (a telomere-binding protein) (253), suggesting a role for the MRE11-NBS1-RAD50 complex in meiotic telomere maintenance, as seen in yeast (289, 290). However, hypomorphic mutations of *Mre11* (*Mre11ATLD*) or *Nbs1 (NbsA*) (289), which recapitulate human ATLD, and NBS, fail to disrupt oocyte development and meiosis (255, 291), in contrast to similar mutations in *S. cerevisiae*, which result in inviable spores (278, 281, 282, 292, 293). Similarly, *mre11* mutations in *C. elegans* result in a failure of crossing over during meiosis, despite the fact that homologous chromosomes pair normally (284).

Hypomorphic alleles of murine Rad50 (Rad50h) also fail to elicit a meiotic phenotype (294, 295). Ovaries and testes appear normal in Rad50h homozygous mice, and both sexes are fertile. These results suggest that the MRN complex may play roles subtly different in mammalian meiosis than in other organisms, or that the function of this complex in facilitating DSB formation is partially fulfilled by other mechanisms. Alternatively, both the *Rad50* and *Mre11* hypomorphs might represent mutant alleles that do not alter regions important for meiosis or that do not drastically diminish the levels of protein in meiotic cells.

### F. A-T

A-T is an autosomal-recessive disorder characterized by progressive cerebellar ataxia, oculocutaneous telangiectasias, immunodeficiency, predisposition to malignancies, chromosomal breakage, incomplete sexual maturation, and infertility (296, 297). The majority of cases of A-T are caused by mutations in the *ATM* gene, which encodes a member of the phosphatidylinositol-3 kinase family of proteins that includes A-T and Rad3-related protein (ATR) and DNA-PKcs (298). Estimations approximate that 6% of A-T cases have
mutations of a different gene, the MRE11A gene (256). A-T and the NBS are similar, caused by the ATM and NBS1 genes, respectively. NBS1 is phosphorylated in an ATM-dependent manner, linking these in a common signaling pathway (271).

The ATM gene spans a 160-kb stretch of chromosome 11q and contains 66 exons, encoding a 350-kDa protein (reviewed in Ref. 299). The phosphatidylinositol 3-kinase domain is located in the C-terminal portion of the proteins, with a modulatory FRAP, ATM, TRAPP domain being upstream of this region. These semiconserved C-terminal motifs are conserved among phosphatidylinositol 3-kinase-related proteins and are essential for recruitment to sites of DNA damage (272). Under normal cellular conditions, ATM exists as an inactive dimer that, upon activation by the presence of DNA damage, cross-phosphorylates and dissociates into kinase-active monomers (300, 301). In yeast, the MRX complex localizes to DNA breaks rapidly in vivo and recruits the ATM homolog, Tel1 (302-305). Similarly, in mammalian cells, NBS1, as part of the MRN complex, may be responsible for unwinding DNA ends at the site of damage and recruiting ATM to those sites, and may also participate in the monomerization of ATM (273). The rapid onset of H2AX phosphorylation after DSB induction is likely the result of this ATM activation, and this signals the further phosphorylation of more ATM targets, including p53, CHK2, BRCA1, BLM, as well as NBS1 (201, 306–316). Thus, activation of ATM is also dependent on the phosphorylation of its downstream effectors.

ATM mRNA is highly expressed in the testis in both mouse and humans (317), whereas the protein is distributed throughout the chromatin in prophase I spermatocyte nuclei in both mice and humans (318). At the SC level, the protein is present in discrete foci on synapsed or synapsing chromosome axes in zygonema and pachynema of mouse spermatocytes (317, 319). The pattern of ATM localization on SCs is complementary to ATR (A-T- and rad3-related), which is found only on unsynapsed axial elements (317) and in a colocalized fashion with RPA (319). Not surprisingly, therefore, the three separate mouse models for A-T, which all exhibit phenotypes similar to those of A-T patients, also display male and female infertility (320–322). Spermatocytes from two of these lines show meiotic arrest at the zygote to pachytene transition and an increase in spermatocytes with bouquet morphology, as a result of disrupted synapsis and chromosome fragmentation (318, 323, 324). The seminiferous tubules of Atm−/− mutant males contain spermatogonia and Sertoli cells, but no normal spermatocytes, spermatids, or mature sperm; and the ovaries of mutant females are devoid of maturing follicles, primordial follicles, and oocytes (323). In Atm-deficient mice, RPA foci appear normally along the SCs of synapsed and synapsing chromosomes, and at sites of fragmentation of the SC, whereas ATR is found normally along unsynapsed axes, and persists on the X and Y nonpseudoautosomal regions (93, 317, 319), suggesting that early synapsis events are ATM independent. RAD51 foci, on the other hand, do not assemble normally on unpaired axial elements in leptotene spermatocytes from Atm-deficient mice, suggesting that ATM is required early in the DSB-processing pathway (91).

ATM induces p53 in response to ionizing radiation, which in turn induces p21 (a cyclin-dependent kinase inhibitor) and BAX (which activates apoptosis) (326). Atm-deficient mice display much higher basal levels of p53, p21, and BAX in the testes (91, 326). Interestingly, in Atm/p53 and Atm/p21 double mutants, the meiotic phenotype is partially rescued compared with Atm single mutants, with spermatogenesis progressing further into pachynema, but not into diplonema (91, 326). These observations point to roles for ATM as early as leptotene, in ensuring RAD51 assembly onto axial elements and in suppressing testicular p53, p21, and BAX.

It is interesting to note that the normal fertility of mice harboring hypomorphic mutations in the MRN complex fail to phenocopy the Atm mutation. This is surprising given that, in somatic cells, it is the MRN complex that recruits ATM to the site of DNA damage. The discrepancy in meiotic phenotypes suggests perhaps that ATM targeting to the SC is independent of the MRN complex, raising the question of how ATM is recruited to the MN.

In summary, the infertility seen in A-T patients is likely the result of multiple meiotic functions of the missing ATM protein, principally in ensuring RAD51 assembly onto unpaired axial elements, regulating testicular p53, p21, and BAX, and in promoting meiotic progression with proper ATR, DMC1, and RAD51 localization to SCs.

G. Fanconi anemia

Fanconi anemia is an autosomal-recessive cancer susceptibility disorder affecting all bone marrow elements and associated with multiple anomalies, including skin pigmentary changes (e.g., cafe-au-lait spots) and malformations of the heart, kidney, and limbs (e.g., aplasia of the radius; thumb deformities). Often, anemia, leukopenia, and thrombocytopenia are all present and patients also develop several types of cancers, including acute myeloid leukemias and solid tumors (327). Fanconi anemia can be caused by a mutation in any one of the nine Fanconi anemia complementation group genes: FANCA, FANCB, FANCC, FANCD1, FANCD2, FANCE, FANCF, FANCG, or FANCL. The FANCG gene has been found to be identical to the XRCC9 gene (328). FANCA, FANCC, FANCE, FANCF, and FANCG form a nuclear complex (329) that is required for the activation of FANC D2 to its active monoubiquitinated isoform, capable of localizing to sites of DNA repair (330).

The interaction and association of the various Fanconi anemia complex proteins with other proteins known to play roles in meiotic recombination and repair, their presence in meiotic cells and on meiotic chromosomes, and the existence of infertility phenotypes all imply that the Fanconi anemia pathway plays a role in meiotic recombination. Indeed, patients exhibit hypergonadotrophic hypogonadism, with its associated abnormalities of sexual development and essential lack of spermatogenesis in males (331, 332). Male Fanconi anemia patients have underdeveloped gonads and defective spermatogenesis, whereas female Fanconi anemia patients can have hypoplastic ovaries, infantile uterus, menstrual irregularities, secondary amenorrhea, and premature ovarian failure (333). Some female Fanconi anemia patients are able to conceive and maintain a pregnancy (334). Consistent infertility phenotypes are seen in mice with targeted disrup-
<table>
<thead>
<tr>
<th>Allele</th>
<th>Gene</th>
<th>MGI no.</th>
<th>Mutation type</th>
<th>Males</th>
<th>Females</th>
<th>Comments</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Atmtm1Bal</em></td>
<td>A-T mutated</td>
<td>1926343</td>
<td>TKO (replaced kinase domain)</td>
<td>Arrest between the zygotene and pachytene stages of meiotic prophase</td>
<td>Meiosis is disrupted before normal meiotic arrest in females, ovaries degenerate in the adult</td>
<td></td>
<td>323</td>
</tr>
<tr>
<td><em>Brca1tm1Arge</em> and <em>Brca1tm2.1Cxd</em></td>
<td>Breast cancer 1</td>
<td>2177209 and 2185470</td>
<td>TKO (lacks BRCT domain)</td>
<td>Normal synapsis and pachynema progression; failure to achieve diplonema; reduced sex body formation; persistent DSBs; no MLH1 accumulation and no chiasmata</td>
<td>Arrest at a zygotene-like stage in which chromosomal synapsis was not initiated</td>
<td>Persistent DSBs with reduced RAD51/DMC1 focus numbers; failure before pachynema</td>
<td>244, 344</td>
</tr>
<tr>
<td><em>Dmc1 htm1Jcs</em> and <em>Dmc1 htm1Tkm</em></td>
<td>Disrupted meiotic cDNA 1 homolog</td>
<td>2176331 and 2179026</td>
<td>TKO (null)</td>
<td>Arrest at a zygotene-like stage in which chromosomal synapsis was not initiated</td>
<td>Arrest at metaphase I</td>
<td>Pre-pachytene disruption of meiosis results in ovaries that are devoid of oocytes</td>
<td>98, 127</td>
</tr>
<tr>
<td><em>Exo1tm1Wed</em></td>
<td>Exonuclease 1</td>
<td>2653925</td>
<td>TKO (I-nuclease domain del)</td>
<td>Arrest at metaphase I</td>
<td>Nondisjunction at first meiotic division</td>
<td>Functions downstream of the MMR pathway</td>
<td>345</td>
</tr>
<tr>
<td><em>Fanca tm1Wong</em></td>
<td>Fanconi anemia, complementation group A</td>
<td>2674094</td>
<td>TKO (partial mRNA detected)</td>
<td>Massive loss of spermatogenic cells (no details of stage of loss)</td>
<td>Large depletion of oocytes during embryogenesis; remaining oocytes are not viable</td>
<td>Fertile</td>
<td>333</td>
</tr>
<tr>
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<td>2183473</td>
<td>TKO (null)</td>
<td>Reduced fertility</td>
<td>Reduced fertility</td>
<td>Also known as Xrc9</td>
<td>337</td>
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<tr>
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<td>2673422</td>
<td>TKO (null)</td>
<td>Reduced fertility</td>
<td>Reduced fertility (more severe than in males)</td>
<td>Fertile</td>
<td>15, 347, 348</td>
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<td><em>Fkbp6tm1Pngr</em></td>
<td>FK506 binding protein 6</td>
<td>2663987</td>
<td>TKO (null)</td>
<td>Reduced fertility</td>
<td>Reduced fertility</td>
<td>Functions downstream of the MMR pathway</td>
<td>345</td>
</tr>
<tr>
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<td>2156048</td>
<td>TKO (null)</td>
<td>Reduced fertility</td>
<td>Reduced fertility</td>
<td>Functions downstream of the MMR pathway</td>
<td>345</td>
</tr>
<tr>
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<td>Heat shock protein 2</td>
<td>2447605</td>
<td>TKO (null)</td>
<td>Reduced fertility</td>
<td>Reduced fertility</td>
<td>Functions downstream of the MMR pathway</td>
<td>345</td>
</tr>
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<td><em>Mei2–5</em></td>
<td>Meiosis defective 2.5</td>
<td>3050560</td>
<td>CIM (EMS)</td>
<td>Arrest at pachynema stage; some postmeiotic spermatid present</td>
<td>Arrest at pachynema stage; some postmeiotic spermatid present</td>
<td>Functions as a heterodimer with MLH3 at meiotic nodules</td>
<td>99, 100</td>
</tr>
<tr>
<td><em>Mei2–7</em></td>
<td>Meiosis defective 2.7</td>
<td>3050584</td>
<td>CIM (EMS)</td>
<td>Arrest at pachynema stage; some postmeiotic spermatid present</td>
<td>Arrest at pachynema stage; some postmeiotic spermatid present</td>
<td>Functions as a heterodimer with MLH3 at meiotic nodules</td>
<td>99, 100</td>
</tr>
<tr>
<td><em>Mei7</em></td>
<td>Meiosis defective 4</td>
<td>3050548</td>
<td>CIM (ENU)</td>
<td>Disruption before first meiotic division</td>
<td>Disruption before first meiotic division</td>
<td>Functions as a heterodimer with MLH3 at meiotic nodules</td>
<td>99, 100</td>
</tr>
<tr>
<td><em>Mlh1tm1Bal</em> and <em>Mlh1tm1Lish</em></td>
<td>MutL homolog-1</td>
<td>3050519 1858054 and 1857946</td>
<td>TKO (null) and TKO (null)</td>
<td>Early prophase I disruption, Premature desynapsis when SC breaks down at diplonema</td>
<td>Early prophase I disruption, Premature desynapsis when SC breaks down at diplonema</td>
<td>Functions as a heterodimer with MLH3 at meiotic nodules</td>
<td>99, 100</td>
</tr>
<tr>
<td>Gene</td>
<td>Allele</td>
<td>Mutation type</td>
<td>Male</td>
<td>Female</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Mlh3</td>
<td>Mlh3tm1Lpkn</td>
<td>TKO (null)</td>
<td>Functions as a heterodimer with MLH1 at meiotic nodules, but functions independently of MLH1 at othersites</td>
<td>Functions as a heterodimer with MLH1 at meiotic nodules, but functions independently of MLH1 at othersites</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mre11a</td>
<td>Mre11atm1Jpt</td>
<td>TKO (loss of 75 amino acids)</td>
<td>Fertile</td>
<td>Severely reduced fertility</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Msh4</td>
<td>Msh4tm1Wed</td>
<td>TKO (null)</td>
<td>Functions as a heterodimer with MSH5 at meiotic nodules</td>
<td>Functions as a heterodimer with MSH5 at meiotic nodules</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Msh5</td>
<td>Msh5tm1Htr</td>
<td>TKO (ATPase mut)</td>
<td>Failure at zygotene to pachytene transition; incomplete synapsis</td>
<td>Failure at zygotene to pachytene transition; incomplete synapsis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pms2</td>
<td>Pms2tm1Lisk</td>
<td>TKO (null)</td>
<td>Fertile</td>
<td>Does not appear to act directly in recombination events</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rec8L1</td>
<td>Rec8L1mei8 and Rec8L1tm1Mjm</td>
<td>TKO (null)</td>
<td>Abnormal SC formation between sister chromatids rather than between homologous chromosomes</td>
<td>Leptotene chromosomes are seen at e16.5–e18.5 but normal pachytene nuclei are never seen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smc1l2</td>
<td>Smc1l2tm1Jess</td>
<td>TKO (null)</td>
<td>Shortened SCs; sister chromatid cohesion is impaired; telomere attachment is impaired, and crossover complexes are not seen; midpachytene arrest</td>
<td>Similar defects to those seen in male meiosis; unlike in males, oocytes undergo meiosis I to dictyate arrest, but defective cohesion results in massive aneuploidy during meiotic divisions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spo11</td>
<td>Spo11tm1Rdco and Spo11/H20851/SK</td>
<td>TKO (null) and TKO (null)</td>
<td>Normal meiosis through leptotene stage; impaired synapsis (about 5–10% of nuclei); lack of RAD51 and DMC1 foci; partial rescue by induction of DSBs by irradiation</td>
<td>Oocyte numbers at birth are 60% of normal, with a rapid decline to 10–20% of normal by d 8 postpartum; females are sterile despite the presence of some antral follicles</td>
<td></td>
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<tr>
<td>Sycp1</td>
<td>Sycp1tm1Aps</td>
<td>TKO (null)</td>
<td>Normal meiosis through leptotene stage; impaired synapsis (about 5–10% of nuclei); lack of RAD51 and DMC1 foci; partial rescue by induction of DSBs by irradiation</td>
<td>Normal meiosis through leptotene stage; impaired synapsis (about 5–10% of nuclei); lack of RAD51 and DMC1 foci; partial rescue by induction of DSBs by irradiation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sycp3</td>
<td>Sycp3tm1Hoog</td>
<td>TKO (null)</td>
<td>Defective chromosomal segregation leading to aneuploidy; decreased fertility with age; embryonic loss due to aneuploidy</td>
<td>Defective chromosomal segregation leading to aneuploidy; decreased fertility with age; embryonic loss due to aneuploidy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Top3b</td>
<td>Top3btm1Jcw</td>
<td>TKO (null)</td>
<td>Increased incidence of mitotic aneuploidy, particularly aneuploidy, resulting from improper resolution of chromosomal transient associations</td>
<td>Increased incidence of mitotic aneuploidy, particularly aneuploidy, resulting from improper resolution of chromosomal transient associations</td>
<td></td>
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</tr>
</tbody>
</table>
tions of Fanc (Fanconi anemia complementation group C gene) (335, 336), mice with targeted disruption of Fancd2 (333), and Fancg knockout mice (337).

In humans, protein expression of FANCD2 is highest in maturing spermatocytes and fetal oocytes, and in the germinal center of the spleen, tonsil, and lymph nodes (338). Activated FANCD2 protein colocalizes with BRCA1, in ionizing radiation-induced nuclear foci, and in SCs of meiotic chromosomes (330). FANCD2 stains the unsynapsed axial elements of the X- and Y-chromosomes of late pachytene and early diplonete mouse spermatocytes and colocalizes with BRCA1 in these regions (330). The proteins of this nuclear Fanconi anemia complex also form a complex with TOPOIIα, RPA, and BLM (206). FANCA itself directly interacts with BRCA1, BRG1 (339), sorting nexin 5, and IκK2 (Iκ kinase-2), as well as colocalizing to nuclear foci with xeroderma pigmentosum complementation group F and nonerythroid aⅡl spectrin (reviewed in Ref. 333). FANCA expression is found at a high level in pachytene spermatocytes, and Fanca<sup>−/−Hsc</sup> homozygous male mice exhibit an elevated frequency of mispaired meiotic chromosomes in pachynema (333).

Although the human FANCD1 and FANCB genes have not yet been cloned, FANCD1- and FANCB-related Fanconi anemia has been shown to be caused by biallelic germline mutations in BRCA2, which do not lead to the typical breast and ovarian cancer-susceptibility phenotypes (340). FANCG binds to BRCA2, and both have been found to colocalize to nuclear foci in human cells together with RAD51 (341). Interestingly, the majority of the BRCA2 mutations that lead to Fanconi anemia remove the C-terminal RAD51-binding domain (329).

VI. Perspectives

Recombination is a common feature of most sexually reproducing organisms, but has evolved to fulfill more than the simple function of segregating chromosomes into haploid progeny cells. Thus in mammals, and possibly in other organisms, recombination is an essential component of homology searching and recognition. These functions are more easily addressed in lower eukaryotes, as testified to by decades of elegant genetic studies in yeast, flies, fungi, worms, and plants, but the unique challenges posed by multicellular mammals and the impact of noncell autonomous factors on meiosis in mammalian germ cells have reinvigorated research in mammalian gametogenesis (Table 2). This is especially relevant when considering the high rate of meiotic errors reported for human female meiosis (5), and the diversity of gene mutations that result in meiotic phenotypes in mice (Table 2) increasing the urgency for research in this area. This increased error rate may be the result of increased complexity associated with larger genome organisms or may be a result of reduced stringency of meiotic checkpoints. Alternatively, or in addition, the higher rates of errors in human meiosis may be the result of environmental toxins, as indicated by studies of Hunt et al. (342), who showed that exposure of mice to Bisphenol A resulted in severe oocyte aneuploidy. Furthermore, it is possible that the declining sperm counts reported for men in Western countries (343) may reflect, at least in part, a reduced ability to progress through meiosis due to activation of the meiotic checkpoints discussed earlier (although clearly there may be other etiological bases for these reduced sperm counts). Those sperm that are produced in men with lower sperm counts are still usually viable, however, again pointing to the selective removal of aberrant sperm and the retention of healthy (haploid) sperm. Thus, studies of mouse mutants for the repair proteins described in this review (Table 2), together with further analysis of toxicological effects on mammalian meiosis, will help in our understanding of the etiological basis for meiotic dysfunction in humans. On the other hand, the identification of meiosis-specific proteins essential for the production of germ cells may also allow the generation of new contraceptive agents that do not disrupt the endocrinology or sexual behavior of the recipients.

Acknowledgments

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