0163-769X/06/$20.00/0
Printed in U.S.A.

Endocrine Reviews 27(4):398–426

doi: 10.1210/er.2005-0017

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

P. E. Cohen, S. E. Pollack, and J. W. Pollard

Center for the Study of Reproduction and Women's Health and Departments of Molecular Genetics (P.E.C.), Obstetrics, Gynecology and Women's Health (P.E.C., S.E.P., J.W.P.), and Developmental and Molecular Biology (J.W.P.), Albert Einstein College of Medicine, Bronx, New York 10461

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 synapsis, 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)

First Published Online March 16, 2006

Abbreviations: A-T, Ataxia-telangiectasia; ATLD, A-T-like disorder; ATM, A-T-mutated; ATR, A-T-related; BLM, BS mutated; BS, Bloom syndrome; CDK, cyclin-dependent kinase; DSB, double-strand break; DSBR, DSB repair; e, embryonic day; FKBP6, FK506 binding protein-6; MCM, minichromosome maintenance; MI, first meiotic division; MLH, MutL homolog; MMR, mismatch repair; MN, meiotic nodule; MPF, maturation-promoting factor; MSH, MutS homolog; NBS, Nijmegen breakage syndrome; pre-RC, prereplicative complex; Rb, retinoblastoma; RPA, replication protein A; SC, synaptonemal complex; SDSA, synthesis-dependent strand annealing; SMC, structural maintenance of chromosome; SYCP, SC protein; TOPOIIIα; topoisomerase IIIα; WRN, Werner syndrome-associated.

I. Introduction

GERM CELL FORMATION in mammals requires a specialized cell division known as meiosis. Originally postulated by August Weissman in 1887, and soon after demonstrated by Edouard van Beneden (reviewed in Ref. 1), meiosis is a double-division cycle that is preceded by only one replication event to produce haploid gametes for fertilization. Meiosis is a common feature of all sexually reproducing organisms, and, although the dynamics of this process differ markedly between species (and sexes), the regulation of meiotic events is highly conserved across plant and animal species.

The defining event of meiosis is prophase I, during which homologous (maternal and paternal) chromosome pairs locate one another and become physically connected in a process termed "synapsis." Two features of prophase I ensure that homologous chromosomes remain tethered together until the appropriate time during metaphase I. The first is a tripartite protein structure called the "synaptonemal complex" (SC), which consists of two lateral elements (aligning each homolog) and a central element that holds sister chromatids together and maintains the homologs in a tethered state. This structure appears early in prophase I and persists until the maturation of recombination events (crossovers) into chiasmata, the second feature that ensures persistent pairing before metaphase I. The formation of crossovers and the appearance of the SC are very much intertwined events and are largely conserved among meiotic species. Interestingly, however, *Drosophila melanogaster* males, *Schizosaccharomyces pombe*, *Tetrahymena thermophila*, and *Aspergillus nidulans*, all of which undergo meiosis, do not assemble SCs and

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 *leptonema* (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: zyogotene), 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 synapsis. 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 pachytene 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

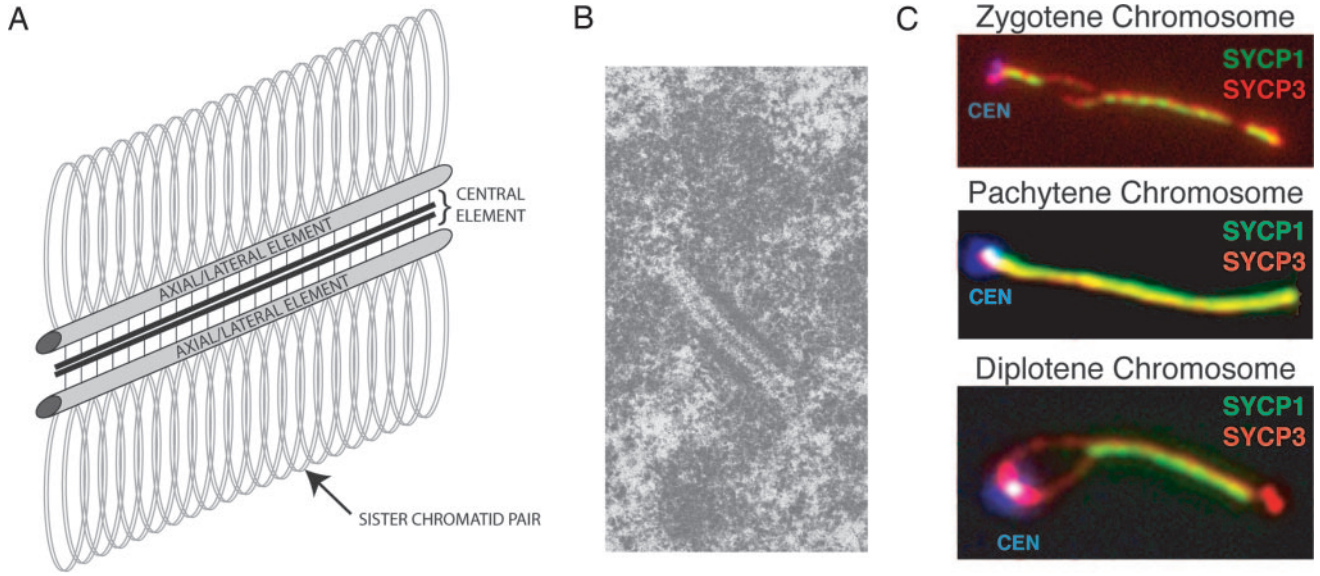

FIG. 1. SC dynamics through prophase I. A, *Cartoon* of SC structure, showing loops of chromatin (paired sister chromatids) extending out from the SC "Core", made up of the two axial/lateral elements and the central element. B, An electron micrograph showing a stretch of SC *in situ* in a mouse spermatocyte. 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 synapsis has occurred, the overlap of the *red and green* colors produces a *yellow* signal. CEN, Centromere.

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 nodule (MN) components and an absence of SYCP1 recruitment at zygonema. However, transverse filaments, 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 FKBP6 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 chromosome 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. *Smc1*$\beta^{-/-}$ 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 synapsis 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 synapsis 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 *Smc1*$\beta^{-/-}$ 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 22–23

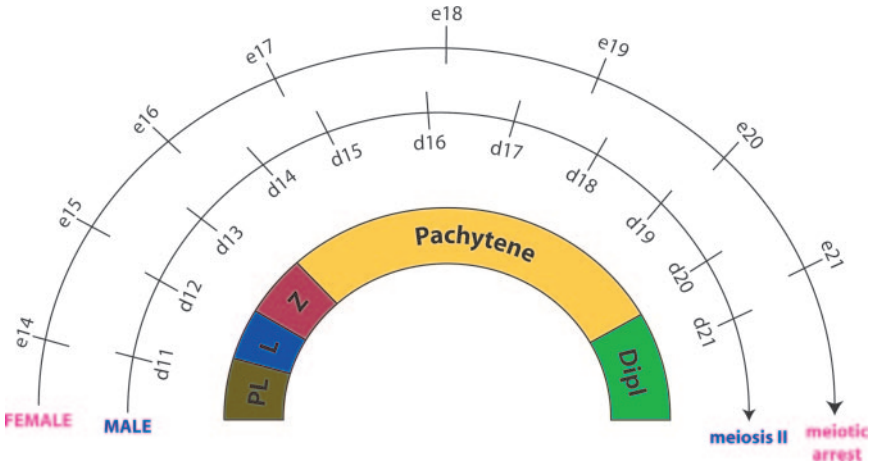

FIG. 2. Timing of prophase I events in male and female mice. e, Embryonic day; d, days postpartum; PL, pre-leptonema; L, leptonema; Z, zygonema; Dipl, diplonema. [Data adapted with permission from Refs. 18 and 19.]

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 I *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 leptonema of prophase I (37, 38). In male mice, the failure to induce DSBs is paired with a failure of chromosome condensation and synapsis in leptonema 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

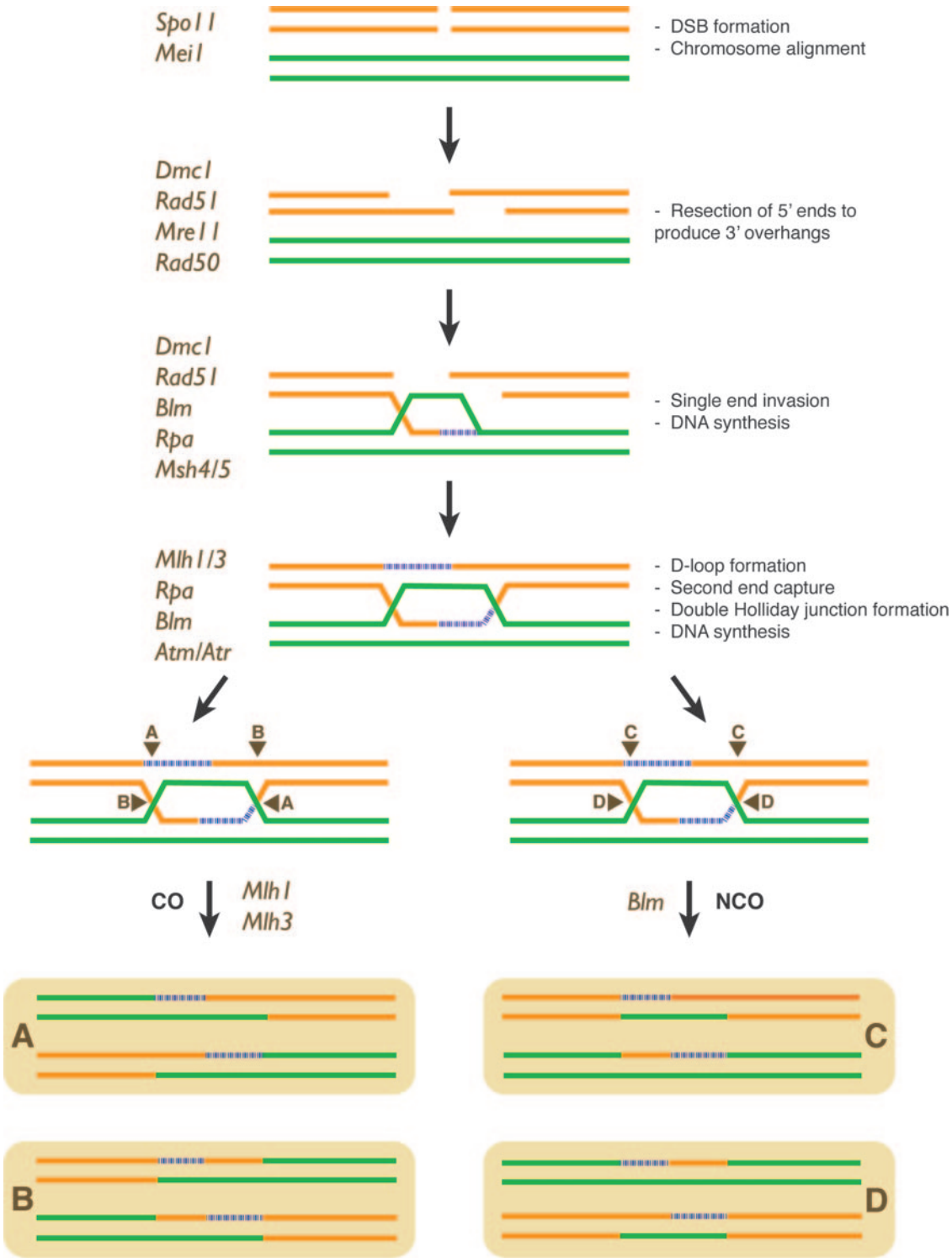

FIG. 3. Summary of the current model for reciprocal recombination according the double Holliday junction model for DSBR. For simplicity, one (two-stranded) sister chromatid of each homologous chromosome is shown (one homolog is *green*; the other is *orange*). A DSB event in the *orange* chromatid results 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* (BS mutated) and *Wrn* (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 G_2 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 G_1 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 G_1 (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 G_1 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

TABLE 1. Meiotic phenotypes of null mutants in cell cycle regulatory −proteins

Cell cycle regulator	Phenotype		Ref.
	Male	Female	
Cyclin			
D1 (*Ccnd1*)	F	F	68
D2 (*Ccnd2*)	F	IF[a]	69
D3 (*Ccnd3*)	F	F	68
No D	EL	EL	68
E1 (*Ccne1*)	F	F	60
E2 (*Ccne2*)	IF, abnormal spermatogenesis	F	60
No E	EL	EL	60
A1 (*Ccna1*)	IF, pre-MI block	F	50
A2 (*Ccna2*)	EL	EL	63
Cyclin-dependent kinases			
CDK1 (*Cdk1*)	EL	EL	57
CDK2 (*Cdk2*)	IF, pachytene arrest	IF, pachytene arrest	61
CDK4 (*Cdk4*)	IF, abnormal spermatogenesis	IF[b]	70
CDK6 (*Cdk6*)	F	F	67
No CDK4 or 6	EL	EL	53
Cyclin-dependent kinase inhibitors			
$P27^{kip1}$ (*Cdkn1b*)	F, hyperplastic testis	IF[b]	75
$P57^{kip2}$ (*Cdkn1c*)	PL	PL	57
$P21^{WAF-1}$ (*Cdkn1a*)	F	F	57
INK4a (p16) (*Cdkn2a*)	F	F	77
INK4b (p15) (*Cdkn2b*)	F	F	77
INK4c (p18) (*Cdkn2c*)	F	F	77
INK4d(p19) (*Cdkn2d*)	Testicular atrophy (F)	F	77
No INK4c or 4d	IF, poor meiotic progression, enhanced cell death, no sperm	F	77

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

through S phase of the cell cycle. At the end of S phase, cyclin A binds to CDK1 for transit into G_2, 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 and Cip/Kip families (57). Cyclin D/CDK4 and CDK6 complexes are subjected to negative regulation by both the INK4 (a–d) and the Cip/Kip ($p21^{Cip/Waf1}$, $p27^{Kip1}$, and $p57^{Kip2}$), families of inhibitors (*Cckn1* and *2*), whereas cyclin E/CDK2 complexes are negatively controlled only by the Cip/Kip family (58). 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 (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 premeiotic 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

die during embryogenesis (67), whereas those lacking a single cyclin D are viable (68). Cyclin D2 (*Ccnd2*) 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 spermatogonia and spermatocytes but exhibiting normal oogeneis 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 *Rb1* result in embryonic lethality (71) because the absence of Rb during meiosis cannot be analyzed without the creation of a conditional *Rb1* 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). $P27^{kip1}$ 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 *Cdkn2c* 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 spermatozoa 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 $p27^{kip1}$ 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 through prophase I including normal chromosome synapsis, 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/R*ad3-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 Nijmengen 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 midpachynema (92), to avoid the inevitable aneuploidy that would follow improper chromosome synapsis. 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 (MSH)4 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 chiasmata 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 synapsis (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 synapsis. 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 $Dmc1^{-/-}$ males show apparently normal progression through leptonema and early zygonema, with no complete pachytene-stage cells being observed (98, 127). No attempts at synapsis 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 zygotene stages, but no diminution of RAD51 focus frequency (that would be indicative of a progression through to pachynema) is observed (98).

In $Dmc1^{-/-}$ 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 $Dmc1^{-/-}$ 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 $Dmc1^{-/-}$ 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 apopototic elimination (37). Interestingly, however, some oocytes from $Spo11^{-/-}$ 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, few or no oocytes from $Dmc1^{-/-}$ females remain to this point; instead they die by diplotene. 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 $Spo11^{-/-}Dmc1^{-/-}$ double-mutant females are a phenocopy of oocytes from $Spo11^{-/-}$ 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 checkpoint to which oocytes from *Dmc1* null females succumb.

By contrast with the situation in females, male meiocytes are wholly dependent on DSB formation and recombination to progress through prophase I. Spermatocytes from *Spo11*$^{-/-}$ and from *Dmc1*$^{-/-}$ males show similar meiotic phenotypes, 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 cisplatin-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 identified 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 homeologous recombination, which could result in potentially lethal chromosomal imbalance. Components of the MMR pathway are highly conserved throughout evolution 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 hereditary polyposis colorectal cancer, characterized by early-onset colorectal cancer, along with cancers of the endometrium, 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 advantage 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 resynthesis (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 discrimination. 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 heterodimers, 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 mutational 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 recombination 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. elegans*, and *Mus musculus* (23, 42, 159–162). For these purposes, 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 protein and forming complete axial elements. The axial elements appear somewhat elongated, however, and stain less intensely for SYCP3 (P. E. Cohen, unpublished observations). Most significantly, chromosome synapsis 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 leptonema 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 synapsis (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 synapsis 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 desynapsis 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 leptonema (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

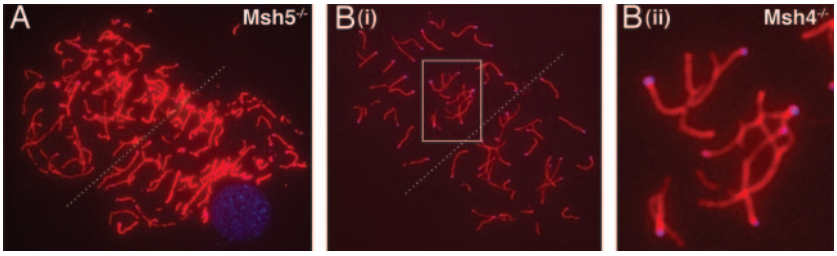

FIG. 4. Examples of mispairing in spermatocytes from *Msh5*$^{-/-}$ (A) and *Msh4*$^{-/-}$ (B) males. Most of the chromosomes remain unsynapsed through to pachynema in both mutants, and any synapse that does occur is often between nonhomologous partners or involves many different chromosomes. Examples of mispairing are shown in the *box* in panel B(i) and are magnified in panel B(ii). In general, more synapsis 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.

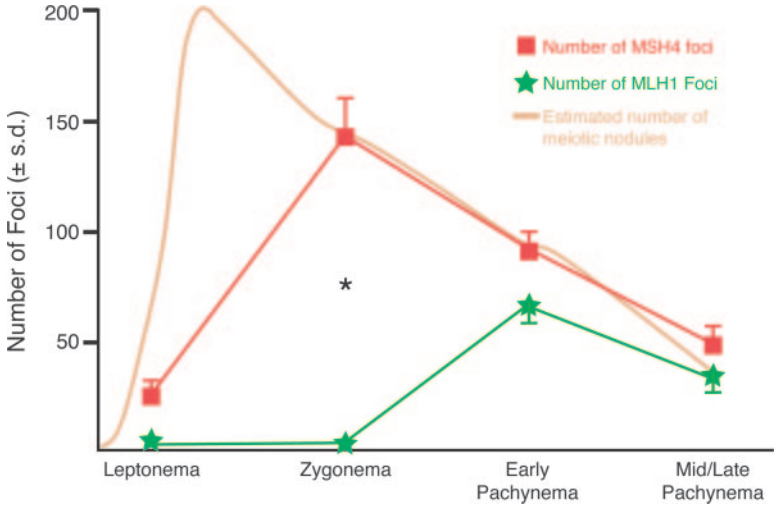

FIG. 5. Correlation of MSH4 and MLH1 focus frequency with DSB processing events. Graphical representation of immunoreactive foci for MSH4 (*orange*) and MLH1 (*green*) through prophase I in mouse spermatocytes. [Data adapted from Ref. 97 and P. E. Cohen, unpublished.] The estimated number of MNs, representing 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).

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).

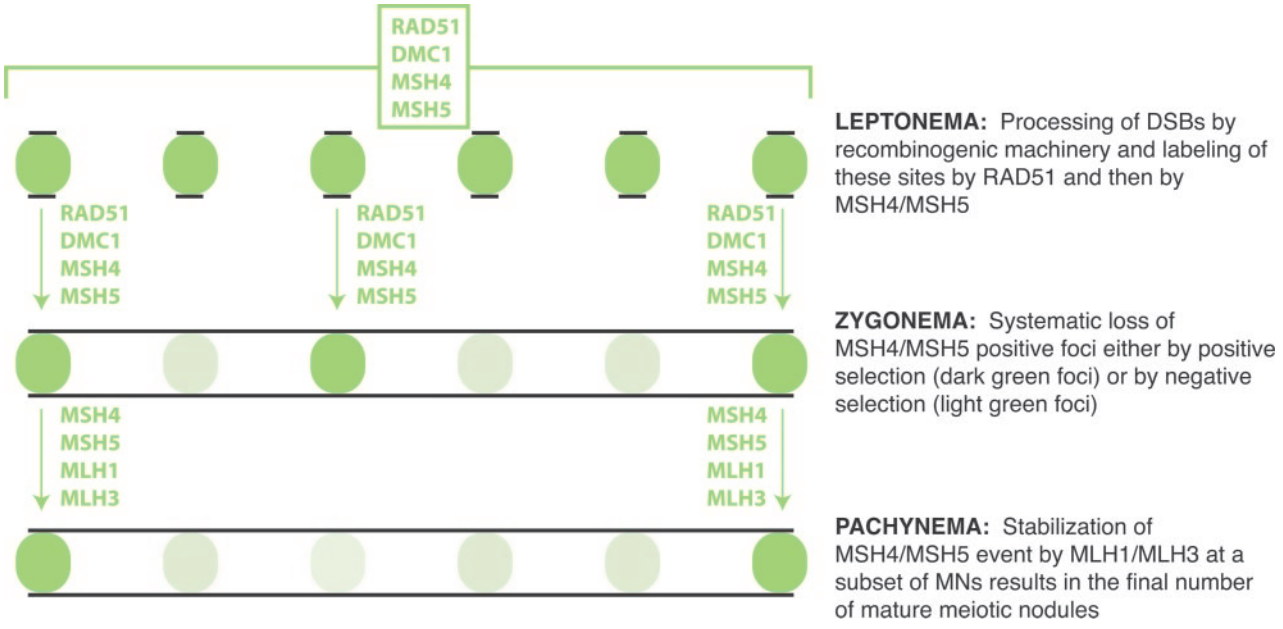

FIG. 6. Sequential selection of MSH4–MSH5 sites for further processing along a DSBR pathway leading to reciprocal recombination. *Cartoon* depicts the progressive selection of DSB sites for further processing down a route that results in crossover formation and is demonstrated by the persistence of MSH4 and MSH5 at these sites.

C. The RecQ helicases, Bloom syndrome (BS) mutated (BLM) and Werner syndrome-associated (WRN)

BS is an autosomal-recessive disorder characterized by growth deficiency, dermatological sun-sensitive pigmentary 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 *Blm* 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). *Blm* 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 IIIα (TOPOIIIα), RAD51, and MLH1, whereas other regions direct binding of BRCA1, ATM, p53, FEN1, NBS1/MRE11, MSH2, and MSH6 (191–200), to name a few. The interaction with ATM is functionally important because ATM may at least partially phosphorylate the helicase (201).

All of these interactions point to a role for BLM in homologous recombination and the maintenance of DNA integrity. Accordingly, BS cells exhibit profound genomic instability, characterized by excessive chromosomal breakage, hyperrecombination, and increased mutation frequency, and are hypersensitive to genotoxic agents (202–204). A defining feature of BS cells is an elevated frequency of sister chromatid exchanges that arise from crossing over of chromatid arms during homologous recombination resulting from damaged replication forks. Recombination in mitotic cells can result in loss of heterozygosity and is thus implicated in the tumorigenic process. BLM, together with TOPOIIIα, can effect the resolution of a recombination intermediate containing a double Holliday junction, resulting in nonreciprocal resolution (205). Whether such structures exist in mitotic cells remains to be seen, but would explain many of the phenotypic observations made in BS cells and in murine $Blm^{-/-}$ cell lines.

Given the antirecombinogenic role of BLM in somatic cells, it is not surprising to find that RecQ helicases are involved in meiosis in many organisms. BLM is also associated with other proteins that play roles in meiotic recombination, including proteins of the nuclear Fanconi anemia complex (see below) and TOPOIIIα (206). In yeast, mutants of the homologs of *BLM* and *TOPOIII*α, *sgs1* and *top3*, respectively, each display sporulation defects that are lost by mutations that abolish meiotic recombination (207, 208). The *sgs1* mutation leads to meiotic chromosome missegregation as a result of increased axial 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 leptonema (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 TOPOIIIα colocalize to human nuclei, can be coimmunoprecipitated from human cell extracts, and bind to each other *in vitro* (193). BLM and TOPOIIIα also colocalize substantially with one another on human meiotic spermatocyte spreads (192). In meiotic human spermatocyte spreads, TOPOIIIα is found on axial or lateral elements during zygonema, before and after synapsis, continues to be associated

with lateral elements as zygonema proceeds, and is concentrated at sites of synapsis (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, sc 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). *Brca1*-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).

Brca1 expression levels increase during late pachynema and diplonema of prophase I (239). In human spermatocytes, BRCA1 colocalizes 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 spermatogonia appeared normal (compared with *p53* null mice), the meiotic spermatids and postmeiotic spermatozoa 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 $Brca1\Delta^{11/\Delta 11}p53^{+/-}$, 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 $Brca1^{\Delta 11/\Delta 11}p53^{+/-}$ males, chromosome synapsis occurs normally, and the cells progress through to 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 hypermorphic. 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 synapsis 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 endonuculease 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 Zn^{2+}-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 Zn^{2+}-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 the 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 functionally, 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

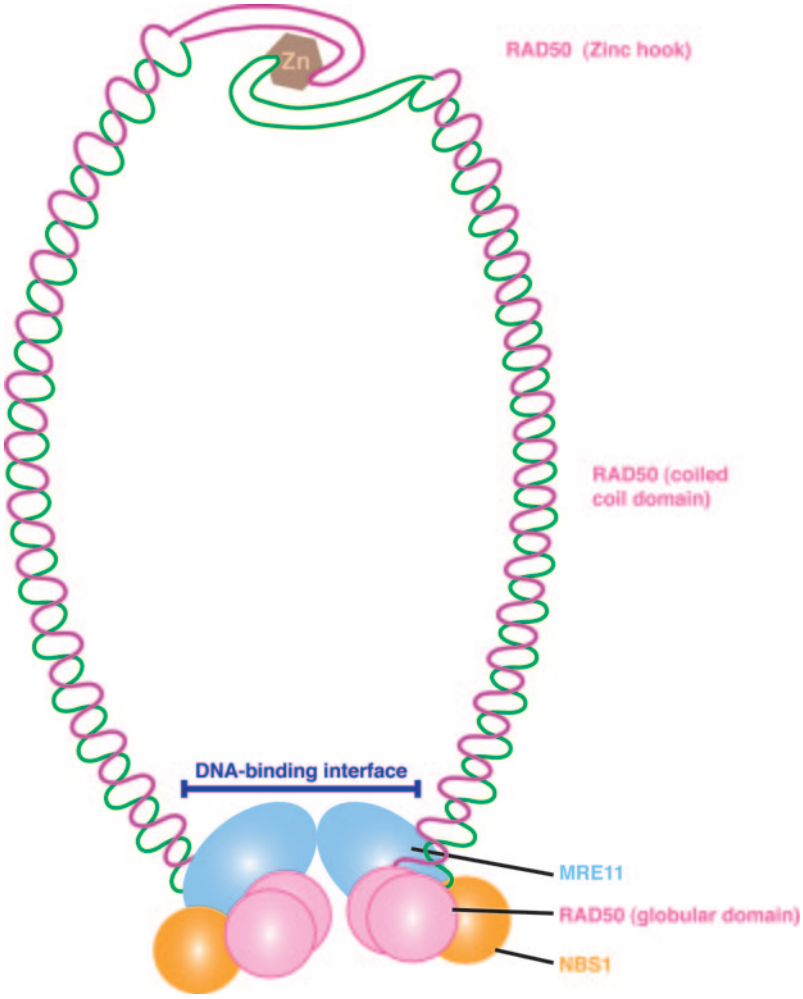

FIG. 7. Postulated structure of the MRE11-RAD50-NBS1 complex. RAD50 consists of two Walker (A and B) motifs (*pink circles*) that together make up a bipatartite ATP-binding cassette. These motifs lie at either end of the protein and are separated by a long chain of heptad coiled-coil repeats (*pink and green*) which, when in their appropriately coiled configuration, bring the two Walker motifs into close apposition. The center of the coiled-coil domain contains a conserved CXXC motif that forms a zinc-binding dimerization domain (the "zinc hook"). RAD50 associates slightly proximal to the bipartite ATPase/Walker domain. MRE11 (*blue ovals*) contains four N-terminal phosphodiesterase motifs, along with the potential NBS1- binding sites, and two DNA-binding domains at the C terminus, together with the RAD50-binding site and MRE11 dimerization domain. NBS1 is shown in *green* and contains two phosphoprotein-binding domains at its N terminus and a putative MRE11 interaction site at the C terminus. This loop arrangement represents one of several ways in which the MRN complex can associate with DNA and recruit other proteins (such as ATM) to this site. Other potential configurations would involve a linear array of MRN, with one DNA-binding interface at one DNA strand linking, via the zinc hook, to another MRN complex interfacing with another DNA strand. Scanning force microscopy predicts other arrangements in which multiple MRN complexes could bind to the ends of a DSB and could thereby provide a tether between a broken end and the intact sister chromatid by virtue of the zinc hook mechanism. [Adapted from Refs. 257 and 262–268.]

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* ($Mre11^{ATLD}$) or *Nbs1* ($Nbs\Delta^{B}$), 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* ($Rad50^{S}$) also fail to elicit a meiotic phenotype (294, 295). Ovaries and testes appear normal in $Rad50^{S/S}$ 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 the *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-PK_{cs} (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 zygotene 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 leptonema, 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.*, café-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 FANCD2 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 spermatogonia in males (331, 332). Male Fanconi anemia patients have underdeveloped gonads and defective spermatogenesis, whereas female Fanconi anemia patients can have hypoplastic ovaries, infantile uteri, 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 2. Summary of mouse mutants exhibiting prophase I defects

Allele	Gene	MGI no.	Mutation type	Males	Females	Comments	Ref.
Atm^{tm1Bal}	A-T mutated	1926343	TKO (replaced kinase domain)	Arrest between the zygotene and pachytene stages of meiotic prophase	Meiosis is disrupted before normal meiotic arrest in females, ovaries degenerate in the adult		323
Brca1^{tm2Arge} and *Brca1*$^{tm2.1Cxd}$	Breast cancer 1	2177209 and 2182470	TKO (lacks BRCT domain)	Normal synapsis and pachynema progression; failure to achieve diplonema; reduced sex body formation; persistent DSBs; no MLH1 accumulation and no chiasmata	Fertile	These phenotypes are only observed in mice that are heterozygous for a mutation in *Trp53* because *Brca1*$^{-/-}$ alone results in embryonic lethality	244, 344
Brca2^{tm1Mak}*.Tg(hBRCA2)*	Breast cancer 2	1930617	TKO (null) plus TG replacement	Persistent DSBs with reduced RAD51/DMC1 focus numbers; failure before pachynema	Loss of many oocytes soon after birth; reduced fertility	Low expression of transgene in the testis	249
Dmc1 h^{tm1Jcs} and *Dmc1 h*^{tm1Tkm}	Disrupted meiotic cDNA 1 homolog	2176331 and 2179026	TKO (null)	Arrest at a zygotene-like stage in which chromosomal synapsis was not initiated	Pre-pachytene disruption of meiosis results in ovaries that are devoid of oocytes	RecA homolog that functions together with RAD51	98, 127
Exo1^{tm1Wed}	Exonuclease 1	2653925	TKO (I-nuclease domain del)	Arrest at metaphase I	Nondisjunction at first meiotic division	Functions downstream of the MMR pathway	345
Fanca^{tm1Wong}	Fanconi anemia, complementation group A	2674094	TKO (partial mRNA detected)	Massive loss of spermatogenic cells (no details of stage of loss)	Large depletion of oocytes during embryogenesis; remaining oocytes are not viable		333
Fancc^{tm1Mab}	Fanconi anemia, complementation group C	1858040	TKO (null)	Meiotic failure	Meiotic failure		335
Fancd2^{tm1Hou}	Fanconi anemia, complementation group D2	2673422	TKO (null)	Failure of complete/appropriate synapsis; failure to progress to pachynema; many mispaired chromosomes	Loss of oocytes before birth indicative of arrest before dictyate		346
Fancg^{tm1Faw}	Fanconi anemia, complementation group G	2183473	TKO (null)	Reduced fertility	Reduced fertility (more severe than in males)	Also known as *Xrcc9*	337
Fkbp6^{tm1Pngr}	FK506 binding protein 6	2663987	TKO (null)	Pachytene arrest	Fertile		15
H2afx^{tm1Nus}	H2A histone family, member X	2156048	TKO (null)	Pachytene arrest of spermatocytes; failure of sex body formation; failure of MLH1 accumulation at meiotic nodules	Fertile		347, 348
Hspa2^{tm1Dix}	Heat shock protein 2	2447605	TKO (null)	Normal SC formation and pachytene progression: loss of spermatocytes before metaphase I	Fertile		349
Mei1^{m1Jcs}	Meiosis defective 1	2176973	CIM (ENU)	Leptotene/zygotene arrest; defective synapsis	Ovaries lack oocytes; presumed	Epistatic to *Dmc1*	213, 325
mei11b	Meiosis defective 11b	3050511	CIM (ENU)	Fertile	Oocytes with normal morphology but resumption of meiosis fails (GV breakdown fails)		169
mei2–5	Meiosis defective 2.5	3050560	CIM (EMS)	Arrest at pachytene stage; some postmeiotic spermatids present	Fertile		169
mei2–7	Meiosis defective 2.7	3050584	CIM (EMS)	Arrest at pachytene stage; some postmeiotic spermatids present	Fertile		169
mei4	Meiosis defective 4	3050548	CIM (ENU)	Disruption before first meiotic division	Disruption before first meiotic division		169
mei7	Meiosis defective 7	3050519	CIM (ENU)	Early prophase I disruption	Fertile		169
Mlh1^{tm1Rak} and *Mlh1*^{tm1Lisk}	MutL homolog-1	1858054 and 1857946	TKO (null) and TKO (null)	Premature desynapsis when SC breaks down at diplonema	Congression failure and nondisjunction at the first meiotic division	Functions as a heterodimer with MLH3 at meiotic nodules	99, 100

TABLE 2. *Continued*

Allele	Gene	MGI no.	Mutation type	Males	Females	Comments	Ref.
Mlh3^{tm1Lpkn}	MutL homolog-3	2384218	TKO (null)	Premature desynapsis when SC breaks down at diplonema	Congression failure and nondisjunction at the first meiotic division	Functions as a heterodimer with MLH1 at meiotic nodules, but functions independently of MLH1 at other sites	148
Mre11a^{tm1Jpt}	Meiotic recombination 11 homolog A (*S. cerevisiae*)	3027036	TKO (loss of 75 amino acids)	Fertile	Severely reduced fertility with occasional offspring; ovarian dysgenesis; phenotype is apparently due to postmeiotic events	Listed in text as Mre11^{ATLD}, recapitulating human ATLD disorder	255
Msh4^{tm1Wed}	MutS homolog-4	2179034	TKO (null)	Failure at zygotene to pachytene transition; incomplete synapsis	Failure at zygotene to pachytene transition; incomplete synapsis	Functions as a heterodimer with MSH5	97
Msh5^{tm1Htr} and *Msh5*^{tm1Rak}	MutS homolog-5	2179022 and 1858057	TKO (ATPase mut) and TKO (null)	Failure at zygotene to pachytene transition; incomplete synapsis	Failure at zygotene to pachytene transition; incomplete synapsis	Functions as a heterodimer with MSH4	96, 164
Pms2^{tm1Lisk}	Postmeiotic segregation 2 (MutL homolog)	1857947	TKO (null)	Early prophase I loss before metaphase I, with some cells escaping this loss to produce nonviable spermatozoa	Fertile	Does not appear to act directly in recombination events	102, 170
Rec8L1^{mei8} and *Rec8L1*^{tm1Mjm}	Rec8-like 1	3512754 and 3583574	CIM (EMS) TKO (null)	Abnormal SC formation between sister chromatids rather than between homologous chromosomes	Leptotene chromosomes are seen at e16.5-e18.5 but normal pachytene nuclei are never seen		19, 20
Smc1l2^{tm1Jess}	SMC (structural maintenance of chromosomes 1)-like 2 (*S. cerevisiae*)	3573932	TKO (null)	Shortened SCs; sister chromatid cohesion is impaired; telomere attachment is impaired, and crossover complexes are not seen; midpachytene arrest	Similar defects to those seen in male meiosis; unlike in males, oocytes progress through meiosis I to dictyate arrest, but defective cohesion results in massive aneuploidy during meiotic divisions	More commonly known as SMC1-β	18, 168
Spo11^{tm1Rdco} and *Spo11* [*SK*]	Sporulation protein, meiosis-specific, SPO11 homolog (*S. cerevisiae*)	3027823 and none	TKO (null) and TKO (null)	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	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		37–39
Sycp1^{tm1Aps}	SYCP-1	3580000	TKO (null)	Axial elements form and chromosomes align, but no synapsis; most spermatocytes arrest at the pachytene stage, 0–3% of spermatocytes reaching diplonema	Loss of growing follicles and oocytes in adult ovaries, indicating early meiotic prophase failure		167
Sycp3^{tm1Hoog}	SYCP-3	2179041	TKO (null)	Defect at zygotene stage of prophase I; impaired chromosomal synapsis	Defective chromosomal segregation leading to aneuploidy; decreased fertility with age; embryonic loss due to aneuploidy		12, 14
Top3b^{tm1Jcw}	Topoisomerase (DNA) III β	2388157	TKO (null)	Increased incidence of chromosomal defects, particularly aneuploidy, resulting from improper resolution of double-Holliday junctions	Age-related decline in litter size and frequency		126

The gene products listed in this table are involved in recombination and/or synapsis and were obtained from the MGI database (http://www.informatics.jax.org/searches/Phat.cgi?id=MP:0001930). The table includes only those alleles for which a meiotic phenotype was reported. TKO, Targeted knockout; CIM, chemically induced mutagenesis; MGI, Mouse genome informatics ID number; EMS, ethyl methansulfonate; ENU, ethylnitrosurea. Allele names include laboratory abbreviations in *superscript* unless the allele has not been registered with MGI, in which case the laboratory source is indicated in *brackets*. mut, Mutation; del, deletion; GV, germinal vesicle.

tions of *Fanc* (Fanconi anemia complementation group C gene) (335, 336), mice with targeted disruption of *Fanca* (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 diplotene mouse spermatocytes and colocalizes with BRCA1 in these regions (330). The proteins of this nuclear Fanconi anemia complex also form a complex with TOPOIIIα, 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 αII spectrin (reviewed in Ref. 333). FANCA expression is found at a high level in pachytene spermatocytes, and *Fanca*^{tmiHsc} 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

Address all correspondence and requests for reprints to: J. W. Pollard, Developmental and Molecular Biology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, New York 10461. E-mail: pollard@aecom.yu.edu

This work was supported by funding from Wyeth and National Institute of Health Grant CA 89617 (to J.W.P.). S.E.P. was a Pfizer Clinical Scholar. Unpublished research discussed in this paper was funded by the March of Dimes Birth Defects Foundation (Grant FY2002-282 to P.E.C.) and by the National Downs Syndrome Society. J.W.P. is the Sheldon and Betty E. Feinberg Senior Faculty Scholar in Cancer Research.

Current affiliation for P.E.C.: Department of Biomedical Sciences, Cornell University, Ithaca, New York 14853.

References

1. **Hamoir G** 2001 [The egg-cell of Edouard Van Beneden and molecular embryology]. Rev Med Liege 56:171–180
2. **Page SL, Hawley RS** 2004 The genetics and molecular biology of the synaptonemal complex. Annu Rev Cell Dev Biol 20:525–558
3. **Loidl J, Scherthan H, Den Dunnen JT, Klein F** 1995 Morphology of a human-derived YAC in yeast meiosis. Chromosoma 104:183–188
4. **von Wettstein D, Rasmussen SW, Holm PB** 1984 The synaptonemal complex in genetic segregation. Annu Rev Genet 18:331–413
5. **Hassold T, Hunt P** 2001 To err (meiotically) is human: the genesis of human aneuploidy. Nat Rev Genet 2:280–291
6. **Schmekel K, Meuwissen RL, Dietrich AJ, Vink AC, van Marle J, van Veen H, Heyting C** 1996 Organization of SCP1 protein molecules within synaptonemal complexes of the rat. Exp Cell Res 226:20–30
7. **Lammers JH, Offenberg HH, van Aalderen M, Vink AC, Dietrich AJ, Heyting C** 1994 The gene encoding a major component of the lateral elements of synaptonemal complexes of the rat is related to X-linked lymphocyte-regulated genes. Mol Cell Biol 14:1137–1146
8. **Heyting C, Dietrich AJ, Moens PB, Dettmers RJ, Offenberg HH, Redeker EJ, Vink AC** 1989 Synaptonemal complex proteins. Genome 31:81–87
9. **Heyting C, Moens PB, van Raamsdonk W, Dietrich AJ, Vink AC, Redeker EJ** 1987 Identification of two major components of the lateral elements of synaptonemal complexes of the rat. Eur J Cell Biol 43:148–154
10. **Offenberg HH, Schalk JA, Meuwissen RL, van Aalderen M, Kester HA, Dietrich AJ, Heyting C** 1998 SCP2: a major protein component of the axial elements of synaptonemal complexes of the rat. Nucleic Acids Res 26:2572–2579
11. **Schalk JA, Dietrich AJ, Vink AC, Offenberg HH, van Aalderen**

M, Heyting C 1998 Localization of SCP2 and SCP3 protein molecules within synaptonemal complexes of the rat. Chromosoma 107:540–548
12. **Yuan L, Liu JG, Zhao J, Brundell E, Daneholt B, Hoog C** 2000 The murine SCP3 gene is required for synaptonemal complex assembly, chromosome synapsis, and male fertility. Mol Cell 5:73–83
13. **Liebe B, Alsheimer M, Hoog C, Benavente R, Scherthan H** 2004 Telomere attachment, meiotic chromosome condensation, pairing, and bouquet stage duration are modified in spermatocytes lacking axial elements. Mol Biol Cell 15:827–837
14. **Yuan L, Liu JG, Hoja MR, Wilbertz J, Nordqvist K, Hoog C** 2002 Female germ cell aneuploidy and embryo death in mice lacking the meiosis-specific protein SCP3. Science 296:1115–1118
15. **Crackower MA, Kolas NK, Noguchi J, Sarao R, Kikuchi K, Kaneko H, Kobayashi E, Kawai Y, Kozieradzki I, Landers R, Mo R, Hui CC, Nieves E, Cohen PE, Osborne LR, Wada T, Kunieda T, Moens PB, Penninger JM** 2003 Essential role of Fkbp6 in male fertility and homologous chromosome pairing in meiosis. Science 300:1291–1295
16. **Costa Y, Speed R, Ollinger R, Alsheimer M, Semple CA, Gautier P, Maratou K, Novak I, Hoog C, Benavente R, Cooke HJ** 2005 Two novel proteins recruited by synaptonemal complex protein 1 (SYCP1) are at the centre of meiosis. J Cell Sci 118:2755–2762
17. **Uhlmann F** 2004 The mechanism of sister chromatid cohesion. Exp Cell Res 296:80–85
18. **Revenkova E, Eijpe M, Heyting C, Hodges CA, Hunt PA, Liebe B, Scherthan H, Jessberger R** 2004 Cohesin SMC1β is required for meiotic chromosome dynamics, sister chromatid cohesion and DNA recombination. Nat Cell Biol 6:555–562
19. **Bannister LA, Reinholdt LG, Munroe RJ, Schimenti JC** 2004 Positional cloning and characterization of mouse mei8, a disrupted allele of the meiotic cohesin Rec8. Genesis 40:184–194
20. **Xu H, Beasley MD, Warren WD, van der Horst GT, McKay MJ** 2005 Absence of mouse REC8 cohesin promotes synapsis of sister chromatids in meiosis. Dev Cell 8:949–961
21. **Carpenter AT** 1975 Electron microscopy of meiosis in *Drosophila melanogaster* females: II. The recombination nodule–a recombination-associated structure at pachytene? Proc Natl Acad Sci USA 72:3186–3189
22. **Moens PB, Kolas NK, Tarsounas M, Marcon E, Cohen PE, Spyropoulos B** 2002 The time course and chromosomal localization of recombination-related proteins at meiosis in the mouse are compatible with models that can resolve the early DNA-DNA interactions without reciprocal recombination. J Cell Sci 115:1611–1622
23. **Kolas NK, Cohen PE** 2004 Novel and diverse functions of the DNA mismatch repair family in mammalian meiosis and recombination. Cytogenet Genome Res 107:216–231
24. **Marcon E, Moens P** 2003 MLH1p and MLH3p localize to precociously induced chiasmata of okadaic-acid-treated mouse spermatocytes. Genetics 165:2283–2287
25. **Bennett MD** 1977 The time and duration of meiosis. Philos Trans R Soc Lond B Biol Sci 277:201–226
26. **Speed RM** 1982 Meiosis in the foetal mouse ovary. I. An analysis at the light microscope level using surface-spreading. Chromosoma 85:427–437
27. **Gondos B, Bhiraleus P, Hobel CJ** 1971 Ultrastructural observations on germ cells in human fetal ovaries. Am J Obstet Gynecol 110:644–652
28. **Motta PM, Nottola SA, Makabe S** 1997 Natural history of the female germ cell from its origin to full maturation through prenatal ovarian development. Eur J Obstet Gynecol Reprod Biol 75:5–10
29. **Peters H, Byskov AG, Grinsted J** 1978 Follicular growth in fetal and prepubertal ovaries of humans and other primates. Clin Endocrinol Metab 7:469–485
30. **Gondos B, Hobel CJ** 1971 Ultrastructure of germ cell development in the human fetal testis. Z Zellforsch Mikrosk Anat 119:1–20
31. **Hirshfield AN** 1991 Development of follicles in the mammalian ovary. Int Rev Cytol 124:43–101
32. **Hunt PA** 1998 The control of mammalian female meiosis: factors that influence chromosome segregation. J Assist Reprod Genet 15:246–252
33. **Hartshorne GM, Barlow AL, Child TJ, Barlow DH, Hulten MA** 1999 Immunocytogenetic detection of normal and abnormal oocytes in human fetal ovarian tissue in culture. Hum Reprod 14: 172–182
34. **Hirshfield AN, DeSanti AM** 1995 Patterns of ovarian cell proliferation in rats during the embryonic period and the first three weeks postpartum. Biol Reprod 53:1208–1221
35. **Morelli MA, Cohen PE** 2005 Not all germ cells are created equal: aspects of sexual dimorphism in mammalian meiosis. Reproduction 130:761–781
36. **Baudat F, Keeney S** 2001 Meiotic recombination: making and breaking go hand in hand. Curr Biol 11:R45–R48
37. **Baudat F, Manova K, Yuen JP, Jasin M, Keeney S** 2000 Chromosome synapsis defects and sexually dimorphic meiotic progression in mice lacking Spo11. Mol Cell 6:989–998
38. **Romanienko PJ, Camerini-Otero RD** 2000 The mouse Spo11 gene is required for meiotic chromosome synapsis. Mol Cell 6:975–987
39. **Di Giacomo M, Barchi M, Baudat F, Edelmann W, Keeney S, Jasin M** 2005 Distinct DNA-damage-dependent and -independent responses drive the loss of oocytes in recombination-defective mouse mutants. Proc Natl Acad Sci USA 102:737–742
40. **Borner GV, Kleckner N, Hunter N** 2004 Crossover/noncrossover differentiation, synaptonemal complex formation, and regulatory surveillance at the leptotene/zygotene transition of meiosis. Cell 117:29–45
41. **Allers T, Lichten M** 2001 Differential timing and control of noncrossover and crossover recombination during meiosis. Cell 106: 47–57
42. **Hoffmann ER, Borts RH** 2004 Meiotic recombination intermediates and mismatch repair proteins. Cytogenet Genome Res 107: 232–248
43. **Boddy MN, Gaillard PH, McDonald WH, Shanahan P, Yates III JR, Russell P** 2001 Mus81-Eme1 are essential components of a Holliday junction resolvase. Cell 107:537–548
44. **De Los Santos T, Hunter N, Lee C, Larkin B, Loidl J, Hollingsworth NM** 2003 The mus81/mms4 endonuclease acts independently of double-holliday junction resolution to promote a distinct subset of crossovers during meiosis in budding yeast. Genetics 164:81–94
45. **Hunter N, Kleckner N** 2001 The single-end invasion: an asymmetric intermediate at the double-strand break to double-holliday junction transition of meiotic recombination. Cell 106:59–70
46. **Kauppi L, Jeffreys AJ, Keeney S** 2004 Where the crossovers are: recombination distributions in mammals. Nat Rev Genet 5:413–424
47. **Kitamura K, Katayama S, Dhut S, Sato M, Watanabe Y, Yamamoto M, Toda T** 2001 Phosphorylation of Mei2 and Ste11 by Pat1 kinase inhibits sexual differentiation via ubiquitin proteolysis and 14–3-3 protein in fission yeast. Dev Cell 1:389–399
48. **Ohno M, Mattaj IW** 1999 Meiosis: MeiRNA hits the spot. Curr Biol 9:R66–R69
49. **Marston AL, Amon A** 2004 Meiosis: cell-cycle controls shuffle and deal. Nat Rev Mol Cell Biol 5:983–997
50. **Yamamoto M** 1996 The molecular control mechanisms of meiosis in fission yeast. Trends Biochem Sci 21:18–22
51. **Yamamoto M** 1996 Regulation of meiosis in fission yeast. Cell Struct Funct 21:431–436
52. **Geijsen N, Horoschak M, Kim K, Gribnau J, Eggan K, Daley GQ** 2004 Derivation of embryonic germ cells and male gametes from embryonic stem cells. Nature 427:148–154
53. **Malumbres M, Barbacid M** 2005 Mammalian cyclin-dependent kinases. Trends Biochem Sci 30:630–641
54. **Sherr CJ** 1996 Cancer cell cycles. Science 274:1672–1677
55. **Sherr CJ, McCormick F** 2002 The RB and p53 pathways in cancer. Cancer Cell 2:103–112
56. **Sherr CJ** 1995 D-type cyclins. Trends Biol Sci 20:187–190
57. **Vidal A, Koff A** 2000 Cell-cycle inhibitors: three families united by a common cause. Gene 247:1–15
58. **Sherr CJ, Roberts JM** 1995 Inhibitors of mammalian G1 cyclin-dependent kinases. Genes Dev 9:1149–1163
59. **Humbert PO, Brumby AM, Quinn LM, Richardson HE** 2004 New tricks for old dogs: unexpected roles for cell cycle regulators revealed using animal models. Curr Opin Cell Biol 16:614–622
60. **Geng Y, Yu Q, Sicinska E, Das M, Schneider JE, Bhattacharya S,**

Rideout WM, Bronson RT, Gardner H, Sicinski P 2003 Cyclin E ablation in the mouse. Cell 114:431–443
61. **Ortega S, Prieto I, Odajima J, Martin A, Dubus P, Sotillo R, Barbero JL, Malumbres M, Barbacid M** 2003 Cyclin-dependent kinase 2 is essential for meiosis but not for mitotic cell division in mice. Nat Genet 35:25–31
62. **Murphy M** 1999 Delayed early embryonic lethality following disruption of the murine cyclin A2 gene. Nat Genet 23:481
63. **Liu D, Matzuk MM, Sung WK, Guo Q, Wang P, Wolgemuth DJ** 1998 Cyclin A1 is required for meiosis in the male mouse. Nat Genet 20:377–380
64. **Sweeney C, Murphy M, Kubelka M, Ravnik SE, Hawkins CF, Wolgemuth DJ, Carrington M** 1996 A distinct cyclin A is expressed in germ cells in the mouse. Development 122:53–64
65. **Wolgemuth DJ, Lele KM, Jobanputra V, Salazar G** 2004 The A-type cyclins and the meiotic cell cycle in mammalian male germ cells. Int J Androl 27:192–199
66. **Kozar K, Ciemerych MA, Rebel VI, Shigematsu H, Zagozdzon A, Sicinska E, Geng Y, Yu Q, Bhattacharya S, Bronson RT, Akashi K, Sicinski P** 2004 Mouse development and cell proliferation in the absence of D-cyclins. Cell 118:477–491
67. **Malumbres M, Sotillo R, Santamaria D, Galan J, Cerezo A, Ortega S, Dubus P, Barbacid M** 2004 Mammalian cells cycle without the D-type cyclin-dependent kinases Cdk4 and Cdk6. Cell 118:493–504
68. **Ciemerych MA, Kenney AM, Sicinska E, Kalaszczynska I, Bronson RT, Rowitch DH, Gardner H, Sicinski P** 2002 Development of mice expressing a single D-type cyclin. Genes Dev 16:3277–3289
69. **Sicinski P, Donaher JL, Geng Y, Parker SB, Gardner H, Park MY, Robker RL, Richards JS, McGinnis LK, Biggers JD, Eppig JJ, Bronson RT, Elledge SJ, Weinberg RA** 1996 Cyclin D2 is an FSH-responsive gene involved in gonadal cell proliferation and oncogenesis. Nature 384:470–474
70. **Tsutsui T, Hesabi B, Moons DS, Pandolfi PP, Hansel KS, Koff A, Kiyokawa H** 1999 Targeted disruption of CDK4 delays cell cycle entry with enhanced p27(Kip1) activity. Mol Cell Biol 19:7011–7019
71. **Jacks T, Fazeli A, Schmitt EM, Bronson RT, Goodell MA, Weinberg RA** 1992 Effects of an Rb mutation in the mouse. Nature 359:295–300
72. **Wu L, de Bruin A, Saavedra HI, Starovic M, Trimboli A, Yang Y, Opavska J, Wilson P, Thompson JC, Ostrowski MC, Rosol TJ, Woollett LA, Weinstein M, Cross JC, Robinson ML, Leone G** 2003 Extra-embryonic function of Rb is essential for embryonic development and viability. Nature 421:942–947
73. **Wu L, Timmers C, Maiti B, Saavedra HI, Sang L, Chong GT, Nuckolls F, Giangrande P, Wright FA, Field SJ, Greenberg ME, Orkin S, Nevins JR, Robinson ML, Leone G** 2001 The E2F1–3 transcription factors are essential for cellular proliferation. Nature 414:457–462
74. **Malumbres M, Ortega S, Barbacid M** 2000 Genetic analysis of mammalian cyclin-dependent kinases and their inhibitors. Biol Chem 381:827–838
75. **Tong W, Kiyokawa H, Soos TJ, Park M, Soares VC, Manova K, Pollard JW, Koff A** 1998 The absence of $p27^{Kip1}$, an inhibitor of G1 cyclin-dependent kinases, uncouples differentiation and growth arrest during the granulosa->luteal transition. Cell Growth Differ 9:787–794
76. **Kiyokawa H, Kineman RD, Manova-Todorova KO, Soares VC, Hoffman ES, Ono M, Khanam D, Hayday AC, Frohman LA, Koff A** 1996 Enhanced growth of mice lacking the cyclin-dependent kinase inhibitor function of p27Kip1. Cell 85:721–732
77. **Zindy F, den Besten W, Chen B, Rehg JE, Latres E, Barbacid M, Pollard JW, Sherr CJ, Cohen PE, Roussel MF** 2001 Control of spermatogenesis in mice by the cyclin D-dependent kinase inhibitors p18(Ink4c) and p19(Ink4d). Mol Cell Biol 21:3244–3255
78. **Ravnik SE, Wolgemuth DJ** 1999 Regulation of meiosis during mammalian spermatogenesis: the A-type cyclins and their associated cyclin-dependent kinases are differentially expressed in the germ-cell lineage. Dev Biol 207:408–418
79. **Lei M, Tye BK** 2001 Initiating DNA synthesis: from recruiting to activating the MCM complex. J Cell Sci 114:1447–1454
80. **Blow JJ, Hodgson B** 2002 Replication licensing–defining the proliferative state? Trends Cell Biol 12:72–78
81. **DePamphilis ML** 2005 Cell cycle dependent regulation of the origin recognition complex. Cell Cycle 4:70–79
82. **Bell SP, Dutta A** 2002 DNA replication in eukaryotic cells. Annu Rev Biochem 71:333–374
83. **Ofir Y, Sagee S, Guttmann-Raviv N, Pnueli L, Kassir Y** 2004 The role and regulation of the preRC component Cdc6 in the initiation of premeiotic DNA replication. Mol Biol Cell 15:2230–2242
84. **Lindner K, Gregan J, Montgomery S, Kearsey SE** 2002 Essential role of MCM proteins in premeiotic DNA replication. Mol Biol Cell 13:435–444
85. **Eward KL, Obermann EC, Shreeram S, Loddo M, Fanshawe T, Williams C, Jung HI, Prevost AT, Blow JJ, Stoeber K, Williams GH** 2004 DNA replication licensing in somatic and germ cells. J Cell Sci 117:5875–5886
86. **Kishimoto T** 2003 Cell-cycle control during meiotic maturation. Curr Opin Cell Biol 15:654–663
87. **Spruck CH, de Miguel MP, Smith AP, Ryan A, Stein P, Schultz RM, Lincoln AJ, Donovan PJ, Reed SI** 2003 Requirement of Cks2 for the first metaphase/anaphase transition of mammalian meiosis. Science 300:647–650
88. **Godet M, Damestoy A, Mouradian S, Rudkin BB, Durand P** 2004 Key role for cyclin-dependent kinases in the first and second meiotic divisions of rat spermatocytes. Biol Reprod 70:1147–1152
89. **Iliakis G, Wang Y, Guan J, Wang H** 2003 DNA damage checkpoint control in cells exposed to ionizing radiation. Oncogene 22:5834–5847
90. **Wang YA, Elson A, Leder P** 1997 Loss of p21 increases sensitivity to ionizing radiation and delays the onset of lymphoma in atm-deficient mice. Proc Natl Acad Sci USA 94:14590–14595
91. **Barlow C, Liyanage M, Moens PB, Deng CX, Ried T, Wynshaw-Boris A** 1997 Partial rescue of the prophase I defects of Atm-deficient mice by p53 and p21 null alleles. Nat Genet 17:462–466
92. **Ashley T, Westphal C, Plug-de Maggio A, de Rooij DG** 2004 The mammalian mid-pachytene checkpoint: meiotic arrest in spermatocytes with a mutation in Atm alone or in combination with a Trp53 (p53) or Cdkn1a (p21/cip1) mutation. Cytogenet Genome Res 107:256–262
93. **Flaggs G, Plug AW, Dunks KM, Mundt KE, Ford JC, Quiggle MR, Taylor EM, Westphal CH, Ashley T, Hoekstra MF, Carr AM** 1997 Atm-dependent interactions of a mammalian chk1 homolog with meiotic chromosomes. Curr Biol 7:977–986
94. **Odorisio T, Rodriguez TA, Evans EP, Clarke AR, Burgoyne PS** 1998 The meiotic checkpoint monitoring synapsis eliminates spermatocytes via p53-independent apoptosis. Nat Genet 18:257–261
95. **Cohen PE, Pollard, JW** 2001 Regulation of meiotic recombination and phosphase I progression in mammals. Bioessays 23:996–1009
96. **Edelmann W, Cohen PE, Kneitz B, Winand N, Lia M, Heyer J, Kolodner R, Pollard JW, Kucherlapati R** 1999 Mammalian MutS homologue 5 is required for chromosome pairing in meiosis. Nat Genet 21:123–127
97. **Kneitz B, Cohen PE, Avdievich E, Zhu L, Kane MF, Hou Jr H, Kolodner RD, Kucherlapati R, Pollard JW, Edelmann W** 2000 MutS homolog 4 localization to meiotic chromosomes is required for chromosome pairing during meiosis in male and female mice. Genes Dev 14:1085–1097
98. **Pittman DL, Cobb J, Schimenti KJ, Wilson LA, Cooper DM, Brignull E, Handel MA, Schimenti JC** 1998 Meiotic prophase arrest with failure of chromosome synapsis in mice deficient for *Dmc1*, a germline-specific RecA homolog. Mol Cell 1:697–705
99. **Edelmann W, Cohen PE, Kane M, Lau K, Morrow B, Bennett S, Umar A, Kunkel T, Cattoretti G, Chaganti R, Pollard JW, Kolodner RD, Kucherlapati R** 1996 Meiotic pachytene arrest in MLH-1-deficient mice. Cell 85:1125–1134
100. **Baker SM, Plug AW, Prolla TA, Bronner CE, Harris AC, Yao X, Christie D-M, Monell C, Arnheim N, Bradley A, Ashley T, Liskay RM** 1996 Involvement of mouse *MIH1* in DNA mismatch repair and meiotic crossing over. Nat Genet 13:336–342
101. **Eaker S, Cobb J, Pyle A, Handel MA** 2002 Meiotic prophase abnormalities and metaphase cell death in MLH1-deficient mouse spermatocytes: insights into regulation of spermatogenic progress. Dev Biol 249:85–95
102. **Baker SM, Bronner CE, Zhang L, Plug AK, Robatzek M, Warren G, Elliott EA, Yu J, Ashley T, Arnheim N, Flavell RA, Liskay RM**

1995 Male mice defective in the DNA mismatch repair gene *PMS2* exhibit abnormal chromosome synapsis in meiosis. Cell 82:309–319
103. **Clark AJ, Margulies AD** 1965 Isolation and characterization of recombination-deficient mutants of *Escherichia coli* K12. Proc Natl Acad Sci USA 53:451–459
104. **Cox MM** 2003 The bacterial RecA protein as a motor protein. Annu Rev Microbiol 57:551–577
105. **Ogawa T, Shinohara A, Nabetani A, Ikeya T, Yu X, Egelman EH, Ogawa H** 1993 RecA-like recombination proteins in eukaryotes: functions and structures of RAD51 genes. Cold Spring Harbor Symp Quant Biol 58:567–576
106. **Ogawa T, Yu X, Shinohara A, Egelman EH** 1993 Similarity of the yeast RAD51 filament to the bacterial RecA filament. Science 259: 1896–1899
107. **Shinohara A, Ogawa H, Matsuda Y, Ushio N, Ikeo K, Ogawa T** 1993 Cloning of human, mouse and fission yeast recombination genes homologous to RAD51 and recA. Nat Genet 4:239–243
108. **Shinohara A, Ogawa H, Ogawa T** 1992 Rad51 protein involved in repair and recombination in *S. cerevisiae* is a RecA-like protein. Cell 69:457–470
109. **Barlow AL, Benson FE, West SC, Hulten MA** 1997 Distribution of the Rad51 recombinase in human and mouse spermatocytes. EMBO J 16:5207–5215
110. **Bishop DK, Park D, Xu L, Kleckner N** 1992 DMC1: a meiosis-specific yeast homolog of *E. coli recA* required for recombination, synaptonemal complex formation, and cell cycle progression. Cell 69:439–456
111. **Plug AW, Peters AH, Keegan KS, Hoekstra MF, de Boer P, Ashley T** 1998 Changes in protein composition of meiotic nodules during mammalian meiosis. J Cell Sci 111:413–423
112. **Bishop DK** 1994 RecA homologs Dmc1 and Rad51 interact to form multiple nuclear complexes prior to meiotic chromosome synapsis. Cell 79:1081–1092
113. **Tarsounas M, Morita T, Pearlman RE, Moens PB** 1999 RAD51 and DMC1 form mixed complexes associated with mouse meiotic chromosome cores and synaptonemal complexes. J Cell Biol 147:207–220
114. **Ashley T, Plug AW, Xu J, Solari AJ, Reddy G, Golub EI, Ward DC** 1995 Dynamic changes in Rad51 distribution on chromatin during meiosis in male and female vertebrates. Chromosoma 104:19–28
115. **Lenzi ML, Smith J, Snowden T, Kim M, Fishel R, Poulos BK, Cohen PE** 2005 Extreme heterogeneity in the molecular events leading to the establishment of chiasmata during meiosis i in human oocytes. Am J Hum Genet 76:112–127
116. **Thacker J** 2005 The RAD51 gene family, genetic instability and cancer. Cancer Lett 219:125–135
117. **Shinohara A, Shinohara M** 2004 Roles of RecA homologues Rad51 and Dmc1 during meiotic recombination. Cytogenet Genome Res 107:201–207
118. **Schwacha A, Kleckner N** 1997 Interhomolog bias during meiotic recombination: meiotic functions promote a highly differentiated interhomolog-only pathway. Cell 90:1123–1135
119. **New JH, Sugiyama T, Zaitseva E, Kowalczykowski SC** 1998 Rad52 protein stimulates DNA strand exchange by Rad51 and replication protein A. Nature 391:407–410
120. **Katagiri T, Saito H, Shinohara A, Ogawa H, Kamada N, Nakamura Y, Miki Y** 1998 Multiple possible sites of BRCA2 interacting with DNA repair protein RAD51. Genes Chromosomes Cancer 21:217–222
121. **Shinohara A, Ogawa T** 1998 Stimulation by Rad52 of yeast Rad51-mediated recombination. Nature 391:404–407
122. **Shinohara A, Shinohara M, Ohta T, Matsuda S, Ogawa T** 1998 Rad52 forms ring structures and co-operates with RPA in single-strand DNA annealing. Genes Cells 3:145–156
123. **Sonoda E, Sasaki MS, Buerstedde JM, Bezzubova O, Shinohara A, Ogawa H, Takata M, Yamaguchi-Iwai Y, Takeda S** 1998 Rad51-deficient vertebrate cells accumulate chromosomal breaks prior to cell death. EMBO J 17:598–608
124. **Benson FE, Baumann P, West SC** 1998 Synergistic actions of Rad51 and Rad52 in recombination and DNA repair. Nature 391:401–404
125. **Anderson LK, Offenberg HH, Verkuijlen WM, Heyting C** 1997 RecA-like proteins are components of early meiotic nodules in lily. Proc Natl Acad Sci USA 94:6868–6873
126. **Kwan KY, Moens PB, Wang JC** 2003 Infertility and aneuploidy in mice lacking a type IA DNA topoisomerase III β. Proc Natl Acad Sci USA 100:2526–2531
127. **Yoshida K, Kondoh G, Matsuda Y, Habu T, Nishimune Y, Morita T** 1998 The mouse *RecA*-like gene *Dmc1* is required for homologous chromosome synapsis during meiosis. Mol Cell 1:707–718
128. **Masson JY, Davies AA, Hajibagheri N, Van Dyck E, Benson FE, Stasiak AZ, Stasiak A, West SC** 1999 The meiosis-specific recombinase hDmc1 forms ring structures and interacts with hRad51. EMBO J 18:6552–6560
129. **Hong EL, Shinohara A, Bishop DK** 2001 *Saccharomyces cerevisiae* Dmc1 protein promotes renaturation of single-strand DNA (ssDNA) and assimilation of ssDNA into homologous super-coiled duplex DNA. J Biol Chem 276:41906–41912
130. **Sehorn MG, Sigurdsson S, Bussen W, Unger VM, Sung P** 2004 Human meiotic recombinase Dmc1 promotes ATP-dependent homologous DNA strand exchange. Nature 429:433–437
131. **Sehorn MG, Sung P** 2004 Meiotic recombination: an affair of two recombinases. Cell Cycle 3:1375–1377
132. **Sung P, Krejci L, Van Komen S, Sehorn MG** 2003 Rad51 recombinase and recombination mediators. J Biol Chem 278:42729–42732
133. **Keeney S, Baudat F, Angeles M, Zhou ZH, Copeland NG, Jenkins NA, Manova K, Jasin M** 1999 A mouse homolog of the *Saccharomyces cerevisiae* meiotic recombination DNA transesterase Spo11p. Genomics 61:170–182
134. **Boyer JC, Umar A, Risinger JI, Lipford JR, Kane M, Yin S, Barrett JC, Kolodner RD, Kunkel TA** 1995 Microsatellite instability, mismatch repair deficiency, and genetic defects in human cancer cell lines. Cancer Res 55:6063–6070
135. **Pearson CE, Ewel A, Acharya S, Fishel RA, Sinden RR** 1997 Human MSH2 binds to trinucleotide repeat DNA structures associated with neurodegenerative diseases. Hum Mol Genet 6:1117–1123
136. **Manley K, Shirley TL, Flaherty L, Messer A** 1999 Msh2 deficiency prevents in vivo somatic instability of the CAG repeat in Huntington disease transgenic mice. Nat Genet 23:471–473
137. **Chaney SG, Campbell SL, Bassett E, Wu Y** 2005 Recognition and processing of cisplatin- and oxaliplatin-DNA adducts. Crit Rev Oncol Hematol 53:3–11
138. **Harfe BD, Jinks-Robertson S** 2000 DNA mismatch repair and genetic instability. Annu Rev Genet 34:359–399
139. **Schofield MJ, Hsieh P** 2003 DNA mismatch repair: molecular mechanisms and biological function. Annu Rev Microbiol 57:579–608
140. **Edelmann L, Edelmann W** 2004 Loss of DNA mismatch repair function and cancer predisposition in the mouse: animal models for human hereditary nonpolyposis colorectal cancer. Am J Med Genet 129C:91–99
141. **de la Chapelle A** 2004 Genetic predisposition to colorectal cancer. Nat Rev Cancer 4:769–780
142. **Au KG, Welsh K, Modrich P** 1992 Initiation of methyl-directed mismatch repair. J Biol Chem 267:12142–12148
143. **Johnson RE, Kovvali GK, Guzder SN, Amin NS, Holm C, Habraken Y, Sung P, Prakash L, Prakash S** 1996 Evidence for involvement of yeast proliferating cell nuclear antigen in DNA mismatch repair. J Biol Chem 271:27987–27990
144. **Pavlov YI, Mian IM, Kunkel TA** 2003 Evidence for preferential mismatch repair of lagging strand DNA replication errors in yeast. Curr Biol 13:744–748
145. **Kearney HM, Kirkpatrick DT, Gerton JL, Petes TD** 2001 Meiotic recombination involving heterozygous large insertions in *Saccharomyces cerevisiae*: formation and repair of large, unpaired DNA loops. Genetics 158:1457–1476
146. **Flores-Rozas H, Kolodner RD** 1998 The *Saccharomyces cerevisiae* MLH3 gene functions in MSH3-dependent suppression of frameshift mutations. Proc Natl Acad Sci USA 95:12404–12409
147. **Harfe BD, Minesinger BK, Jinks-Robertson S** 2000 Discrete in vivo roles for the MutL homologs Mlh2p and Mlh3p in the removal of frameshift intermediates in budding yeast. Curr Biol 10:145–148
148. **Lipkin SM, Moens PB, Wang V, Lenzi M, Shanmugarajah D, Gilgeous A, Thomas J, Cheng J, Touchman JW, Green ED, Schwartzberg P, Collins FS, Cohen PE** 2002 Meiotic arrest and aneuploidy in MLH3-deficient mice. Nat Genet 31:385–390

149. **Lipkin SM, Wang V, Jacoby R, Banerjee-Basu S, Baxevanis AD, Lynch HT, Elliott RM, Collins FS** 2000 MLH3: a DNA mismatch repair gene associated with mammalian microsatellite instability. Nat Genet 24:27–35
150. **Wang TF, Kung WM** 2002 Supercomplex formation between Mlh1-Mlh3 and Sgs1-Top3 heterocomplexes in meiotic yeast cells. Biochem Biophys Res Commun 296:949–953
151. **Wang TF, Kleckner N, Hunter N** 1999 Functional specificity of MutL homologs in yeast: evidence for three Mlh1-based heterocomplexes with distinct roles during meiosis in recombination and mismatch correction. Proc Natl Acad Sci USA 96:13914–13919
152. **Loukola A, Vilkki S, Singh J, Launonen V, Aaltonen LA** 2000 Germline and somatic mutation analysis of MLH3 in MSI-positive colorectal cancer. Am J Pathol 157:347–352
153. **Wu Y, Berends MJ, Sijmons RH, Mensink RG, Verlind E, Kooi KA, van der Sluis T, Kempinga C, van dDer Zee AG, Hollema H, Buys CH, Kleibeuker JH, Hofstra RM** 2000 Germline hMLH3 mutations in patients with suspected HNPCC. J Am Soc Hum Genet 67(Suppl):39
154. **Wu Y, Berends MJ, Sijmons RH, Mensink RG, Verlind E, Kooi KA, van der Sluis T, Kempinga C, van dDer Zee AG, Hollema H, Buys CH, Kleibeuker JH, Hofstra RM** 2001 A role for MLH3 in hereditary nonpolyposis colorectal cancer. Nat Genet 29:137–138
155. **Lipkin SM, Wang V, Stoler DL, Anderson GR, Kirsch I, Hadley D, Lynch HT, Collins FS** 2001 Germline and somatic mutation analyses in the DNA mismatch repair gene MLH3: evidence for somatic mutation in colorectal cancers. Hum Mutat 17:389–396
156. **de Jong MM, Hofstra RM, Kooi KA, Westra JL, Berends MJ, Wu Y, Hollema H, van der Sluis T, van der Graaf WT, de Vries EG, Schaapveld M, Sijmons RH, te Meerman GJ, Kleibeuker JH** 2004 No association between two MLH3 variants (S845G and P844L)and colorectal cancer risk. Cancer Genet Cytogenet 152:70–71
157. **Hienonen T, Laiho P, Salovaara R, Mecklin JP, Jarvinen H, Sistonen P, Peltomaki P, Lehtonen R, Nupponen NN, Launonen V, Karhu A, Aaltonen LA** 2003 Little evidence for involvement of MLH3 in colorectal cancer predisposition. Int J Cancer 106:292–296
158. **Liu HX, Zhou XL, Liu T, Werelius B, Lindmark G, Dahl N, Lindblom A** 2003 The role of hMLH3 in familial colorectal cancer. Cancer Res 63:1894–1899
159. **Surtees JA, Argueso JL, Alani E** 2004 Mismatch repair proteins: key regulators of genetic recombination. Cytogenet Genome Res 107:146–159
160. **Kelly KO, Dernburg AF, Stanfield GM, Villeneuve AM** 2000 *Caenorhabditis elegans* msh-5 is required for both normal and radiation-induced meiotic crossing over but not for completion of meiosis. Genetics 156:617–630
161. **Zalevsky J, MacQueen AJ, Duffy JB, Kemphues KJ, Villeneuve AM** 1999 Crossing over during *Caenorhabditis elegans* meiosis requires a conserved MutS-based pathway that is partially dispensable in budding yeast. Genetics 153:1271–1283
162. **Higgins JD, Armstrong SJ, Franklin FC, Jones GH** 2004 The Arabidopsis MutS homolog AtMSH4 functions at an early step in recombination: evidence for two classes of recombination in Arabidopsis. Genes Dev 18:2557–2570
163. **Culligan KM, Meyer-Gauen G, Lyons-Weiler J, Hays JB** 2000 Evolutionary origin, diversification and specialization of eukaryotic MutS homolog mismatch repair proteins. Nucleic Acids Res 28:463–471
164. **de Vries SS, Baart EB, Dekker M, Siezen A, de Rooij DG, de Boer P, te Riele H** 1999 Mouse MutS-like protein Msh5 is required for proper chromosome synapsis in male and female meiosis. Genes Dev 13:523–531
165. **Santucci-Darmanin S, Walpita D, Lespinasse F, Desnuelle C, Ashley T, Paquis-Flucklinger V** 2000 MSH4 acts in conjunction with MLH1 during mammalian meiosis. FASEB J 14:1539–1547
166. **Santucci-Darmanin S, Neyton S, Lespinasse F, Saunieres A, Gaudray P, Paquis-Flucklinger V** 2002 The DNA mismatch-repair MLH3 protein interacts with MSH4 in meiotic cells, supporting a role for this MutL homolog in mammalian meiotic recombination. Hum Mol Genet 11:1697–1706
167. **de Vries FA, de Boer E, van den Bosch M, Baarends WM, Ooms M, Yuan L, Liu JG, van Zeeland AA, Heyting C, Pastink A** 2005 Mouse Sycp1 functions in synaptonemal complex assembly, meiotic recombination, and XY body formation. Genes Dev 19:1376–1389
168. **Hodges CA, Revenkova E, Jessberger R, Hassold TJ, Hunt PA** 2005 SMC1β-deficient female mice provide evidence that cohesins are a missing link in age-related nondisjunction. Nat Genet 37: 1351–1355
169. **Ward JO, Reinholdt LG, Hartford SA, Wilson LA, Munroe RJ, Schimenti KJ, Libby BJ, O'Brien M, Pendola JK, Eppig J, Schimenti JC** 2003 Toward the genetics of mammalian reproduction: induction and mapping of gametogenesis mutants in mice. Biol Reprod 69:1615–1625
170. **Kolas NK, Svetlanov A, Lenzi ML, Macaluso FP, Lipkin SM, Liskay RM, Greally J, Edelmann W, Cohen PE** 2005 Localization of MMR proteins on meiotic chromosomes in mice indicates distinct functions during prophase I. J Cell Biol 171:447–458
171. **Guillon H, Baudat F, Grey C, Liskay RM, de Massy B** 2005 Crossover and noncrossover pathways in mouse meiosis. Mol Cell 20: 563–573
172. **Woods LM, Hodges CA, Baart E, Baker SM, Liskay RM, Hunt PA** 1999 Chromosomal influence on meiotic spindle assembly: abnormal meiosis I in female Mlh1 mutant mice. J Cell Biol 145:1395–1406
173. **Neyton S, Lespinasse F, Moens PB, Paul R, Gaudray P, Paquis-Flucklinger V, Santucci-Darmanin S** 2004 Association between MSH4 (MutS homologue 4) and the DNA strand-exchange RAD51 and DMC1 proteins during mammalian meiosis. Mol Hum Reprod 10:917–924
174. **Snowden T, Acharya S, Butz C, Berardini M, Fishel R** 2004 hMSH4-hMSH5 recognizes Holliday junctions and forms a meiosis-specific sliding clamp that embraces homologous chromosomes. Mol Cell 15:437–451
175. **Acharya S, Foster PL, Brooks P, Fishel R** 2003 The coordinated functions of the *E. coli* MutS and MutL proteins in mismatch repair. Mol Cell 12:233–246
176. **Argueso JL, Wanat J, Gemici Z, Alani E** 2004 Competing crossover pathways act during meiosis in *Saccharomyces cerevisiae*. Genetics 168:1805–1816
177. **Anderson LK, Reeves A, Webb LM, Ashley T** 1999 Distribution of crossing over on mouse synaptonemal complexes using immunofluorescent localization of MLH1 protein. Genetics 151:1569–1579
178. **German J** 1993 Bloom syndrome: a mendelian prototype of somatic mutational disease. Medicine (Baltimore) 72:393–406
179. **Ellis NA, Groden J, Ye TZ, Straughen J, Lennon DJ, Ciocci S, Proytcheva M, German J** 1995 The Bloom's syndrome gene product is homologous to RecQ helicases. Cell 83:655–666
180. **German J, Ellis NA** 1998 Bloom syndrome. In: Vogelstein B, Kenzler KW, eds. The genetic basis of human cancer. New York: McGraw-Hill; 301–316
181. **Martin RH, Rademaker A, German J** 1994 Chromosomal breakage in human spermatozoa, a heterozygous effect of the Bloom syndrome mutation. Am J Hum Gen 55:1242–1246
182. **Chisholm CA, Bray MJ, Karns LB** 2001 Successful pregnancy in a woman with Bloom syndrome. Am J Med Genet 102:136–138
183. **Mulcahy MT, French M** 1981 Pregnancy in Bloom's syndrome. Clin Genet 19:156–158
184. **Neff NF, Ellis NA, Ye TZ, Noonan J, Huang K, Sanz M, Proytcheva M** 1999 The DNA helicase activity of BLM is necessary for the correction of the genomic instability of Bloom syndrome cells. Mol Biol Cell 10:665–676
185. **Seki T, Wang WS, Okumura N, Seki M, Katada T, Enomoto T** 1998 cDNA cloning of mouse BLM gene, the homologue to human Bloom's syndrome gene, which is highly expressed in the testis at the mRNA level. Biochim Biophys Acta 1398:377–381
186. **Mohaghegh P, Karow JK, Brosh Jr RM, Bohr VA, Hickson ID** 2001 The Bloom's and Werner's syndrome proteins are DNA structure-specific helicases. Nucleic Acids Res 29:2843–2849
187. **Brosh Jr RM, Majumdar A, Desai S, Hickson ID, Bohr VA, Seidman MM** 2001 Unwinding of a DNA triple helix by the Werner and Bloom syndrome helicases. J Biol Chem 276:3024–3030
188. **Karow JK, Constantinou A, Li JL, West SC, Hickson ID** 2000 The Bloom's syndrome gene product promotes branch migration of holliday junctions. Proc Natl Acad Sci USA 97:6504–6508

189. **Sun H, Karow JK, Hickson ID, Maizels N** 1998 The Bloom's syndrome helicase unwinds G4 DNA. J Biol Chem 273:27587–27592
190. **Guo RB, Rigolet P, Zargarian L, Fermandjian S, Xi XG** 2005 Structural and functional characterizations reveal the importance of a zinc binding domain in Bloom's syndrome helicase. Nucleic Acids Res 33:3109–3124
191. **Wang XW, Tseng A, Ellis NA, Spillare EA, Linke SP, Robles AI, Seker H, Yang Q, Hu P, Beresten S, Bemmels NA, Garfield S, Harris CC** 2001 Functional interaction of p53 and BLM DNA helicase in apoptosis. J Biol Chem 276:32948–32955
192. **Johnson FB, Lombard DB, Neff NF, Mastrangelo MA, Dewolf W, Ellis NA, Marciniak RA, Yin Y, Jaenisch R, Guarente L** 2000 Association of the Bloom syndrome protein with topoisomerase IIIα in somatic and meiotic cells. Cancer Res 60:1162–1167
193. **Wu L, Davies SL, North PS, Goulaouic H, Riou JF, Turley H, Gatter KC, Hickson ID** 2000 The Bloom's syndrome gene product interacts with topoisomerase III. J Biol Chem 275:9636–9644
194. **Franchitto A, Pichierri P** 2002 Bloom's syndrome protein is required for correct relocalization of RAD50/MRE11/NBS1 complex after replication fork arrest. J Cell Biol 157:19–30
195. **Davalos AR, Campisi J** 2003 Bloom syndrome cells undergo p53-dependent apoptosis and delayed assembly of BRCA1 and NBS1 repair complexes at stalled replication forks. J Cell Biol 162:1197–1209
196. **Braybrooke JP, Li JL, Wu L, Caple F, Benson FE, Hickson ID** 2003 Functional interaction between the Bloom's syndrome helicase and the RAD51 paralog, RAD51L3 (RAD51D). J Biol Chem 278:48357–48366
197. **Pedrazzi G, Bachrati CZ, Selak N, Studer I, Petkovic M, Hickson ID, Jiricny J, Stagljar I** 2003 The Bloom's syndrome helicase interacts directly with the human DNA mismatch repair protein hMSH6. Biol Chem 384:1155–1164
198. **Sharma S, Sommers JA, Wu L, Bohr VA, Hickson ID, Brosh Jr RM** 2004 Stimulation of flap endonuclease-1 by the Bloom's syndrome protein. J Biol Chem 279:9847–9856
199. **Yang Q, Zhang R, Wang XW, Linke SP, Sengupta S, Hickson ID, Pedrazzi G, Perrera C, Stagljar I, Littman SJ, Modrich P, Harris CC** 2004 The mismatch DNA repair heterodimer, hMSH2/6, regulates BLM helicase. Oncogene 23:3749–3756
200. **Pedrazzi G, Perrera C, Blaser H, Kuster P, Marra G, Davies SL, Ryu GH, Freire R, Hickson ID, Jiricny J, Stagljar I** 2001 Direct association of Bloom's syndrome gene product with the human mismatch repair protein MLH1. Nucleic Acids Res 29:4378–4386
201. **Beamish H, Kedar P, Kaneko H, Chen P, Fukao T, Peng C, Beresten S, Gueven N, Purdie D, Lees-Miller S, Ellis N, Kondo N, Lavin MF** 2002 Functional link between BLM defective in Bloom's syndrome and the ataxia-telangiectasia-mutated protein, ATM. J Biol Chem 277:30515–30523
202. **Bischof O, Kim SH, Irving J, Beresten S, Ellis NA, Campisi J** 2001 Regulation and localization of the Bloom syndrome protein in response to DNA damage. J Cell Biol 153:367–380
203. **Hickson ID, Davies SL, Li JL, Levitt NC, Mohaghegh P, North PS, Wu L** 2001 Role of the Bloom's syndrome helicase in maintenance of genome stability. Biochem Soc Trans 29:201–204
204. **Sengupta S, Linke SP, Pedeux R, Yang Q, Farnsworth J, Garfield SH, Valerie K, Shay JW, Ellis NA, Wasylyk B, Harris CC** 2003 BLM helicase-dependent transport of p53 to sites of stalled DNA replication forks modulates homologous recombination. EMBO J 22:1210–1222
205. **Wu L, Hickson ID** 2003 The Bloom's syndrome helicase suppresses crossing over during homologous recombination. Nature 426:870–874
206. **Meetei AR, Sechi S, Wallisch M, Yang D, Young MK, Joenje H, Hoatlin ME, Wang W** 2003 A multiprotein nuclear complex connects Fanconi anemia and Bloom syndrome. Mol Cell Biol 23:3417–3426
207. **Gangloff S, de Massy B, Arthur L, Rothstein R, Fabre F** 1999 The essential role of yeast topoisomerase III in meiosis depends on recombination. EMBO J 18:1701–1711
208. **Watt PM, Louis EJ, Borts RH, Hickson ID** 1995 Sgs1: a eukaryotic homolog of *E. coli* RecQ that interacts with topoisomerase II in vivo and is required for faithful chromosome segregation. Cell 81:253–260
209. **Rockmill B, Fung JC, Branda SS, Roeder GS** 2003 The Sgs1 helicase regulates chromosome synapsis and meiotic crossing over. Curr Biol 13:1954–1962
210. **Watt PM, Hickson ID, Borts RH, Louis EJ** 1996 SGS1, a homologue of the Bloom's and Werner's syndrome genes, is required for maintenance of genome stability in *Saccharomyces cerevisiae*. Genetics 144:935–945
211. **Moens PB, Freire R, Tarsounas M, Spyropoulos B, Jackson SP** 2000 Expression and nuclear localization of BLM, a chromosome stability protein mutated in Bloom's syndrome, suggest a role in recombination during meiotic prophase. J Cell Sci 113:663–672
212. **Walpita D, Plug AW, Neff NF, German J, Ashley T** 1999 Bloom's syndrome protein, BLM, colocalizes with replication protein A in meiotic prophase nuclei of mammalian spermatocytes. Proc Natl Acad Sci USA 96:5622–5627
213. **Munroe RJ, Bergstrom RA, Zheng QY, Libby B, Smith R, John SW, Schimenti KJ, Browning VL, Schimenti JC** 2000 Mouse mutants from chemically mutagenized embryonic stem cells. Nat Genet 24:318–321
214. **Bohr VA** 2005 Deficient DNA repair in the human progeroid disorder, Werner syndrome. Mutat Res 577:252–259
215. **Bohr VA, Cooper M, Orren D, Machwe A, Piotrowski J, Sommers J, Karmakar P, Brosh R** 2000 Werner syndrome protein: biochemical properties and functional interactions. Exp Gerontol 35:695–702
216. **Lee JW, Harrigan J, Opresko PL, Bohr VA** 2005 Pathways and functions of the Werner syndrome protein. Mech Ageing Dev 126:79–86
217. **Shevelev IV, Hubscher U** 2002 The 3′ 5′ exonucleases. Nat Rev Mol Cell Biol 3:364–376
218. **Huang S, Beresten S, Li B, Oshima J, Ellis NA, Campisi J** 2000 Characterization of the human and mouse WRN 3′–>5′ exonuclease. Nucleic Acids Res 28:2396–2405
219. **Sharma S, Sommers JA, Driscoll HC, Uzdilla L, Wilson TM, Brosh Jr RM** 2003 The exonucleolytic and endonucleolytic cleavage activities of human exonuclease 1 are stimulated by an interaction with the carboxyl-terminal region of the Werner syndrome protein. J Biol Chem 278:23487–23496
220. **Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W, Bell R, Rosenthal J, Hussey C, Tran T, McClure M, Frye C, Hattier T, Phelps R, Haugen-Strano A, Katcher H, Yakumo K, Gholami Z, Shaffer D, Stone S, Bayer S, Wray C, Bogden R, Dayananth P, Ward J, Tonin P, Narod S, Bristow PK, Norris FH, Helvering L, Morrison P, Rosteck P, Lai M, Barrett JC, Lewis C, Neuhauen S, Cannon-Albright L, Goldgar D, Wiseman R, Kamb A, Skolnick MH** 1994 A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 266:66–71
221. **Hall JM, Lee MK, Newman B, Morrow JE, Anderson LA, Huey B, King MC** 1990 Linkage of early-onset familial breast cancer to chromosome 17q21. Science 250:1684–1689
222. **Stratton MR, Ford D, Neuhasen S, Seal S, Wooster R, Friedman LS, King MC, Egilsson V, Devilee P, McManus R, Daly PA, Smyth E, Ponder BAJ, Peto J, Cannon-Albright L, Easton DF, Goldgar DE** 1994 Familial male breast cancer is not linked to the BRCA1 locus on chromosome 17q. Nat Genet 7:103–107
223. **Wooster R, Neuhausen SL, Mangion J, Quirk Y, Ford D, Collins N, Nguyen K, Seal S, Tran T, Averill D, Fields P, Marshall G, Narod S, Lenoir GM, Lynch H, Feunteun J, Devilee P, Cornelisse CJ, Menko FH, Daly PA, Ormiston W, McManus R, Pye C, Lewis CM, Cannon-Albright LA, et al** 1994 Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12–13. Science 265:2088–2090
224. **Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, Collins N, Gregory S, Gumbs C, Micklem G** 1995 Identification of the breast cancer susceptibility gene BRCA2. Nature 378:789–792
225. **Zhang H, Tombline G, Weber BL** 1998 BRCA1, BRCA2, and DNA damage response: collision or collusion? Cell 92:433–436
226. **Ford D, Easton DF, Stratton M, Narod S, Goldgar D, Devilee P, Bishop DT, Weber B, Lenoir G, Chang-Claude J, Sobol H, Teare MD, Struewing J, Arason A, Scherneck S, Peto J, Rebbeck TR, Tonin P, Neuhausen S, Barkardottir R, Eyfjord J, Lynch H, Ponder BA, Gayther SA, Zelada-Hedman M, et al** 1998 Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. The Breast Cancer Linkage Consortium. Am J Hum Genet 62:676–689
227. **Struewing JP, Hartge P, Wacholder S, Baker SM, Berlin M, McAdams M, Timmerman MM, Brody LC, Tucker MA** 1997 The risk

of cancer associated with specific mutations of BRCA1 and BRCA2 among Ashkenazi Jews. N Engl J Med 336:1401–1408
228. **Thorlacius S, Struewing JP, Hartge P, Olafsdottir GH, Sigvaldason H, Tryggvadottir L, Wacholder S, Tulinius H, Eyfjord JE** 1998 Population-based study of risk of breast cancer in carriers of BRCA2 mutation. Lancet 352:1337–1339
229. **Hopper JL, Southey MC, Dite GS, Jolley DJ, Giles GG, McCredie MR, Easton DF, Venter DJ** 1999 Population-based estimate of the average age-specific cumulative risk of breast cancer for a defined set of protein-truncating mutations in BRCA1 and BRCA2. Australian Breast Cancer Family Study. Cancer Epidemiol Biomarkers Prev 8:741–747
230. **Easton DF, Ford D, Bishop DT** 1995 Breast and ovarian cancer incidence in BRCA1-mutation carriers. Breast Cancer Linkage Consortium. Am J Hum Genet 56:265–271
231. **Welcsh PL, Owens KN, King MC** 2000 Insights into the functions of BRCA1 and BRCA2. Trends Genet 16:69–74
232. **Narod SA, Foulkes WD** 2004 BRCA1 and BRCA2: 1994 and beyond. Nat Rev Cancer 4:665–676
233. **Morris JR, Solomon E** 2004 BRCA1: BARD1 induces the formation of conjugated ubiquitin structures, dependent on K6 of ubiquitin, in cells during DNA replication and repair. Hum Mol Genet 13:807–817
234. **Wang Y, Cortez D, Yazdi P, Neff N, Elledge SJ, Qin J** 2000 BASC, a super complex of BRCA1-associated proteins involved in the recognition and repair of aberrant DNA structures. Genes Dev 14:927–939
235. **Scully R, Chen J, Plug A, Xiao Y, Weaver D, Feunteun J, Ashley T, Livingston DM** 1997 Association of BRCA1 with Rad51 in mitotic and meiotic cells. Cell 88:265–275
236. **Chen J, Silver DP, Walpita D, Cantor SB, Gazdar AF, Tomlinson G, Couch FJ, Weber BL, Ashley T, Livingston DM, Scully R** 1998 Stable interaction between the products of the BRCA1 and BRCA2 tumor suppressor genes in mitotic and meiotic cells. Mol Cell 2:317–328
237. **Moynahan ME, Chiu JW, Koller BH, Jasin M** 1999 Brca1 controls homology-directed DNA repair. Mol Cell 4:511–518
238. **Snouwaert JN, Gowen LC, Latour AM, Mohn AR, Xiao A, DiBiase L, Koller BH** 1999 BRCA1 deficient embryonic stem cells display a decreased homologous recombination frequency and an increased frequency of non-homologous recombination that is corrected by expression of a brca1 transgene. Oncogene 18:7900–7907
239. **Zabludoff SD, Wright WW, Harshman K, Wold BJ** 1996 BRCA1 mRNA is expressed highly during meiosis and spermiogenesis but not during mitosis of male germ cells. Oncogene 13:649–653
240. **Sharan SK, Morimatsu M, Albrecht U, Lim DS, Regel E, Dinh C, Sands A, Eichele G, Hasty P, Bradley A** 1997 Embryonic lethality and radiation hypersensitivity mediated by Rad51 in mice lacking Brca2. Nature 386:804–810
241. **Hakem R, de la Pompa JL, Elia A, Potter J, Mak TW** 1997 Partial rescue of Brca1 (5–6) early embryonic lethality by p53 or p21 null mutation. Nat Genet 16:298–302
242. **Harvey M, McArthur MJ, Montgomery Jr CA, Butel JS, Bradley A, Donehower LA** 1993 Spontaneous and carcinogen-induced tumorigenesis in p53-deficient mice. Nat Genet 5:225–229
243. **Cressman VL, Backlund DC, Avrutskaya AV, Leadon SA, Godfrey V, Koller BH** 1999 Growth retardation, DNA repair defects, and lack of spermatogenesis in BRCA1-deficient mice. Mol Cell Biol 19:7061–7075
244. **Xu X, Aprelikova O, Moens P, Deng CX, Furth PA** 2003 Impaired meiotic DNA-damage repair and lack of crossing-over during spermatogenesis in BRCA1 full-length isoform deficient mice. Development 130:2001–2012
245. **Moynahan ME, Pierce AJ, Jasin M** 2001 BRCA2 is required for homology-directed repair of chromosomal breaks. Mol Cell 7:263–272
246. **Connor F, Bertwistle D, Mee PJ, Ross GM, Swift S, Grigorieva E, Tybulewicz VLJ, Ashworth A** 1997 Tumorigenesis and a DNA repair defect in mice with a truncated *Brca2* mutation. Nat Genet 17:423–430
247. **Suzuki A, de la Pompa JL, Hakem R, Elia A, Yoshida R, Mo R, Nishina H, Chuang T, Wakeham A, Itie A, Koo W, Billia P, Ho A, Fukumoto M, Hui CC, Mak TW** 1997 Brca2 is required for embryonic cellular proliferation in the mouse. Genes Dev 11:1242–1252
248. **Ludwig T, Chapman DL, Papaioannou VE, Efstratiadis A** 1997 Targeted mutations of breast cancer susceptibility gene homologs in mice: lethal phenotypes of Brca1, Brca2, Brca1/Brca2, Brca1/p53, and Brca2/p53 nullizygous embryos. Genes Dev 11:1226–1241
249. **Sharan SK, Pyle A, Coppola V, Babus J, Swaminathan S, Benedict J, Swing D, Martin BK, Tessarollo L, Evans JP, Flaws JA, Handel MA** 2004 BRCA2 deficiency in mice leads to meiotic impairment and infertility. Development 131:131–142
250. **Resnick IB, Kondratenko I, Togoev O, Vasserman N, Shagina I, Evgrafov O, Tverskaya S, Cerosaletti KM, Gatti RA, Concannon P** 2002 Nijmegen breakage syndrome: clinical characteristics and mutation analysis in eight unrelated Russian families. J Pediatr 140:355–361
251. **Shiloh Y** 1997 Ataxia-telangiectasia and the Nijmegen breakage syndrome: related disorders but genes apart. Annu Rev Genet 31:635–662
252. **Franchitto A, Pichierri P** 2002 Protecting genomic integrity during DNA replication: correlation between Werner's and Bloom's syndrome gene products and the MRE11 complex. Hum Mol Genet 11:2447–2453
253. **Lombard DB, Guarente L** 2000 Nijmegen breakage syndrome disease protein and MRE11 at PML nuclear bodies and meiotic telomeres. Cancer Res 60:2331–2334
254. **Carney JP, Maser RS, Olivares H, Davis EM, Le Beau M, Yates III JR, Hays L, Morgan WF, Petrini JH** 1998 The hMre11/hRad50 protein complex and Nijmegen breakage syndrome: linkage of double-strand break repair to the cellular DNA damage response. Cell 93:477–486
255. **Theunissen JW, Kaplan MI, Hunt PA, Williams BR, Ferguson DO, Alt FW, Petrini JH** 2003 Checkpoint failure and chromosomal instability without lymphomagenesis in Mre11(ATLD1/ATLD1) mice. Mol Cell 12:1511–1523
256. **Stewart GS, Maser RS, Stankovic T, Bressan DA, Kaplan MI, Jaspers NG, Raams A, Byrd PJ, Petrini JH, Taylor AM** 1999 The DNA double-strand break repair gene hMRE11 is mutated in individuals with an ataxia-telangiectasia-like disorder. Cell 99:577–587
257. **Assenmacher N, Hopfner KP** 2004 MRE11/RAD50/NBS1: complex activities. Chromosoma 113:157–166
258. **Stracker TH, Theunissen JW, Morales M, Petrini JH** 2004 The Mre11 complex and the metabolism of chromosome breaks: the importance of communicating and holding things together. DNA Repair (Amst) 3:845–854
259. **Connelly JC, de Leau ES, Leach DR** 2003 Nucleolytic processing of a protein-bound DNA end by the *E. coli* SbcCD (MR) complex. DNA Repair (Amst) 2:795–807
260. **Connelly JC, Leach DR** 2002 Tethering on the brink: the evolutionarily conserved Mre11-Rad50 complex. Trends Biochem Sci 27:410–418
261. **Hopfner KP, Craig L, Moncalian G, Zinkel RA, Usui T, Owen BA, Karcher A, Henderson B, Bodmer JL, McMurray CT, Carney JP, Petrini JH, Tainer JA** 2002 The Rad50 zinc-hook is a structure joining Mre11 complexes in DNA recombination and repair. Nature 418:562–566
262. **Arthur LM, Gustausson K, Hopfner KP, Carson CT, Stracker TH, Karcher A, Felton D, Weitzman MD, Tainer J, Carney JP** 2004 Structural and functional analysis of Mre11–3. Nucleic Acids Res 32:1886–1893
263. **de Jager M, Trujillo KM, Sung P, Hopfner KP, Carney JP, Tainer JA, Connelly JC, Leach DR, Kanaar R, Wyman C** 2004 Differential arrangements of conserved building blocks among homologs of the Rad50/Mre11 DNA repair protein complex. J Mol Biol 339:937–949
264. **Hopfner KP, Karcher A, Craig L, Woo TT, Carney JP, Tainer JA** 2001 Structural biochemistry and interaction architecture of the DNA double-strand break repair Mre11 nuclease and Rad50-ATPase. Cell 105:473–485
265. **Hopfner KP, Putnam CD, Tainer JA** 2002 DNA double-strand break repair from head to tail. Curr Opin Struct Biol 12:115–122
266. **Hopfner KP, Tainer JA** 2003 Rad50/SMC proteins and ABC transporters: unifying concepts from high-resolution structures. Curr Opin Struct Biol 13:249–255
267. **Moncalian G, Lengsfeld B, Bhaskara V, Hopfner KP, Karcher A, Alden E, Tainer JA, Paull TT** 2004 The rad50 signature motif: essential to ATP binding and biological function. J Mol Biol 335:937–951
268. **de Jager M, van Noort J, van Gent DC, Dekker C, Kanaar R,**

Wyman C 2001 Human Rad50/Mre11 is a flexible complex that can tether DNA ends. Mol Cell 8:1129–1135
269. **Paull TT, Lee JH** 2005 The Mre11/Rad50/Nbs1 Complex and Its Role as a DNA Double-Strand Break Sensor for ATM. Cell Cycle 4:737–740
270. **Bressan DA, Baxter BK, Petrini JH** 1999 The Mre11-Rad50-Xrs2 protein complex facilitates homologous recombination-based double-strand break repair in *Saccharomyces cerevisiae*. Mol Cell Biol 19:7681–7687
271. **Lim DS, Kim ST, Xu B, Maser RS, Lin J, Petrini JH, Kastan MB** 2000 ATM phosphorylates p95/nbs1 in an S-phase checkpoint pathway. Nature 404:613–617
272. **Falck J, Coates J, Jackson SP** 2005 Conserved modes of recruitment of ATM, ATR and DNA-PKcs to sites of DNA damage. Nature 434:605–611
273. **Lee JH, Paull TT** 2005 ATM activation by DNA double-strand breaks through the Mre11-Rad50-Nbs1 complex. Science 308:551–554
274. **Lavin MF, Khanna KK** 1999 ATM: the protein encoded by the gene mutated in the radiosensitive syndrome ataxia-telangiectasia. Int J Radiat Biol 75:1201–1214
275. **Cheng WH, Sakamoto S, Fox JT, Komatsu K, Carney J, Bohr VA** 2005 Werner syndrome protein associates with γH2AX in a manner that depends upon Nbs1. FEBS Lett 579:1350–1356
276. **Keeney S** 2001 Mechanism and control of meiotic recombination initiation. Curr Top Dev Biol 52:1–53
277. **Haber JE** 1998 The many interfaces of Mre11. Cell 95:583–586
278. **Nairz K, Klein F** 1997 mre11S–a yeast mutation that blocks double-strand-break processing and permits nonhomologous synapsis in meiosis. Genes Dev 11:2272–2290
279. **Borde V, Lin W, Novikov E, Petrini JH, Lichten M, Nicolas A** 2004 Association of Mre11p with double-strand break sites during yeast meiosis. Mol Cell 13:389–401
280. **Usui T, Ohta T, Oshiumi H, Tomizawa J, Ogawa H, Ogawa T** 1998 Complex formation and functional versatility of Mre11 of budding yeast in recombination. Cell 95:705–716
281. **Tsubouchi H, Ogawa H** 1998 A novel mre11 mutation impairs processing of double-strand breaks of DNA during both mitosis and meiosis. Mol Cell Biol 18:260–268
282. **Ohta K, Nicolas A, Furuse M, Nabetani A, Ogawa H, Shibata T** 1998 Mutations in the MRE11, RAD50, XRS2, and MRE2 genes alter chromatin configuration at meiotic DNA double-stranded break sites in premeiotic and meiotic cells. Proc Natl Acad Sci USA 95:646–651
283. **Farah JA, Cromie G, Steiner WW, Smith GR** 2005 A novel recombination pathway initiated by the Mre11/Rad50/Nbs1 complex eliminates palindromes during meiosis in *Schizosaccharomyces pombe*. Genetics 169:1261–1274
284. **Chin GM, Villeneuve AM** 2001 *C. elegans* mre-11 is required for meiotic recombination and DNA repair but is dispensable for the meiotic G(2) DNA damage checkpoint. Genes Dev 15:522–534
285. **Bleuyard JY, Gallego ME, White CI** 2004 Meiotic defects in the Arabidopsis rad50 mutant point to conservation of the MRX complex function in early stages of meiotic recombination. Chromosoma 113:197–203
286. **Bannister LA, Schimenti JC** 2004 Homologous recombinational repair proteins in mouse meiosis. Cytogenet Genome Res 107:191–200
287. **Zhu J, Petersen S, Tessarollo L, Nussenzweig A** 2001 Targeted disruption of the Nijmegen breakage syndrome gene NBS1 leads to early embryonic lethality in mice. Curr Biol 11:105–109
288. **Goedecke W, Eijpe M, Offenberg HH, van Aalderen M, Heyting C** 1999 Mre11 and Ku70 interact in somatic cells, but are differentially expressed in early meiosis. Nat Genet 23:194–198
289. **Nugent CI, Bosco G, Ross LO, Evans SK, Salinger AP, Moore JK, Haber JE, Lundblad V** 1998 Telomere maintenance is dependent on activities required for end repair of double-strand breaks. Curr Biol 8:657–660
290. **Le S, Moore JK, Haber JE, Greider CW** 1999 RAD50 and RAD51 define two pathways that collaborate to maintain telomeres in the absence of telomerase. Genetics 152:143–152
291. **Williams BR, Mirzoeva OK, Morgan WF, Lin J, Dunnick W, Petrini JH** 2002 A murine model of Nijmegen breakage syndrome. Curr Biol 12:648–653
292. **Furuse M, Nagase Y, Tsubouchi H, Murakami-Murofushi K, Shibata T, Ohta K** 1998 Distinct roles of two separable in vitro activities of yeast Mre11 in mitotic and meiotic recombination. EMBO J 17:6412–6425
293. **Ivanov EL, Korolev VG, Fabre F** 1992 XRS2, a DNA repair gene of *Saccharomyces cerevisiae*, is needed for meiotic recombination. Genetics 132:651–664
294. **Bender CF, Sikes ML, Sullivan R, Huye LE, Le Beau MM, Roth DB, Mirzoeva OK, Oltz EM, Petrini JH** 2002 Cancer predisposition and hematopoietic failure in Rad50(S/S) mice. Genes Dev 16:2237–2251
295. **Luo G, Yao MS, Bender CF, Mills M, Bladl AR, Bradley A, Petrini JH** 1999 Disruption of mRad50 causes embryonic stem cell lethality, abnormal embryonic development, and sensitivity to ionizing radiation. Proc Natl Acad Sci USA 96:7376–7381
296. **Boder E** 1975 Ataxia-telangiectasia: some historic, clinical and pathologic observations. Birth Defects Orig Artic Ser 11:255–270
297. **Sedgewick R, Boder E** 1991 Ataxia-telangiectasia. In: Vinken P, Bruyn G, Klawans H, eds. Handbook of clinical neurology. New York: Elsevier Scientific Publishers; 347–423
298. **Savitsky K, Bar-Shira A, Gilad S, Rotman G, Ziv Y, Vanagaite L, Tagle DA, Smith S, Uziel T, Sfez S, Ashkenazi M, Pecker I, Frydman M, Harnik R, Patanjali SR, Simmons A, Clines GA, Sartiel A, Gatti RA, Chessa L, Sanal O, Lavin MF, Jaspers NGJ, Taylor AMR, et al** 1995 A single ataxia telangiectasia gene with a product similar to PI-3 kinase. Science 268:1749–1753
299. **Lavin M, Birrell G, Chen P, Kozlov S, Scott S, Gueven N** 2005 ATM signaling and genomic stability in response to DNA damage. Mutat Res 569:123–132
300. **Bakkenist CJ, Kastan MB** 2003 DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. Nature 421:499–506
301. **Kozlov S, Gueven N, Keating K, Ramsay J, Lavin MF** 2003 ATP activates ataxia-telangiectasia mutated (ATM) in vitro. Importance of autophosphorylation. J Biol Chem 278:9309–9317
302. **Nakada D, Matsumoto K, Sugimoto K** 2003 ATM-related Tel1 associates with double-strand breaks through an Xrs2-dependent mechanism. Genes Dev 17:1957–1962
303. **Usui T, Ogawa H, Petrini JH** 2001 A DNA damage response pathway controlled by Tel1 and the Mre11 complex. Mol Cell 7:1255–1266
304. **Shroff R, Arbel-Eden A, Pilch D, Ira G, Bonner WM, Petrini JH, Haber JE, Lichten M** 2004 Distribution and dynamics of chromatin modification induced by a defined DNA double-strand break. Curr Biol 14:1703–1711
305. **Lisby M, Barlow JH, Burgess RC, Rothstein R** 2004 Choreography of the DNA damage response: spatiotemporal relationships among checkpoint and repair proteins. Cell 118:699–713
306. **Lavin MF** 2004 The Mre11 complex and ATM: a two-way functional interaction in recognising and signaling DNA double strand breaks. DNA Repair (Amst) 3:1515–1520
307. **Khanna KK, Keating KE, Kozlov S, Scott S, Gatei M, Hobson K, Taya Y, Gabrielli B, Chan D, Lees-Miller SP, Lavin MF** 1998 ATM associates with and phosphorylates p53: mapping the region of interaction. Nat Genet 20:398–400
308. **Gatei M, Young D, Cerosaletti KM, Desai-Mehta A, Spring K, Kozlov S, Lavin MF, Gatti RA, Concannon P, Khanna K** 2000 ATM-dependent phosphorylation of nibrin in response to radiation exposure. Nat Genet 25:115–119
309. **Gatei M, Scott SP, Filippovitch I, Soronika N, Lavin MF, Weber B, Khanna KK** 2000 Role for ATM in DNA damage-induced phosphorylation of BRCA1. Cancer Res 60:3299–3304
310. **Dasika GK, Lin SC, Zhao S, Sung P, Tomkinson A, Lee EY** 1999 DNA damage-induced cell cycle checkpoints and DNA strand break repair in development and tumorigenesis. Oncogene 18:7883–7899
311. **Hirao A, Cheung A, Duncan G, Girard PM, Elia AJ, Wakeham A, Okada H, Sarkissian T, Wong JA, Sakai T, De Stanchina E, Bristow RG, Suda T, Lowe SW, Jeggo PA, Elledge SJ, Mak TW** 2002 Chk2 is a tumor suppressor that regulates apoptosis in both an ataxia telangiectasia mutated (ATM)-dependent and an ATM-independent manner. Mol Cell Biol 22:6521–6532
312. **Buscemi G, Savio C, Zannini L, Micciche F, Masnada D, Nakanishi M, Tauchi H, Komatsu K, Mizutani S, Khanna K, Chen P, Concannon P, Chessa L, Delia D** 2001 Chk2 activation dependence on Nbs1 after DNA damage. Mol Cell Biol 21:5214–5222
313. **Falck J, Mailand N, Syljuasen RG, Bartek J, Lukas J** 2001 The

ATM-Chk2-Cdc25A checkpoint pathway guards against radioresistant DNA synthesis. Nature 410:842–847
314. **Matsuoka S, Rotman G, Ogawa A, Shiloh Y, Tamai K, Elledge SJ** 2000 Ataxia telangiectasia-mutated phosphorylates Chk2 in vivo and in vitro. Proc Natl Acad Sci USA 97:10389–10394
315. **Chaturvedi P, Eng WK, Zhu Y, Mattern MR, Mishra R, Hurle MR, Zhang X, Annan RS, Lu Q, Faucette LF, Scott GF, Li X, Carr SA, Johnson RK, Winkler JD, Zhou BB** 1999 Mammalian Chk2 is a downstream effector of the ATM-dependent DNA damage checkpoint pathway. Oncogene 18:4047–4054
316. **Matsuoka S, Huang M, Elledge SJ** 1998 Linkage of ATM to cell cycle regulation by the Chk2 protein kinase. Science 282:1893–1897
317. **Keegan KS, Holtzman DA, Plug AW, Christenson ER, Brainerd EE, Flaggs G, Bentley NJ, Taylor EM, Meyn MS, Moss SB, Carr AM, Ashley T, Hoekstra MF** 1996 The Atr and Atm protein kinases associate with different sites along meiotically pairing chromosomes. Genes Dev 10:2423–2437
318. **Scherthan H, Jerratsch M, Dhar S, Wang YA, Goff SP, Pandita TK** 2000 Meiotic telomere distribution and Sertoli cell nuclear architecture are altered in Atm- and Atm-p53-deficient mice. Mol Cell Biol 20:7773–7783
319. **Plug AW, Peters AH, Xu Y, Keegan KS, Hoekstra MF, Baltimore D, de Boer P, Ashley T** 1997 ATM and RPA in meiotic chromosome synapsis and recombination. Nat Genet 17:457–461
320. **Barlow C, Hirotsune S, Paylor R, Liyanage M, Eckhaus M, Collins F, Shiloh Y, Crawley JN, Ried T, Tagle D, Wynshaw-Boris A** 1996 Atm-deficient mice: a paradigm of ataxia telangiectasia. Cell 86: 159–171
321. **Elson A, Wang Y, Daugherty CJ, Morton CC, Zhou F, Campos-Torres J, Leder P** 1996 Pleiotropic defects in ataxia-telangiectasia protein-deficient mice. Proc Natl Acad Sci USA 93:13084–13089
322. **Xu Y, Baltimore D** 1996 Dual roles of ATM in the cellular response to radiation and in cell growth control. Genes Dev 10:2401–2410
323. **Xu Y, Ashley T, Brainerd EE, Bronson RT, Meyn MS, Baltimore D** 1996 Targeted disruption of ATM leads to growth retardation, chromosomal fragmentation during meiosis, immune defects, and thymic lymphoma. Genes Dev 10:2411–2422
324. **Barlow C, Liyanage M, Moens PB, Tarsounas M, Nagashima K, Brown K, Rottinghaus S, Jackson SP, Tagle D, Ried T, Wynshaw-Boris A** 1998 Atm deficiency results in severe meiotic disruption as early as leptonema of prophase I. Development 125:4007–4017
325. **Libby BJ, De La Fuente R, O'Brien MJ, Wigglesworth K, Cobb J, Inselman A, Eaker S, Handel MA, Eppig JJ, Schimenti JC** 2002 The mouse meiotic mutation mei1 disrupts chromosome synapsis with sexually dimorphic consequences for meiotic progression. Dev Biol 242:174–187
326. **Brown KD, Barlow C, Wynshaw-Boris A** 1999 Multiple ATM-dependent pathways: an explanation for pleiotropy. Am J Hum Genet 64:46–50
327. **Alter BP** 2003 Cancer in Fanconi anemia, 1927–2001. Cancer 97: 425–440
328. **de Winter JP, Waisfisz Q, Rooimans MA, van Berkel CG, Bosnoyan-Collins L, Alon N, Carreau M, Bender O, Demuth I, Schindler D, Pronk JC, Arwert F, Hoehn H, Digweed M, Buchwald M, Joenje H** 1998 The Fanconi anaemia group G gene FANCG is identical with XRCC9. Nat Genet 20:281–283
329. **Stewart G, Elledge SJ** 2002 The two faces of BRCA2, a FANCtastic discovery. Mol Cell 10:2–4
330. **Garcia-Higuera I, Taniguchi T, Ganesan S, Meyn MS, Timmers C, Hejna J, Grompe M, D'Andrea AD** 2001 Interaction of the Fanconi anemia proteins and BRCA1 in a common pathway. Mol Cell 7:249–262
331. **Bargman GJ, Shahidi NT, Gilbert EF, Opitz JM** 1977 Studies of malformation syndromes of man XLVII: disappearance of spermatogonia in the Fanconi anemia syndrome. Eur J Pediatr 125:163–168
332. **Berkovitz GD, Zinkham WH, Migeon CJ** 1984 Gonadal function in two siblings with Fanconi's anemia. Horm Res 19:137–141
333. **Wong JC, Alon N, McKerlie C, Huang JR, Meyn MS, Buchwald M** 2003 Targeted disruption of exons 1 to 6 of the Fanconi anemia group A gene leads to growth retardation, strain-specific microphthalmia, meiotic defects and primordial germ cell hypoplasia. Hum Mol Genet 12:2063–2076
334. **Alter BP, Frissora CL, Halperin DS, Freedman MH, Chitkara U, Alvarez E, Lynch L, Adler-Brecher B, Auerbach AD** 1991 Fanconi's anaemia and pregnancy. Br J Haematol 77:410–418
335. **Chen M, Tomkins DJ, Auerbach W, McKerlie C, Youssoufian H, Liu L, Gan O, Carreau M, Auerbach A, Groves T, Guidos CJ, Freedman MH, Cross J, Percy DH, Dick JE, Joyner AL, Buchwald M** 1996 Inactivation of Fac in mice produces inducible chromosomal instability and reduced fertility reminiscent of Fanconi anaemia. Nat Genet 12:448–451
336. **Whitney MA, Royle G, Low MJ, Kelly MA, Axthelm MK, Reifsteck C, Olson S, Braun RE, Heinrich MC, Rathbun RK, Bagby GC, Grompe M** 1996 Germ cell defects and hematopoietic hypersensitivity to γ-interferon in mice with a targeted disruption of the Fanconi anemia C gene. Blood 88:49–58
337. **Koomen M, Cheng NC, van de Vrugt HJ, Godthelp BC, van der Valk MA, Oostra AB, Zdzienicka MZ, Joenje H, Arwert F** 2002 Reduced fertility and hypersensitivity to mitomycin C characterize Fancg/Xrcc9 null mice. Hum Mol Genet 11:273–281
338. **Holzel M, van Diest PJ, Bier P, Wallisch M, Hoatlin ME, Joenje H, de Winter JP** 2003 FANCD2 protein is expressed in proliferating cells of human tissues that are cancer-prone in Fanconi anaemia. J Pathol 201:198–203
339. **Otsuki T, Furukawa Y, Ikeda K, Endo H, Yamashita T, Shinohara A, Iwamatsu A, Ozawa K, Liu JM** 2001 Fanconi anemia protein, FANCA, associates with BRG1, a component of the human SWI/SNF complex. Hum Mol Genet 10:2651–2660
340. **Howlett NG, Taniguchi T, Olson S, Cox B, Waisfisz Q, De Die-Smulders C, Persky N, Grompe M, Joenje H, Pals G, Ikeda H, Fox EA, D'Andrea AD** 2002 Biallelic inactivation of BRCA2 in Fanconi anemia. Science 297:606–609
341. **Hussain S, Witt E, Huber PA, Medhurst AL, Ashworth A, Mathew CG** 2003 Direct interaction of the Fanconi anaemia protein FANCG with BRCA2/FANCD1. Hum Mol Genet 12:2503–2510
342. **Hunt PA, Koehler KE, Susiarjo M, Hodges CA, Ilagan A, Voigt RC, Thomas S, Thomas BF, Hassold TJ** 2003 Bisphenol a exposure causes meiotic aneuploidy in the female mouse. Curr Biol 13:546–553
343. **Swan SH, Elkin EP, Fenster L** 2000 The question of declining sperm density revisited: an analysis of 101 studies published 1934–1996. Environ Health Perspect 108:961–966
344. **Ludwig T, Fisher P, Ganesan S, Efstratiadis A** 2001 Tumorigenesis in mice carrying a truncating Brca1 mutation. Genes Dev 15:1188–1193
345. **Wei K, Clark AB, Wong E, Kane MF, Mazur DJ, Parris T, Kolas NK, Russell R, Hou Jr H, Kneitz B, Yang G, Kunkel TA, Kolodner RD, Cohen PE, Edelmann W** 2003 Inactivation of Exonuclease 1 in mice results in DNA mismatch repair defects, increased cancer susceptibility, and male and female sterility. Genes Dev 17:603–614
346. **Houghtaling S, Timmers C, Noll M, Finegold MJ, Jones SN, Meyn MS, Grompe M** 2003 Epithelial cancer in Fanconi anemia complementation group D2 (Fancd2) knockout mice. Genes Dev 17:2021–2035
347. **Celeste A, Petersen S, Romanienko PJ, Fernandez-Capetillo O, Chen HT, Sedelnikova OA, Reina-San-Martin B, Coppola V, Meffre E, Difilippantonio MJ, Redon C, Pilch DR, Olaru A, Eckhaus M, Camerini-Otero RD, Tessarollo L, Livak F, Manova K, Bonner WM, Nussenzweig MC, Nussenzweig A** 2002 Genomic instability in mice lacking histone H2AX. Science 296:922–927
348. **Fernandez-Capetillo O, Mahadevaiah SK, Celeste A, Romanienko PJ, Camerini-Otero RD, Bonner WM, Manova K, Burgoyne P, Nussenzweig A** 2003 H2AX is required for chromatin remodeling and inactivation of sex chromosomes in male mouse meiosis. Dev Cell 4:497–508
349. **Dix DJ, Allen JW, Collins BW, Mori C, Nakamura N, Poorman-Allen P, Goulding EH, Eddy EM** 1996 Targeted gene disruption of Hsp70–2 results in failed meiosis, germ cell apoptosis, and male infertility. Proc Natl Acad Sci USA 93:3264–3268

Endocrine Reviews **is published by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**