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The Hop2 Protein Has a Direct Role in Promoting Interhomolog Interactions during Mouse Meiosis

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Summary

The *S. cerevisiae* Hop2 protein and its fission yeast homolog Meu13 are required for proper homologous chromosome pairing and recombination during meiosis. The mechanism of this requirement is, however, not understood. The previous studies in *Saccharomyces* suggested that Hop2 is a guardian of meiotic chromosome synapsis with the ability to prevent or resolve deleterious associations between nonhomologous chromosomes. We have generated a *Hop2* knockout mouse that shows profound meiotic defects with a distinct and novel phenotype. *Hop2*$^{-/-}$ spermatocytes arrest at the stage of pachytene-like chromosome condensation. Axial elements are fully developed, but synapsis of any kind is very limited. Immunofluorescence analysis of meiotic chromosome spreads indicates that while meiotic double-stranded breaks are formed and processed in the *Hop2* knockout, they fail to be repaired. In aggregate, the *Hop2* phenotype is consistent with a direct role for the mouse Hop2 protein in promoting homologous chromosome synapsis.

Introduction

During meiosis, diploid precursor cells give rise to haploid gametes through the precisely controlled segregation of homologous chromosomes. In order for reductional division to occur, the homologs must first pair and engage in a transient physical association, required for the subsequent segregation to the different poles of the cell. This physical linkage is ensured by homologous recombination. Another important outcome of this event is the genetic exchange between parental chromosomes. Arguably the most puzzling question in the field is how homologs find each other in genomes of very high complexity, harboring up to thousands of megabases of genetic information. In most organisms, chromosome pairing culminates in the formation of the synaptonemal complex (SC). This proteinacious ribbon-like structure holds homologs closely apposed along their entire length. Homologous chromosome pairing, recombination, and SC formation are closely interconnected in most organisms, but their precise role has been difficult to determine since distinct mechanisms to promote and/or stabilize these homologous interactions have developed in the course of evolution.

During meiosis, a type II topoisomerase Spo11 cleaves the chromosomal DNA at many sites (200 or more in mammals) in each and every nucleus (Keeney, 2001). The repair of these DNA double-stranded breaks (DSBs) is mediated by homologous recombination (HR). In most organisms, including *S. cerevisiae* (reviewed in Roeder, 1997; Zickler and Kleckner, 1999) and mice (Baudat et al., 2000; Romanienko and Camerini-Otero, 2000), but not in *D. melanogaster* and *C. elegans* (Dernburg et al., 1998; McKim et al., 1998), initiation of HR is required for proper synapsis of the homologs in the context of the SC. Flies and worms have developed alternative mechanisms to stabilize pairing interactions and promote SC formation (reviewed in Roeder, 1997; Villeneuve and Hillers, 2001; Walker and Hawley, 2000; Zickler and Kleckner, 1999; also see MacQueen et al., 2002). Curiously, the genes encoding three conservative meiosis-specific proteins—Dmc1, Hop2, and Mnd1—are only present in the genomes of organisms that require DSBs (hence Spo11) for meiotic chromosome synapsis (that is, they are absent from the genomes of flies and worms). At the same time, these three genes are among two dozen genes with the greatest changes in their level of expression in Spo11$^{-/-}$ mice (N. Smirnova and R.D.C.-O., unpublished).

The *hop2* deletion mutant in *S. cerevisiae* fails to sporulate and undergoes a uniform arrest at the pachytene stage of meiosis I (Leu et al., 1998). SC formation is strongly delayed but ultimately proceeds to almost wild-type level of synapsis, albeit mostly between nonhomologous chromosomes. The mutant sustains wild-type levels of meiotic DSBs that are processed to expose single-stranded tails but not repaired. Formation of mature crossover recombinants drops to 0%–7% of the wild-type level, depending on the sensitivity of the assay (Leu et al., 1998; Tsubouchi and Roeder, 2002).

The *MND1* gene was first discovered in a screen for meiotically expressed genes (Rabitsch et al., 2001) and was later identified as a multicopy suppressor of a *hop2-ts* allele (Tsubouchi and Roeder, 2002), and an *mnd1* null mutant has a phenotype similar to that of the *hop2* deletion (Gerton and DeRisi, 2002; Tsubouchi and Roeder, 2002). Co-immunoprecipitation of Mnd1 and Hop2 from meiotic extracts suggests the involvement of the two in the same meiotic process (Tsubouchi and Roeder, 2002).

In budding yeast, *dmc1* mutants arrest (or experience a substantial delay, depending on the genetic background) at the same stage as *hop2* mutants (Bishop et al., 1992; Rockmill and Roeder, 1994; Rockmill et al., 1995). The chromosomes at this point are maximally condensed and correspond in length to the pachytene stage of wild-type meiosis (Roeder and Bailis, 2000). Overall, the recombination-defective phenotype of the *hop2* null mutation is remarkably reminiscent of the *dmc1* deletion, but with a more severe defect when both are compared in the same genetic background. However, a few important differences suggest distinct

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roles for these two proteins in the same pairing/recombination pathway (Leu et al., 1998; Roeder and Bailis, 2000; Shimada et al., 2002).

Dmc1 knockout mice revealed the conserved function of Dmc1 throughout evolution (Pittman et al., 1998; Yoshida et al., 1998). To define the function of the mammalian *Hop2*, we have generated a knockout mouse. The animals did not show any gross anomalies in somatic tissues but developed a profound deficiency in gametogenesis. Evaluation of the chromosomal spreads of knockout spermatocytes revealed striking meiotic defects, consistent with a failure in recombination. Our data strongly suggest that Hop2 has a direct role in promoting early steps of interhomolog interactions.

Results

Disruption and Inactivation of the Mouse *Hop2* Gene

The mouse *Hop2* gene (*mTBPIP*) was disrupted by gene targeting in embryonic stem (ES) cells. The targeting vector was designed to replace the first three exons of Hop2 with the Neomycin-resistance gene (NEO) marker (see Supplemental Figure S1A at http://www.developmentalcell.com/cgi/content/full/5/6/927/DC1). A correctly targeted clone was injected into blastocysts, and the first progeny of chimeric mice was genotyped by Southern blot analysis (Supplemental Figure S1B). The ability to generate homozygous knockout mice indicates that Hop2 is not essential for mouse development. A polyclonal antibody raised against the intact mouse Hop2 protein revealed a band with an apparent size of 29 kDa on Western blots of wild-type and heterozygote but not knockout testes extracts (Supplemental Figure S1C, left panel). Even though the remaining four C-terminal exons could potentially code for three partial Hop2 polypeptides of 108, 92, or 39 amino acids, a polyclonal antibody raised against the C terminus peptide failed to detect any protein products in the knockout mouse (Supplemental Figure S1C, right panel). Thus, we have generated a mouse Hop2 knockout strain that produces no part of the Hop2 protein. Since the introduction of the NEO gene targeting marker could result in the silencing of adjacent promoters (Artelt et al., 1991), we examined the transcription of genes located up to 8 kb up- and downstream of the inserted NEO gene (GenBank mRNA accession numbers NM 026501 and NM 011550, respectively). We found no significant difference between wild-type and knockout mRNA levels for either of these genes using PCR (data not shown).

Defects in Gametogenesis of the *Hop2*$^{-/-}$ Mice Are Consistent with a Role for the Protein in Meiosis

In good agreement with the role of the yeast Hop2 protein in meiosis (Leu et al., 1998; Nabeshima et al., 2001), the *Hop2* knockout mice are infertile. Males develop testicular hypoplasia with hyperplasia of interstitial cells and lack of spermatozoa (Figure 1A). Knockout testes are approximately 30% of the size of the wild-type. This reduction in size is similar to that observed in other mouse knockouts of early meiotic genes such as *Dmc1* and *Spo11* (Baudat et al., 2000; Pittman et al., 1998; Romanienko and Camerini-Otero, 2000; Yoshida et al., 1998). The number of seminiferous tubules is not reduced, but the diameter is 25%–30% of normal. Primary spermatocytes represent the most advanced spermatogenic cells in the *Hop2* knockout, indicating that spermatogenesis is blocked at prophase of meiosis I. Arrested cells undergo extensive apoptosis that could be detected in a fraction of the tubules (Figure 1C). Female *Hop2* knockouts show ovarian tubulostromal hyperplasia with no follicles present (Figure 1B). The lack of mature gametes in both sexes of *Hop2* knockout mice is consistent with a role for the Hop2 protein in meiosis.

Heterozygous mice of both sexes are fertile with no obvious reproductive abnormalities. 6-month-old knockout mice do not show gross changes in any of the 32 somatic tissues examined (including thymus and spleen). Out of the first 374 pups born, 27.8% are homozygous, a number that is close to a normal Mendelian distribution and suggests that *Hop2* is not an essential gene.

Hop2 Knockout Spermatocytes Arrest prior to Pachytene and Show a Marked Failure in Chromosome Synapsis

To investigate the defect in meiosis I in greater detail, we prepared chromosomal spreads from *Hop2*$^{-/-}$ spermatocytes (Figures 2A, 2B, and 3). The cells proceed normally through leptotene and early zygotene with 40 centromeres clearly identified by CREST antisera (Brenner et al., 1981). The correct number of centromeres and chromosome cores indicates that sister chromatid cohesion is not affected in the absence of Hop2 (Figure 2A). Immunostaining for the Scp3/Cor1 protein of axial/lateral elements (Dobson et al., 1994) revealed that homologous chromosomes are not assembled into the synaptonemal complex. Even though the chromosomes in arrested *Hop2* spermatocytes look very compact and relatively stiff (pachytene-like), only limited synapsis could be seen in a fraction of cells (Figure 2B, panels II and III). Synapsed regions could be detected by staining for the Scp1/Syn1 protein, a component of the central element of the synaptonemal complex (Dobson et al., 1994). The most advanced *Hop2* spermatocytes show short stretches of synapsed regions, mostly between nonhomologous chromosomes (Figure 2B, see below). However, chromosomes appear longer in the cells with maximal synapsis compared to those without it. The extent of nonhomologous synapsis varies from none (Figure 2A) to rather extensive synapsis (Figure 2B, panel III), but does not exceed 33% of the total length of a chromosome complement (Figure 2C). The extent of synapsis in the Hop2-arrested spermatocytes is significantly lower than in *Spo11*$^{-/-}$ mice (data not shown) or *Dmc1*$^{-/-}$ mice (Figure 2C). This difference could not be attributed to the different genetic background of the knockout mice, since pairs of littermates from double heterozygote crosses were used in this analysis. With respect to the extent of synapsis, the double *Hop2*$^{-/-}$*Dmc1*$^{-/-}$ knockout mice display the *Hop2*$^{-/-}$ phenotype (Figure 2C). This indicates an epistatic relationship between these two genes and argues for an earlier role of Hop2 in meiosis compared to Dmc1.

At earlier stages, Scp1 protein could be seen on the

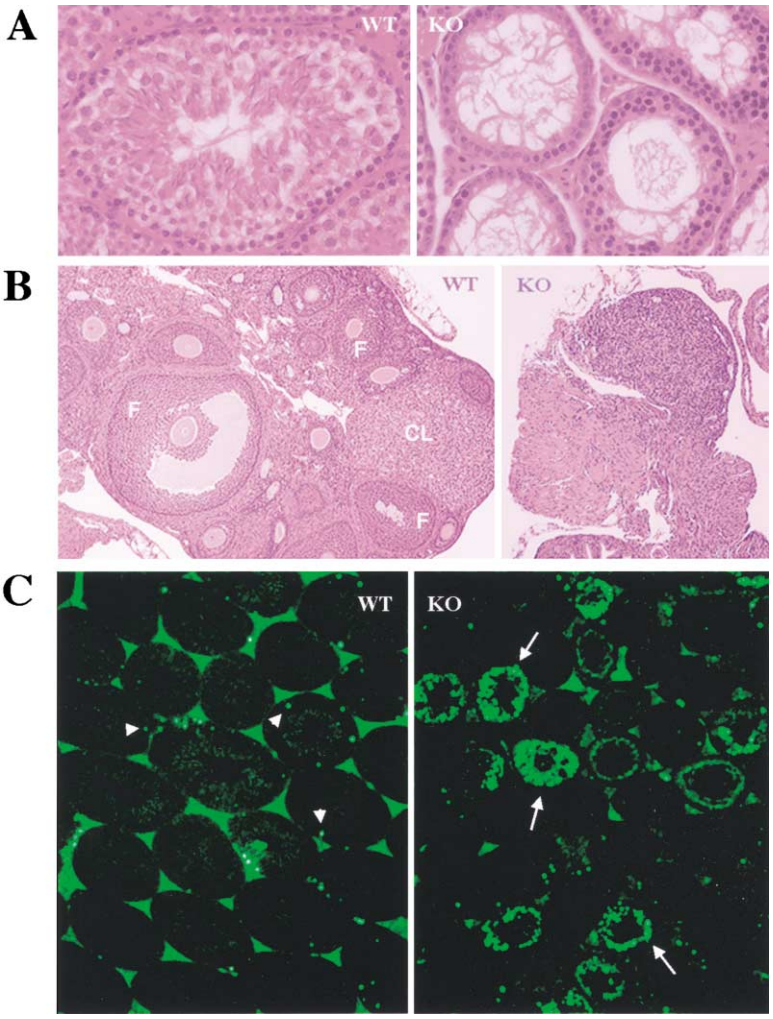


Figure 1. *Hop2* Knockout Mice Show Profound Defects in Gametogenesis

(A) Histological sections of wild-type (left) and knockout (right) testes. Note the reduced size of the seminiferous tubules in the mutant and the lack of cells beyond the primary spermatocyte stage.

(B) Histological sections of wild-type (left) and knockout (right) ovaries. Note the numerous follicles (F) at various development stages and the corpa lutea (CL) in the wild-type ovary. No follicles are present in the knockout ovary, and it is greatly reduced in size.

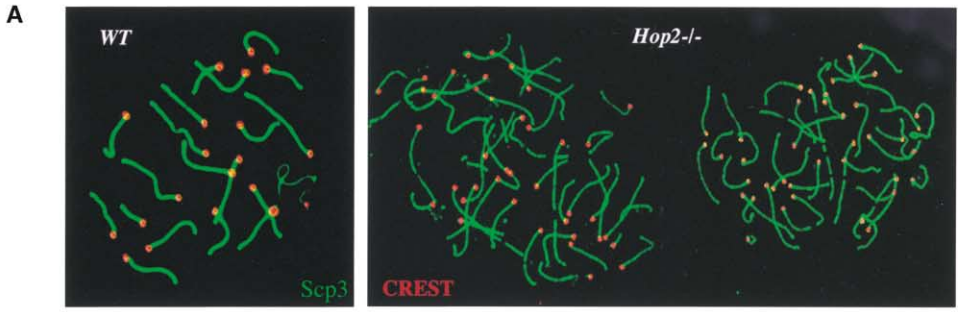
(C) TUNEL assay for apoptotic cells within the wild-type (left) and knockout (right) seminiferous tubules. Occasional apoptotic cells (arrowheads) are present in wild-type testes, whereas extensive apoptosis is seen in a fraction of the seminiferous tubules of knockout mice (arrows).

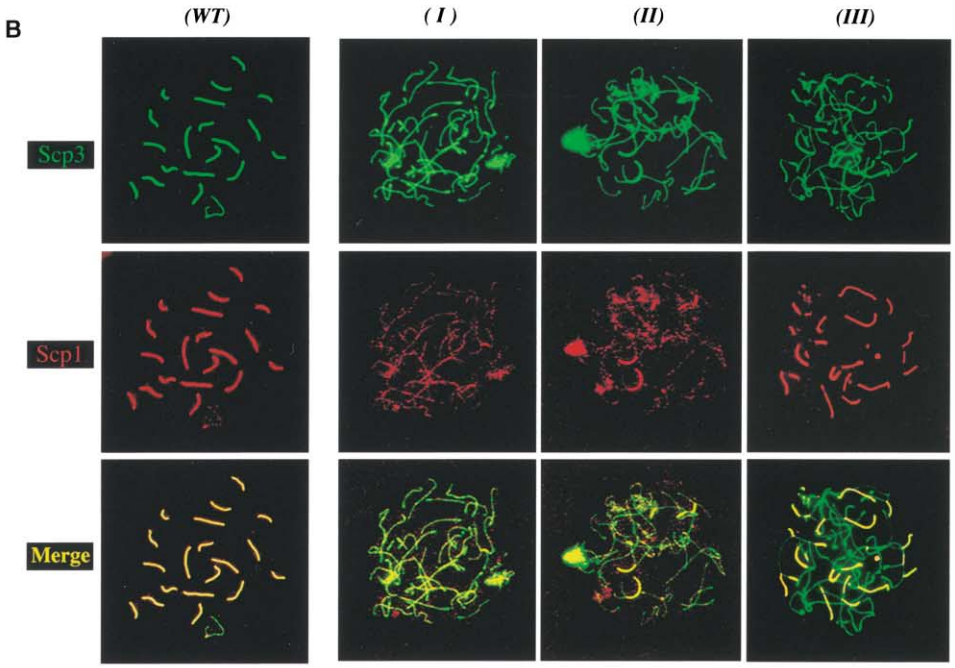
axial elements of the asynapsed *Hop2* chromosomes (Figure 2B, panels I and II). This staining pattern is unusual for wild-type cells, although a limited number of transverse filaments (Scp1) has been previously reported in association with the partially synapsed chromosome cores in Locusta (Moens, 1969). Most likely, the binding of Scp1 to the axial elements promotes the fast assembly of the SC. This step may be too transient to detect in the normal course of mouse meiosis but is rather obvious in the arrested *Hop2* mutant (Figure 2B). A similar pattern of Scp1 staining could be observed in *Dmc1* knockout spermatocytes (data not shown). However, reduced levels of Scp1 are also present on the unpaired regions of X and Y chromosomes in wild-type pachytene spermatocytes (Figure 2B, panel WT). It is possible that a significant accumulation of the Scp1 protein at asynapsed chromosome cores takes place only if synapsis is delayed or impossible.

Careful analysis of the synapsed regions shows that synapsis in *Hop2*$^{-/-}$ spermatocytes takes place mostly between nonhomologous chromosomes (Figure 2B, panel III). Some chromosomes are engaged in more then one interaction, leading to the formation of a large chromosomal network. To determine if any of the chromosomes are actually synapsed homologously, we used chromosome painting (Figure 2D). Hybridization of the *Hop2* knockout spreads with the chromosome *V*-specific probe showed no significant alignment in the majority of the cells. In 88% of spermatocytes with a strong hybridization signal, two copies of chromosome *V* were clearly separated, and in 10% of the cells they partially overlapped (data not shown). Thus, the synapsis in *Hop2* knockout spermatocytes is not only decreased, but also nonhomologous.

DSBs Are Formed but not Repaired in *Hop2*$^{-/-}$ Spermatocytes

Spo11-mediated DSBs initiate meiotic homologous recombination, and the repair of these breaks is required for chromosome synapsis in most organisms including the mouse (Keeney, 2001; Roeder, 1997; Villeneuve and Hillers, 2001; Zickler and Kleckner, 1999). The appearance of the breaks in leptotene is accompanied by the phosphorylation of histone H2ax over megabase-sized chromatin regions (Mahadevaiah et al., 2001). In wild-type mouse spermatocytes, this stage can be easily recognized by anti-γH2ax (phosphorylated H2ax) antibodies that produce a cloud-like staining pattern all over the chromatin. The Rad50/Mre11/Nbs1 protein complex processes the breaks in preparation for repair by the





C

Synapsis	>10%	>20%	>30%	Total
Hop2-/-	16	5	4	25
Dmc1-/-	32	19	10	61
Hop2-/-Dmc-/-	15	9	2	26

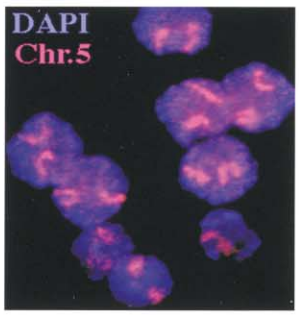


Figure 2. Homologous Chromosomes Fail to Synapse in the *Hop2* Knockout

Chromosomal spreads were prepared from adult mouse spermatocytes and immunostained (A and B) with anti-SCP3, anti-SCP1, or CREST antisera, as indicated.

(A) Left: Pachytene wild-type spermatocyte (WT) showing complete synapsis. Right: Arrested *Hop2*$^{-/-}$ spermatocytes showing no synapsis.

(B) Left column: A wild-type (WT) pachytene spermatocyte. Right columns: *Hop2* knockout spermatocytes at different stages after arrest. (I) An early stage with fully developed axial elements but completely asynapsed chromosomes. (II) An intermediate stage. (III) A cell with maximum synapsis. SCP1 staining localizes to the asynapsed chromosome cores at the earlier stages and later relocates to the synapsed regions. Note the nonhomologous nature of the synapsis in panel III.

(C) Chromosomes in *Hop2* knockout spermatocytes show less synapsis than in *Dmc1* knockout spermatocytes. The cells with the indicated degree of synapsis were counted in populations of 100 randomly picked arrested spermatocytes from *Hop2*$^{-/-}$, *Dmc1*$^{-/-}$, or *Hop2*$^{-/-}$*Dmc1*$^{-/-}$ mice. Total indicates the number of cells with significant (more than 10%) synapsis. The majority of the scored synaptic events involve nonhomologous chromosomes.

(D) Fluorescent in situ hybridization with a chromosome *V*-specific probe shows no homologous chromosome alignment in the *Hop2* spermatocytes.

homologous recombination machinery. In wild-type spermatocytes, these proteins can be detected all over the nucleus up until early zygotene (Eijpe et al., 2000; and data not shown). By pachytene, Rad50/Mre11/Nbs1 and γH2ax staining remains only in the sex body region (Eijpe et al., 2000; Mahadevaiah et al., 2001). Current models (Allers and Lichten, 2001; Villeneuve and Hillers, 2001) suggest that the major event in meiotic recombination is the Rad51/Dmc1-mediated homology search followed by the single end invasion (Hunter and Kleckner, 2001) of the intact homologous chromosome. Rad51 and Dmc1 are believed to load onto the single-stranded ends of resected DSBs and can be observed as numerous foci on the chromosome cores. Replication Protein A (RPA) is known to assist Rad51 in the strand exchange reaction in vitro (Sigurdsson et al., 2001; Sung and Robberson, 1995). In mouse meiosis, RPA foci form on chromosomal cores shortly after Rad51 and Dmc1 are loaded and colocalizes with them (Moens et al., 2002; Plug et al., 1998). These foci disappear in pachytene, when the breaks are successfully repaired.

Hop2$^{-/-}$ spermatocytes show extensive γH2ax staining that persists to the most advanced stages with the maximum extent of synapsis (Figure 3A). Similarly, Nbs1 staining remains distributed all over the nuclei (Figure 3B). Rad51, Dmc1, and Rpa foci are highly abundant in *Hop2*$^{-/-}$ spermatocytes and localize to synapsed as well as asynapsed regions (Figures 3C–3H). These results indicate that DSBs are generated in the *Hop2* knockout spermatocytes but are not repaired. Furthermore, the presence of the Rad51/Dmc1/Rpa foci also strongly suggests that the DNA ends are already processed and prepared for strand invasion but that in the absence of the Hop2 protein the repair cannot proceed. Thus, the chromosomes have DSBs, and the proper RecA homologs are loaded onto them as if they are primed for and on the cusp of synapsis but can't initiate this process. These findings suggest that the Hop2 protein might play a heretofore-unrecognized central role in bringing meiotic chromosomes together.

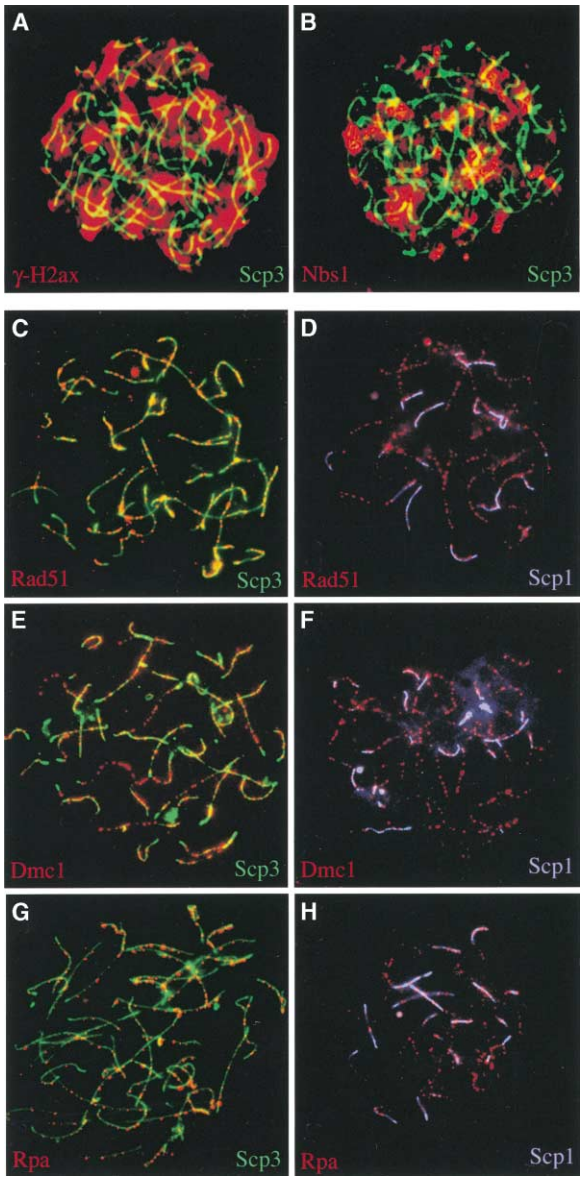


Figure 3. Meiotic DSBs Are Formed but not Repaired in *Hop2* Knockout Spermatocytes

Chromosome spreads were prepared from adult *Hop2* knockout spermatocytes and immunostained with specific antibodies as indicated. See text for discussion.

Discussion

The Meiotic Function of the Hop2 Protein Is Conserved in Yeast and Mammals

Genetic studies in yeast suggested a role for the Hop2 protein in homologous pairing and recombination (Leu et al., 1998; Nabeshima et al., 2001; Shimada et al., 2002; Tsubouchi and Roeder, 2002). In *Hop2* knockout male mice, meiosis is arrested at the stage that could be defined as pachytene with respect to the level of chromosome compaction. Axial elements are complete, but no synapsis takes place at the appropriate time: on the 15th day after birth, when 60% of wild-type spermatocytes are in pachytene with complete synapsis, no *Hop2* spermatocytes show any degree of synapsis. Arrested spermatocytes eventually accumulate Scp1/Cor1 on asynapsed chromosome cores and undergo nonhomologous synapsis that could reach as much as 33%. Since meiosis in mammals is not synchronized except for the first wave of spermatogenesis in juvenile mice, adult animals have a mixed population of arrested spermatocytes with variable degrees of synapsis. Nonhomologous synapsis seems to be a common feature among yeast mutants with delayed synapsis (Zickler and Kleckner, 1999). The *S. cerevisiae hop2* null mutation is rather unique in this regard because of the very high degree (up to wild-type levels) of SC assembly between nonhomologous chromosomes (Leu et al., 1998). We did not, however, observe such a phenomenon in mice. In fact, we see noticeably less synapsis in *Hop2* spermatocytes than in other meiotic gene knockouts (Figure 2C and data not shown). This discrepancy could be attributed to the rapid apoptosis of arrested spermatocytes in mice: arrested cells simply could not survive till the corresponding stage described in yeast. Furthermore, after 28 hr in sporulation media, when synapsis in pachytene-blocked yeast *ndt80* cells is complete, the degree of

synapsis in a *hop2* mutant is not as striking as was described for later time points (Tsubouchi and Roeder, 2002). Thus, the synaptic defect in *Hop2*$^{-/-}$ mice is similar to that in budding yeast *hop2*: SC formation is delayed and aberrant.

Phosphorylation of the H2ax histone (Mahadevaiah et al., 2001) in *Hop2* knockout spermatocytes indicates that meiotic DSB formation does not require Hop2 function. However, the repair of the breaks must be postponed, since γH2ax staining persists until the latest stages in arrested spermatocytes. Similarly, since numerous Rad51/Dmc1 foci form and stay along the chromosomal cores, the removal of Spo11 and resection of the DSB ends must not be affected. The accumulation of Rad51/Dmc1 foci in knockout spermatocytes has been shown for a number of genes involved in homologous pairing and recombination (see for example Pittman et al., 1998, for *Dmc1*$^{-/-}$; Edelmann et al., 1999, for *Msh5*$^{-/-}$; Kneitz et al., 2000, for *Msh4*$^{-/-}$; or Yuan et al., 2000, for *Scp3*$^{-/-}$). The striking difference in the *Hop2* knockout is the very high degree of chromosome compaction combined with the complete lack of even nonhomologous synapsis in a large fraction of cells.

What could be the role of Hop2 that a lack of this protein disrupts meiosis in such a profound and unique manner? In principle, there are several possibilities. First, mouse Hop2 could be a component of the synaptonemal complex. Since our numerous attempts to raise anti-Hop2 antibody suitable for immunofluorescence studies were not fruitful, the localization of the protein in spermatocytes is still unknown. Nevertheless, the following two reasons make the structural role of Hop2 in SC formation highly unlikely: (1) in *S. cerevisiae*, the protein localizes to the meiotic chromatin, rather than chromosome cores, as would be expected for an SC component; and (2) the *meu13* (*hop2*) mutation results in a recombination deficient phenotype even in *S. pombe*, where SC does not form in the normal course of meiosis. Second, Hop2 could be involved in HR indirectly, by regulating the expression of gene(s) involved in homologous pairing or recombination. The function of Hop2 as a transciptional modulator has been reported (Ijichi et al., 2000; Ko et al., 2002; Tanaka et al., 1997). However, as discussed in the next section, the phenotype of the Hop2$^{-/-}$ mice could not be easily explained by the absence of this activity. Thus, we favor the third possibility, that Hop2 is directly involved in meiotic homologous recombination (see below).

The Meiotic Phenotype of the *Hop2*$^{-/-}$ Mice Is Consistent with a Failure in Recombination

The human counterpart of the Hop2 protein, GT198, was found to interact with a number of nuclear hormone receptors and to stimulate the transcription mediated by some of these receptors (Ko et al., 2002). However, mice with targeted disruptions of nuclear receptor genes show phenotypes that are very different from that of the *Hop2*$^{-/-}$ mice (Cooke and Saunders, 2002; Couse and Korach, 1998; Gothe et al., 1999; Reichardt et al., 1998). This indicates that there is a different basis for the fertility defects in the Hop2 knockout. So far, we have been unable to discern any somatic defects in the *Hop2* knockout mice. In addition, the meiotic defects of the *Hop2* disruption in unicellular organisms like yeast are similar to the mouse knockout phenotype (see Results and Discussion). Thus, the meiosis-specific phenotype observed in the *Hop2* knockout mouse is most likely the consequence of a lack of a recombinational Hop2 function rather than its role as a transcription factor. Curiously, the expression of GT198 was reported to be restricted to the tissues with developmentally regulated recombination events (primarily testis, but also thymus, spleen, and ovaries) (Ko et al., 2002).

Essential Role of Hop2 in Meiotic Recombination

As opposed to only one RecA protein in bacteria, unicellular eukaryotes have four RecA homologs (Rad51, Rad55, Rad57, and Dmc1 in *S. cerevisiae*) and mammals have seven (Rad51, Rad51B, Rad51C, Rad51D, Xrcc2, Xrcc3, and Dmc1 proteins). Still, *E. coli* RecA remains the most robust recombinase in the family, whereas its eukaryoic homologs either do not possess canonical recombinational activities in vitro or rely on a number of accessory proteins for their function (Sung et al., 2000; Symington, 2002). Most of these factors act in mitotic as well as meiotic recombination. The meiosis-specific RecA homolog, Dmc1 protein, is a rather inefficient recombinase, and no proteins so far have been found to stimulate its activity in vitro (Hong et al., 2001; Li et al., 1997; Masson et al., 1999). Rad51 protein requires RPA, Rad52, and Rad55/Rad57 proteins to carry out efficient strand exchange, whereas for D-loop formation, there is an additional requirement for the Rad54 or Rdh54 proteins (reviewed in Raoul Tan et al., 2003; Sung et al., 2000). Whereas single deletions of these last two proteins in yeast cause only mild sporulation defects (Symington, 2002), sporulation in the *rad54*/*rdh54* double mutants is severely impeded (Klein, 1997; Shinohara et al., 1997). On the other hand, single knockouts of both mouse *Rad54* homologs (*Rad54* and *Rad54B*) as well as the double *Rad54*/*Rad54B* knockout mice are not sterile (Essers et al., 1997; and R. Kanaar, personal communication). Since neither Dmc1 nor Rad51 can promote efficient strand invasion by itself, this implies that there might be additional factor(s) playing a critical role in meiotic recombination in mammals. Rad52 protein is the only of the Rad51 partners shown to promote D-loop formation on its own (Kagawa et al., 2001). Nevertheless, while *rad52* deletion yeast produce inviable spores (Game et al., 1980; Prakash et al., 1980), the knockout mice are fertile (Rijkers et al., 1998). This again suggests a missing essential player in mammalian meiotic recombination. We propose that Hop2 is the heretofore-unrecognized recombinase that either initiates the Rad51- and/or Dmc1-dependent heteroduplex formation by promoting single strand invasion or is an accessory factor for one or both these proteins.

Rad51 and Dmc1 form overlapping foci on the late leptotene-zygotene chromosome cores and are believed to act at the same stage of meiosis. We propose that Hop2 acts upstream of Rad51 and Dmc1 for the following reasons. First, epistatic analysis suggests an earlier role of Hop2 protein compared to Dmc1 (Figure 2C). A Rad51 deletion is lethal in mice (Tsuzuki et al., 1996), but both *S. cerevisae rad51* and *dmc1* mutants have a less severe meiotic defect than a *hop2* mutant

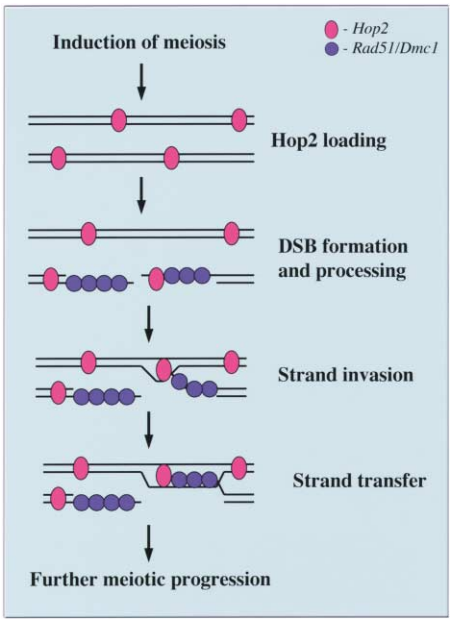


Figure 4. A Model for the Role of Mouse Hop2 in Meiotic Recombination

The Hop2 protein binds to meiotic chromosomes and promotes invasion of the intact homolog by a single-stranded tail of the resected DSB. This initial interaction is stabilized by Rad51/Dmc1-mediated strand exchange, leading to the formation of the first recombination intermediate, single end invasion (SEI). In addition, Hop2 binding to chromosomes may promote alignment between the homologs and further stabilization of the homologous pairing as described in the Discussion.

when compared in the same genetic background (Rockmill et al., 1995; Leu et al., 1998). Second, Hop2 localizes to chromatin before the loading of Rad51 and Dmc1 and even in the absence of DSBs (Tsubouchi and Roeder, 2002). Hop2 localization to the chromatin rather than chromosome cores is important, since most DSBs occur in the chromatin loops (Blat et al., 2002) and only later are pulled to the chromosome axes where RAD51/DMC1 foci are later observed. Hop2 might be localized to chromatin regions that will eventually be susceptible to the action of Spo11, thus ensuring that Hop2 will be in the vicinity of the ssDNA exposed on the resected ends. And finally, biochemical data indicate that mouse Hop2 can be involved in single strand invasion, the initial step in heteroduplex formation during homologous recombination (G.V.P., M.C. Gauthier, J.Y. Masson, and R.D.C.-O., unpublished).

The data from yeast suggest that the Hop2 (Meu13) protein could be involved in DSB-independent pairing during meiosis (Nabeshima et al., 2001; Tsubouchi and Roeder, 2002). However, the existence of recombination-independent homologous pairing in mammals has yet to be established. In budding yeast, homologous chromosome alignment could be detected in premeiotic cells. After a transient dissociation, pairing is restored by middle to late prophase (Weiner and Kleckner, 1994). In mammals, homologs are spatially separated in premeiotic cells, and high levels of homologous alignment is first observed in zygotene nuclei (Pfeifer et al., 2001). Thus, in mammals DSB-independent pairing should occur in the same time frame as meiotic DSB repair. If Hop2 is involved in such interactions, this activity could coincide with the recombinational function of the protein described above. It would be interesting to see if Hop2 alone or in combination with other proteins could promote duplex-duplex pairing interactions that would contribute to the pairing process in the absence of DSBs.

Based on our findings and data from studies in yeast, we propose the following model for Hop2 function in meiosis (Figure 4). The protein can bind to chromosomes regardless of meiotic DSB formation. In the normal course of meiosis Hop2 binds to chromosomes before the loading of Rad51/Dmc1 proteins onto the single-stranded tails of the resected breaks (Leu et al., 1998; Tsubouchi and Roeder, 2002). Since there is an indication for a role of Hop2 in DSB-independent homologous pairing (Leu et al., 1998; Nabeshima et al., 2001), the protein could ensure such associations by homologous duplex-duplex interactions or otherwise. Regardless of this, Hop2 may promote strand invasion of the intact chromosome by a broken homolog. This initial interaction could be stabilized by strand exchange promoted by Rad51/Dmc1 and other proteins required for formation and further processing of heteroduplexes.

Experimental Procedures

Generation and Characterization of the Knockout Mice

The targeting construct was based on the ploxPneo vector (Yang et al., 1998) (a gift from Dr. Chu-Xia Deng). A 5.7 kb BsaBI/NotI fragment containing the 5′ region of the mouse *Hop2* gene was cloned in the ploxPneo plasmid between the *TK* and *neo* genes in an opposite orientation to the *neo* gene. A 6.9 kb PacI/Eco47III fragment containing introns 4–8 of the *Hop2* gene was cloned on the other side of the *neo* cassette. The plasmid was linearized and transfected into 1.8×10^7 TC1 ES cells (Deng et al., 1996) (a gift from Dr. Chu-Xia Deng) by electroporation. The cells were seeded onto mitomycin C-treated mouse embryonic feeder cells (Incyte Genomics), and the colonies were selected in media containing LIF (GIBCO-BRL), 350 μg/ml G418, and 5 μM gancyclovir. Colonies were picked and expanded, and genomic DNA was extracted for Southern blot analysis. The DNA was digested with EcoRI and XhoI restriction enzymes and hybridized with either 5′ or 3′ probes (see Supplemental Figure S1A at http://www.developmentalcell.com/cgi/content/full/5/6/927/DC1). Cells from one of the correctly targeted ES clones were injected into C57/B6 blastocysts to obtain germline transmission. For genotyping the tips of the tails, 1–1.5 mm was incubated in 200 μl of lysis buffer (20 ml TrisHCl [pH 8.0], 2.5 mM $MgCl_2$, 50 mM KCl, 0.45% NP-40, 0.45% Tween 20, and 1.2 mg/ml Proteinase K) for at least 4 hr. The protease was inactivated for 15 min at 95°C, and 1 μl of the DNA mixture was used for further analysis. The genotyping was done by PCR using primers 1 (5′-CCGTCTTTCTTGGGCGTTCAAATG) and 2 (5′-CTAGTAGCGGCTG TCAAGAAGTCG) to amplify the wild-type allele (a 410 bp fragment) and primers 1 and 3 (5′-CAGCTCATTCCTCCCACTCATGATC) to amplify the knockout allele (a 770 bp fragment). The Advantage 2 DNA Polymerase Mix (Clontech) was used in 20 μl reactions. Tissues for histological examination were removed and fixed overnight in 10% neutral-buffered formalin (Sigma). Tissues were embedded in paraffin and 6 μm sections were cut (American Histolabs). Gross and histopathologic diagnoses of the knockout mice were carried out by the NIH ORS/VRP Mouse Phenotyping Service.

Antibodies

Anti-mHop2 rabbit polyclonal antibodies were raised against the full-length protein or the 40 amino acid C-terminal peptide. Anti-Scp3 mouse polyclonal antibodies were raised against a GST fusion of the full-length rat Scp3 protein. Commercial primary antibodies

against γH2AX (Trevigen), Rad51, and Dmc1 (Santa Cruz Biotechnology, H92 and C-20, respectively) were used for immunofluorescence. Rabbit anti-Rpa was a gift from P. Moens (Moens et al., 2002), mouse anti-Scp1 antibodies was a gift from C. Heyting (Eijpe et al., 2003), rabbit anti-Scp1 antibody was a gift from C. Hoog (Liu et al., 1996), anti-Nbs1 antibody was a gift from J. Petrini (Williams et al., 2002), and CREST antisera was a gift from B. Brinkley (Brenner et al., 1981). Secondary antibodies were from Jackson IR laboratories. The primary antibodies were used at the following dilutions: anti-Hop2, 1:5000 (Westerns); anti-SCP3, 1:800; anti-γH2AX, 1:100; anti-Rad51, 1:50; anti-Dmc1, 1:100; anti-Rpa, 1:200; mouse anti-Scp1, 1:20; rabbit anti-Scp1, 1:100; anti-Nbs1, 1:100; and CREST antiserum, 1:500. Rad51 and Dmc1 antibodies did not cross-react on a Western blot prepared from testis extracts (1:100–1:200 dilutions). Though unlikely, we cannot, however, exclude the possibility that the Rad51 antibody could recognize Dmc1 protein on the chromosomal spreads. Since the anti-Dmc1 antibody did not generate a signal in the *Dmc1* knockout mice, the Dmc1 antibodies can be considered specific.

Meiotic Chromosome Spreads and Immunofluorescence

The protocol was modified from the procedure described by Moens and coauthors (Moens et al., 1998). One wild-type testis was dissected and the capsule was removed. Seminiferous tubules were finely chopped with a scalpel in a Petri dish containing 10 ml of RPMI 1640 high-glucose media (GIBCO-BRL). The cells were released from the tubules by gently pipetting in and out and filtered through a 40 μm cell strainer (Falcon). The suspension was centrifuged for 8 min at 800 × g, and the cells were resuspended in 10 ml of RPMI and pelleted again. The resulting pellet was resuspended in 1 ml of 0.5% NaCl after any residual media had been removed. 15 μl of the cell suspension was added to the glass slides with hydrophobic rings (Becton Dickinson), and the cells were allowed to adhere without drying out for 10–15 min. The slides were fixed in 2% paraformaldehyde and 0.03% SDS for 3 min, 2% paraformaldehyde for 3 min, washed 3 times in 0.4% Photo-Flo 200 (Kodak) for 1 min, and air dried. The slides were stored at −20°C.

For chromosome painting, the cell pellet was prepared as described above and the cells were resuspended in 10 ml of 0.075 M KCl. The suspension was incubated at 37°C for 15 min followed by addition of a few drops of freshly prepared fixative (methanol:glacial acetic acid, 3:1). The cells were pelleted, resuspended in 10 ml of fixative solution, and pelleted again. The last step was repeated one more time and the resulting pellet was resuspended in 1 ml of fixative solution. 10 μl of the suspension was dropped on the glass slides and let air dry.

For immunostaining, the slides were incubated with blocking solution (10% goat or donkey serum, 3% BSA, and 0.05% Triton X-100 in PBS) for 20 min at 37°C in a humidity chamber. Primary antibodies were diluted in blocking buffer and incubated under the same conditions for 1–2 hr. After two 5 min washes in 0.4% Photo-Flo/PBS solution, slides were blocked for an additional 5 min and incubated with secondary antibodies for 20 min at 37°C. The slides were washed twice with 0.4% Photo-Flo in PBS, rinsed twice with 0.4% Photo-Flo, and allowed to air dry. Vectashield Mounting Medium with DAPI (Vector Laboratories) was added and the slides were covered with cover slips and viewed with a Leica fluorescent microscope. The images were captured at 1000× magnification with OpenLab software and processed using Adobe Photoshop.

Chromosome Painting

The chromosome *V*-specific probe was purchased from Applied Genetics Laboratories, Inc., and hybridization was carried out according to the protocol provided.

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References

Allers, T., and Lichten, M. (2001). Differential timing and control of noncrossover and crossover recombination during meiosis. Cell *106*, 47–57.

Artelt, P., Grannemann, R., Stocking, C., Friel, J., Bartsch, J., and Hauser, H. (1991). The prokaryotic neomycin-resistance-encoding gene acts as a transcriptional silencer in eukaryotic cells. Gene *99*, 249–254.

Baudat, F., Manova, K., Yuen, J.P., Jasin, M., and Keeney, S. (2000). Chromosome synapsis defects and sexually dimorphic meiotic progression in mice lacking Spo11. Mol. Cell *6*, 989–998.

Bishop, D.K., Park, D., Xu, L., and 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.

Blat, Y., Protacio, R.U., Hunter, N., and Kleckner, N. (2002). Physical and functional interactions among basic chromosome organizational features govern early steps of meiotic chiasma formation. Cell *111*, 791–802.

Brenner, S., Pepper, D., Berns, M.W., Tan, E., and Brinkley, B.R. (1981). Kinetochore structure, duplication, and distribution in mammalian cells: analysis by human autoantibodies from scleroderma patients. J. Cell Biol. *91*, 95–102.

Cooke, H.J., and Saunders, P.T. (2002). Mouse models of male infertility. Nat. Rev. Genet. *3*, 790–801.

Couse, J.F., and Korach, K.S. (1998). Exploring the role of sex steroids through studies of receptor deficient mice. J. Mol. Med. *76*, 497–511.

Deng, C., Wynshaw-Boris, A., Zhou, F., Kuo, A., and Leder, P. (1996). Fibroblast growth factor receptor 3 is a negative regulator of bone growth. Cell *84*, 911–921.

Dernburg, A.F., McDonald, K., Moulder, G., Barstead, R., Dresser, M., and Villeneuve, A.M. (1998). Meiotic recombination in *C. elegans* initiates by a conserved mechanism and is dispensable for homologous chromosome synapsis. Cell *94*, 387–398.

Dobson, M.J., Pearlman, R.E., Karaiskakis, A., Spyropoulos, B., and Moens, P.B. (1994). Synaptonemal complex proteins: occurrence, epitope mapping and chromosome disjunction. J. Cell Sci. *107*, 2749–2760.

Edelmann, W., Cohen, P.E., Kneitz, B., Winand, N., Lia, M., Heyer, J., Kolodner, R., Pollard, J.W., and Kucherlapati, R. (1999). Mammalian MutS homologue 5 is required for chromosome pairing in meiosis. Nat. Genet. *21*, 123–127.

Eijpe, M., Offenberg, H., Goedecke, W., and Heyting, C. (2000). Localisation of RAD50 and MRE11 in spermatocyte nuclei of mouse and rat. Chromosoma *109*, 123–132.

Eijpe, M., Offenberg, H., Jessberger, R., Revenkova, E., and Heyting, C. (2003). Meiotic cohesin REC8 marks the axial elements of rat synaptonemal complexes before cohesins SMC1beta and SMC3. J. Cell Biol. *160*, 657–670.

Essers, J., Hendriks, R.W., Swagemakers, S.M., Troelstra, C., de Wit, J., Bootsma, D., Hoeijmakers, J.H., and Kanaar, R. (1997). Disruption of mouse RAD54 reduces ionizing radiation resistance and homologous recombination. Cell *89*, 195–204.

Game, J.C., Zamb, T.J., Braun, R.J., Resnick, M., and Roth, R.M. (1980). The role of the radiation (rad) genes in meiotic recombination in yeast. Genetics *94*, 51–68.

Gerton, J.L., and DeRisi, J.L. (2002). Mnd1p: an evolutionarily conserved protein required for meiotic recombination. Proc. Natl. Acad. Sci. USA *99*, 6895–6900.

Gothe, S., Wang, Z., Ng, L., Kindblom, J.M., Barros, A.C., Ohlsson, C., Vennstrom, B., and Forrest, D. (1999). Mice devoid of all known thyroid hormone receptors are viable but exhibit disorders of the

pituitary-thyroid axis, growth, and bone maturation. Genes Dev. *13*, 1329–1341.

Hong, E.L., Shinohara, A., and Bishop, D.K. (2001). *Saccharomyces cerevisiae* Dmc1 protein promotes renaturation of single-strand DNA (ssDNA) and assimilation of ssDNA into homologous supercoiled duplex DNA. J. Biol. Chem. *276*, 41906–41912.

Hunter, N., and 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.

Ijichi, H., Tanaka, T., Nakamura, T., Yagi, H., Hakuba, A., and Sato, M. (2000). Molecular cloning and characterization of a human homologue of TBPIP, a BRCA1 locus-related gene. Gene *248*, 99–107.

Kagawa, W., Kurumizaka, H., Ikawa, S., Yokoyama, S., and Shibata, T. (2001). Homologous pairing promoted by the human Rad52 protein. J. Biol. Chem. *276*, 35201–35208.

Keeney, S. (2001). Mechanism and control of meiotic recombination initiation. Curr. Top. Dev. Biol. *52*, 1–53.

Klein, H.L. (1997). RDH54, a RAD54 homologue in *Saccharomyces cerevisiae*, is required for mitotic diploid-specific recombination and repair and for meiosis. Genetics *147*, 1533–1543.

Kneitz, B., Cohen, P.E., Avdievich, E., Zhu, L., Kane, M.F., Hou, H., Jr., Kolodner, R.D., Kucherlapati, R., Pollard, J.W., and 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.

Ko, L., Cardona, G.R., Henrion-Caude, A., and Chin, W.W. (2002). Identification and characterization of a tissue-specific coactivator, GT198, that interacts with the DNA-binding domains of nuclear receptors. Mol. Cell. Biol. *22*, 357–369.

Leu, J.Y., Chua, P.R., and Roeder, G.S. (1998). The meiosis-specific Hop2 protein of *S. cerevisiae* ensures synapsis between homologous chromosomes. Cell *94*, 375–386.

Li, Z., Golub, E.I., Gupta, R., and Radding, C.M. (1997). Recombination activities of HsDmc1 protein, the meiotic human homolog of RecA protein. Proc. Natl. Acad. Sci. USA *94*, 11221–11226.

Liu, J.G., Yuan, L., Brundell, E., Bjorkroth, B., Daneholt, B., and Hoog, C. (1996). Localization of the N-terminus of SCP1 to the central element of the synaptonemal complex and evidence for direct interactions between the N-termini of SCP1 molecules organized head-to-head. Exp. Cell Res. *226*, 11–19.

MacQueen, A.J., Colaiacovo, M.P., McDonald, K., and Villeneuve, A.M. (2002). Synapsis-dependent and -independent mechanisms stabilize homolog pairing during meiotic prophase in *C. elegans*. Genes Dev. *16*, 2428–2442.

Mahadevaiah, S.K., Turner, J.M., Baudat, F., Rogakou, E.P., de Boer, P., Blanco-Rodriguez, J., Jasin, M., Keeney, S., Bonner, W.M., and Burgoyne, P.S. (2001). Recombinational DNA double-strand breaks in mice precede synapsis. Nat. Genet. *27*, 271–276.

Masson, J.Y., Davies, A.A., Hajibagheri, N., Van Dyck, E., Benson, F.E., Stasiak, A.Z., Stasiak, A., and West, S.C. (1999). The meiosis-specific recombinase hDmc1 forms ring structures and interacts with hRad51. EMBO J. *18*, 6552–6560.

McKim, K.S., Green-Marroquin, B.L., Sekelsky, J.J., Chin, G., Steinberg, C., Khodosh, R., and Hawley, R.S. (1998). Meiotic synapsis in the absence of recombination. Science *279*, 876–878.

Moens, P.B. (1969). The fine structure of meiotic chromosome polarization and pairing in *Locusta migratoria* spermatocytes. Chromosoma *28*, 1–25.

Moens, P.B., Pearlman, R.E., Heng, H.H., and Traut, W. (1998). Chromosome cores and chromatin at meiotic prophase. Curr. Top. Dev. Biol. *37*, 241–262.

Moens, P.B., Kolas, N.K., Tarsounas, M., Marcon, E., Cohen, P.E., and 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.

Nabeshima, K., Kakihara, Y., Hiraoka, Y., and Nojima, H. (2001). A novel meiosis-specific protein of fission yeast, Meu13p, promotes homologous pairing independently of homologous recombination. EMBO J. *20*, 3871–3881.

Pfeifer, C., Thomsen, P.D., and Scherthan, H. (2001). Centromere and telomere redistribution precedes homologue pairing and terminal synapsis initiation during prophase I of cattle spermatogenesis. Cytogenet. Cell Genet. *93*, 304–314.

Pittman, D.L., Cobb, J., Schimenti, K.J., Wilson, L.A., Cooper, D.M., Brignull, E., Handel, M.A., and Schimenti, J.C. (1998). Meiotic prophase arrest with failure of chromosome synapsis in mice deficient for Dmc1, a germline-specific RecA homolog. Mol. Cell *1*, 697–705.

Plug, A.W., Peters, A.H., Keegan, K.S., Hoekstra, M.F., de Boer, P., and Ashley, T. (1998). Changes in protein composition of meiotic nodules during mammalian meiosis. J. Cell Sci. *111*, 413–423.

Prakash, S., Prakash, L., Burke, W., and Montelone, B. (1980). Effects of the RAD52 gene on recombination in *Saccharomyces cerevisiae*. Genetics *94*, 31–50.

Rabitsch, K.P., Toth, A., Galova, M., Schleiffer, A., Schaffner, G., Aigner, E., Rupp, C., Penkner, A.M., Moreno-Borchart, A.C., Primig, M., et al. (2001). A screen for genes required for meiosis and spore formation based on whole-genome expression. Curr. Biol. *11*, 1001–1009.

Raoul Tan, T.L., Kanaar, R., and Wyman, C. (2003). Rad54, a Jack of all trades in homologous recombination. DNA Repair (Amst.) *2*, 787–794.

Reichardt, H.M., Kaestner, K.H., Tuckermann, J., Kretz, O., Wessely, O., Bock, R., Gass, P., Schmid, W., Herrlich, P., Angel, P., and Schutz, G. (1998). DNA binding of the glucocorticoid receptor is not essential for survival. Cell *93*, 531–541.

Rijkers, T., Van Den Ouweland, J., Morolli, B., Rolink, A.G., Baarends, W.M., Van Sloun, P.P., Lohman, P.H., and Pastink, A. (1998). Targeted inactivation of mouse RAD52 reduces homologous recombination but not resistance to ionizing radiation. Mol. Cell. Biol. *18*, 6423–6429.

Rockmill, B., and Roeder, G.S. (1994). The yeast med1 mutant undergoes both meiotic homolog nondisjunction and precocious separation of sister chromatids. Genetics *136*, 65–74.

Rockmill, B., Sym, M., Scherthan, H., and Roeder, G.S. (1995). Roles for two RecA homologs in promoting meiotic chromosome synapsis. Genes Dev. *9*, 2684–2695.

Roeder, G.S. (1997). Meiotic chromosomes: it takes two to tango. Genes Dev. *11*, 2600–2621.

Roeder, G.S., and Bailis, J.M. (2000). The pachytene checkpoint. Trends Genet. *16*, 395–403.

Romanienko, P.J., and Camerini-Otero, R.D. (2000). The mouse Spo11 gene is required for meiotic chromosome synapsis. Mol. Cell *6*, 975–987.

Shimada, M., Nabeshima, K., Tougan, T., and Nojima, H. (2002). The meiotic recombination checkpoint is regulated by checkpoint rad+ genes in fission yeast. EMBO J. *21*, 2807–2818.

Shinohara, M., Shita-Yamaguchi, E., Buerstedde, J.M., Shinagawa, H., Ogawa, H., and Shinohara, A. (1997). Characterization of the roles of the *Saccharomyces cerevisiae* RAD54 gene and a homologue of RAD54, RDH54/TID1, in mitosis and meiosis. Genetics *147*, 1545–1556.

Sigurdsson, S., Trujillo, K., Song, B., Stratton, S., and Sung, P. (2001). Basis for avid homologous DNA strand exchange by human Rad51 and RPA. J. Biol. Chem. *276*, 8798–8806.

Sung, P., and Robberson, D.L. (1995). DNA strand exchange mediated by a RAD51-ssDNA nucleoprotein filament with polarity opposite to that of RecA. Cell *82*, 453–461.

Sung, P., Trujillo, K.M., and Van Komen, S. (2000). Recombination factors of *Saccharomyces cerevisiae*. Mutat. Res. *451*, 257–275.

Symington, L.S. (2002). Role of RAD52 epistasis group genes in homologous recombination and double-strand break repair. Microbiol. Mol. Biol. Rev. *66*, 630–670.

Tanaka, T., Nakamura, T., Takagi, H., and Sato, M. (1997). Molecular cloning and characterization of a novel TBP-1 interacting protein (TBPIP):enhancement of TBP-1 action on Tat by TBPIP. Biochem. Biophys. Res. Commun. *239*, 176–181.

Tsubouchi, H., and Roeder, G.S. (2002). The Mnd1 protein forms a complex with hop2 to promote homologous chromosome pairing and meiotic double-strand break repair. Mol. Cell. Biol. *22*, 3078–3088.

Tsuzuki, T., Fujii, Y., Sakumi, K., Tominaga, Y., Nakao, K., Sekiguchi, M., Matsushiro, A., Yoshimura, Y., and Morita, T. (1996). Targeted disruption of the Rad51 gene leads to lethality in embryonic mice. Proc. Natl. Acad. Sci. USA *93*, 6236–6240.

Villeneuve, A.M., and Hillers, K.J. (2001). Whence meiosis? Cell *106*, 647–650.

Walker, M.Y., and Hawley, R.S. (2000). Hanging on to your homolog: the roles of pairing, synapsis and recombination in the maintenance of homolog adhesion. Chromosoma *109*, 3–9.

Weiner, B.M., and Kleckner, N. (1994). Chromosome pairing via multiple interstitial interactions before and during meiosis in yeast. Cell *77*, 977–991.

Williams, B.R., Mirzoeva, O.K., Morgan, W.F., Lin, J., Dunnick, W., and Petrini, J.H. (2002). A murine model of Nijmegen breakage syndrome. Curr. Biol. *12*, 648–653.

Yang, X., Li, C., Xu, X., and Deng, C. (1998). The tumor suppressor SMAD4/DPC4 is essential for epiblast proliferation and mesoderm induction in mice. Proc. Natl. Acad. Sci. USA *95*, 3667–3672.

Yoshida, K., Kondoh, G., Matsuda, Y., Habu, T., Nishimune, Y., and Morita, T. (1998). The mouse RecA-like gene Dmc1 is required for homologous chromosome synapsis during meiosis. Mol. Cell *1*, 707–718.

Yuan, L., Liu, J.G., Zhao, J., Brundell, E., Daneholt, B., and Hoog, C. (2000). The murine SCP3 gene is required for synaptonemal complex assembly, chromosome synapsis, and male fertility. Mol. Cell *5*, 73–83.

Zickler, D., and Kleckner, N. (1999). Meiotic chromosomes: integrating structure and function. Annu. Rev. Genet. *33*, 603–754.