Proteins Involved in Meiotic Recombination: A Role in Male Infertility?

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INTRODUCTION

Meiotic recombination occurs in prophase I of meiosis and involves the induction of double strand breaks (DSB), the pairing of parental (homologous) chromosomes and the subsequent repair of DSB using an intact homologous chromosome as a template. This differs from somatic homologous recombination, which uses the sister chromatid as a template for repair. Meiotic recombination results in the reciprocal exchange of genetic sequences between homologous chromosomes and a complement of alleles that are not associated with each other on either of the parental chromosomes.

Meiotic recombination requires the careful coordination of several groups of proteins that direct genetic crossover between homologous chromosomes during prophase I. Prophase I of meiosis is divided into 4 stages: leptotene, zygotene, pachytene and diplotene. These stages are defined by the...
morphology of the XY bivalent and the synaptic progression of the autosomal bivalents [Solari and Tres 1970; Solari 1980]. During the leptotene stage, programmed DSB are formed and marked for repair. Events in the zygotene stage include homology search between homologous chromosomes and the initiation of synopsis. The completion of synopsis of homologous chromosomes and the repair of DSB, using homologous chromosomes as templates, occur from the mid-zygotene through the pachytene stages. The culmination of this process results in the reciprocal exchange of genetic information between homologs, which is completed through the mid-pachytene to diplotene stages. The location of each of these reciprocal exchanges is a chiasma or crossover. Alternately, DSB formation and subsequent repair can result in gene conversion by other mechanisms.

Two important genetic benefits are provided by successful meiotic crossover. From an evolutionary perspective, meiotic recombination contributes to genetic variability of a species in order for it to withstand the pressure of natural selection. Mechanistically, it provides physical connections through chiasmata, ensuring proper alignment and segregation of chromosomes during cell division [Koren et al. 2002]. While genetic variability is commonly cited as the benefit of recombination, the benefits obtained from proper alignment and segregation of the chromatids during meiosis are just as essential.

The number of crossovers (measured as the number of MLH1 foci) in a spermatocyte or oocyte varies by sex [Lynn et al. 2002]. Human males have an average of 50 crossover sites per spermatocyte while females have about 70 per oocyte [Tease and Hulten 2004]. Variation in the number of crossover foci exists within (40–60 per spermatocyte) and between fertile men with a mean range of 46.2 ± 3.3 to 55.3 ± 3.7 [Hassold et al. 2004]. Each bivalent should have a minimum of one crossover focus to ensure proper alignment at the metaphase plate prior to segregation of homologous chromosomes during cell division.

Several studies have shown significantly lower rates of meiotic recombination in infertile men [Gonsalves et al. 2004; Sun et al. 2005a; 2007; Topping et al. 2006]. Men with obstructive azoospermia have an overall mean of 46.3 ± 6.3 crossover points with a range of 43.0 ± 8.8 to 52.6 ± 4.4. Non-obstructive azoospermic (NOA) men have a more extreme phenotype with an overall mean of 40.4 ± 6.1 and a broader range of 32.3 ± 15.1 to 48.9 ± 7.4 [Sun et al. 2005a]. Men with NOA also have a significant increase in the number of bivalents lacking the requisite crossover per bivalent [Gonsalves et al. 2004; Sun et al. 2005a].

Synapsis is also impaired in infertile men. Although meiotic chromosomes from both obstructive and non-obstructive azoospermic patients are able to synapse, there is an increase in the number of gaps and splits in synaptonemal complexes in spermatocytes from these men [Gonsalves et al. 2004; Sun et al. 2004b; 2005a; Codina-Pascual et al. 2006; Topping et al. 2006]. These gaps and splits are often found in noncentromeric heterochromatic regions, which are transcriptionally and recombinationally inactive, and interfere with the placement and number of crossover foci, by a mechanism that is not yet understood [Sun et al. 2005b; Codina-Pascual et al. 2006]. These heterochromatic regions are the last to synapse in both controls and infertile patients [Codina-Pascual et al. 2006], which could suggest inefficient checkpoint activation in some infertile patients.

Interestingly, transcription of heterochromatic regions does occur at the lamp-brush stage in cres-ted newt oocytes [Varley et al. 1980; Baldwin and Macgregor 1985]. These transcripts come from highly repetitive microsatellite regions and are transcribed by a possible read through mechanism. The transcription of the histone gene clusters, which are flanked by these microsatellite regions, fails to stop at the end of each of the histone genes, but continues through the flanking microsatellite regions [Diaz and Gall 1985].

Differences have been recently found in pericentric heterochromatin organization between somatic cells and developing mouse oocytes, which could provide a mechanism for somatically transcriptionally silent heterochromatin to become transcriptionally active [Meglicki et al. 2007]. Whether or not this occurs in spermatocytes has not yet been determined. It is well established that differences exist between the genetic programs involved in male and female germ cell development, which could account for differences.
seen in the heterochromatin behavior of oocytes and spermatocytes.

Progression through prophase I is impaired among men with NOA, with significantly fewer cells at the pachytene stage and more at the leptotene and zygotene stages than in controls. One study showed that 48% of NOA patients had a complete absence of meiotic cells [Sun et al. 2007]. Another study identified 3 out of 26 NOA individuals with synaptic defects [Topping et al. 2006].

Reduced recombination has been shown to be a strong contributing factor in the production of aneuploid gametes [Hassold 1998; Martin 2005; Hall et al. 2006; Martin 2006], with a higher incidence occurring in infertile males than case controls [Carrell et al. 2004; Gonsalves et al. 2004; Ma et al. 2006]. Bernardini et al. [2000; 2005] found a significant increase in the aneuploidy and diploidy rates in spermatozoa of men with abnormal semen parameters [Bernardini et al. 2000; Bernardini et al. 2005]. Globozoospermia, teratozoospermia, and oligoasthenoteratozoospermia have all been shown to be factors associated with increased aneuploidy rates [Pfeffer et al. 1999; Carrell et al. 2001; Ditzel et al. 2005; Faure et al. 2007].

If these imbalanced gametes participate in fertilization, early embryonic death or pregnancy loss can occur [Hassold and Hunt 2001; Lamb et al. 2005]. An increased frequency of aneuploidy in sperm cells has been shown to be a contributing factor for couples with recurrent pregnancy loss [Carrell et al. 2003; Rubio et al. 2005]. Aneuploid gametes can also lead to the production of offspring with chromosome imbalances and developmental disabilities, such as trisomy 21 and the array of sex chromosome imbalances [Hassold and Hunt 2001]. This may contribute to the increased rate of sex chromosome aneuploidies, birth defects and miscarriages which has been reported in IVF/ICSI offspring [Carrell et al. 2001; Dunn et al. 2001; Hansen et al. 2002; Van Steirteghem et al. 2002; Kurinczuk et al. 2004; Seller et al. 2004; Hansen et al. 2005; Macas et al. 2006].

Faulty meiotic recombination can also contribute to infertility through the activation of meiotic checkpoints. If the cell is unable to correct the error, it will trigger the initiation of apoptotic pathways, which in extreme cases can lead to global testicular failure [Roeder and Bailis 2000; Gonsalves et al. 2004]. Approximately 5–10% of cases of non-obstructive azoospermia are due to meiotic arrest [Gonsalves et al. 2004; Sun et al. 2005; Topping et al. 2006]. Zygotene and pachytene appear to be the stages when many cases of meiotic arrest occur [Gonsalves et al. 2004; Sun et al. 2007].

Recent advances, such as the completion of the Human Genome Project and improvements in Fluorescent In-Situ Hybridization (FISH) technology, have greatly enhanced our understanding of the processes involved in meiosis. However, infertility remains an elusive, complex and diverse disorder. It can result from a number of improperly functioning mechanisms and processes. This review will focus on the current understanding of the proteins and mechanisms involved in meiotic recombination and their potential role in male infertility. We will focus on the human orthologs of the proteins studied in other organisms, their roles in meiotic recombination and the clinical manifestations of infertility that can result when these proteins and mechanisms do not function properly. Specifically, we will look at proteins involved in DSB formation, the mechanism of strand exchange and the role of mismatch repair proteins in the maintenance of chromosome structure through the meiotic process and the formation and role of the synaptonemal complex (SC). Refer to Table 1 for a list of proteins, their known function and related animal/molecular and human/infertility studies.

**HOMOLOGOUS RECOMBINATION**

**Double Strand Break Formation**

The formation of a DSB signals the onset of recombination. These DSBs can occur as a result of a genotoxic event, which is usually the case in mitotic cells [Mills et al. 2003], or as a programmed event which occurs in meiotic cells. DSB formation occurs in the early leptotene stage, and is a requirement for proper synopsis to occur [Mahadevaiah et al. 2001]. The SPO11 protein, a type II topoisomerase is required for DSB formation and the eventual synopsis of chromosomes [Baudat and de Massy 2004]. Yeast spo11 mutants show a range of phenotypes from partial loss of function, to complete loss of DSB formation. Varying levels of SC defects are also observed, highlighting the importance of DSB formation in homologous chromosome synopsis [Henderson and Keeney 2004].
TABLE 1  Studies of Proteins Involved in Meiotic Recombination

<table>
<thead>
<tr>
<th>Protein</th>
<th>Known function</th>
<th>Animal/molecular studies</th>
<th>Human/infertility studies</th>
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<tbody>
<tr>
<td>Spo11</td>
<td>DSB formation</td>
<td>Spermatocytes from null mice undergo apoptosis before pachytene. Oocytes from null mice undergo apoptosis at diplotene.</td>
<td>Mutation study identified 2 non-obstructive azoospermic patients with missense mutations in exons 1 and 9 &amp; 16 SNPs in intron regions.</td>
</tr>
<tr>
<td>SMC1α</td>
<td>Cohesion of sister chromatids</td>
<td>None</td>
<td>Mutations associated with Cornelia de Lange Syndrome.</td>
</tr>
<tr>
<td>SMC1β</td>
<td>Meiosis specific cohesion of sister chromatids</td>
<td>Null male and female mice viable but sterile with arrest at mid-pachytene in males and metaphase II in females.</td>
<td>None</td>
</tr>
<tr>
<td>SMC3</td>
<td>Cohesion of sister chromatids</td>
<td>Knockdown in zebrafish and mouse leads to increased aneuploidy, genome instability.</td>
<td>None</td>
</tr>
<tr>
<td>Rad21</td>
<td>Cohesion of sister chromatids</td>
<td>N-terminal truncated hRad21 caused cohesion failure and spindle-assembly checkpoint activation in cultured mitotic human cells.</td>
<td>None</td>
</tr>
<tr>
<td>Rec8</td>
<td>Meiosis specific cohesion of sister chromatids</td>
<td>Knockout in mice leads to synopsis of sister chromatids and loss of Mlh1 foci.</td>
<td>None</td>
</tr>
<tr>
<td>Stag3</td>
<td>Meiosis specific cohesion of sister chromatids</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>SMC2</td>
<td>Condensation of chromosomes</td>
<td>Knockdown in C. elegens results in chromosome bridges at anaphase of mitosis.</td>
<td>None</td>
</tr>
<tr>
<td>SMC4</td>
<td>Condensation of chromosomes</td>
<td>Knockdown in C. elegens results in chromosome bridges at anaphase of mitosis.</td>
<td>None</td>
</tr>
<tr>
<td>SCP3</td>
<td>Lateral element of SC required for synopsis of homologous chromosomes</td>
<td>Knockout in mice leads to meiotic arrest by zygotene in males, and higher aneuploidy rates in oocytes.</td>
<td>Truncated C-terminus found in 2 men caused by single base-pair deletion. Forty-three percent of azoospermic men in another study showed no expression of SCP3.</td>
</tr>
<tr>
<td>SCP2</td>
<td>Lateral element of SC required for synopsis of homologous chromosomes</td>
<td>Knockout in mice leads to meiotic arrest in zygotene-like stage in males. Females sub-fertile compared to wild-type littermates.</td>
<td>None</td>
</tr>
<tr>
<td>SCP1</td>
<td>Central element of SC. Required for synopsis of homologous chromosomes</td>
<td>Knockout in mice results in infertility in males and females. Persistence of DSB and inability to complete synopsis.</td>
<td>None</td>
</tr>
<tr>
<td>Rad51</td>
<td>Genomic stability. Strand invasion phase of homologous recombination</td>
<td>Knockout in mice results in early embryonic death.</td>
<td>None</td>
</tr>
<tr>
<td>Dmc1</td>
<td>Meiosis specific. Strand invasion phase of homologous recombination</td>
<td>Knockout in mice causes meiotic arrest and apoptosis in zygotene/pachytene in males and during pachytene in females. Failure to properly synapse.</td>
<td>None</td>
</tr>
<tr>
<td>Rad54</td>
<td>Involved in homology search phase of strand invasion and branch migrations. Stabilizes Rad51-ssDNA filament</td>
<td>Mild meiotic phenotype in S. cerevisia, extreme mitotic phenotype. In knockout mice, decrease recombination, but otherwise fertile.</td>
<td>Mutation of and aberrant splicing of RAD54 gene associated with several primary cancers.</td>
</tr>
<tr>
<td>MSH4</td>
<td>Mts homolog. Binds along with MSH5 to double Holliday junctions in sliding clamp fashion. Expressed exclusively in meiotic cells</td>
<td>Knockout in mice leads to loss of recombination intermediates and infertility in males and females, but otherwise normal development and health.</td>
<td>None</td>
</tr>
<tr>
<td>MSH5</td>
<td>Mts homolog. Binds along with MSH4 to double</td>
<td>Knockout in mice leads to aberrant SC formation and infertility in males and females.</td>
<td>Expression of variant form in lung and breast carcinomas.</td>
</tr>
</tbody>
</table>
The interaction of SPO11 with the protein products of other genes is also important in DSB formation [Malone et al. 1991; Keeney et al. 1997]. The N and C- terminals are non-conserved regions of the SPO11 protein that are important in protein-protein interactions with the other proteins involved in DSB formation [Nag et al. 2006]. Some cases of infertility could be explained by failure of SPO11 to interact properly with these proteins, or from mutations in affiliated genes. For example, MRE11, RAD50 and NBS1 which comprise the MRN complex, form a scaffold structure that stabilizes a DSB and has been implicated to play a role with SPO11 in the formation of a DSB [Borde et al. 2004; Williams and Tainer 2005].

*S. cerevisiae* mutations in *Mre11*, *Rad50* or *Nbs1*, greatly reduce the efficiency of DSB formation [Haber 1998]. However, hypomorphic mutations of *Mre11* and *Nbs1* in mice do not appear to affect DSB formation, although repair of DSB, synopsis of chromosomes and placement of MLH1 foci are affected. A sexually dimorphic reversal in the number of crossover foci is also apparent in these mice. Hypomorphic males have an increase of crossover foci, while females have a decrease in crossover foci [Cherry et al. 2007].

In *Spo11−/−* mice, there is a complete lack of DSB and RAD51/D.MC1 DNA repair foci [Baudat et al. 2000; Romanienko and Camerini-Otero 2000]. This phenotype is partially rescued by treatment with the genotoxic agent Cisplatin, which induces DSB formation and the localization of RAD51/D.MC1 foci to these DSB sites [Romanienko and Camerini-Otero 2000]. Both male and female *Spo11−/−* mice are infertile, with smaller gonads than their wild type or heterozygous littermates. Spermatocytes arrest and undergo apoptotic death before the pachytenne stage. Oocytes progress through the end of prophase I, when they arrest and enter apoptosis at the diplotene stage [Baudat et al. 2000].

A genetic screening study identified 2 non-obstructive azoospermic men with missense mutations in exon 1 and exon 9 of the *SPO11* gene and 16 single nucleotide polymorphisms (SNPs) in intron regions. One of the missense mutations identified corresponds to an amino acid residue shown to play a crucial role in DSB formation. Three of the SNPs were located in the 3′ untranslated region, which could have an effect on transcription of this gene [Christensen et al. 2005]. Although mutations in *SPO11* and its regulatory sequences do not appear to be a common contributor to male infertility, when they are present, formation of DSB appears to be greatly affected.

### Proteins Involved in Strand Exchange and Synapsis

Homologous recombination requires the invasion of an intact partner by the 3′ overhang resulting from a processed DSB. In mitotic cells, the intact partner that serves as a template for correction is the sister chromatid; in meiotic cells, this intact partner is the homologous chromosome. In both cases, this process takes place under the direction of the highly conserved RecA family of proteins [Sugiyama et al. 1997; Bianco et al. 1998].

Replication Protein A (RPA) has been shown in *S. cerevisiae* to bind to and remove the secondary hairpin structure of the 3′ ssDNA overhang to a more compatible conformation. This stimulates the ATP-dependent loading of the RecA homolog, RAD51 onto the ssDNA. RAD51 will bind to dsDNA as well as ssDNA and destabilization of the secondary

<table>
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<tbody>
<tr>
<td>MLH3</td>
<td>MutL homolog. Localizes with MLH1 to cross-over sites after MSH4/MSH5 localization</td>
<td>Knockout in male mice lose chiasmata during pachytene and are sterile. Knockout females fail to complete meiosis I and are sterile.</td>
<td>Mutations associated with HPNCC.</td>
</tr>
<tr>
<td>MLH1</td>
<td>MutL homolog. Localizes with MLH3 to cross-over sites</td>
<td>Knockout in mice causes pachytene arrest in spermatocytes. Oocyte development fails after meiosis I.</td>
<td>Mutations associated with HPNCC.</td>
</tr>
</tbody>
</table>
structure by RPA prevents the formation of dsDNA hairpins which can interrupt RAD51 directed strand invasion [Sung and Robberson 1995]. However, this interaction is complex and loading RAD51 onto the 3' ssDNA can also be inhibited by RPA, suggesting that both proteins share a competing ssDNA binding site [Sugiyama et al. 1997].

RAD52 interacts both with RAD51 and RPA to stimulate the replacement of RPA on the ssDNA filament with RAD51. RAD52 forms a co-complex with RPA and Rad51, displacing RPA from the ssDNA, while targeting RAD51 to the ssDNA in an ATP dependent process [Sung and Robberson 1995; New et al. 1998; Sugiyama and Kowalczykowski 2002].

The ssDNA-RAD51 complex can then invade an intact segment of a potentially homologous partner. This is believed to occur through a mechanism of transient binding, with the help of RAD54, which stabilizes the ssDNA-RAD51 complex [Mazin et al. 2003]. RAD54 modifies chromatin structure by interacting with both ssDNA and dsDNA and opening up the dsDNA of a potential homologous chromosome. This allows for rapid Watson-Crick sampling between the RAD51-ssDNA and its potential homologous partner [Van Komen et al. 2000; Alexeev et al. 2003; Lee et al. 2006] (Fig. 1). Differences in base pairs between the two DNA strands affect the stability and efficiency of strand-exchange [Sagi et al. 2006].

DMCI, a meiosis specific RecA homolog, is also required for strand exchange to occur in meiosis. Both DMCI and RAD51 are required for meiotic recombinant activity in most organisms, although Drosophila melanogaster, C. elegans and Neurospora crassa lack a dmc1 ortholog [Stahl et al. 2004]. Human DMCI and RAD51 are greater than 50 percent identical and co-localize with each other at the site of a DSB in a juxtaposed manner, possibly interacting with different ends of the DSB [Masson et al. 1999; Shinohara et al. 2000].

DMCI forms ring like structures with a preference for interacting with and forming helical nucleoprotein filaments with ssDNA [Masson et al. 1999; Sehorn et al. 2004]. This strongly supports a role for DMCI in strand exchange in meiosis, although the exact mechanisms involved in the assembly of DMCI onto ssDNA are poorly understood.

After homology has been established, RAD54 is important in the extension of heteroduplex DNA and the establishment of the joint molecules which are key intermediates of homologous recombination (Fig. 1) [Solinger and Heyer 2001; Bugreev et al. 2006]. In S. cerevisiae, Rad54 has been shown to stimulate strand exchange and D-loop formation of heteroduplex DNA [Solinger et al. 2001].

RAD51 is an absolute requirement for genomic stability, making it difficult to determine differences in function between mitosis and meiosis. Rad51/C0/C0 mice die early in embryonic development, before day 8.5 post coitus via the initiation of a p53 mediated pathway [Lim and Hasty 1996; Tsuzuki et al. 1996]. Although tissue samples from heterozygous mice contain about half of the RAD51 protein that is found in wild-type tissues, these mice appear to be normal and fertile [Tsuzuki et al. 1996]. With its recombinant activity, over expression of RAD51 has strong transformation capabilities and has been
found in several tumor lines [Xia et al. 1997; Maacke et al. 2000a; b; Orre et al. 2006].

Male and female Dmc1−/− mice both display defective meiotic recombination, with arrest and cell death occurring from the late zygotene to early pachytene stages in males and during the pachytene stage in females [Pittman et al. 1998]. Supporting a role in the synaptic phase of strand exchange, Dmc1 null mutants also fail to properly synapse, and mature SCs are absent in these mice [Pittman et al. 1998; Yoshida et al. 1998]. In a recent genetic screening study in mice [Bannister et al. 2007], a missense mutation was found in the L2 binding domain of the Dmc1 gene. This mutation causes a single amino acid change that results in synaptic defects, loss of recombination and complete sterilization in males. Interestingly, females with this same mutation show some signs of synaptic defects and have a reduced oocyte pool, but are otherwise fertile [Bannister et al. 2007].

The meiotic phenotype of rad54 mutants is relatively mild in budding yeast. Deletion of rad54 in S. cerevisiae causes a small reduction in spore viability, but non-disjunction during meiosis I is not increased [Schmuckli-Maurer and Heyer 2000]. This is in contrast to the mitotic phenotype in which rad54−/− vegetative cells have increased chromosome loss compared to controls, and general genomic instability [Schmuckli-Maurer et al. 2003]. This difference between meiotic and mitotic phenotypes is intriguing and suggests a much more minor role for Rad54 in meiotic recombination than mitotic recombination.

Mouse Rad54−/− cells display decreased homologous recombination and an increased sensitivity to ionizing radiation, mitomycin C and methyl methanesulfonate, but are otherwise viable [Essers et al. 1997]. Spermatocytes in Rad54−/− mice display aberrant RAD51 staining with foci and threadlike localization well into the diplotene stage when they should no longer be present. In spite of the effects on RAD51 foci, both Rad54−/− males and females are still fertile, suggesting a small role in meiotic recombination similar to that found in budding yeast [Wesoly et al. 2006].

**Mismatch Repair Proteins**

Strand exchange results in the formation of double Holliday-Junctions (DHJ) which are then resolved with the aid of the Mismatch Repair (MMR) protein family to produce either crossover or non-crossover products. The MMR protein family is a highly conserved group of proteins with diverse functions that include the excision of mismatched nucleotides following replication, response to DNA damage, participation in V(D)J recombination [Martomo et al. 2004; Larson et al. 2005] and meiotic recombination. The MMR protein family is crucial in ensuring genome integrity and stability. Mutations in several proteins in this family are responsible for microsatellite instability and various forms of cancer, in particular Hereditary Nonpolyposis Colorectal Cancer (HNPCC) [Harfe and Jinks-Robertson 2000; Wang et al. 2003; van der Klift et al. 2005]. This is not surprising given the ubiquitous nature of these proteins and their multiple functions, including their namesake function of correcting mismatches that occur during replication [Kunkel and Erie 2005; Jun et al. 2006]. A complete discussion of the roles of MMR is reviewed in Kunkel and Erie [2005] and Jun et al. [2006] but is briefly summarized here. The model for MMR interaction with a DSB involves recognition of the DSB by a MutS heterodimer, which signals a MutL heterodimer to the site. The MutL heterodimer coordinates further downstream events leading to the correction of the DSB [Kunkel and Erie 2005].

In meiosis, at least five MMR proteins (MSH4, MSH5, MLH1, MLH3 and PMS2) act to facilitate homologous recombination. The first four are particularly important and are reviewed below. The MutS heterodimer consists of MSH4 and MSH5, which interact with each other exclusively during meiosis [Bocker et al. 1999]. Like other MutS homologs, MSH4 and MSH5 belong to the Walker A/B family of proteins with ATP hydrolysis activity [Fishel 1998]. This is important when considering what is known about the mechanism of action of this heterodimer. The MSH4/MSH5 heterodimer binds to DHJ to form a sliding clamp that embraces the homologous chromosomes in their intermediate state following strand invasion and branch migration. The ATPase activity of MSH4/MSH5 is involved in its initial interaction with and clamp formation around the duplex DNA. After the formation of the MSH4/MSH5 clamp around duplex DNA, the heterodimer is able to slide along and stabilize DHJ in an ATP independent fashion [Snowden et al. 2004].
In human immunostained spermatocytes, MSH4 appears early in the zygotene stage and disappears beginning early in the pachytene stage [Oliver-Bonet et al. 2005]. MSH4 interacts with both RAD51 and DMC1 [Neyton et al. 2004]. This provides a confluence between strand exchange and mismatch repair functions in meiotic recombination. The loss of chromosome pairing that is seen in Msh4−/− male and female mice indicates that MSH4 is important in maintaining strand invasion intermediates and synopsis of homologous chromosomes [Kneitz et al. 2000]. Similarly, Msh5−/− male and female mice show aberrant SC formation and synaptic defects [de Vries et al. 1999]. Msh4−/− and Msh5−/− mice develop normally and display normal sexual and mating behavior, but the gonads of both males and females are significantly smaller in size than their wild-type littermates and are completely devoid of germ cells [de Vries et al. 1999; Edelmann et al. 1999; Kneitz et al. 2000].

Following the formation of the MSH4/MSH5 sliding clamp, the MutL heterodimer, consisting of MLH1/MLH3 is signaled to the sight of the DSB early in the pachytene stage [Oliver-Bonet et al. 2005]. MLH3 appears first, co-localizing with MSH4, then is followed by the co-localization of MLH1 in mid-pachytene [Kolas et al. 2005]. The localization of MLH1 foci on meiotic chromosomes is dependent on the presence of MLH3 [Lipkin et al. 2002]. Mlh3−/− mice lack both Mlh3 and Mlh1 foci. Homologous chromosomes from spermatocytes of Mlh3−/− male mice are able to synapse correctly, but lose chiasmata during pachytene, becoming unstable and separating abnormally at metaphase I. They then become aneuploid and enter an apoptotic pathway. Oocytes from Mlh3−/− female mice fail to complete meiosis I before arresting [Lipkin et al. 2002].

Both male and female mice deficient in Mlb1 are sterile. Testes from Mlb1−/− males are about half the size as those of their wild type littermates and spermatocytes arrest at the pachytene stage. Ovaries from Mlb1−/− females show no overt phenotypic difference from their wild type littermates, with follicles at all stages and an ability to ovulate. However, oocytes from these Mlb1−/− mice fail to suggest that they never complete meiosis II. Both male and female Mlb1−/− mice display normal mating behavior and the estrous cycle of females is unaffected by the loss of Mlb1 [Edelmann et al. 1996]. The loss of Mlb1 does not preclude the formation of Mlb3 foci, although the number of foci is significantly lower in Mlb1−/− mice than in their wild-type litter mates [Kolas et al. 2005].

The overall function of the MMR protein family in meiosis is not well understood, but appears to include the stabilization of crossovers. In mice, approximately 250 DSB are introduced to meiocytes during the leptotene stage. About 150 of these sites will become associated with MSH4/MSH5. Of these 150, about 105 MSH4/MSH5 foci will resolve by means other than a reciprocal crossover and the remaining will obtain MLH1/MLH3 foci. The eventual addition of MLH1/MLH3 to these sites is required to ensure that the DSB resolves into a crossover rather than through another repair pathway [Kolas and Cohen 2004]. The mechanisms by which MSH4/MSH5 and MLH1/MLH3 stabilize strand invasion intermediates are poorly understood at this point, but future biochemical and mutational studies should provide a greater understanding.

Because MLH1 is present at crossover foci in mid to late pachytene cells, it has become an important tool in investigating meiotic recombination in both animal and human studies. Each MLH1 foci on a pachytene stage bivalent is counted as a crossover and correlates with chiasmata and linkage-based studies involving meiotic recombination (Fig. 2) [Barlow and Hulten 1998; Marcon and Moens 2003].

FIGURE 2 Pachytene stage human spermatocyte. SCP3 (red) anti-sheep antibodies provided by Peter Moens’ lab, York University, Toronto, Canada. CREST (blue) anti-human antibodies provided by Allen Sawitzke’s lab, University of Utah, Salt Lake City, Utah. MLH1 (green) anti-mouse antibodies commercially available at Biocare Medical.
PROTEINS INVOLVED IN THE STRUCTURAL MAINTENANCE OF CHROMOSOMES

During both mitosis and meiosis, chromosomes undergo complex interactions with each other, and extreme changes in their morphology. Meiotic recombination requires that chromosomes condense from their G-phase morphology, synapse with their homologous partner and exchange genetic information. After recombination has occurred, homologous chromosomes segregate at anaphase I, while sister chromatids stay closely associated with each other through metaphase II. The structural maintenance of chromosomes (SMC) family of proteins plays a crucial role in these dynamic processes [Pelttari et al. 2001; Taylor et al. 2001; Jessberger 2002; Revenkova et al. 2004; Firooznia et al. 2005]. There are 6 types of SMC proteins, (SMC1-SMC6), forming heterodimer complexes with specialized function.

The SMC1/SMC3 complex associates with 2 non-SMC proteins, forming the cohesin complex, which participates in holding sister chromatids together during mitosis and meiosis [Revenkova et al. 2001; 2004; Firooznia et al. 2005]. Importantly, even after cohesin subunits are removed from the chromosome arms as the cell moves into metaphase I, sister chromatids maintain their close association through metaphase II by the continued presence of cohesin proteins at the centromeres, ensuring that segregation of sister chromatids takes place at the correct time [Pelttari et al. 2001; Eijpe et al. 2003; Prieto et al. 2004; Firooznia et al. 2005; Revenkova and Jessberger 2005]. The SMC2/SMC1 heterodimer associates with 3 non-SMC proteins to form the condensin complex, which condenses chromatin and promotes proper segregation of homologous chromosomes following recombination [Ono et al. 2003; Losada and Hirano 2005]. The SMC5/SMC6 complex interacts with 3 non-SMC proteins and may also play a role in the segregation of homologs following recombination [Ono et al. 2003; Firooznia et al. 2005].

SMC proteins share common structural features with two coiled-coil domains flanking a hinge domain, which allows the protein to fold back onto itself (Fig. 3). In heterodimer complexes, two SMC proteins interact at the hinge domain forming a ring in the case of cohesin or a closed V-shaped structure in condensin. The non-SMC subunits attach to one of the catalytic ends in condensin and to both catalytic ends in cohesin [Anderson et al. 2002; Jessberger 2002; Chiu et al. 2004; Firooznia et al. 2005].

Cohesin

Three forms of the cohesin complex are known to be present in meiotic cells [Revenkova and Jessberger 2006]. One consists of SMC3, the meiotic specific SMC1B and the non-SMC somatic subunits RAD21 and SA1/SA2. The other two complexes contain SMC3 and the non-SMC meiosis specific subunits REC8 and STAG3, but differ in their SMC1 protein. One contains the meiotic form (SMC1B) and one contains the mitotic form (SMC1A) [Prieto et al. 2001; Anderson et al. 2002; Hirano 2002; Firooznia et al. 2005; Revenkova and Jessberger 2005; 2006].

There are two possible mechanisms that explain how cohesin interacts with DNA. In one mechanism, when cohesin interacts with DNA, one of the catalytic domains is freed from the non-SMC subunits, increasing the angle of the hinge, and allowing the free end to connect with a DNA segment of the sister chromatid (Fig. 4A) [Anderson et al. 2002; Gruber et al. 2003; Firooznia et al. 2005]. The other mechanism involves the brief disassociation of the hinge domains of SMC1/SMC3 upon interaction with DNA, which opens the complex and allows it to encircle the sister chromatids [Gruber et al. 2006] (Fig. 4B).

SMC1B is the meiosis specific isoform of SMC1 [Revenkova et al. 2001]. This protein is expressed...
exclusively in the gonads and appears along the chromosomes later than SMC1A, during the leptotene stage. It remains present along the chromosome arms until metaphase I when it relocates exclusively to the centromeres until anaphase II [Jessberger 2002; Firooznia et al. 2005].

Both male and female mice with a null mutation in SMC1B are viable but sterile [Revenkova et al. 2004]. Spermatocytes from these mice arrest and enter apoptosis at the mid-pachytene stage, but oocytes continue until metaphase II. Testes of 5-week-old SMC1B<sup>−/−</sup> male mice are half the size of their heterozygous and wild type littermates. Although oocytes of SMC1B<sup>−/−</sup> mice are able to develop up to metaphase II, they are highly prone to aneuploidy, due to faulty sister chromatid cohesion. In both sexes, the SC is shorter in length than in wild type mice and the synopsis of homologous chromosomes is incomplete, indicating the importance of proper sister chromatid cohesion for appropriate SC formation and recombination [Revenkova et al. 2004].

Knockdown of smc3 in zebrafish embryos and human cells leads to increased rates of aneuploidy, genome instability and activation of the p53 apoptosis pathway [Ghiselli 2006]. Due to the importance of these proteins in normal somatic cell function, it has been difficult to experimentally dissect how their meiotic functions differ from their mitotic functions. Testes specific knockdown studies using RNA-interference (RNAi) could help in defining the meiotic roles of these proteins while not interfering with the developmental potential of the animal model being studied.

SMC1A and SMC3 are both expressed in mitosis and meiosis. Mutations in these genes are associated with the multi-system developmental disorder Cornelia de Lange syndrome, which is characterized by facial dysmorphisms, cognitive disability, growth delay and other diverse phenotypes [Musio et al. 2006; Deardorff et al. 2007]. A high percentage of human colon tumors over express SMC3 [Ghiselli and Iozzo 2000]. In mouse fibroblast cells, induced

**FIGURE 4** Cohesin. The cohesin complex consists of the SMC1/SMC3 heterodimer and non-SMC subunits. A) Upon interaction with sister chromatids, a non-SMC subunit detaches from the catalytic domains which increases the angle of the hinge, allowing the complex to wrap around them. B) Alternately, the hinge domains may separate slightly to enfold the sister chromatids.
over expression of SMG3 causes the over expression of several oncogenic factors and the transformation of these cells [Ghiselli and Iozzo 2000; Ghiselli and Liu 2005].

The non-SMC subunit, RAD21 is present in the somatic and one of the meiotic complexes of cohesin. RNAi knockdown of Rad21 in Drosophila D3 cells, results in loss of sister chromatid cohesion and longer than normal spindle fibers at metaphase of mitosis [Goshima et al. 2005]. Although the cells being investigated are mitotic, given the localization of RAD21 to the centromeres in late prophase I of meiosis [Parra et al. 2004], a similar response might be seen in meiotic cells.

There is some conflicting data regarding the expression and localization patterns of RAD21. Using FISH probes for RAD21 in mice, it was shown to be present in somatic cells and spermatogonia, but not in spermatocytes [Lee et al. 2002]. However, other studies (also in the mouse), have shown that RAD21 associates with the lateral elements of the SC and has a temporal and spatial distribution similar to SMG3 and SMG1B, changing localization from along the entire chromatid arms to the centromeres at metaphase I, where it remains until anaphase II [Xu et al. 2004; Firooznia et al. 2005]. In further contrast, RAD21 was shown in another study to disassociate from the centromeres at anaphase I with no localization in meiosis II in mouse spermatocytes [Parra et al. 2004]. Testes specific RNAi would be a useful tool in better establishing the role of RAD21 in meiotic cells.

REC8 is a non-SMC meiosis specific subunit of cohesin with a well established expression and localization pattern that is similar to SMG3 [Eijpe et al. 2003]. It first appears associated with the SC in the pre-meiotic S phase, before other elements of cohesin are present, and is located along the entire chromosome into the leptotene phase. REC8 localizes exclusively to the centromeres at metaphase I, where it remains until anaphase II [Eijpe et al. 2003; Firooznia et al. 2005]. In budding yeast, condensin facilitates the removal of cohesin from areas of crossover between homologous chromosomes so that proper segregation can occur at anaphase I [Yu and Koshland 2005]. In budding yeast, condensin facilitates the removal of cohesin from areas of crossover between homologous chromosomes so that proper segregation can occur at anaphase I [Yu and Koshland 2005]. In budding yeast, condensin facilitates the removal of cohesin from areas of crossover between homologous chromosomes so that proper segregation can occur at anaphase I [Yu and Koshland 2005]. Mutant condensin in budding yeast results in the formation of chromosome bridges between separating chromosomes during both anaphase I and anaphase II. After the formation of these chromosome bridges, fragmentation of chromosomes and cell death occur.

Condensin

Condensin is a ubiquitous protein complex with functions in both mitosis and meiosis. RNAi of condensin subunits in HeLa cells results in instability of the chromosomes at anaphase when they become unable to withstand opposing spindle forces and display chromosome bridges [Gerlich et al. 2006]. In budding yeast, condensin facilitates the removal of cohesin from areas of crossover between homologous chromosomes so that proper segregation can occur at anaphase I [Yu and Koshland 2005]. Mutant condensin in budding yeast results in the formation of chromosome bridges between separating chromosomes during both anaphase I and anaphase II. After the formation of these chromosome bridges, fragmentation of chromosomes and cell death occur.
Knockdown of either SMC-4 or MIX-1 (a homolog of SMC2) via RNAi in C. elegens also results in this chromosome bridge phenotype [Hagstrom et al. 2002]. This is not surprising given the role of condensin in maintaining chromosome compaction during cell division. The V-shaped structure of this heterodimer provides it with the ability to bind adjacent DNA segments, serving as an intra-molecular connector (Fig. 5) [Anderson et al. 2002]. In the budding yeast S. cerevisiae, it was shown that condensin compacts DNA into chiral structures and is able to bind multiple DNA duplexes, providing a mechanism for further compaction [Stray et al. 2005]. It is likely that the mitotic and meiotic functions of condensin are similar.

Two forms of condensin have been identified in meiotic cells. Both forms contain the same SMC2/SMC4 subunits, but differ in their non-SMC subunits. The non-SMC subunits for condensin I are CAP-D2, CAP-G and CAP-H while the non-SMC subunits for condensin II are CAP-D3, CAP-G2 and CAP-H2 [Hirano 2002]. Condensin II appears first, in early prophase, and is believed to be involved with early restructuring and condensation of meiotic chromosomes. Condensin I does not appear on the chromosomes until metaphase I, when it associates with condensin II [Hirano 2002; Hirota et al. 2004; Losada and Hirano 2005].

SYNAPTONEMAL COMPLEX

The SC and SMC complexes interact with each other to organize chromatin during meiotic recombination [Eijpe et al. 2003; Page and Hawley 2004]. The SC is a proteinaceous structure that serves as a physical scaffold to facilitate and stabilize the synapsis of homologous chromosomes so that recombination can occur. The SC consists of two axial/lateral elements which contain Synaptonemal Complex Protein 2 (SCP2) and Synaptonemal Complex Protein 3 (SCP3) connected by transverse filaments which include Synaptonemal Complex Protein 1 (SCP1) (Fig. 6) [Vidal et al. 1987; Lynn et al. 2002; de Vries et al. 2005; Ollinger et al. 2005]. The complete SC resembles a ladder-like structure with SCP3 and SCP2 forming the legs and SCP1 forming the rungs.

Proper formation of the SC is necessary for meiotic recombination to occur and failure to construct a functional SC is associated with necrozoospermia, oligoasthenoteratozoospermia and azoospermia [Vidal et al. 1982; 1987; Lange et al. 1997]. As discussed earlier, SC anomalies such as gaps and splits are significantly more common in infertile patients than case controls [Gonsalves et al. 2004; Sun et al. 2004; Sun et al. 2005a; b; Codina-Pascual et al. 2006; Topping et al. 2006]. Meiotic arrest, due to SC fragmentation or improper assembly usually occurs in the zygotene stage when all parts of the SC should be present, but can sometimes occur as late as the pachytene stage [Yuan et al. 2000; Miyamoto et al. 2003; Judis et al. 2004; de Vries et al. 2005].

Axial/Lateral Elements of the SC

SCP3 (Fig. 6) is a major component of the axial element (AE) of the SC and is expressed exclusively in the gonads [Miyamoto et al. 2003]. Initiation of the SC occurs in the leptotene stage, when SCP3 forms filaments along unsynapsed chromosomes [Page and Hawley 2004]. SCP2 localization is SCP3
dependent and in the absence of \( \text{SCP3} \) fibers fail to properly localize to the AE [Yuan et al. 2000; Pelttari et al. 2001]. As mentioned previously, the AE also contains cohesin complex proteins REC8, SMC1A, SMC1B, RAD21 and STAG3 [Eijpe et al. 2003; Prieto et al. 2004; Firooznia et al. 2005; Revenkova and Jessberger 2006]. Mutation of these proteins results in various anomalies in the SC structure and meiotic breakdown [Revenkova et al. 2004; Goshima et al. 2005; Xu et al. 2005].

Unlike males, female \( \text{SCP3} \) male knockout mice are sterile, with a testes size that is 30% the size of heterozygous or wild type mice but otherwise healthy [Yuan et al. 2000]. Pachytene spermatocytes are absent in \( \text{SCP3} \) male mice 14 days after birth, when they first appear in the testes of wild-type mice. TUNEL from the testes of null mice show a high number of apoptotic cells, indicating that upon failure to form a SC, meiotic arrest and apoptosis occurs [Yuan et al. 2000].

Not surprisingly, synopsis of homologous chromosomes is impaired in \( \text{SCP3} \) male mice. Staining for cohesin subunits, which co-localize with SCP3, reveals that homologous chromosome alignment occurs, but in a non-specific manner and synopsis is never completed. Chromosome compaction is also impaired with a four-fold increase in length compared to wild-type mice [Kolas et al. 2004]. An overabundance of CREST signals (stain for the centromeres) in these mice also indicates synaptic failure [Yuan et al. 2000].

Unlike males, female \( \text{SCP3}^{-/-} \) mice are fertile, although they do exhibit higher aneuploidy rates and embryo death than their wild-type littermates [Yuan et al. 2002]. Approximately 50% of oocytes from \( \text{SCP3}^{-/-} \) mice have one or more univalents, suggesting that they either fail to synapse or are unable to maintain synopsis. These oocytes have fewer chiasmata at the diplotene stage than wild-type cells and this effect shows an age related increase [Yuan et al. 2002].

Some studies addressing \( \text{SCP3} \) have been performed in humans. Miyamoto et al. [2003] identified two azoospermic males with a single base pair deletion, resulting in a premature stop codon and truncated version of the C-terminal coiled-coil region [Miyamoto et al. 2003] which mediates protein-protein interactions [Yuan et al. 1998]. A recent study of 110 azoospermic men in which testicular biopsies were assayed by real time PCR (RT-PCR) for expression of SCP3, found that 43 (39.1%) of these men showed no expression of SCP3. No expression was seen in cases of spermatogonial arrest or sertoli cell only syndrome. Two patients with very few spermatocytes also showed no expression of SCP3 [Aarabi et al. 2006].

\( \text{SCP2} \) (Fig. 6) is another component of the AE of the SC. It is a large, meiosis specific DNA-binding protein with a coiled-coil domain, coded by exons 39–43 that is responsible for interaction with \( \text{SCP3} \) [Offenberg et al. 1998]. Deletion of this region in mice leads to failure of synapsis and meiotic arrest at a zygotene-like stage in males [Yang et al. 2006]. Testes size of \( \text{SCP2} \) male mice are 70% the size of their wild type or heterozygous counterparts. Both male and female heterozygotes are fertile. A less dramatic effect is seen in \( \text{SCP2} \) female mice, which are less fertile with much smaller litter sizes than wild-type mice [Yang et al. 2006].

Transverse Filaments of the SC

\( \text{SCP1} \) (Fig. 6) is a required protein of the Transverse Filaments (TF) of the SC and connects the two AE of homologous chromosomes. The presence of \( \text{SCP1} \) along the SC indicates regions in which synopsis between homologous chromosomes is complete.
After localization of SCP1 between the two AE is complete, the AE are termed lateral elements (LE). SCP1 first appears in subtelomeric regions during the zygotene stage, closing inward towards the centromeres [Brown et al. 2005].

SCP1 has three domains: a DNA-binding C-terminus, a middle coiled-coil domain and a protein interacting N-terminus [Meuwissen et al. 1992; Liu et al. 1996; Schmekel et al. 1996]. In the mature SC, the DNA-binding C-terminus of SCP1 is found within the LE, the coiled-coil domain extends between the two. The N-termini of two opposing SCP1 molecules interact at the center of the TF. Yeast two-hybrid assays have established that the N-terminus of SCP1 interacts strongly with itself [Liu et al. 1996].

Although disruption of SCP3 and SCP2 in mouse models leads to sexually dimorphic effects, with infertility in males and milder effects in females, Scp1 disruption in mice causes infertility in both sexes [Yuan et al. 2000; Bannister and Schimenti 2004; Kolas et al. 2004; de Vries et al. 2005; Yang et al. 2006]. Scp1+/– males and females are fertile, but Scp1–/– testes and ovaries are smaller than wild type or Scp1+/–. The formation of AE is not inhibited in Scp1–/– mice, but homologous chromosomes are unable to synapse. DSB persist through a pachytene-like stage and MLH1 foci are altogether absent in the meiocytes of these mice, suggesting the critical role of SCP1 in the synapsis of homologous chromosomes in order for crossover to occur [de Vries et al. 2005].

CONCLUSIONS

Infertility is a common event with many etiologies. However, most cases of male infertility are idiopathic and may be due to genetic causes [Carrell 2007]. This review has discussed targeted proteins and their genes that are essential for meiotic recombination. Due to the complex timing and interaction of the proteins involved in this process, tight regulation is necessary, including cell cycle check-points, and apoptotic elimination of abnormal spermatocytes [Hochwagen and Amon 2006; Joshi and Dighe 2006]. Mutations of genes involved in meiosis, abnormal expression patterns, defects in translation, and errors in post-translational modifications to these proteins may all lead to aberrant meiosis, which ultimately results in infertility [Matzuk and Lamb 2002; Carrell 2007]. This infertility can manifest itself in different ways, from generalized meiotic arrest, leading to azoospermia, to increased aneuploidy in gametes, leading to early embryonic death or developmental problems in potential offspring. In light of the above, future studies should focus on the screening of infertile patients for proper expression of the proteins and gene mutations that may alter meiotic recombination.

REFERENCES


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Proteins Involved in Meiotic Recombination


