Chromosoma (2006) 115: 220–234
DOI 10.1007/s00412-006-0057-5

REVIEW

Esther de Boer · Christa Heyting

# The diverse roles of transverse filaments of synaptonemal complexes in meiosis

Received: 23 December 2005 / Revised: 30 January 2006 / Accepted: 30 January 2006 / Published online: 8 March 2006


**Abstract** In most eukaryotes, homologous chromosomes (homologs) are closely apposed during the prophase of the first meiotic division by a ladderlike proteinaceous structure, the synaptonemal complex (SC) [Fawcett, J Biophys Biochem Cytol 2:403–406, 1956; Moses, J Biophys Biochem Cytol 2:215–218, 1956]. SCs consist of two proteinaceous axes, which each support the two sister chromatids of one homolog, and numerous transverse filaments (TFs), which connect the two axes. Organisms that assemble SCs perform meiotic recombination in the context of these structures. Although much information has accumulated about the composition of SCs and the pathways of meiotic crossing over, several questions remain about the role of SCs in meiosis, in particular, about the role of the TFs. In this review, we focus on possible role(s) of TFs. The interest in TF functions received new impulses from the recent characterization of TF-deficient mutants in a number of species. Intriguingly, the phenotypes of these mutants are very different, and a variety of TF functions appear to be hidden behind a façade of morphological conservation. However, in all TF-deficient mutants a specific class of crossovers that display interference is affected. TFs appear to create suitable preconditions for the formation of these crossovers in most species, but are most likely not directly involved in the interference process itself. Furthermore, TFs are important for full-length homolog alignment.

Communicated by R. Benavente

The synaptonemal complex—50 years.

E. de Boer · C. Heyting (✉)
Botanical centre, Molecular genetics group,
Wageningen University,
Arboretumlaan 4,
6703 BD Wageningen, The Netherlands
e-mail: Christa.Heyting@wur.nl
Tel.: +31-317-483140
Fax: +31-317-483146

## Assembly and composition of synaptonemal complexes

### Axial elements

Synaptonemal complexes (SCs) are proteinaceous structures that closely appose homologs during the prophase of the first meiotic division (meiosis I). The assembly, structure, and composition of SCs have been reviewed recently (Page and Hawley 2004), and are only briefly summarized here. SC assembly starts during premeiotic S-phase and early meiotic prophase with the formation of a single proteinaceous axis, the axial element (AE), along the two sister chromatids of each chromosome. Then, following homolog recognition and alignment, the AEs of homologs are closely apposed along their length by numerous transverse filaments (TFs): a process called synapsis. The SC is the ladder-like structure formed by the two AEs [named lateral elements in the context of the SC] and the TFs (reviewed by Page and Hawley 2004) (Fig. 1).

Cohesins, which are proteins that mediate cohesion between sister chromatids (reviewed by Nasmyth 2005), are important for AE assembly (Klein et al. 1999; Pelttari et al. 2001). During S-phase, both in the mitotic cycle and in premeiotic S-phase, cohesins are installed in such a way that they hold the newly synthesized sister chromatids together. In the mitotic cycle, all sister chromatid cohesion is lost at the metaphase-to-anaphase transition, so that sister chromatids can disjoin. In meiosis, two chromosome segregations follow a single round of DNA-replication, and this is possible because cohesion is released in two steps (reviewed by Nasmyth 2001, 2005).

Cohesins not only provide sister chromatid cohesion, but also participate in homologous recombination, both in the mitotic cycle and in meiosis. In mitosis, they enhance sister chromatid-based recombinational repair. In meiosis, their role is modified in such a way that homologous recombination occurs preferentially between chromatids of homologs, in most species, mainly or exclusively by a TF-dependent pathway of crossing over (reviewed by Page and Hawley 2004; and van Heemst and Heyting 2000). The participation of AE-associated proteins, includ-

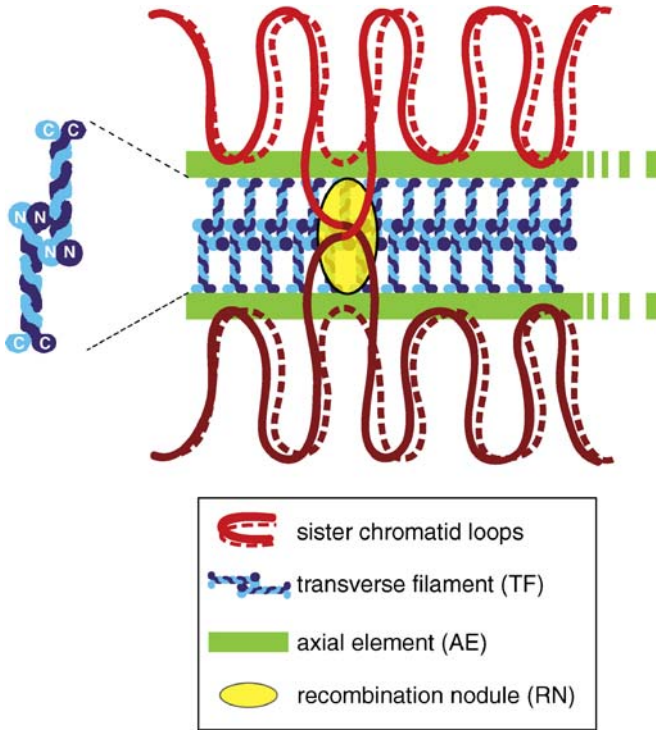


**Fig. 1** Position of transverse filament protein molecules within a synaptonemal complex. A detail of TF protein molecules, which are long, coiled-coil proteins, is shown to the left. The molecules are attached by their C terminus (*C*) to the axial element (*AE*) of one chromosome, and interact with their N terminus (*N*) with TF protein molecules that are attached to the homologous chromosome. In *C. elegans*, two different short coiled-coil proteins bridge the space between the two *AEs*. Reciprocal exchange between nonsister chromatids (crossing over) has already occurred in the stage shown here (late pachytene), but is not yet visible as a chiasma. The site of crossing over is marked by a recombination nodule (*RN*)

ing cohesins, in recombination manifests itself by the formation of ultrastructurally recognizable protein complexes (recombination nodules or RNs) along AEs or SCs (reviewed by Carpenter 1987), and the localization of recombination-related proteins within RNs (Anderson et al. 1997; Moens et al. 2002) and along AEs and SCs (reviewed by Ashley and Plug 1998; Bishop 1994). Given these adaptations in cohesin functions, it is not surprising that all species analyzed thus far express one or more specific cohesins during meiosis (reviewed by Nasmyth 2001).

Besides cohesins, various other proteins participate in AE formation. Some of these help create a preference for recombination between homologs rather than sister chromatids (Schwacha and Kleckner 1997). Most AE components analyzed thus far are required for full synapsis between homologs, and some AE components are absolutely essential for the incorporation of TF proteins in the SC structure (reviewed by Page and Hawley 2004).

### Transverse filaments

Whereas the ultrastructural appearance of the SC is highly conserved, many of its components, including TF proteins, are ill-conserved at the amino acid sequence level. TF proteins have therefore been identified independently in only a limited number of species, namely, mammals (SYCP1) (Dobson et al. 1994; Meuwissen et al. 1992; Meuwissen et al. 1997; Sage et al. 1999), budding yeast (Zip1) (Sym et al. 1993), *Drosophila* [C(3)G] (Page and Hawley 2001), and *Caenorhabditis* (Syp-1 and Syp-2) (Colaiácovo et al. 2003; MacQueen et al. 2002). Despite their lack of amino acid sequence conservation, SYCP1 (Meuwissen et al. 1992), Zip1 (Sym et al. 1993), and C(3)G (Page and Hawley 2001) have similar structures (discussed by Bogdanov et al. 2002; Higgins et al. 2005): they are long, coiled-coil proteins with globular domains at both ends. The C-terminal domain is basic, and has S/TPXX motifs, which are possibly involved in DNA-binding (Suzuki 1989). The Zip1 C terminus is essential for attachment of TFs to AEs (Tung and Roeder 1998). Within SCs, TF proteins form parallel coiled-coil homodimers, which are embedded with their C termini in the AEs, whereas the N termini of TF protein molecules from opposite AEs overlap in the narrow region (called central region) between the AEs of the two homologs (Anderson et al. 2005; Dong and Roeder 2000; Liu et al. 1996; Schmekel et al. 1996; Tung and Roeder 1998) (Fig. 1). *Caenorhabditis* Syp-1 and Syp-2 are two short coiled-coil proteins, which possibly take the place of a single longer one in other species (Colaiácovo et al. 2003; MacQueen et al. 2002). Based on the structural properties of the known TF proteins, Higgins et al. (2005) identified a number of candidate TF proteins in the *Arabidopsis* proteome, and then showed that the closely related proteins ZYP1a and ZYP1b, encoded by two genes resulting from a recent duplication, were TF proteins. ZYP1a and ZYP1b are partially functionally redundant (Higgins et al. 2005), and are collectively indicated as ZYP1.

## Phenotypes of TF-deficient mutants

Table 1 summarizes the phenotypes of the TF-deficient mutants of the species analyzed so far with respect to crossover (CO) formation and homolog alignment. Most striking in this table are the differences between the phenotypes of TF-deficient mutants in various species. On the one hand, it is not possible to point out a single function for which TFs are absolutely required in all organisms, albeit that all TF-protein mutants are deficient in CO formation to at least some extent. On the other hand, TF proteins are required for meiosis in nearly all analyzed species, although the number of exceptions is growing slowly but steadily (discussed in Loidl and Scherthan 2004; see also Richard et al. 2005). This combination of TF protein features (structural conservation but no detectable amino acid sequence conservation, pleiotropic effects of TF-protein deficiency, and a strong dependence of the deficiency phenotype on the setting in which the deficiency occurs) points to structural roles of TF proteins rather than a catalytic function in one specific process.

Table 1 also shows that TFs are not required for the initiation of meiotic recombination or homolog recognition

**Table 1** Crossover formation and homolog alignment in TF-deficient mutants of various species

| A. Some relevant features of analyzed species | | | | | | |
|---|---|---|---|---|---|---|
| Feature | *S. cer.* | *D. mel.* | *C. el.* | *M. mus.* | *A. thal.* | |
| Homolog alignment and synapsis depend on recombination (1) | Yes | No | No | Yes | Yes | |
| Interfering COs only (1) | No | Yes | Yes | Yes[a] | No | |
| B. Phenotypes of TF-deficient mutants | | | | | | |
| Phenotypic trait | *zip1* (23°C) (*S. cer.*) | *zip1* (33°C) (*S. cer.*) | *c(3)G* (*D. mel.*[b]) | *syp-1, syp-2* (*C. el.*[c]) | *sycp1* (*M. mus.*) | *zyp1* (*A. thal.*) |
| Initiation of recombination | + (2) | + (2,3[d],4[d]) | Reduced (8) | + (10,11) | + (12) | + (13) |
| Homolog alignment | + (2) | + (2,3[d]) | + (9) | + (10,11) | + (12) | ± (13)[e] |
| Msh4 foci | n.d. | Weak[d] (5) | n.d. | n.d. | + | n.d. |
| Mlh1 foci | n.d. | n.d. | n.d. | n.d. | − (12) | + (13) |
| Crossing over | Reduced (2) | −[f] (2) | − (9) | − (10,11) | − (12) | Reduced (13)[g] |
| CO interference | n.d. | −[h] (6); +[i] (7) | n.a. | n.a. | n.a. | n.d. |

Numbers in parentheses represent the following references: 1, reviewed by Stahl et al. (2004); 2, Börner et al. (2004); 3, Sym et al. (1993); 4, Storlazzi et al. (1996); 5, Novak et al. (2001); 6, Sym and Roeder (1994b); 7, Fung et al. (2004); 8, Jang et al. (2003); 9, Page and Hawley (2001); 10, MacQueen et al. (2002); 11, Colaiácovo et al. (2003); 12, de Vries et al. (2005); and 13, Higgins et al. (2005)
*S. cer.Saccharomyces cerevisiae*, *D. mel.Drosophila melanogaster*, *C. el.Caenorhabditis elegans*, *M. mus.Mus musculus*, *A. thal. Arabidopsis thaliana*, *CO* crossover, *n.d.* not determined, *n.a.* not applicable
[a]Less than 10% of the crossovers might belong to a noninterfering class (Baker et al. 1996; de Vries et al. 2005; Housworth and Stahl 2003)
[b]Phenotype of $c(3)G^{1}$ and $c(3)G^{68}$ mutants, which might express short truncated C(3)G protein
[c]The phenotype of *syp-1* and *syp-2* mutants is the same for the traits considered here
[d]Phenotype at 30°C
[e]Extensive alignment in less than 1% of the pollen mother cells
[f]Assayed at DNA level
[g]Part of the crossovers is nonhomologous
[h]Genetic analysis at 30°C
[i]Cytological interference between Zip3 foci (30°C)

and alignment. Various mechanisms, including recombinational interactions and chromosomal pairing centers, are used for homolog recognition and alignment, and which mechanism(s) prevails it depends on the species (reviewed by Burgess 2004; and Gerton and Hawley 2005). Furthermore, synapsis appears to be insensitive to homology: nonhomologous synapsis can occur under a variety of conditions (reviewed by Zickler and Kleckner 1999), and given the ease with which TF proteins self-assemble into regular stacks of SC-like structures (polycomplexes) (Öllinger et al. 2005; Sym and Roeder 1994b), there are more reasons to wonder what prevents nonhomologous synapsis than what causes homologous synapsis. However, at least in some species, TFs are important for the maintenance of homolog alignment (Colaiácovo et al. 2003; MacQueen et al. 2002; Sherizen et al. 2005).

Because the TF-deficiency phenotypes differ so strongly between species, we will not discuss them collectively, but first consider the *zip1* mutants of budding yeast (*Saccharomyces cerevisiae*), which have been characterized in most detail, and then compare these with TF deficiency mutants in other species.

## Budding yeast (*Saccharomyces cerevisiae*)

### *Yeast TF protein Zip1 contributes to the formation of one class of COs*

Meiotic recombination is initiated by the induction of DNA double-strand breaks (DSBs) (Cao et al. 1990; Sun et al. 1989). In yeast, two major pathways have been proposed for the formation of meiotic COs by repair of these DSBs (reviewed by Hollingsworth and Brill 2004; and Whitby 2005). One pathway, named the Class I or ZMM pathway, largely proceeds via the Szostak model for DSB repair (Szostak et al. 1983). It includes resection of the 5′-ended strands at the broken DNA ends, so that two 3′-ended single stranded tails are generated at each DSB. One of the two 3′-ended tails invades a homologous double-strand DNA molecule and forms a D-loop structure (also called single-end invasion or SEI; Hunter and Kleckner 2001), which is extended by DNA synthesis from the invaded 3′ end. The displaced strand then anneals with the other 3′-ended tail of the same DSB, and after DNA synthesis from this annealed 3′ end and ligation, a double Holliday junction (dHJ) is

formed (Schwacha and Kleckner 1995). dHJs are responsible for about 60 of the 90 COs per wild-type yeast meiotic nucleus (Agarwal and Roeder 2000; Börner et al. 2004; Chua and Roeder 1998). Most or all dHJs formed through this pathway are resolved as COs (reviewed by Bishop and Zickler 2004). The next major meiotic CO pathway in yeast, the Class II or Mus81 pathway, does not include dHJs, and is characterized by a dependence of the Mus81–Mms4 endonuclease (Argueso et al. 2004; de los Santos et al. 2003; reviewed by Hollingsworth and Brill 2004; and Whitby 2005). Zip1 is essential for the formation of Class I COs, together with a group of proteins that are collectively indicated as ZMM proteins, and, besides Zip1, include Zip2, Zip3, Msh5, and Mer3 (Börner et al. 2004; reviewed by Hollingsworth and Brill 2004; and Whitby 2005). Msh4, which forms a heterodimer with Msh5 (Pochart et al. 1997; Snowden et al. 2004) and is required for wild type levels of crossing over (Ross-Macdonald and Roeder 1994), also belongs to this group. The ZMM proteins promote the formation of stable SEIs and dHJs (Börner et al. 2004), which yield COs in yeast. Mlh1 (Hunter and Borts 1997) and Mlh3 (Wang et al. 1999) also contribute to the formation of Class I COs, but act in a later step than the ZMM proteins (Argueso et al. 2004).

Class II COs do not require Zip1, Msh4/Msh5, or Mlh1/Mlh3 (Argueso et al. 2004; de los Santos et al. 2003). Most DSBs in yeast are repaired as non-COs (NCOs), which arise through a separate pathway (Börner et al. 2004; reviewed by Whitby 2005), and possibly primarily serve homolog alignment (discussed by Burgess 2004; and Carpenter 1987).

The ZMM proteins form complexes that are detectable as immunofluorescent foci (Agarwal and Roeder 2000; Chua and Roeder 1998; Novak et al. 2001). Normally, Zip3 is incorporated first, followed by Zip2 and then Zip1 (Agarwal and Roeder 2000). Zip3 promotes and/or stabilizes the association of Zip2 containing recombination complexes with AEs, attracts Zip1, and paves the way for Zip1 polymerization along the AEs (synapsis) (Agarwal and Roeder 2000; Chua and Roeder 1998). Zip2–Zip3 foci are therefore also called synapsis initiation complexes (Agarwal and Roeder 2000). The Zip2–Zip3 foci form independently of Zip1. In *zip1* mutants, the AEs of homologs align, but they are farther apart than within an SC (Sym et al. 1993), and are connected by a limited number of ultrastructurally recognizable axial associations (AAs) at sites of AE convergence (Sym et al. 1993), whereas Zip2–Zip3 foci are found at the sites of convergence between the aligned AEs, presumably on the AAs (Agarwal and Roeder 2000; Chua and Roeder 1998). Zip3 interacts with proteins involved both in early steps in meiotic recombination (Rad51) and later steps (Msh5) (Agarwal and Roeder 2000). Msh4 (and probably Msh5) also forms foci, most of which colocalize with Zip2–Zip3 foci in wild type. In *zip1* mutants, Msh4 foci are fainter and colocalize less strictly with Zip2 than in wild type (Novak et al. 2001). Apparently, Zip1 is required for stable ZMM complexes in yeast, and perhaps it facilitates some specific step that is accompanied by the incorporation of more Msh4/Msh5 into foci (Börner et al. 2004). Zip1-stabilized ZMM complexes might then act as synapsis initiation sites. The numbers of ZMM foci per wild-type nucleus and the CO frequencies in *zmm* and mus81 mutants are consistent with the idea that most or all ZMM foci represent future sites of Class I COs, at least in wild-type yeast (Argueso et al. 2004; discussed in Fung et al. 2004).

In short, in yeast, stable homolog alignment and synapsis initiation depend on recombinational interactions between homologs, and part of the interactions develop into COs. Most COs (Class I) depend on TF proteins plus a number of other proteins that are collectively indicated as ZMM proteins. The ZMM proteins form foci, which, in wild-type yeast, appear to coincide with sites of AE convergence, AAs, synapsis initiation, and future COs.

### *Zip1-dependent (Class I) COs display interference*

In organisms that assemble SCs, meiotic COs are not randomly distributed: every bivalent gets at least one CO (obligate CO), even if the average number of COs per bivalent is close to one, and, if multiple COs occur on a bivalent, they are placed in such a way that simultaneous crossing over in two nearby chromosomal intervals occurs less frequently than expected if COs were placed independently of each other: a phenomenon called (positive CO) interference. As a result, COs become more evenly spaced along the bivalent than if they would not influence each other's position. The obligate CO and interference are probably two manifestations of the same regulatory mechanism, because they are usually lost together.

Interference was originally defined genetically (discussed in Foss et al. 1993), but has also been analyzed cytologically by the study of chiasma positions (reviewed by Jones, 1987), immunofluorescent foci (Fig. 2) (Froenicke et al. 2002; Fung et al. 2004), or RNs (Sherman and Stack 1995) (Table 2). The various methods of determination of CO positions each have their own advantages and disadvantages: chiasmata can only be analyzed in organisms with favorable chromosome morphology; analysis of recombinant progeny usually covers only part of the genome, has a low spatial resolution, and requires that meiosis is completed and yields viable products; and there is no guarantee that all foci or RNs will yield COs. We will therefore distinguish between estimations of interference based on analyses of recombinant progeny or tetrads (genetic interference), chiasma positions (chiasma interference), or positions of foci or RNs (cytological interference) (Fung et al. 2004).

The mechanism(s) of interference have not yet been elucidated. One early model ascribes an essential role to TFs and synapsis (reviewed in Egel 1995): synapsis would initiate at sites of (future) COs, and the establishment of new CO sites within the synapsed stretches would be precluded (reviewed by Fung et al. 2004; Roeder 1997). That would explain why CO interference is not observed in two known TF-less species, *Aspergillus nidulans* and *Schizosaccharomyces pombe* (but see Loidl and Scherthan 2004), and why

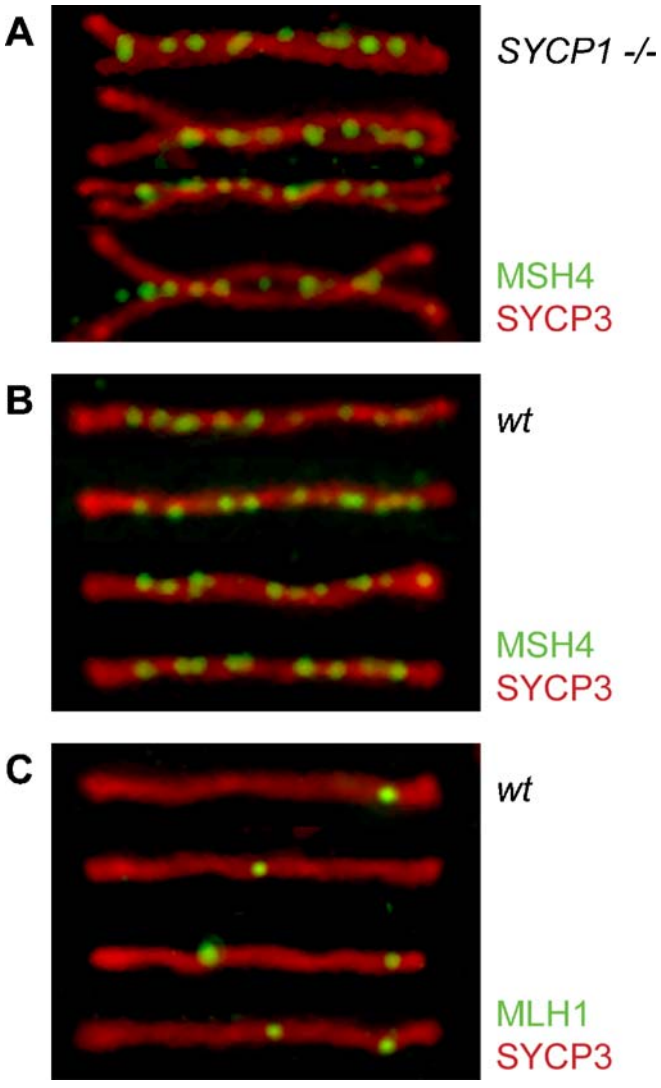


**Fig. 2** Cytological interference in the mouse. Shown are MSH4 foci along SCs or AEs of chromosome 2 of SYCP1-deficient mice (**a**) or wild-type mice (**b**), and MLH1 foci along SCs of chromosome 2 of wild type mice (**c**). Two aspects of interference can be read from these figures: (1) the *strength* of interference: the stronger interference is, the more evenly the foci will be distributed along the chromosome; and (2) the *range of action* of the interference mechanism: the average interfocus distance can be used as a measure for this. The figures suggest that the strength of interference between MSH4 foci is about the same in wild-type and SYCP1-deficient mice, and that the range of action of the mechanism that causes interference between MSH4 foci is also about the same in the two genotypes. Furthermore, the figure shows that interference between MLH1 has a longer range of action than interference between MSH4 foci. This will be worked out quantitatively elsewhere

genetic interference is abolished in yeast *zip1* mutants (Sym and Roeder 1994a), at least under conditions (genetic background; low temperature) that allow *zip1* mutants to complete meiosis and produce COs (Börner et al. 2004). The correlation between the extent of synapsis and the strength of interference in a series of nonnull *zip1* mutants (Tung and Roeder 1998) is also consistent with this model. However, the following observations in yeast undermined the synapsis model of interference:

1. Zip1 contributes to CO formation in a *red1* mutant, which does not form SC (Storlazzi et al. 1996), so positions of Zip1-mediated COs can be determined in the absence of synapsis. If Zip1-mediated COs in a *red1* background would still display interference, this would be a strong argument against the synapsis model of interference, but this has not been (cannot be) determined.
2. *zmm* mutants, including *zip1Δ* mutants, make wild-type levels of NCOs, whereas COs are decreased. Börner et al. (2004) concluded from this that the differentiation between COs and NCOs does not depend on TFs or synapsis and that, therefore, interference does not depend on TFs or synapsis. This presupposes that, in a *zip1* background, the designated but failed COs display interference, which is not known (but see below, Fung et al. 2004).
3. In wild type, stable SEIs (which are virtually quantitatively converted into COs in yeast; see above) are formed in zygotene/early pachytene (Hunter and Kleckner 2001). This is earlier than expected if full synapsis would be required for interference, but does not rule out that partial synapsis contributes to interference.
4. *zip1* mutants have not only lost CO interference, but also make fewer COs than wild type (Sym et al. 1993). In the context of models like the synapsis model, which ascribe interference to a local CO-inhibiting mechanism, COs should *increase* if interference is lost. However, this can be explained because Zip1 has a role in CO formation (Börner et al. 2004).
5. In *zip1* mutants, Zip2 foci still display cytological interference (Fung et al. 2004). If, as seems likely, these Zip2 foci represent designated CO sites that fail to yield COs (Börner et al. 2004), this shows that interference can occur without Zip1. However, Fung et al. (2004) point out that it does not rule out that Zip1 contributes to CO interference in wild type.

Taken together, these observations strongly suggest that TF protein Zip1, and thus, synapsis, has no role in interference, but the synapsis model of interference cannot yet be definitively dismissed. However, observations in other organisms also argue against a direct role of TF proteins in interference (see below).

Most COs found in *zmm* mutants (at low temperature, see above) depend on Mus81, and thus belong to Class II (Argueso et al. 2004). Mus81-dependent COs predominate in *S. pombe*, where interference is not observed (reviewed in Hollingsworth and Brill 2004). The loss of genetic interference in *zip1* mutants can therefore be interpreted outside the context of the synapsis model for interference by assuming that the Zip1-dependent (Class I) COs in wild-type yeast display interference, whereas the Zip1-independent, but Mus81-dependent, (Class II) COs do not (de los Santos et al. 2003). Loss of interference in *zip1* mutants is then primarily due to loss of interfering COs. In the above-mentioned series

**Table 2** Various methods of determining crossover positions

| Advantage/ disadvantage of method | Method | | | |
|---|---|---|---|---|
| | Analysis of recombinant progeny (1) | Chiasma positions (1–3) | Positions of late RNs (4,5) | Positions of MLH1 foci (6) |
| Conforms with the original definition of interference | + | − | − | − |
| Genetic markers required | + | − | − | − |
| Depends on good cytology | − | + | + | + |
| Depends on the completion of meiosis, or on the production of viable meiotic products | + | − | − | − |
| Spatial resolution | Limited by the size of the intervals between the analyzed genetic markers | Limited by the size and morphology of the chromosomes in diakinesis or metaphase I | High | High |
| Covers the entire genome | No; only intervals between markers are analyzed | Yes; but requires cytological markers for recognition of chromosomes | Yes; but requires cytological markers for recognition of chromosomes | Yes; but requires cytological markers for recognition of chromosomes |
| Problems specific to the method | Misclassification of phenotypes may erroneously suggest the occurrence of two closely spaced crossovers | Closely spaced chiasmata can be mistaken for a single chiasma | No guarantee that all crossovers are marked by late RNs | No guarantee that all crossovers are marked by Mlh1 foci |
| | | No guarantee that the chiasma stays at its original position | No guarantee that all late RNs will develop into crossovers | No guarantee all MLH1 foci will develop into crossovers |
| | | Disproportionate local condensation or stretching of chromatin may hamper the determination of chiasma positions (3) | Late RNs may be missed because of technical problems, e.g., variation in stainability (4) | MLH1 foci may be missed because of technical problems (e.g., accessibility to anti-Mlh1 antibodies) |
| | | In some situations, associations of chromosomes can be mistaken for chiasmata | In some organisms, early RNs can be mistaken for late RNs | Imunofluorescence background labeling can be mistaken for MLH1 foci |
| | | Perhaps closely spaced chiasmata involving the same two chromatids disappear by loss of sister chromatid cohesion between the chiasmata (7) | | |
| | | In some mutants, chiasmata close to the telomeres may be missed because of loss of sister chromatid cohesion distal to the chiasma (8) | | |

Numbers in parentheses represent the following references: 1, reviewed by Sybenga (1996); 2, reviewed by Sybenga (1975); 3, reviewed by Jones (1987); 4, reviewed by Anderson and Stack (2005); 5, Anderson et al. (2003); 6, Froenicke et al. (2002); 7, Maguire (1980); and 8, Hodges et al. (2005)

of nonnull *zip1* mutants (Tung and Roeder 1998), decreased synapsis is not only correlated with loss of interference, but also with decreased crossing over, which would fit this interpretation. However, this interpretation does not solve all problems, because cytological interference is unaffected in the yeast *ndj1* mutant, which displays wild-type levels of crossing over but reduced genetic interference (Chua and Roeder 1997; Conrad et al. 1997). Fung et al. (2004)

therefore suggested that there might be more than one interference mechanism: First, an unknown Zip1-independent mechanism would determine the position of ZMM complexes along the bivalent, and subsequently, synapsis, starting from the ZMM complexes, would prevent the formation of ZMM-independent COs near the ZMM complexes. In this view, ZMM-independent (Class II) COs would experience interference, but not exert it. This should result in a nonrandom distribution (negative interference) of Class II COs in wild type, but this cannot yet be verified, because Class II CO sites cannot (yet) be visualized as foci. Mutants with delayed synapsis, like *ndj1*, would then display decreased genetic interference. Strictly speaking, such mutants should have increased rather than wild-type levels of crossing over, but it is possible that an increase (if any) is too small to be detected, or that *ndj1* mutants have problems with CO formation besides the interference defect.

### *Does Zip1 prevent/remove AAs/ZMM complexes?*

Sgs1 is a RecQ helicase with a possible role in homologous recombinational repair (reviewed by Rockmill et al. 2003; and Thompson and Schild 2002). In yeast *sgs1* mutants, the formation of Zip2/Zip3 foci and COs is increased about 1.4-fold, whereas cytological interference between ZMM foci is unaffected (Fung et al. 2004). Rockmill et al. (2003) proposed that Sgs1 unwinds meiotic recombination intermediates that have not (yet) committed to crossing over. In *zip1* mutants, the number of AAs (as detected ultrastructurally) (Sym et al. 1993) per nucleus is roughly similar to the number of AAs (as estimated from the number of Zip2 foci) (Rockmill et al. 2003) in wild type. However, in *zip1sgs1* double mutants, the number of AAs (as estimated from AE convergence sites) (Rockmill et al. 2003) has increased dramatically. This suggests that there are two different mechanisms of AA removal or prevention, one dependent on Sgs1 and one on Zip1 (Rockmill et al. 2003). Both AA removal/prevention activities might bring about interference if they would spread from a limited number of sites along the AEs, for instance, from precursors of AAs that have reached a certain step in their assembly. If so, then the ranges of action of the two mechanisms should either be similar, or the Sgs1-dependent mechanism should have a slightly longer range of action than the Zip1-dependent mechanism (Fig. 3). Such a (hypothetical) scenario would still leave some room for a role of Zip1 in interference.

### *Homology-independent centromere coupling by Zip1*

An unexpected role of Zip1 was discovered in yeast *spo11* mutants, which do not form meiotic DSBs (Keeney 2001), and therefore, do not perform meiotic recombination. In these mutants, Zip1 localizes to the centromeric regions in such a way that most centromeres are coupled in pairs. Centromere coupling is nonhomologous, depends on Zip1, and also occurs in early meiosis of wild type (Tsubouchi and Roeder 2005). In wild type, nonhomologous centromere coupling is gradually replaced by homologous centromere associations (Tsubouchi and Roeder 2005), presumably by extension of synapsis initiated at homologous recombination sites (discussed by Henderson and Keeney 2005). It is possible that Zip1-mediated centromere coupling makes part of the distributive disjunction system, which can promote disjunction of occasional pairs of chromosomes that failed to form COs (it cannot ensure proper chromosome disjunction if all chromosomes fail to form COs, see Klein et al. 1999). Various species have such a backup system for meiotic chromosome disjunction (discussed by Tsubouchi and Roeder 2005), and contributing to such a system might represent an ancient role of TFs (see below). It is not clear, however, how the coupling of centromeres of nonexchange chromosomes can persist until anaphase, while TFs are shed from the rest of the chromosomes at the end of meiotic prophase.

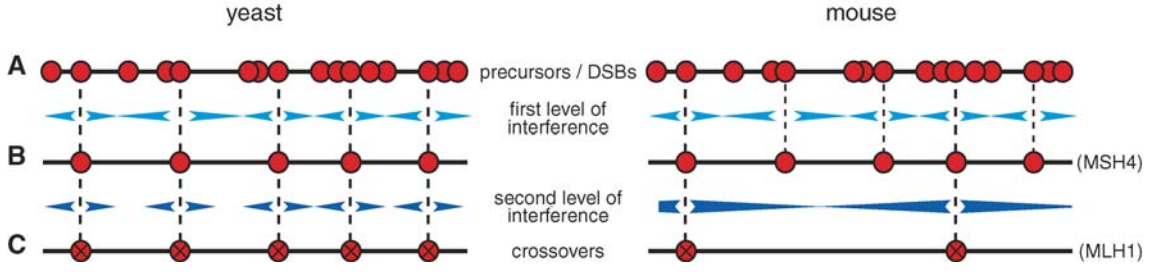


**Fig. 3** The possibility of interference in two successive steps in meiosis. Early recombination intermediates or DSBs are randomly distributed along the chromosome (**a**); if an intermediate reaches a certain stage in its development, it emits an interference effect along the chromosome that prohibits surrounding intermediates to reach that same stage (**b**); in a later stage of CO formation this process can repeat itself (**c**). In the mouse, the second interference step has a much longer range of action than the first step, whereas in yeast there might also be two steps, but both interference steps should have about the same range of action. An alternative possibility is not shown in this figure but is discussed in the text: it is also possible that interference is not imposed in two steps, but that there are two independent interference mechanisms (at least in the mouse), which need not necessarily act in the same stage of meiosis. The possibility of interference phenomena in more than one stage of meiotic prophase has been proposed in one model (Kleckner 1996; Kleckner et al. 2004)

## Mouse

### *Mouse TF protein SYCP1 is essential for CO formation*

In the mouse, homolog alignment and synapsis depend on recombinational interactions, as in yeast (Baudat et al. 2000; reviewed by Hunter 2003; Romanienko and Camerini-Otero 2000). The distribution of foci of recombination-related proteins along SCs can be analyzed in detail in the mouse (Fig. 2), and possible roles of TF proteins in the distribution of recombination-related events can therefore be analyzed by immunocytology.

A minimum estimate of mouse meiotic DSBs is provided by the number of RAD51/DMC1 foci: in early male mouse meiosis (leptotene), there are up to 300 RAD51/DMC1 foci per nucleus along nascent AEs (Moens et al. 2002). In zygotene, their number declines to about 200 foci, which acquire, successively, RPA, BLM, and MSH4, and lose RAD51/DMC1. In late zygotene, there are about 200 MSH4 foci, most of which also contain RPA and BLM. BLM is a RecQ helicase, like yeast Sgs1 (Thompson and Schild 2002), which might help resolving interhomolog DNA interactions that will not become COs (discussed by Guillon et al. 2005; and Moens et al. 2002), perhaps by dissolving dHJs in cooperation with topoisomerase IIIα (Wu and Hickson 2003). Most MSH4 foci are on synapsed segments of SCs, or exceptionally in the synapsis fork (de Vries et al. 2005; Moens et al. 2002), which indicates that the formation of MSH4 foci in the wild-type mouse is rapidly followed by synapsis. Possibly, MSH4 foci serve as synapsis initiation sites, or they appose homologs sufficiently closely for synapsis to initiate. Differently from that in yeast, synapsis initiation is not confined to CO sites in the mouse: Only a minority of the mouse MSH4 foci yield COs (see below), and synapsis initiation is not confined to the sites of these foci (de Boer et al., unpublished observations).

Whereas most or all yeast Msh4 foci yield COs, only 10–15% (20–25 per cell) of the mouse MSH4 foci incorporate MLH1 (Santucci-Darmanin et al. 2000) and MLH3 (Lipkin et al. 2002; Santucci-Darmanin et al. 2002) and yield COs (Froenicke et al. 2002; Koehler et al. 2002). What the other 85–90% of the MSH4 foci represent is not known. The COs that arise through the MSH4–MLH1 pathway comprise more than 90% of the mouse COs (Baker et al. 1996; Guillon et al. 2005; Woods et al. 1999). They correspond most likely to yeast Class I COs, because they also depend on TF protein SYCP1. MLH1 or MLH3 foci have not been found in *Sycp1*$^{-/-}$ spermatocytes (de Vries et al. 2005), although a small proportion of these cells proceed beyond mid–late pachytene, when MLH1/MLH3 foci occur in wild type (Moens et al. 2002 and references therein). Furthermore, the few *Sycp1*$^{-/-}$ spermatocytes that reach metaphase I do not form chiasmata (de Vries et al. 2005), whereas pachytene *Sycp1*$^{-/-}$ spermatocytes that were forced to condense their chromosomes by okadaic acid treatment show less than 10% of the wild-type level of chiasmata (de Vries et al. 2005), which is all consistent with the idea that >90% of the mouse COs correspond to yeast Class I COs, which depend both on TFs and Mlh1. These COs display cytological (Froenicke et al. 2002; de Boer et al., unpublished observations) and genetic (Broman et al. 2002) interference in wild-type mice, like Class I COs in yeast.

### *Cytological interference occurs in the absence of mouse SYCP1*

In the wild-type mouse, the RPA and MSH4 foci already display cytological interference in zygotene (Fig. 2). The strength of cytological interference can be inferred from the frequency distribution of distances between foci: the stronger interference is, the more evenly the foci are spaced. Furthermore, the average interfocus distance gives an indication of the range of action of the interference mechanism. Although the cytological interference between RPA or MSH4 foci is unmistakable, it is weaker and has a shorter range of action than interference between MLH1 foci (Fig. 2; de Boer et al., unpublished observations). This suggests either two interference mechanisms, or two successive steps in the imposition of interference between mouse MLH1 foci (and thus, COs): If the CO-yielding MSH4 foci have already differentiated from other MSH4 foci in late zygotene, then there are two interference mechanisms acting in parallel, one causing weak interference between MSH4 foci that do not yield COs, and one causing stronger interference between MSH4 foci that yield COs. On the other hand, if it has not yet been decided in late zygotene which MSH4 foci will yield MLH1 foci, then interference between MLH1 foci develops in two steps: The first step would cause weak interference between all MSH4 foci, and the second step, stronger interference between those MSH4 foci that (will) acquire MLH1 and yield COs (Fig. 2). The possibility of interference phenomena in more than one stage of meiosis has been suggested in one model (Kleckner 1996; Kleckner et al. 2004).

In the TF-deficient *Sycp1*$^{-/-}$ mice, RAD51/DMC1, RPA, and MSH4 foci appear in similar numbers as in wild type, and have the same intensity as in wild type (de Vries et al. 2005). Along the autosomal bivalents, the RPA and MSH4 foci occur predominantly between homologously aligned AEs, without obvious convergence sites, and display equally strong interference as in wild type (Fig. 2; de Boer et al., unpublished observations). Thus, TFs are neither important for the homologous alignment of autosomes in the mouse, nor for the formation of RAD51/DMC1, RPA, and MSH4 foci, nor for cytological interference between RPA or MSH4 foci. However, the short pseudoautosomal regions of the X and Y chromosomes are not aligned in part of the *Sycp1*$^{-/-}$ spermatocytes (de Vries et al. 2005). This might suggest that SYCP1 is required for stable homolog apposition if the homologous chromosomal region is short and/or the number of recombinational interactions is small. SYCP1/synapsis would thus turn the local, unstable homology

recognition based on MSH4-marked interactions into at least a regional recognition. Whether SYCP1 is required for the strong interference between MLH1 foci or COs is not known, because *Sycp1*$^{-/-}$ spermatocytes do not make these structures (see above) (de Vries et al. 2005).

### *Mouse SYCP1 is directly or indirectly required for removal of recombination-related proteins*

In early mouse meiosis, DSB induction is accompanied by phosphorylation of histone variant H2AX to γH2AX (Mahadevaiah et al. 2001). In leptotene, ATM phosphorylates H2AX throughout the nucleus (Bellani et al. 2005), while another kinase, ATR, is recruited to the AEs (Moens et al. 1999; Perera et al. 2004). ATR becomes gradually confined to distinct sites along AEs, presumably sites where DSB repair has progressed and RPA-coated single-strand DNA has been generated (Jazayeri et al. 2006). ATR maintains H2AX phosphorylation at these sites (Turner et al. 2004), until it disappears in zygotene from the synapsed portions of AEs (Turner et al. 2004), presumably after the underlying DNA lesions have been repaired (Jazayeri et al. 2006). In *Sycp1*$^{-/-}$ mice, γH2AX appears on schedule, but disappears delayed. The H2AX phosphorylation throughout the nucleus, which is only seen in leptotene in wild type, persists into zygotene in *Sycp1*$^{-/-}$, whereas the distinct H2AX domains, which disappear during zygotene in wild type, persist into pachytene and diplotene in *Sycp1*$^{-/-}$ (de Vries et al. 2005). The persisting γH2AX domains correlate with AE-associated patches of ATR (de Vries et al. 2005). Thus, SYCP1 is somehow required for the timely disappearance of ATR from the AEs.

Similarly, in wild type, all MSH4 and RPA foci have disappeared by midpachytene (Moens et al. 2002), whereas in *Sycp1*$^{-/-}$ spermatocytes, 50–70% of the MSH4 and RPA foci persist into late pachytene/diplotene; some of these foci colocalize with γH2AX domains (de Vries et al. 2005). SYCP1 is therefore directly or indirectly required for the disappearance of these foci. This is reminiscent of us of the possible requirement of Zip1 for the removal/prevention of AAs in *sgs1* yeast (see above) (Rockmill et al. 2003), but because the role of Zip1 and SYCP1 in the removal of AAs or foci is not known (and can be very indirect), this correspondence may be superficial.

Summarizing, there are no indications that yeast Zip1 and mouse SYCP1 fulfill fundamentally different roles. In both species, TF proteins are dispensable for the initiation of recombination and homolog alignment, but are indispensable for wild-type levels of crossing over. Cytological interference is possible in the absence of TFs in both species. However, a detailed comparison of TF functions in the two species is hampered by uncertainty about the interpretation of foci, in particular of the ZMM protein MSH4/Msh4. A conservative interpretation of MSH4/Msh4 foci would be that mouse MSH4 foci represent steps in meiotic recombination similar to those of yeast Msh4 foci, but are less dependent on TFs (SYCP1) for their stability, and give a lower yield of COs. Why the CO yield of MSH4 foci is low in the mouse and whether TF proteins have a role in this remain important unanswered questions. One possibility could be that interference has a role in the CO yield of MSH4/Msh4 foci. The two mechanisms or steps in the imposition of cytological interference found in the mouse (first, short-range interference between MSH4 foci and then longer-range interference between MLH1 foci/COs) might also exist in yeast, but if so, then the two mechanisms should have similar ranges of action in yeast, so that nearly all Msh4 foci would yield COs (Fig. 3). As argued above, SYCP1 is not required for the (short-range) interference between MSH4 foci in mouse, but it has not yet been ruled out that it has a role in the (long-range) interference between mouse MLH1 foci (but see below for *Arabidopsis*).

In both yeast and mouse, MSH4/Msh4 enhances homologous synapsis. Because most MSH4/Msh4 foci yield COs in yeast but not in mouse, it is not surprising that synapsis initiation coincides with sites of CO formation in yeast (Agarwal and Roeder 2000; Chua and Roeder 1998; Henderson and Keeney 2004), but not necessarily in the mouse. This implies that, in the mouse, either synapsis can start by association of TFs with MSH4 foci that do not yield COs, or that the close apposition of homologs provided by MSH4 foci suffices for synapsis initiation.

## Arabidopsis

### *CO formation is only moderately affected in TF-deficient Arabidopsis*

Recently, TF-deficient mutants have been characterized in a third species that relies on recombinational interactions for homolog alignment; *Arabidopsis thaliana* (Higgins et al. 2005). Unexpectedly, there are some important differences between these mutants and TF-deficient mutants in yeast and mouse.

*Arabidopsis* requires DSBs (Grelon et al. 2001) and AtMSH4 (the *Arabidopsis* protein homologous to yeast Msh4) for homolog alignment, synapsis, and wild-type levels of COs (Higgins et al. 2004). AtMSH4 possibly fulfills roles in CO formation similar to those of its homologues in yeast and mouse, albeit that the timing of AtMSH4 focus assembly and disassembly suggests additional roles for this protein in early *Arabidopsis* meiosis (discussed in Higgins et al. 2004). It is not known whether AE-associated AtMSH4 foci display cytological interference. AtMSH4 is required for about 85% of the COs in *Arabidopsis* (Higgins et al. 2004), and a similar percentage depends on another ZMM protein, AtMER3 (also known as RCK) (Chen et al. 2005; Mercier et al. 2005). In AtMSH4- or AtMER3-deficient *Arabidopsis*, COs are randomly distributed among meiotic nuclei, which indicates that the obligate CO mechanism either does not function or does not influence the COs that still occur. Because obligate COs and interference are probably two manifestations of the same regulatory mechanism, it seems likely that the AtMSH4 and AtMER3 independent COs do

not display interference; they might correspond to yeast Class II COs. Because total COs in wild-type *Arabidopsis* display interference (Copenhaver et al. 2002), there must be interference between AtMSH4/AtMER3-dependent COs, which, therefore, likely correspond to yeast Class I COs. However, as is explained below, it appears that AtMSH4- and AtMER3-dependent COs do not depend to the same extent on TFs as yeast and mouse Class I COs.

Two closely related genes have been identified in *Arabidopsis* that encode TF proteins: *ZYP1a* and *ZYP1b*. The *zyp1a* or *zyp1b* single mutants have indistinguishable, mild phenotypes: they complete synapsis, albeit that meiotic prophase is somewhat delayed, and display about 90% of the weight level of crossing over (chiasmata). However, in the *zyp1a zyp1b* double knockout (further indicated as *zyp1*), meiotic prophase is severely disturbed. *zyp1* pollen mother cells (PMCs) pass through meiotic prophase with considerable delay, whereas extensive alignment of AEs occurs in less than 1% of the PMCs. Nevertheless, *zyp1* PMCs assemble substantial numbers of AtMLH1 foci (seven to eight per nucleus, compared with ten in wild type), which yield chiasmata. Apparently, AtMLH1 foci are less dependent on TF proteins for their stability than the corresponding foci in yeast and mouse. Whether TF proteins are important for chiasma formation in wild-type *Arabidopsis* is undecided because in *zyp1* mutants, chiasmata appear delayed, and therefore arise possibly via pathways that are not normally used (cf. Börner et al. 2004). Higgins et al. (2005) reported preliminarily that chiasmata in *zyp1Arabidopsis* still display interference. If this can be confirmed, and the strength of chiasma interference in *zyp1* mutants would be similar to that in wild type, it would virtually rule out the synapsis model of interference unless two interference mechanisms with similar ranges of action would act in parallel.

### *Many chiasmata are nonhomologous in TF-deficient Arabidopsis*

Many chiasmata in *zyp1* mutants involve nonhomologous chromosomes (Higgins et al. 2005). This contrasts observations in yeast and mouse where TF-deficient mutants nicely align their homologs and do not show obvious signs of nonhomologous interactions (see above). Higgins et al. (2005) propose that ZYP1 is required for the disruption of interactions between nonhomologous chromosomes, which represents another hint that TFs might have a role in the prevention or removal of interchromosomal recombinational interactions. Because of the widespread occurrence of genome duplications in plants, the loss of such a function might affect homologous alignment more in plant species than in yeast or mouse (discussed in Higgins et al. 2005). However, removal of recombinational interactions between nonhomologous or homologous chromosomes by itself cannot ensure the alignment of homologs; it must be accompanied by some mechanism that distinguishes full length from local or regional homology. Possibly, regions of synapsis are unstable until they are fastened at both ends. If fastening happens only at the telomeres, it would automatically favor full-length synapsis of homologs. Mature COs should form late in such a scenario.

In short, the TF-deficiency phenotype in *Arabidopsis* further undermines the idea that TFs or synapsis have a role in chiasma interference, and reveals one possible function of TFs that was less evident in yeast and mouse: the promotion of full-length homologous apposition of chromosomes at the cost of regional or local homologous or nonhomologous interhomolog interactions.

## Drosophila

In *Drosophila*, SCs are only formed in female meiosis (reviewed by Page and Hawley 2004). Homolog synapsis does not depend on meiotic DSBs or recombination in *Drosophila* females (McKim et al. 1998), but on *cis*-acting pairing sites (reviewed by McKee et al. 2000), whereas DSBs are likely induced after completion of synapsis (Jang et al. 2003).

One *Drosophila* gene has been identified that encodes a TF protein, namely, *c(3)G* (Bogdanov et al. 2002; Gowen and Gowen 1922; Page and Hawley 2001). Females homozygous for $c(3)G^{68}$, which is effectively a null allele, still display homolog alignment; however, maintenance of alignment in euchromatic regions and sister chromatid arm cohesion appear to be affected (Gong et al. 2005; Sherizen et al. 2005). Furthermore, meiotic DSBs are reduced, though not eliminated (Jang et al. 2003), and CO formation is abolished (Page and Hawley 2001, and references therein). Because all COs in *Drosophila* depend on TFs and likely all exert/experience interference (discussed in Copenhaver et al. 2002; Muller 1916), they probably all correspond to yeast Class I COs. Because $c(3)G^{68}$ mutants yield offspring, the DSBs that should have yielded COs are eventually repaired without yielding COs in these mutants. Remarkably, $c(3)G^{68}$ mutants can produce some COs in a *c(2)M* background (Manheim and McKim 2003). C(2)M is an AE component (Anderson et al. 2005) that is distantly related to meiotic cohesin Rec8 of other species (Schleiffer et al. 2003). Possibly, C(2)M forces DSB repair into a TF-dependent CO pathway, whereas abnormal ways of CO formation become available in a *c(2)M* background. In hypomorphic mutants that express C(3)G protein lacking part of the coiled-coil region, synapsis is severely affected and crossing over is reduced, particularly in the centromere-distal regions (Page and Hawley 2001). In contrast, interference between the COs that still occur is only moderately reduced, which further weakens the idea that synapsis is important for CO interference.

Thus, differently from the species discussed above, DSBs are likely formed after completion of synapsis in *Drosophila*, which would imply that the process of synapsis cannot influence CO positions. Possibly, the independence of homolog alignment on DSBs in *Drosophila* has led to altered regulation of DSB induction (discussed in Page and Hawley 2001), and this need not

necessarily include altered roles of TFs. Similar to other species, TFs appear to create favorable preconditions for CO formation in *Drosophila*. Specifically, uninterrupted synapsis between defined boundaries on *Drosophila* chromosomes appear to promote CO formation (Gong et al. 2005; Sherizen et al. 2005).

## Caenorhabditis

Like *Drosophila*, *Caenorhabditis elegans* does not require DSBs for recognition, alignment, and synapsis of homologs (Dernburg et al. 1998). Before meiosis, homologs are not aligned, but upon entering meiosis they align rapidly (MacQueen et al. 2002). *Caenorhabditis* chromosomes each have one pairing center (McKim et al. 1993), which probably accounts for initial homolog recognition. In *Caenorhabditis*, DSBs are induced before synapsis is complete; RAD-51 foci start to increase from leptotene/early zygotene on, peak in late zygotene/early pachytene, and disappear during late pachytene (Alpi et al. 2003; Colaiácovo et al. 2003). Two TF proteins have been identified in *Caenorhabditis*, SYP-1 (MacQueen et al. 2002) and SYP-2 (Colaiácovo et al. 2003), which are both short, coiled-coil proteins. *syp-1* and *syp-2* mutants do not form SC, but in early meiosis, homologs align at least locally; however, this initial alignment is largely lost in late meiotic prophase (MacQueen et al. 2002). In *syp-1* and *syp-2* mutants, RAD-51 foci appear on schedule, but they persist (Colaiácovo et al. 2003). Another feature that persists abnormally long in *syp-1* and *syp-2* germ cells is the clustering of chromatin in a crescent-shaped body in one half of the nucleus (Colaiácovo et al. 2003). In wild type, this polarized nuclear organization is normally lost upon completion of synapsis, whereas it is at least partially retained in *syp-1* and *syp-2* germ cells (Colaiácovo et al. 2003; MacQueen et al. 2002). Possibly, synapsis produces either a mechanical force or a regulatory signal that promotes redispersal of the chromosomes through the nucleus (discussed in Colaiácovo et al. 2003; MacQueen et al. 2002). Such a correlation between TF proteins/synapsis and chromatin organization might exist in other organisms as well (e.g., in the tomato) (Havekes 1999).

Most *syp-2* germ cells undergo apoptosis in late pachytene, but some proceed to diakinesis, and these cells do not display chiasmata but only univalents without (visible) breaks (Colaiácovo et al. 2003), as has also been observed in mouse *sycp1* mutants (above). Probably, the persisting RAD-51 foci in *syp-2* mutants represent DNA-lesions that are eventually repaired on the sister chromatids (discussed by Colaiácovo et al. 2003). All COs in *Caenorhabditis* thus depend on TFs, and probably correspond to yeast Class I COs. Accordingly, interference is strong in *Caenorhabditis*: Wild-type *Caenorhabditis* has six pairs of chromosomes, and in almost every meiotic cell the six bivalents each develop a single CO (reviewed by Hillers 2004). If two or three *Caenorhabditis* chromosomes are fused end-to-end, the bivalent of the fusion products shows usually only one CO (Hillers and Villeneuve 2003; discussed by van Veen and Hawley 2003). In heterozygotes for an end-to-end fusion, the trivalent consisting of the fusion product and the two homologous unfused chromosomes develop either one or two COs, and if two COs occur, each unfused chromosome has one. This suggests that the spreading of an interference effect requires at least uninterrupted chromosomes or AEs, but does not preclude that TFs or an intact SC structure contribute to the spreading of the interference signal.

## Species without TFs/SCs

A limited number of eukaryotes, including *S. pombe* and *A. nidulans*, do not form SC during meiosis. *S. pombe* does not assemble SC, but makes discontinuous linear elements (reviewed by Kohli and Bähler 1994), which are related to AEs (Lorenz et al. 2004; Molnar et al. 1995), whereas *A. nidulans* makes rudimentary SC fragments, but no recognizable linear structures (Egel-Mitani et al. 1982). These species do not display CO interference (Munz 1994; Strickland 1958), and originally, this was considered an argument in favor of a role of TFs/the SC in interference. However, because these species do not make continuous AEs, it is also possible that interference requires uninterrupted AEs. Moreover, it seems now more likely that at least *S. pombe* makes a noninterfering type of CO (see above).

Recently, meiosis has been analyzed in another TF-less eukaryote, *Tetrahymena thermophila*, which makes neither detectable AEs nor SCs, whereas candidate TF genes have not been identified in the genome (Loidl and Scherthan 2004 and references therein). During meiotic prophase, the meiotic nuclei of this species elongate to form threadlike structures, with the telomeres at one end and the remainder of the chromosomes arranged in parallel to the length axis of the elongated nucleus, presumably in a bouquet conformation (i.e., both telomeres at one end of the nucleus). During this process, homologous loci come into close proximity and Rad51 foci increase in number, which suggests that the apposition of homologous loci is accompanied/followed by recombinational interactions. Loidl and Scherthan (2004) propose that a prolonged bouquet conformation, together with extreme nuclear elongation, replaces SC formation for the initial apposition of homologs along their length in *Tetrahymena*. Furthermore, they suggest that in *S. pombe* a prolonged bouquet state, together with moderate nuclear elongation and nuclear movements, might fulfill a similar role to that in *Tetrahymena* (reviewed by Yamamoto and Hiraoka 2001). However, in the organisms considered above, except *Arabidopsis*, SC formation was not important for full-length homolog alignment, even though the meiotic nucleus did not elongate. Perhaps other mechanisms act in parallel with synapsis in these species to ensure homolog alignment. Interestingly, preliminary evidence suggests that COs interfere in *Tetrahymena* (Loidl and Scherthan 2004). If confirmed, this would not only cast doubt upon the role of SCs, but also upon that of AEs in interference.

To summarize the characterizations of TF-deficient mutants and species lacking detectable TFs: Evidence is accumulating that TFs are not involved in CO interference. Furthermore, whereas several functions were identified that require TFs in part of the species, it was not possible to pinpoint a single function for which TFs are essential in all species.

## Evolutionary considerations and speculation

Besides comparisons of mutants of living species, evolutionary considerations might help us to understand what TFs do, and why nearly all eukaryotes have SCs.

It is usually assumed that meiosis evolved in organisms that already performed mitosis (Bogdanov 2003; Kleckner 1996; Maguire 1992; Maynard Smith and Szathmáry 1995). Maguire (1992) argues that subsequent steps in the evolution of meiosis were centromere stickiness, followed by association of homologs, increased crossing over, and, finally, chiasma maintenance. TF proteins might have contributed to centromere stickiness, thanks to their ability to bind to the basis of chromatin loops and/or kinetochore proteins at their C terminus, and to other TF molecules at their N terminus (Fig. 1); therefore, TFs might initially have evolved separately from crossing over. In some protozoa, SCs are limited to the centromere region (reviewed by Bogdanov 2003), which might reflect this presumed early role of TFs. Zip1-mediated centromere coupling in yeast (Tsubouchi and Roeder 2005) might also represent a relict of this role of TFs. However, because TFs are homology-insensitive, TF-mediated centromere stickiness can at best ensure the gradual loss of chromosomes by a series of nondisjunctions, and the efficiency of such a reductional process would be greatly enhanced if homologs (perhaps originally sister chromosomes) would recognize each other. DNA-based interactions were available for this purpose (discussed in Kleckner 1996), but it is conceivable that other recognition mechanisms have also worked. The sites of homolog recognition have presumably acquired the ability (1) to attract TFs, to ensure homolog association after resolution of the initial DNA (or other recognition) interactions, and (2) to bring about some change in the chromosomal region that surrounds them so that further recombinational interactions in that region are blocked [this might result in interference (cf. Börner et al. 2004), e.g., between MSH4 foci in the mouse] and TF binding to that region is facilitated, in other words, extension of synapsis from the homology recognition site is enhanced; see also the discussion above about synapsis initiation sites in yeast (Agarwal and Roeder 2000; Bogdanov 2003; Kleckner 1996; Maguire 1992). This would, stepwise, drive back ectopic interactions and convert strictly local homology recognition into regional and ultimately full-length homolog association; this is particularly important for organisms with duplications in their genome (see discussion above about the *zyp1* phenotype of *Arabidopsis*). In this view, TFs are not an intrinsic part of the recombination machinery, although they might stabilize and then replace recombinational interactions.

The coordination of TF-mediated homology-insensitive chromosome stickiness with recombination and/or other homology recognition mechanisms ensures, in principle, an efficient reductional division, provided that TFs stay in position until anaphase and are released upon some cell cycle signal. However, modern "standard" meiosis differs from this set-up in several respects, and various additional changes must have occurred (reviewed by Maynard Smith and Szathmáry 1995). For instance, TFs are now shed from the chromosomes before metaphase I, and connection of the homologs during the first meiotic division is now ensured by chiasmata (and sister chromatid cohesion). Why and how these changes might have occurred is beyond the scope of this review. However, they must have been accompanied/preceded by the development of efficient mechanisms of CO formation, and controls of the distribution of COs among and along bivalents. Based on the evidence available now, it seems likely that TFs contribute to efficient CO formation, and are less important for the controls of the number and distribution of COs. Thus, one of the key questions in meiosis research remains unanswered: "What determines the number and distribution of COs along bivalents?" Perhaps we will have to wait until the next jubilee year of the SC to learn more about this.

## References


Agarwal S, Roeder GS (2000) Zip3 provides a link between recombination enzymes and synaptonemal complex proteins. Cell 102:245–255

Alpi A, Pasierbek P, Gartner A, Loidl J (2003) Genetic and cytological characterization of the recombination protein RAD-51 in *Caenorhabditis elegans*. Chromosoma 112:6–16

Anderson LK, Offenberg HH, Verkuijlen WC, Heyting C (1997) RecA-like proteins make part of early meiotic nodules in lily. Proc Natl Acad Sci U S A 94:6868–6873

Anderson LK, Doyle GG, Brigham B, Carter J, Hooker KD, Lai A, Rice M, Stack SM (2003) High-resolution crossover maps for each bivalent of Zea mays using recombination nodules. Genetics 165:849–865

Anderson LK, Royer SM, Page SL, McKim KS, Lai A, Lilly MA, Hawley RS (2005) Juxtaposition of C(2)M and the transverse filament protein C(3)G within the central region of *Drosophila* synaptonemal complex. Proc Natl Acad Sci U S A 102:4482–4487

Anderson LK, Stack SM (2005) Recombination nodules in plants. Cytogenet Genome Res 109:198–204

Argueso JL, Wanat J, Gemici Z, Alani E (2004) Competing crossover pathways act during meiosis in *Saccharomyces cerevisiae*. Genetics 168:1805–1816

Ashley T, Plug AW (1998) Caught in the act: deducing meiotic function from protein immunolocalization. Curr Top Dev Biol 37:201–239

Baker SM, Plug AW, Prolla TA, Bronner CE, Harris AC, Yao X, Christie D-M, Monell C, Arnheim N, Bradley A, Ashley T, Liskay RM (1996) Involvement of mouse Mlh1 in DNA mismatch repair and meiotic crossing over. Nat Genet 13:336–342

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

Bellani MA, Romanienko PJ, Cairatti DA, Camerini-Otero RD (2005) SPO11 is required for sex-body formation, and Spo11 heterozygosity rescues the prophase arrest of Atm−/− spermatocytes. J Cell Sci 118:3233–3245

Bishop D (1994) RecA homologs Dmc1 and Rad51 interact to form multiple nuclear complexes prior to meiotic chromosome synapsis. Cell 79:1081–1092

Bishop DK, Zickler D (2004) Early decision: Meiotic crossover interference prior to stable strand exchange and synapsis. Cell 117:9–15

Bogdanov YF (2003) Variation and evolution of meiosis. Russ J Genet 39:363–381

Bogdanov YF, Grishaeva TM, Dadashev SY (2002) Gene CG17604 of *Drosophila melanogaster* may be a functional homolog of yeast gene ZIP1 and mammal gene SCP1 (SYCP1) encoding proteins of the synaptonemal complex. Russ J Genet 38:90–94

Börner GV, Kleckner N, Hunter N (2004) Crossover/noncrossover differentiation, synaptonemal complex formation, and regulatory surveillance at the leptotene/zygotene transition of meiosis. Cell 117:29–45

Broman KW, Rowe LB, Churchill GA, Paigen K (2002) Crossover interference in the mouse. Genetics 160:1123–1131

Burgess SM (2004) The ends are the means. Mol Cell 13:766–768

Cao L, Alani E, Kleckner N (1990) A pathway for generation and processing of double-strand breaks during meiotic recombination in *S. cerevisiae*. Cell 61:1089–1101

Carpenter ATC (1987) Gene conversion, recombination nodules, and the initiation of meiotic synapsis. Bioessays 6:232–236

Chen C, Zhang W, Timofejeva L, Gerardin Y, Ma H (2005) The *Arabidopsis* ROCK-N-ROLLERS gene encodes a homolog of the yeast ATP-dependent DNA helicase MER3 and is required for normal meiotic crossover formation. Plant J 43:321–334

Chua PR, Roeder GS (1997) Tam1, a telomere associated meiotic protein, functions in chromosome synapsis and crossover interference. Genes Dev 11:1786–1800

Chua PR, Roeder GS (1998) Zip2, a meiosis-specific protein required for the initiation of chromosome synapsis. Cell 93:349–359

Colaiácovo MP, MacQueen AJ, Martinez PE, McDonald K, Adamo A, La Volpe A, Villeneuve AM (2003) Synaptonemal complex assembly in *C. elegans* is dispensable for loading strand-exchange proteins but critical for proper completion of recombination. Dev Cell 5:463–474

Conrad MN, Dominguez AM, Dresser ME (1997) Ndj1p, a meiotic telomere protein required for normal chromosome synapsis and segregation in yeast. Science 276:1252–1255

Copenhaver GP, Housworth EA, Stahl FW (2002) Crossover interference in *Arabidopsis*. Genetics 160:1631–1639

de los Santos T, Hunter N, Lee C, Larkin B, Loidl J, Hollingsworth NM (2003) The Mus81/Mms4 endonuclease acts independently of double-Holliday junction resolution to promote a distinct subset of crossovers during meiosis in budding yeast. Genetics 164:81–94

de Vries FAT, de Boer E, van den Bosch M, Baarends WM, Ooms M, Yuan L, Liu J-G, Heyting C, Pastink A (2005) Mouse Sycp1 functions in synaptonemal complex assembly, meiotic recombination, and XY body formation. Genes Dev 19:1376–1389

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

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

Dong HJ, Roeder GS (2000) Organization of the yeast Zip1 protein within the central region of the synaptonemal complex. J Cell Biol 148:417–426

Egel R (1995) The synaptonemal complex and the distribution of meiotic recombination events. Trends Genet 11:206–208

Egel-Mitani M, Olson LW, Egel R (1982) Meiosis in *Aspergillus nidulans*: another example for lacking synaptonemal complexes in the absence of crossover interference. Hereditas 97:179–187

Fawcett DW (1956) The fine structure of chromosomes in the meiotic prophase of vertebrate spermatocytes. J Biophys Biochem Cytol 2:403–406

Foss E, Lande R, Stahl FW, Steinberg CM (1993) Chiasma interference as a function of genetic distance. Genetics 133:681–691

Froenicke L, Anderson LK, Wienberg J, Ashley T (2002) Male mouse recombination maps for each autosome identified by chromosome painting. Am J Hum Genet 71:1353–1368

Fung JC, Rockmill B, Odell M, Roeder GS (2004) Imposition of crossover interference through the nonrandom distribution of synapsis initiation complexes. Cell 116:795–802

Gerton JL, Hawley RS (2005) Homologous chromosome interactions in meiosis: diversity amidst conservation. Nat Rev Genet 6:477–487

Gong WJ, Mckim KS, Hawley RS (2005) All paired up with no place to go: pairing, synapsis and DSB formation in a balancer heterozygote. PLoS Genet 1:589–602

Gowen MS, Gowen JW (1922) Complete linkage in *Drosophila melanogaster*. Am Nat 56:286–288

Grelon M, Vezon D, Gendrot G, Pelletier G (2001) AtSPO11-1 is necessary for efficient meiotic recombination in plants. EMBO J 20:589–600

Guillon H, Baudat F, Grey C, Liskay RM, de Massy B (2005) Crossover and noncrossover pathways in mouse meiosis. Mol Cell 20:563–573

Havekes, FWJ (1999) Homologous chromosome pairing and recombination during meiosis in wild type and synaptic mutants of tomato. Thesis, Wageningen University, Wageningen

Henderson KA, Keeney S (2004) Tying synaptonemal complex initiation to the formation and programmed repair of DNA double-strand breaks. Proc Natl Acad Sci U S A 101:4519–4524

Henderson KA, Keeney S (2005) Synaptonemal complex formation: where does it start? Bioessays 27:995–998

Higgins JD, Armstrong SJ, Franklin FCH, Jones GH (2004) The *Arabidopsis* MutS homolog AtMSH4 functions at an early step in recombination: evidence for two classes of recombination in *Arabidopsis*. Genes Dev 18:2557–2570

Higgins JD, Sanchez-Moran E, Armstrong SJ, Jones GH, Franklin FC (2005) The *Arabidopsis* synaptonemal complex protein ZYP1 is required for chromosome synapsis and normal fidelity of crossing over. Genes Dev 19:2488–2500

Hillers KJ (2004) Crossover interference. Curr Biol 14: R1036–R1037

Hillers KJ, Villeneuve AM (2003) Chromosome-wide control of meiotic crossing over in *C. elegans*. Curr Biol 13:1641–1647

Hodges CA, Revenkova E, Jessberger R, Hassold T, Hunt P (2005) The SMC1b-deficient female mouse: evidence that cohesins are a missing link in age-related nondisjunction. Nat Genet 37:1351–1355

Hollingsworth NM, Brill SJ (2004) The Mus81 solution to resolution: generating meiotic crossovers without Holliday junctions. Genes Dev 18:117–125

Housworth EA, Stahl FW (2003) Crossover interference in humans. Am J Hum Genet 73:188–197

Hunter N (2003) Synaptonemal complexities and commonalities. Mol Cell 12:533–535

Hunter N, Borts RH (1997) Mlh1 is unique among mismatch repair proteins in its ability to promote crossing over during meiosis. Genes Dev 11:1573–1582

Hunter N, Kleckner N (2001) The single-end invasion. An asymmetric intermediate at the double-strand break to double-Holliday junction transition of meiotic recombination. Cell 106:59–70

Jang JK, Sherizen DE, Bhagat R, Manheim EA, McKim KS (2003) Relationship between DNA double-strand breaks to synapsis in *Drosophila*. J Cell Sci 116:3069–3077

Jazayeri A, Falck J, Lukas C, Bartek J, Smith GCM, Lukas J, Jackson SP (2006) ATM- and cell cycle-dependent regulation of ATR in response to DNA double-strand breaks. Nat Cell Biol 8:37–45

Jones GH (1987) Chiasmata. In: Moens PB (ed) Meiosis. Academic, Orlando, pp 213–244

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

Kleckner N (1996) Meiosis: How could it work? Proc Natl Acad Sci U S A 93:8167–8174

Kleckner N, Zickler D, Jones GH, Dekker J, Padmore R, Henle J, Hutchinson J (2004) A mechanical basis for chromosome function. Proc Natl Acad Sci U S A 101:12592–12597

Klein F, Mahr P, Galova M, Buonomo SB, Michaelis C, Nairz K, Nasmyth K (1999) A central role for cohesins in sister chromatid cohesion, formation of axial elements, and recombination during yeast meiosis. Cell 98:91–103

Koehler KE, Cherry JP, Lynn A, Hunt PA, Hassold TJ (2002) Genetic control of mammalian meiotic recombination. I. Variation in exchange frequencies among males from inbred mouse strains. Genetics 162:297–306

Kohli J, Bähler J (1994) Homologous recombination in fission yeast: absence of crossover interference and synaptonemal complex. Experientia 50:295–306

Lipkin SM, Moens PB, Wang V, Lenzi M, Shanmugarajah D, Gilgeous A, Thomas J, Cheng J, Touchman JW, Green ED, Schwartzberg P, Collins FS, Cohen PE (2002) Meiotic arrest and aneuploidy in MLH3-deficient mice. Nat Genet 31:385–390

Liu J-G, Yuan L, Brundell E, Björkroth B, Daneholt B, Höög 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

Loidl J, Scherthan H (2004) Organization and pairing of meiotic chromosomes in the ciliate *Tetrahymena thermophila*. J Cell Sci 117:5791–5801

Lorenz A, Wells JL, Pryce DW, Novatchkova M, Eisenhaber F, McFarlane RJ, Loidl J (2004) *S. pombe* meiotic linear elements contain proteins related to synaptonemal complex components. J Cell Sci 117:3343–3351

MacQueen AJ, Colaiácovo MP, McDonald K, Villeneuve AM (2002) Synapsis-dependent and -independent mechanisms stabilize homolog pairing during meiotic prophase in *C. elegans*. Genes Dev 16:2428–2442

Maguire MP (1980) Adaptive advantage for chiasma interference: a novel suggestion. Heredity 45:127–131

Maguire MP (1992) The evolution of meiosis. J Theor Biol 154:43–55

Mahadevaiah SK, Turner JMA, Baudat F, Rogakou EP, de Boer P, Blanco-Rodriguez J, Jasin M, Keeney S, Bonner WM, Burgoyne PS (2001) Recombinational DNA double-strand breaks in mice precede synapsis. Nat Genet 27:271–276

Manheim EA, McKim KS (2003) The synaptonemal complex component C(2)M regulates meiotic crossing over in *Drosophila*. Curr Biol 13:276–285

Maynard Smith J, Szathmáry E (1995) The major transitions in evolution. Freeman, Oxford

McKee B, Hong C, Das S (2000) On the roles of heterochromatin and euchromatin in meiosis in *Drosophila*: mapping chromosomal pairing sites and testing candidate mutations for effects on X–Y nondisjunction and meiotic drive in male meiosis. Genetica 109:77–93

McKim KS, Peters K, Rose AM (1993) Two types of sites required for meiotic chromosome pairing in *Caenorhabditis elegans*. Genetics 134:749–768

McKim KS, Green-Marroquin BL, Sekelsky JJ, Chin G, Steinberg C, Khodosh R, Hawley RS (1998) Meiotic synapsis in the absence of recombination. Science 279:876–878

Mercier R, Jolivet S, Vezon D, Huppe E, Chelysheva L, Giovanni M, Nogue F, Doutriaux MP, Horlow C, Grelon M, Mezard C (2005) Two meiotic crossover classes cohabit in *Arabidopsis*: one is dependent on MER3, whereas the other one is not. Curr Biol 15:692–701

Meuwissen RLJ, Offenberg HH, Dietrich AJJ, Riesewijk A, van Iersel M, Heyting C (1992) A coiled-coil related protein specific for synapsed regions of meiotic prophase chromosomes. EMBO J 11:5091–5100

Meuwissen RLJ, Meerts I, Hoovers JMN, Leschot NJ, Heyting C (1997) Human synaptonemal complex protein 1 (SCP1): isolation and characterization of the cDNA and chromosomal localization of the gene. Genomics 39:377–384

Moens PB, Tarsounas M, Morita T, Habu T, Rottinghaus ST, Freire R, Jackson SP, Barlow C, Wynshaw BA (1999) The association of ATR protein with mouse meiotic chromosome cores. Chromosoma 108:95–102

Moens PB, Kolas NK, Tarsounas M, Marcon E, Cohen PE, Spyropoulos B (2002) The time course and chromosomal localization of recombination-related proteins at meiosis in the mouse are compatible with models that can resolve the early DNA–DNA interactions without reciprocal recombination. J Cell Sci 115:1611–1622

Molnar M, Bähler J, Sipiczki M, Kohli J (1995) The rec8 gene of *Schizosaccharomyces pombe* is involved in linear element formation, chromosome pairing and sister-chromatid cohesion during meiosis. Genetics 141:61–73

Moses MJ (1956) Chromosome structures in crayfish spermatocytes. J Biophys Biochem Cytol 2:215–218

Muller HJ (1916) The mechanism of crossing-over. Am Nat 50:193–221

Munz P (1994) Lack of crossover and chromatid interference in *Schizosaccharomyces pombe*. Genetics 137:701–707

Nasmyth K (2001) Disseminating the genome: joining, resolving, and separating sister chromatids during mitosis and meiosis. Annu Rev Genet 35:673–745

Nasmyth K (2005) How might cohesin hold sister chromatids together? Philos Trans R Soc Lond B Biol Sci 360:483–496

Novak JE, Ross-Macdonald PB, Roeder GS (2001) The budding yeast Msh4 protein functions in chromosome synapsis and the regulation of crossover distribution. Genetics 158:1013–1025

Öllinger R, Alsheimer M, Benavente R (2005) Mammalian protein SCP1 forms synaptonemal complex-like structures in the absence of meiotic chromosomes. Mol Biol Cell 16:212–217

Page SL, Hawley RS (2001) c(3)G encodes a *Drosophila* synaptonemal complex protein. Genes Dev 15:3130–3143

Page SL, Hawley RS (2004) The genetics and molecular biology of the synaptonemal complex. Annu Rev Cell Dev Biol 20:525–558

Pelttari J, Hoka M-R, Yuan L, Liu J-G, Brundell E, Moens P, Santucci-Darmanin S, Jessberger R, Barbero JL, Heyting C, Höög C (2001) Meiotic chromosomal core consisting of cohesin complex proteins recruits DNA recombination proteins and promotes synapsis in the absence of an axial element in mammalian meiotic cells. Mol Cell Biol 22:5667–5677

Perera D, Perez-Hidalgo L, Moens PB, Reini K, Lakin N, Syväoja JE, San-Segundo PA, Freire R (2004) TopBP1 and ATR colocalization at meiotic chromosomes: role of TopBP1/Cut5 in the meiotic recombination checkpoint. Mol Biol Cell 15:1568–1579

Pochart P, Woltering D, Hollingsworth NM (1997) Conserved properties between functionally distinct MutS homologs in yeast. J Biol Chem 272:30345–30349

Richard GF, Kerrest A, Lafontaine I, Dujon B (2005) Comparative genomics of hemiascomycete yeasts: genes involved in DNA replication, repair and recombination. Mol Biol Evol 22:1011–1023

Rockmill B, Fung JC, Branda SS, Roeder GS (2003) The Sgs1 helicase regulates chromosome synapsis and meiotic crossing over. Curr Biol 13:1954–1962

Roeder GS (1997) Meiotic chromosomes: it takes two to tango. Genes Dev 11:2600–2621

Romanienko PJ, Camerini-Otero RD (2000) The mouse SPO11 gene is required for meiotic chromosome synapsis. Mol Cell 6:975–987

Ross-Macdonald P, Roeder GS (1994) Mutation of a meiosis-specific MutS homolog decreases crossing over but not mismatch correction. Cell 79:1069–1080

Sage J, Martin L, Meuwissen R, Heyting C, Cuzin F, Rassoulzadegan M (1999) Temporal and spatial control of the Sycp1 gene transcription in the mouse meiosis: regulatory elements active in the male are not sufficient for expression in the female gonad. Mech Dev 80:29–39

Santucci-Darmanin S, Walpita D, Lespinasse F, Desnuelle C, Ashley T, Paquis-Flucklinger V (2000) MSH4 acts in conjunction with MLH1 during mammalian meiosis. FASEB J 14:1539–1547

Santucci-Darmanin S, Neyton S, Lespinasse F, Saunieres A, Gaudray P, Paquis-Flucklinger V (2002) The DNA mismatch-repair MLH3 protein interacts with MSH4 in meiotic cells, supporting a role for this MutL homolog in mammalian meiotic recombination. Hum Mol Genet 11:1697–1706

Schleiffer A, Kaitna S, Maurer SS, Glotzer M, Nasmyth K, Eisenhaber F (2003) Kleisins: a superfamily of bacterial and eukaryotic SMC protein partners. Mol Cell 11:571–575

Schmekel K, Meuwissen RLJ, Dietrich AJJ, Vink ACG, van Marle J, van Veen H, Heyting C (1996) Organization of SCP1 protein molecules within synaptonemal complexes of the rat. Exp Cell Res 226:20–30

Schwacha A, Kleckner N (1995) Identification of double Holliday junctions as intermediates in meiotic recombination. Cell 83:783–791

Schwacha A, Kleckner N (1997) Interhomolog bias during meiotic recombination: meiotic functions promote a highly differentiated interhomolog only pathway. Cell 90:1123–1135

Sherizen D, Jang JK, Bhagat R, Kato N, McKim KS (2005) Meiotic recombination in *Drosophila* females depends on chromosome continuity between genetically defined boundaries. Genetics 169:767–781

Sherman JD, Stack SM (1995) Two-dimensional spreads of synaptonemal complexes from solanaceous plants. IV. High-resolution recombination nodule map for tomato (*Lycopersicon esculentum*). Genetics 141:683–708

Snowden T, Acharya S, Butz C, Berardini M, Fishel R (2004) hMSH4–hMSH5 recognizes Holliday junctions and forms a meiosis-specific sliding clamp that embraces homologous chromosomes. Mol Cell 15:437–451

Stahl FW, Foss HM, Young LS, Borts RH, Abdullah MFF, Copenhaver GP (2004) Does crossover interference count in *Saccharomyces cerevisiae*? Genetics 168:35–48

Storlazzi A, Xu L, Schwacha A, Kleckner N (1996) Synaptonemal complex (SC) component Zip1 plays a role in meiotic recombination independent of SC polymerization along the chromosomes. Proc Natl Acad Sci U S A 93:9043–9048

Strickland WN (1958) An analysis of interference in *Aspergillus nidulans*. Proc Roy Soc B 149:82–101

Sun H, Treco D, Szostak JW (1989) Initiation of meiotic recombination by double-strand DNA-scission. Nature 250:150–153

Suzuki M (1989) SPXX, a frequent sequence motif in gene regulatory proteins. J Mol Biol 207:61–84

Sybenga J (1975) Meiotic configurations. Springer, Berlin Heidelberg New York

Sybenga J (1996) Recombination and chiasmata: few but intriguing discrepancies. Genome 39:473–484

Sym M, Engebrecht JA, Roeder GS (1993) Zip1 is a synaptonemal complex protein required for meiotic chromosome synapsis. Cell 72:365–378

Sym M, Roeder GS (1994) Crossover interference is abolished in the absence of a synaptonemal complex protein. Cell 79:283–292

Sym M, Roeder GS (1994b) Zip1-induced changes in synaptonemal complex structure and polycomplex assembly. J Cell Biol 128:455–466

Szostak JW, Orr-Weaver TL, Rothstein RJ, Stahl FW (1983) The double-strand-break repair model for recombination. Cell 33:25–35

Thompson LH, Schild D (2002) Recombinational DNA repair and human disease. Mutat Res 509:49–78

Tsubouchi T, Roeder GS (2005) A synaptonemal complex protein promotes homology-independent centromere coupling. Science 308:870–873

Tung KS, Roeder GS (1998) Meiotic chromosome morphology and behavior in zip1 mutants of *Saccharomyces cerevisiae*. Genetics 149:817–832

Turner JMA, Aprelikova O, Xu X, Wang R, Kim S, Chandramouli GVR, Barrett JC, Burgoyne PS, Deng CX (2004) BRCA1, histone H2AX phosphorylation, and male meiotic sex chromosome inactivation. Curr Biol 14:2135–2142

van Heemst D, Heyting C (2000) Sister chromatid cohesion and recombination in meiosis. Chromosoma 109:10–26

van Veen JE, Hawley RS (2003) Meiosis: when even two is a crowd. Curr Biol 13:R831–R833

Wang TF, Kleckner N, Hunter N (1999) Functional specificity of MutL homologs in yeast: evidence for three Mlh1-based heterocomplexes with distinct roles during meiosis in recombination and mismatch correction. Proc Natl Acad Sci U S A 96:13914–13919

Whitby MC (2005) Making crossovers during meiosis. Biochem Soc Trans 33:1451–1455

Woods LM, Hodges CA, Baart E, Baker SM, Liskay M, Hunt PA (1999) Chromosomal influence on meiotic spindle assembly: abnormal meiosis I in female Mlh1 mutant mice. J Cell Biol 145:1395–1406

Wu L, Hickson ID (2003) The Bloom's syndrome helicase suppresses crossing over during homologous recombination. Nature 426:870–874

Yamamoto A, Hiraoka Y (2001) How do meiotic chromosomes meet their homologous partners?: lessons from fission yeast. Bioessays 23:526–533

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