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REVIEW

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From early homologue recognition to synaptonemal complex formation

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Abstract This review focuses on various aspects of chromosome homology searching and their relationship to meiotic and vegetative pairing and to the silencing of unpaired copies of genes. Chromosome recognition and pairing is a prominent characteristic of meiosis; however, for some organisms, this association (complete or partial) is also a normal part of nuclear organization. The multiple mechanisms suggested to contribute to homologous pairing are analyzed. Recognition of DNA/DNA homology also plays an important role in detecting DNA segments that are present in inappropriate number of copies before and during meiosis. In this context, the mechanisms of methylation induced premeiotically, repeat-induced point mutation, meiotic silencing by unpaired DNA, and meiotic sex chromosome inactivation will be discussed. Homologue juxtaposition during meiotic prophase can be divided into three mechanistically distinct steps, namely, recognition, presynaptic alignment, and synapsis by the synaptonemal complex (SC). In most organisms, these three steps are distinguished by their dependence on DNA double-strand breaks (DSBs). The coupling of SC initiation to (and downstream effects of) DSB formation and the exceptions to this dependency are discussed. Finally, this review addresses the specific factors that appear to promote chromosome movement at various stages of meiotic prophase, most particularly at the bouquet stage, and on their significance for homologue pairing and/or achieving a final pachytene configuration.

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The synaptonemal complex - 50 years

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Introduction

As stated by Barbara McClintock in 1933, “there is a tendency for chromosomes to associate 2-by-2 in the prophase of meiosis”. Two-by-two association of homologues involves a long-distance recognition of “self” from “non-self” followed by a gradual alignment of homologous axes. Association culminates in the formation of a prominent structure called the synaptonemal complex (SC) that finally mediates the intimate connection of homologue axes along their lengths at pachytene.

Another fascinating aspect of meiosis is that two homologous DNA regions, which are meant to recombine, must locate one another in a vast surrounding of non-homologous sequences. Even more striking is the fact that recombination can discriminate between allelic and ectopic homology (e.g., Goldman and Lichten 1996). For a long time, it was thought that one solution to the problem would be to restrict recombination to regions that are already brought together by the SC, but it is now clear that this is not the solution. Instead, in several organisms, DNA double-strand breaks (DSBs) and recombination are major determinants of close and stable juxtaposition of homologues, before and prerequisite to SC formation (e.g., Tesse et al. 2003; Henderson and Keeney 2004 and references therein). In some organisms, SC can form in the absence of recombination [e.g., in *spo11* mutants of *Drosophila melanogaster* and *Caenorhabditis elegans* where DSBs do not occur (Dernburg et al. 1998; McKim et al. 1998) and apparently also in *Bombyx mori* females where crossing-over is absent (Rasmussen 1977)]. While it is not yet known whether, in wild-type meiosis, recombination still plays a significant role, multiple mechanisms involving *cis*-acting elements have been suggested to contribute to homologous recognition and synapsis in these cases (see review in McKee 2004; below). However, in all organisms where SC occurs, including *Drosophila* female and *C. elegans*, SC plays a functional role in the recombination process as suggested (1) by the close physical localization of the biochemical complexes of recombination to the SC and (2) by the fact that mutations in the structural components

of the SC cause defects in crossing-over maturation (Page and Hawley 2001; Hunter and Kleckner 2001; MacQueen and Villeneuve 2001; Jang et al. 2003; Colaiacovo et al. 2003; Couteau et al. 2004; Börner et al. 2004; de Vries et al. 2005; Higgins et al. 2005).

Two further peculiarities relating to homologue recognition and juxtaposition are worthy of note. One is "nonhomologous synapsis": although synapsis is mostly confined to homologous parts of chromosomes, SC can form also between non-homologous chromosomes or chromosomal regions at late pachytene (see review in Zickler and Kleckner 1999). The other is the fact that, in some organisms, homologous pairing can occur throughout the life cycle ("somatic pairing") as in Dipterans (e.g., Fung et al. 1998). The relationship between somatic and meiotic pairing remains another unresolved question. However, it is often supposed that, mechanistically, somatic pairing occurs by the same mechanism as the earliest stages of meiotic pairing (e.g., Brown and Stack 1968; see discussions in Kleckner and Weiner 1993; Vazquez et al. 2002; McKee 2004).

This review will concentrate on recent developments that have revealed some of the intricacies of the diverse processes involved in recognition and juxtaposition of homologous chromosomes during meiosis. The term "pairing" will be used to describe all aspects of homologous associations before the initiation of the SC. The term "synapsis" will only be used to describe the connection of homologues via the SC. One important distinction is the differentiation between features involved in the establishment of pairing and/or SC formation and features involved in their maintenance. The inter-homologue interaction process during meiosis also involves a progression of events, many of which likely interact functionally.

Studies combining fluorescence in situ hybridization (FISH), green fluorescent protein (GFP)-tagging of loci, genetic and molecular analysis of mutants, and physical recombination assays in synchronous budding yeast cells have yielded a great deal of new information about how pairing and SC formation relate to the recombination process, and the field is progressing at a rapid speed. However, studying pairing remains difficult because this highly dynamic process occurs only between some regions of a chromosome in a nonsynchronized way from one nucleus to the other. Moreover, when co-localizations are seen they can in fact be remnants of contacts that occurred earlier; vice versa, absence of visible contacts may not mean that none happened. We are also beginning to gain some understanding of the dramatic spatial nuclear reorganization and chromosome movements needed to pair homologues, the importance of which was for a long time underestimated. Nonetheless, despite major advances in our understanding of pairing and synapsis since the discovery of the SC by Moses and by Fawcett in 1956, we still remain rather ignorant of the mechanisms that regulate both homologous chromosome recognition and SC function.

When homologous pairing and/or synapsis is DSB independent

DSB-independent pairing/synapsis during meiosis

During meiosis, recombination initiates by programmed DSBs generated by the evolutionarily conserved Spo11 protein at sites determined by both higher-order chromosome structure and local chromatin changes (reviewed in Keeney 2001). Important steps in meiotic chromosome pairing are specifically dependent upon DSBs in most organisms (below). However, DSB-independent mechanisms for aligning meiotic chromosomes are widespread in some taxonomic groups such as Dipterans and Lepidopterans as well as in *C. elegans* (below). Two categories must be distinguished. *C. elegans* normally exhibits recombination and uses chiasmata for segregation of homologues. In this case, a mutant lacking DSBs can still undergo homologue pairing and SC formation, implying the existence of a DSB-independent pairing mechanism, but ensuing homologue segregation is aberrant, implying a requirement for recombination-mediated chiasma formation in wild-type meiosis (Dernburg et al. 1998). The analogous situation is also observed in *Drosophila* female (McKim et al. 1998).

In contrast, in organisms like *B. mori* and *D. melanogaster*, one sex (respectively, female and male) is completely achiasmate. In *B. mori* female, homologues form SCs in the absence of crossovers and, interestingly, this SC provides a substitute for chiasmata by persisting in a modified form until the onset of anaphase (Rasmussen 1977). In *Drosophila* male, homologues pair, remain connected, and then segregate independent of both recombination and SCs. Even in this latter case, however, the frequency with which the homologous loci are paired is always higher during meiosis than during mitosis, indicating that meiotic homologous pairing is not just a continuation of the prominent somatic pairing (Vazquez et al. 2002). An analysis of living spermatocytes suggests that pairing occurs in two steps. When cells enter meiosis, homologues are already efficiently aligned through premeiotic pairing (euchromatic regions are paired at G1 and their pairing persists through S-phase into G2). Homologous chromosomes are then sequestered into physically distinct territories within the nucleus, each of which is associated with the nuclear envelope (NE): this likely maintains their physical proximity (Vazquez et al. 2002). Similar chromosome sorting and maintenance of homologue association through sequestration into distinct territories is also seen for the *C. elegans* X chromosomes in a mutant background that abolishes SC formation (Martinez-Perez and Villeneuve 2005). At least three genes are required for the maintenance of meiotic pairing in *Drosophila* male. Mutations in *teflon* cause dissociation of the autosomes but not of the X–Y pair, whereas *mnn* (coding a BTB-domain protein) and the stromalin-*SCC3* homologue *snm* are required for proper segregation of all pairs (Tomkiel et al. 2001; Thomas et al. 2005).

Are particular sites involved in recombination-independent pairing? The best information in this regard comes from an analysis of *Drosophila* male meiosis. In this case, the X and Y chromosomes have a clear, molecularly identified, *cis*-acting pairing site: a 240-bp repeated sequence in the intergenic spacer of the rDNA repeats operates as a pairing center for this pair during meiosis. Similar heterochromatic pairing sites were not found on the autosomes 2 and 3, which pair by the interactions of a large number of euchromatic sites (see review in McKee et al. 1992, 1993; McKee 2004). In other cases, however, pairing occurs preferentially in heterochromatic regions, e.g., in female *Drosophila* meiosis when recombination is absent (Hawley et al. 1992; Dernburg et al. 1996). The mechanical basis for homologue pairing and segregation remains, however, largely unknown.

Premeiotic pairing

Vegetative/somatic pairing was often thought to be a regular preliminary to meiotic pairing, especially in plants (Brown and Stack 1968). However, the existence of vegetative and/or premeiotic pairing in organisms other than Dipterans is still controversial. The only spatial organization of chromosomes that is widely conserved in mitotically dividing cells is the bipolar Rabl orientation with centromeres clustered on top of the nucleus and telomeres pointing inwards. Such organization is seen in nuclei of fission and budding yeasts (e.g., Chikashige et al. 1997; Jin et al. 1998; Fuchs et al. 2002; Bystricky et al. 2005), higher plants (e.g., Martinez-Perez et al. 2000; Mikhailova et al. 2001; see review in Shaw et al. 2002), *Drosophila* (Marshall et al. 1996), and mammal cells (e.g., Cremer and Cremer 2001). There is also a domain organization with chromosomes tending to occupy discrete separate territories (e.g., Tanabe et al. 2002).

Premeiotic associations of homologues, defined as associations that precede the onset of meiotic S-phase and usually studied by FISH or GISH analyses, are less clearly universal. Higher-plants reports show differences not only from one species to another but also in the same organism from one study to the other. No premeiotic pairing is seen in maize (Bass et al. 1997) and *Arabidopsis* (Pecinka et al. 2004), but premeiotic chromosome associations are observed in two rice species (Prieto et al. 2004). Some studies have failed to detect any homologous associations in cereals (Heslop-Harrison et al. 1988), while others indicate that homologues are closer than nonhomologous chromosomes especially in centromeric regions (Schwarzacher 1997; Aragon-Alcaide et al. 1997; Mikhailova et al. 1998; Martinez-Perez et al. 1999). Similar preferential homologous pericentric associations are also observed in *Arabidopsis* mitotic cells (e.g., Fransz et al. 2002; Lysak et al. 2003; Kato and Lam 2003). In contrast, Pecinka et al. (2004) found that the only chromosomes that associate more often than randomly in *Arabidopsis* are the two chromosomes bearing the nucleolar-organizing region. Even when present, there is no proof yet that the mitotic/premeiotic pairing contributes to meiotic recognition and pairing.

Unstable and transient homologous associations are detected in vegetative cycling and premeiotic G1 diploid budding yeast cells when analyzed by FISH, Cre/*loxP* recombination reporter system, and crosslinking plus PCR analyses (Weiner and Kleckner 1994; Loidl et al. 1994; Burgess et al. 1999; Burgess and Kleckner 1999; Dekker et al. 2002). In premeiotic cells, pairing involves multiple interstitial interactions along chromosomes, but their molecular nature remains unknown. Other groups found that mitotic associations are exclusively due to spatial constraints linked to the centromere clustering in the Rabl orientation (Jin et al. 1998; Lorenz et al. 2003). When chromosomal loci with transgenic repeats are analyzed, they are always interestingly more often associated with each other than normal loci, whatever their location along the chromosomes in both budding yeast and *Arabidopsis* (Fuchs et al. 2002; Pecinka et al. 2005). In budding yeast, such associations are strong enough to perturb the normal Rabl arrangement in interphase nuclei by, e.g., displacing telomere regions to the centromeric pole (Fuchs et al. 2002). The mechanisms involved may resemble the mechanisms that promote the clustering of heterochromatic chromosomal regions (see review in McKee 2004).

In all cases, premeiotic contacts are lost during DNA replication. They reappear at the onset of leptotene, independently of SC formation, which plays later roles in homologue juxtaposition (Weiner and Kleckner 1994; Burgess et al. 1999; Burgess and Kleckner 1999; Cha et al. 2000; Peoples et al. 2002; Dekker et al. 2002). Premeiotic pairing is also independent of RecA homologues, implying that identification of homology at the chromosomal level is determined by processes other than those involved in searching for homology at the DNA level during recombinational repair (Weiner and Kleckner 1994; Cha et al. 2000). Some mutants defective for DSB formation are also defective for pairing (Weiner and Kleckner 1994; Loidl et al. 1994; Peoples et al. 2002; Neale et al. 2002), most likely because of effects on the DSB-dependent mechanism (below). In fission yeast, homologues normally arrive in a common nucleus just before premeiotic S-phase. However, stable diploids can be created and can be induced to undergo mitotic divisions and undergo meiosis by manipulating genetic background and media. In such diploids, homologues are in joined domains and centromeric probes are paired in 60% of the cells (Scherthan et al. 1994).

Drosophila somatic pairing interestingly shares a number of features with premeiotic pairing as defined in other organisms, including multiple interstitial interactions (Fung et al. 1998; Gemkow et al. 1998) and disruption of pairing during S-phase of the mitotic cell cycle (Csink and Henikoff 1998), in accord with the possibility of a direct mechanistic relationship (above).

What mechanism governs vegetative or premeiotic pairing? Hypothesis abound: (1) Studies by Keeney and Kleckner (1996) suggest that homology is sensed directly

at the DNA level, guided by direct physical interactions between DNA duplexes in accessible regions such as nuclease-hypersensitive or/and nucleosome-free regions. However, protein–protein interactions cannot be excluded. (2) In view of the strong pairing abilities seen between the highly transcribed histone and rDNA loci during *Drosophila* male meiosis, McKee (1996) suggested that transcriptional activity might correlate with pairing ability. Involvement of allelic transcription units is also suggested for pairing in polyploids (Wilson et al. 2005). (3) High-resolution cytology on GFP-tagged loci of living *Drosophila* cells showed that homologous interactions correspond to random, diffuse walk motion. Moreover, each chromatin segment is constrained to move only in a limited area within 1% of the nuclear volume (Marshall et al. 1997). This constraint being eliminated by nocodazole, the authors attributed the constraint to cytoskeleton, although recent studies suggest that microtubule-inhibiting drugs can have diverse unexpected consequences that may not involve the cytoskeleton (Hochwagen et al. 2005). (4) Kleckner et al. (2004), considering each aspect of the chromosome (chromatin fiber and axis) as a physical system, suggested that chromatin expansion could put stress on higher-order organizational features that constrain the chromosomes within the nucleus. For example, expansion might increase the separateness of different chromosome masses while, oppositely, contraction might allow more stable associations. Furthermore, variations in expansion and contraction status might cause different chromatin regions to "push one another around", thereby contributing "stirring forces" (Maguire 1984) that might help to bring homologous regions together. In conclusion, although it is becoming increasingly apparent that the spatial organization of chromosomes in the nucleus is often nonrandom and that, at least, in some organisms interactions between homologues occur in vegetative and premeiotic cells, the processes leading to those interactions remain to be discovered.

Premeiotic DNA–DNA homology sensing

The existence of communication between homologous DNA sequences outside of the recombination process is documented in several organisms (e.g., see review in Henikoff 1997). The presence of such *trans* effects is revealed when the status of one chromosome or chromosomal region varies when the nature of its homologue varies. This is best illustrated in the fungi *Neurospora crassa*, *Ascobolus immersus*, *Podospora anserina*, *Magnaporthe grisea*, and *Coprinus cinereus*, where duplication of a sequence greater than 400 bp triggers its inactivation during the sexual life cycle, regardless of the origin of the sequence (i.e., endo- or exogenous). Only the rDNA repeats located in the nucleolus-organizer region evade inactivation (see review in Rossignol and Faugeron 1994; Galagan and Selker 2004; Bouhouche et al. 2004 and references therein). After fertilization, when the two haploid nuclei of opposite mating type are in the dikaryotic cells preceding karyogamy, the duplicated DNA sequences present in one of the two parental nuclei are specifically recognized and methylated at the cytosine residues. An analysis of the four meiotic products of individual meioses indicates that the two products issued from premeiotic replication display identical pattern of changes, a clear indication that methylation occurs after fertilization but before karyogamy/S-phase. Both duplicated sequences are silenced in a reversible way by affecting transcription in *Ascobolus* (MIP, methylation induced premeiotically; see review in Rossignol and Faugeron 1994). In contrast, both sequences are irreversibly mutated by transitions from C-G to T-A in *Neurospora* and the number of transition mutations is usually large enough to disrupt their original coding potential (RIP, repeat-induced point mutation; see review in Galagan and Selker 2004). Moreover, as mutations can extend beyond the duplicated regions, RIP can have epigenetic effects on adjacent sequences. Consequently, the *N. crassa* genome shows almost no duplicated genes or active transposons. Therefore, RIP and MIP mechanisms are remarkably efficient in defending those fungi against foreign DNA.

How do RIP and MIP occur? They may both involve a direct homologous DNA–DNA interaction. First, inactivation requires the presence of two or more copies of a gene in the same haploid nucleus. Second, there is co-extension between the length of homology and the length of the sequence affected by methylation or point mutation. When an *Ascobolus* strain contains three unlinked copies of the integrated gene, all three copies are also inactivated and never only one. This suggests that inactivation occurs through a pairing process in which one copy can undergo more than one cycle of pairing–inactivation and that a MIPed copy of the gene can pair with the unmodified copy and inactivate it (Faugeron et al. 1990). One component of the molecular machinery for RIP and MIP is a DNA methyltransferase-like enzyme, but the factors that control the targeting and activity of these enzymes remain unknown (Malagnac et al. 1997; Freitag et al. 2002). The high efficiency of MIP and RIP implies that the premeiotic cells have the capacity to search the entire genome for homology. This can be compared to the capacity of meiotic budding yeast cells to recombine allelic and ectopic artificial inserts (e.g., Goldman and Lichten 1996). Moreover, as shown in *Ascobolus*, a methylated allele can be transferred at meiosis to an active allele at the same frequencies as the wild-type conversion events of the gene, and this transfer is polarized from 5′ to 3′ as is gene conversion (Colot et al. 1996). Thus, methylation transfer can occur between homologous nonsister chromatids during meiosis without accompanying exchange, which may be suggestive of DNA–DNA recognition without a previous break.

Trans effects have also been described in higher plants via their effects on gene expression, but with apparently several types of mechanisms (see review in Jorgensen 1990; Bender 2004). Multiple-copy integrations are needed for transgene inactivation in tobacco, *Petunia*, and *Arabidopsis* (see review in Martienssen and Colot 2001). *Trans*-silencing can be an asymmetrical process: one transgenic locus, the silenced locus, is methylated by the presence of the other, the silencing locus, which continues to be expressed (see review

in Matzke and Matzke 1998). In contrast, co-suppression involves silencing of both the transgene and the resident gene(s), illustrated by the loss of *Petunia* flower color when both are silenced. Expression is restored as soon as the transgene and the resident genes are segregated in a cross (see review in Jorgensen 1990). It was proposed that recognition of DNA repeats could be mediated by either of two types of interactions: direct DNA–DNA interactions depending on homology between the repeats or indirect recognition process involving, for example, DNA-binding proteins. Genes could also be sequestered in the same nuclear compartment, related to the transvection and dominant position-effect variegation situation (Pirrotta 1999; Henikoff 1997). However, most of the evidence now points to RNA playing a central role in directing DNA methylation to specific regions of the genome in plants. In this mechanism, an aberrant RNA trigger species is proposed to pair with homologous DNA sequences in the genome and provide an attractive substrate for cytosine methyltransferases. The mechanism by which the RNA trigger seeks out and aligns with matching DNA sequences remains unknown (see review in Bender 2004).

Meiotic silencing of unpaired DNA

An extra copy of a gene not found on its partner chromosome during meiotic prophase of *N. crassa* generates a sequence-specific signal that scans the genome (Shiu et al. 2001; Shiu and Metzenberg 2002). When an asymmetrical situation is detected, such as a deletion or an insertion in one of the homologous chromosomes, the presence of this extra sequence of DNA activates a process called meiotic silencing by unpaired DNA (MSUD). This extra sequence results in silencing not only of itself but of any other copy (ies) of the same sequence present elsewhere in the genome regardless of whether they do or do not have a homologous sequence present at the allelic position (and thus presumptively are paired) or not. It is not known whether silencing is transcriptional or post-transcriptional, and nothing is known about the time frame in which it operates. Self-silencing must occur after homologues have a chance to detect their "pairing status", e.g., during meiotic prophase, and must have ended not later than the time of ascospore formation because the affected genes are then re-expressed in vegetative life. Also, self-silencing must occur after karyogamy and, thus, at a different time than RIP, which occurs in haploid premeiotic nuclei before their fusion (above).

The fact that a single extra copy of a gene can silence expression from all copies in the genome suggests a similarity between MSUD and other RNA-based silencing systems such as quelling and co-suppression. In addition, the fact that *sad-1* coding for a RNA-dependent RNA polymerase is required for MSUD suggests that the synthesis of dsRNA, its amplification, or both are essential for the process (Shiu et al. 2001; Shiu and Metzenberg 2002). Production of the silencing signal (e.g., small interfering RNAs) does not affect the expression of adjacent genes (Kutil et al. 2003). MSUD involves at least three more genes: *sms-2* coding for an Argonaute-like protein, *sms-3* coding for a Dicer-like protein, and *sad-2* with unknown function (Lee et al. 2003; Kutil et al. 2003; Shiu et al. 2006).

MSUD exerts surveillance over proliferated invasive elements that are not established in homologous positions of both parents when meiosis starts. MSUD is interestingly important also from an evolutionary point of view. Shiu et al. (2001) showed that *sad-1* mutants suppress the interbreeding inability between *N. crassa* and three related species: *Neurospora sitophila*, *Neurospora tetrasperma*, and *Neurospora intermedia*. This increase in fertility suggests that an important barrier between two closely related species is in fact the existence of numerous small rearrangements in the genome.

Silencing of unsynapsed chromosome regions also takes place in male and female mouse during meiosis (MSCI, meiotic sex chromosome inactivation) and the tumor suppressor protein BRCA1 is implicated in this silencing (Turner et al. 2005). It is not yet clear whether this process is or is not related to MSUD in *Neurospora* (Schimenti 2005).

When homologous pairing and/or synapsis is DSB dependent

In contrast to *C. elegans* and *Drosophila* female (above), the catalytic function of the Spo11 DSB transesterase protein is essential for full pairing and SC formation in three fungi (budding yeast, *Coprinus*, and *Sordaria*), in the higher plant *Arabidopsis thaliana*, and in mouse (Keeney et al. 1997; Celerin et al. 2000; Baudat et al. 2000; Romanienko and Camerini-Otero 2000; Grelon et al. 2001; Storlazzi et al. 2003). SC formation in these organisms is also dependent upon the other proteins known to be required for DSB formation (Keeney 2001). Although no SCs are formed in fission yeast, *SPO11/REC12* is nevertheless required for wild-type level of pairing in that organism (Nabeshima et al. 2001; Molnar et al. 2003).

The timing of events of DNA recombination to pairing and synapsis has so far been directly defined only in primarily synchronized cultures of budding yeast and, to some extent, in mouse. Yeast studies (Padmore et al. 1991; Schwacha and Kleckner 1995; Hunter and Kleckner 2001 and references therein) reveal the following progression. DSBs appear at leptotene, thus, long before the SC. At zygotene, one end of a resected DSB undergoes strand-exchange with a partner DNA duplex giving a single-end invasion (SEI). SEI and SC formation are contemporaneous processes. The fully ligated strand-exchange intermediates in the form of double-Holliday junctions (dHJs) form around mid-pachytene after full-length SC formation. Mature crossover products are observed in middle–late pachytene in both budding yeast (references above) and mouse (Guillon et al. 2005).

Börner et al. (2004) showed that, in budding yeast, the noncrossover/crossover decision is made at a very early step of recombination, independent of and, most likely, before synapsis. The same appears to be true in *Arabidopsis*

(Higgins et al. 2004). After the decision is made, actual formation of crossovers depends on the ZMM class of proteins Zip1, Zip2, Zip3, Mer3, and Msh5 (de los Santos et al. 2003; Börner et al. 2004). Most crossovers (approximately 85% in budding yeast) are formed through this pathway and are dependent on Msh4/Msh5p. There are also secondary ways of generating crossovers that do not involve SEI and dHJ intermediates, as seen also in *Arabidopsis* (de los Santos et al. 2003; Higgins et al. 2004). The relationship of these events to SC formation remains unclear. One proposal is that these crossovers may occur as minority products along a pathway that produces primarily noncrossovers (Börner et al. 2004). Another proposal involves a third DSB-dependent process (Whitby 2005).

Homologous pairing occurs in three steps distinguishable by their differential dependence on DSBs

Observations in a number of organisms suggest that, at least in fungi, animals and plants, homologue pairing involves three sequential steps: an early recognition process that is independent of recombination, DSB-mediated juxtaposition of homologue axes to a distance of ~400 nm via local linkages, and, finally, formation of SC via nucleation and spreading from a subset of DSB-mediated linkages, namely, those that are destined to eventually mature into crossovers/chiasmata.

A number of unique insights into this progression and, most particularly, the earlier stages are provided by mycelial fungi like *Sordaria*, *Neurospora*, and *Coprinus*. First, the two sets of homologous chromosomes are in separated nuclei before entering meiosis, with their first contacts occurring only after karyogamy (e.g., Lu and Raju 1970; Zickler 1977; Li et al. 1999). Therefore, homologue juxtaposition cannot involve simple retention of pre-meiotic pairing. Second, the progression of pairing can be conveniently described with respect to two landmarks: karyogamy and SC formation. Moreover, ascus growth and nuclear volume increase during prophase giving easy landmarks for staging (Fig. 1). Third, SC axial element formation is complete at early leptotene. This allows an accurate electron microscopy (EM) three-dimensional (3D) analysis of chromosomes at stages where such observations are impossible in most organisms (e.g., Holm et al. 1981; Rasmussen et al. 1981; Zickler 1977; Bojko 1988; 1989; Zickler et al. 1992). As chromosome identities can be assigned by length difference and centromere index in *Sordaria*, spatial relationships among different chromosomes can be followed through all pairing steps in wild type and mutants (Figs. 1, 2, 3, 4, 5, 6, and 7). Finally, as in higher plants, meiosis usually proceeds to its end even when severe recombination or SC defects are present, rather than arresting as in budding yeast and mammals.

In early wild-type leptotene nuclei, *Sordaria* homologues are far apart with only rare contacts (Figs. 1a, 2a–f, and 3a–f). By mid-leptotene, homologues have moved into rough, long-range alignment, with a tendency for telomeres to align first, but interstitial alignment is also found (Figs. 1b and 4a–f). There is no individual chromosome rule for alignment from one nucleus to another (e.g., compare homologues 1 in Figs. 4 and 7). However, a rotation of the nuclei reveals that a given pair of chromosomes tends to occupy a fairly well-defined territory, e.g., the longer pair 1 (pink and red) in Figs. 4c–e and 6b,d and pair 3 (green) in Figs. 5 and 6. This territory is maintained during bouquet formation [e.g., pair 1 (small arrow) is always located above the smaller chromosomes as illustrated in Fig. 1e,f]. At the end of leptotene, all pairs are co-aligned at a distance of ~400 nm (Fig. 1c). Similar complete alignment before SC formation is also observed in *Neurospora*, *Coprinus*, and higher plants (Pukkila and Lu 1985; Bojko 1989; Lu 1993; reviewed in Loidl 1990), but not in budding yeast and mammals where segmental alignment and SC initiation coincide (see review in Zickler and Kleckner 1999). SC initiates often at telomere regions but also at several interstitial sites along all pairs, and there is no specific site at which SC always initiates (see examples in Zickler et al. 1992). From early leptotene onward, chromosome ends are attached to the NE. They remain dispersed throughout the nuclear periphery through the time of presynaptic alignment (Figs. 1b,c, 2, 3, and 4). Rad51 foci appear on axes at mid-leptotene before alignment and either on or, often, between axes in aligned pairs. They are no longer visible by the beginning of pachytene, in accord with the timing observed in other organisms (Bishop 1994; Tarsounas et al. 1999; Franklin et al. 1999; Moens et al. 2002).

Phenotypes of *Sordaria spo11* and *ski8* mutants with varying endogenous and exogenous DSB frequencies, monitored by EM and immunofluorescence, provide coherent evidence that progressive juxtaposition of homologues is established by three mechanistically distinct processes that occur in overlapping succession. First, in the absence of *SPO11* or its catalytic activity or of *SKI8*, axial elements are built along all chromosomes, but homologues show only rare signs of recognition, and do not form SCs (Figs. 5 and 6; Storlazzi et al. 2003; Tesse et al. 2003). Second, in *spo11* or *ski8* deletion null mutants after low levels of irradiation, segments of homologue pairs are placed into joint spaces in the nucleus, indicating that DSBs endogenously induced by Spo11 or exogenously induced by irradiation are required for presynaptic alignment of homologues. Moreover, the number of co-aligned segments directly correlates with the level of DSBs measured by Rad51 foci, which are mainly observed between the axes at the sites of alignment. It, thus, appears that DSBs directly mediate presynaptic alignment via formation of specific inter-axis connections (Tesse et al. 2003). Further synapsis is, however, not observed in these mutants, suggesting that formation of a presynaptic connection is not sufficient for SC nucleation. Analogous DSB-dependent homologue pairing was also detected in budding yeast by FISH and Lox/Cre recombination (Peoples et al. 2002; Neale et al. 2002). Third, mutants with even higher levels of DSBs or higher rates of exogenous DSBs not only align homologues but do also form pieces of SCs. However, nearly normal levels of DSBs (*SPO11*-induced or exogenous) are required for complete alignment at a distance of 100 nm and SC formation (Tesse et al. 2003). A similar correlation between the level of DSBs and level of SC

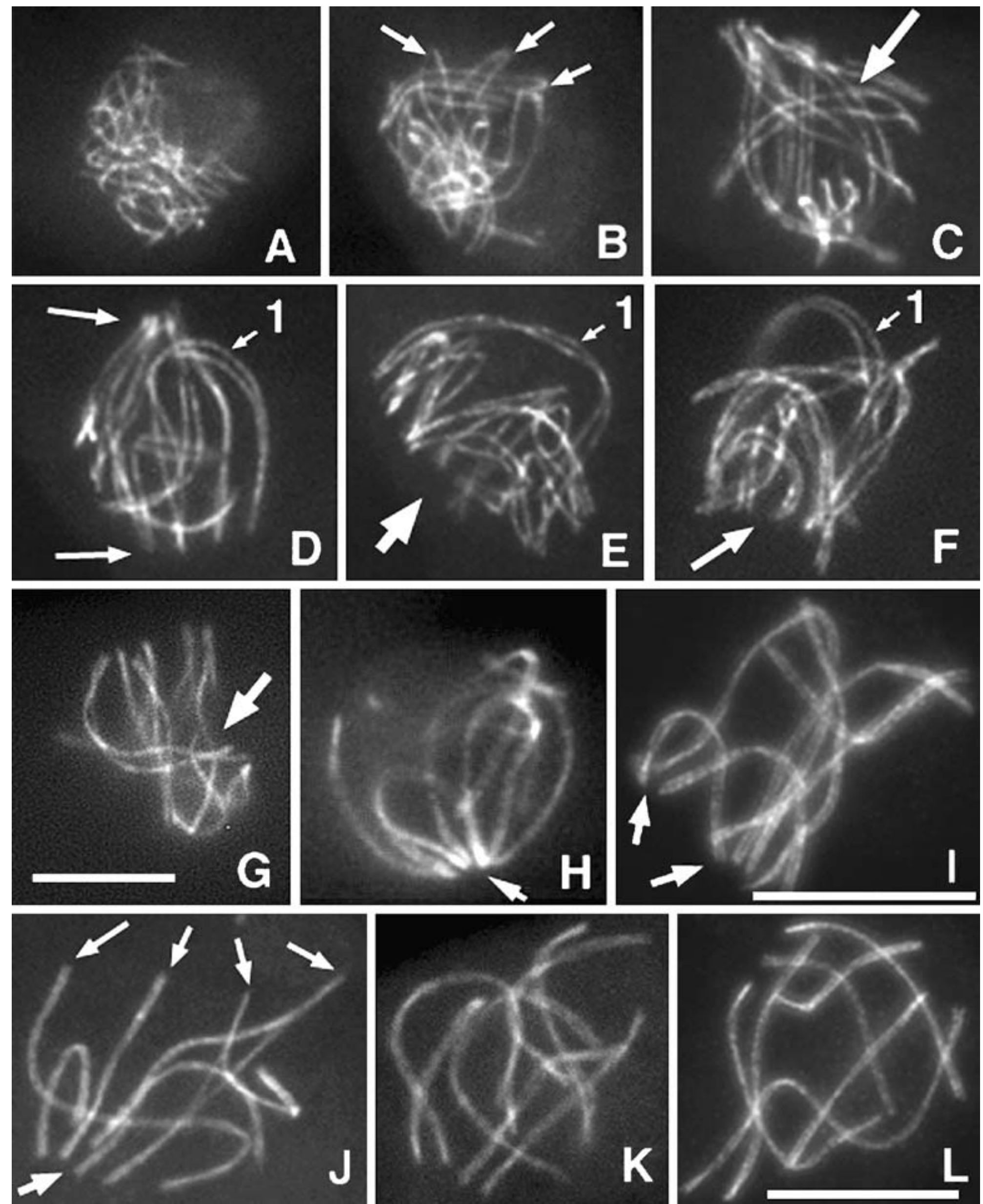

Fig. 1 **a**–**l** Leptotene to late pachytene nuclei of *Sordaria*. Chromosome axes are stained by Spo76/Pds5-GFP. **a** Early leptotene. **b** At mid-leptotene, telomere regions start pairing (*arrows*). **c** At end leptotene, all homologues are aligned. *Arrow* points to an interlocking between two pairs of homologues. **d** Early bouquet with two clusters of telomeres (*large arrows*). **e** Loose bouquet (*large arrow*). **f** Tight bouquet with almost all telomeres grouped (*arrow*). Homologues 1 are indicated by *small arrows* in **d** through **f**. **g** Zygotene bouquet. *Arrow* points to two interlocked pairs. **h** At early pachytene, bouquet is very tight (*arrow*). **i** Looser bouquet at mid-pachytene (*arrows*). **j** At mid-pachytene, release from the bouquet starts mostly at one telomere of each pair (*arrows*). **k**, **l** Chromosome ends disperse completely at mid-end pachytene. *Bars* represent 5 μm; *bar* in **g** applies to **a**–**g**, *bar* in **l** applies to **j**–**l**

formation is also seen in budding yeast *spo11* missense mutants that exhibit a range of different DSB frequencies (Henderson and Keeney 2004). Spo11p may, in fact, participate in DSB site selection (Diaz et al. 2002; Pecina et al. 2002). These findings imply that SC formation requires more DSBs than presynaptic alignment does. This is probably because SC is nucleated specifically at only a specific subset of DSB-mediated presynaptic connections, i.e., those that have been designated for future maturation into chiasmata (below). The same considerations can explain why the extent of synapsis directly correlates with the number of Rad51 foci in several maize mutants (Pawlowski et al. 2003). Studies of *Sordaria* have revealed an additional, unexpected finding: initiation of a single, short segment of SC does not permit SC polymerization along the entire length of the chromosome (Zickler et al. 1992; Tesse et al. 2003).

A variety of cytological studies in other organisms support and extend this picture. A good correspondence can be made between the local interactions that mediate presynaptic alignment and the sum of DSB-mediated recombinational interactions. (1) Rad51 and Dmc1 foci appear mainly on axes but also between aligned chromosomes in plants and mammals (e.g., Tarsounas et al. 1999; Franklin et al. 1999; de Vries et al. 2005) and accumulate between aligned axial elements in mutants of yeast and mouse that are blocked specifically in SC formation (de Vries et al. 2005 and references therein). (2) Immunoelectron-microscope studies in mouse localize Rad51/Dmc1 and RPA to bridges that link homologue axes during presynaptic alignment (e.g., Moens et al. 2002). (3) In mammals and plants, the number of early recombination nodules and Rad51 or Msh4 foci highly exceeds the

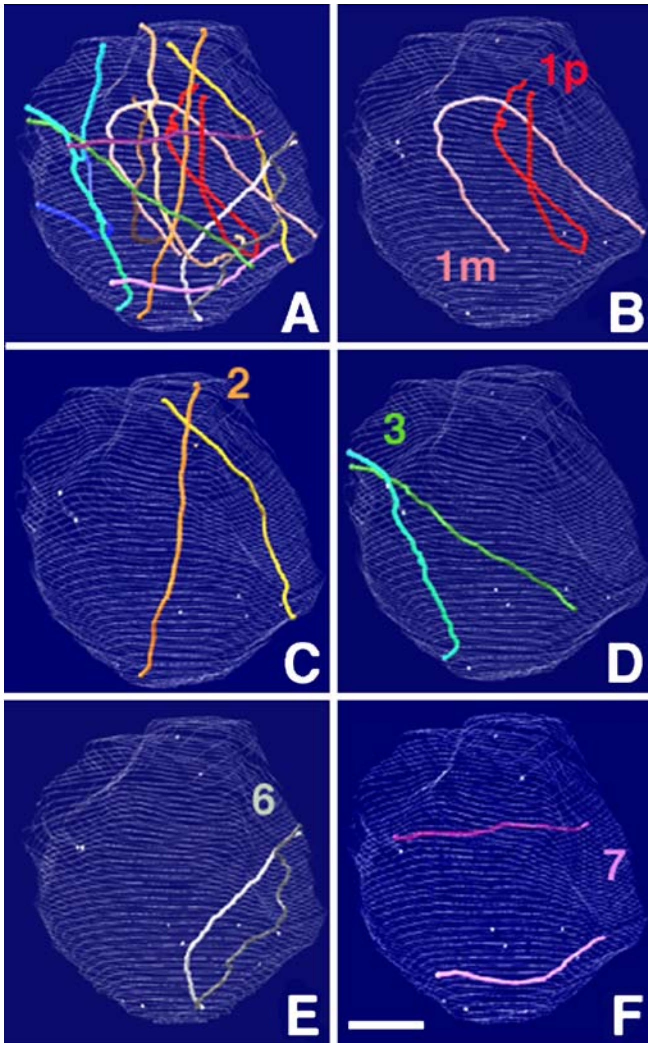

Fig. 2 a–**f** Three-dimensional representation of a reconstructed early leptotene nucleus (from 52 EM serial sections/pictures). The seven homologues are represented with matching colors. **a** Overview of the nucleus. **b** Homologues 1 (*1m pink* and *1p red* in **b**), 2 (**c**), and 7 (**f**) are far apart when homologues 3 (**d**) and 6 (**e**) show telomere alignment. *Bar* represents 1 μm

number of crossovers/chiasmata (Albini and Jones 1987; Santucci-Darmanin et al. 2000; Higgins et al. 2004; Neyton et al. 2004; Anderson and Stack 2005; de Vries et al. 2005). They likely mark global recombinational interactions that serve to align the homologues. In *Allium*, it can be seen that inter-homologue bridges disappear at sites where SC nucleates (Albini and Jones 1987), implying a direct precursor–product relationship.

Synaptonemal complex initiates at recombination sites that will give rise to crossovers

A variety of observations suggest that SC initiates specifically at sites of crossovers in most organisms, with the possible exceptions of *Drosophila* female and *C. elegans* (see review in Henderson and Keeney 2005; above). First, SC formation is directly dependent upon recombination initiation via DSBs. SCs do not form in the absence of Spo11p in budding yeast, *Arabidopsis*, mouse, *Coprinus*, and *Sordaria* (Keeney et al. 1997; Celerin et al. 2000; Baudat et al. 2000; Grelon et al. 2001; Storlazzi et al. 2003). Occasional SCs are observed in the absence of Spo11 in budding yeast, especially in a *ndt80* background, in which cells arrest at pachytene (Bhuiyan and Schmekel 2004). SCs are also seen in mouse *spo11* mutants, but they are nonhomologous (Baudat et al. 2000; Romanienko and Camerini-Otero 2000). In both cases, they likely correspond to the late SCs observed in several mutants with delayed synapsis (see review in Zickler and Kleckner 1999). While *Coprinus spo11* mutants do not form SCs, they form SCs if premeiotic replication is prevented, thus, in the absence of a normal sister chromatid (Merino et al. 2000). Those examples show that SC formation can be temporally different in a mutant condition. They raise the possibility that the unique behavior of the *spo11* mutants of *Drosophila*

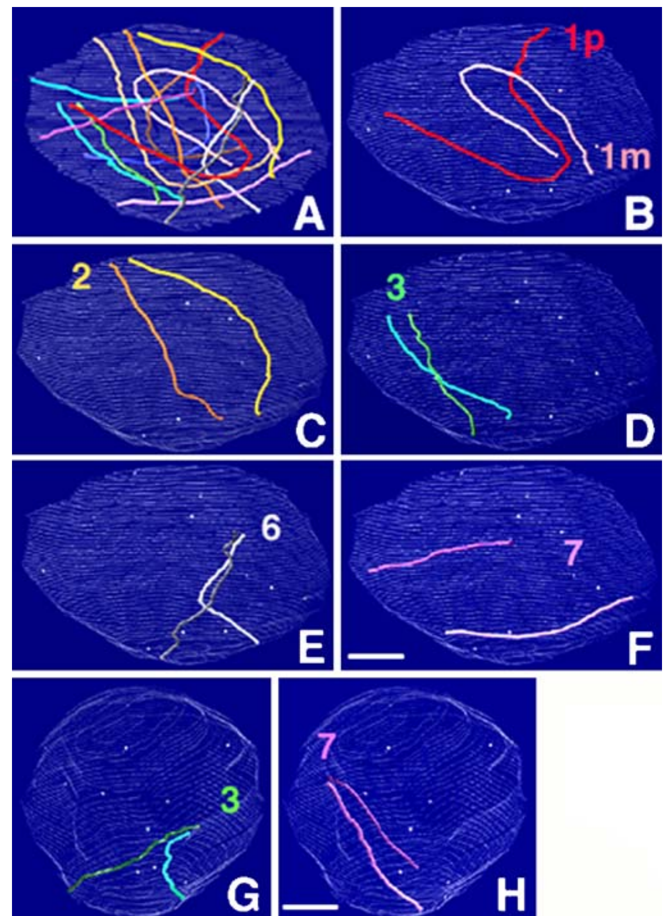

Fig. 3 a–**h** Two successive rotations of the nucleus shown in Fig. 2. **a** Overview. Computer rotation of the nucleus confirms that homologues 1 (**b**), 2 (**c**), and 7 (**f**) are not aligned, whereas homologues 3 (**d**) and 6 (**e**) are roughly co-localized at telomere regions. A third rotation (**g**, **h**) shows that homologues 3 (**g**) have in fact only one telomere region aligned and homologues 7 (**h**) appear now closely aligned when they are, in fact, far apart as seen in **f**. *Bars* represent 1 μm

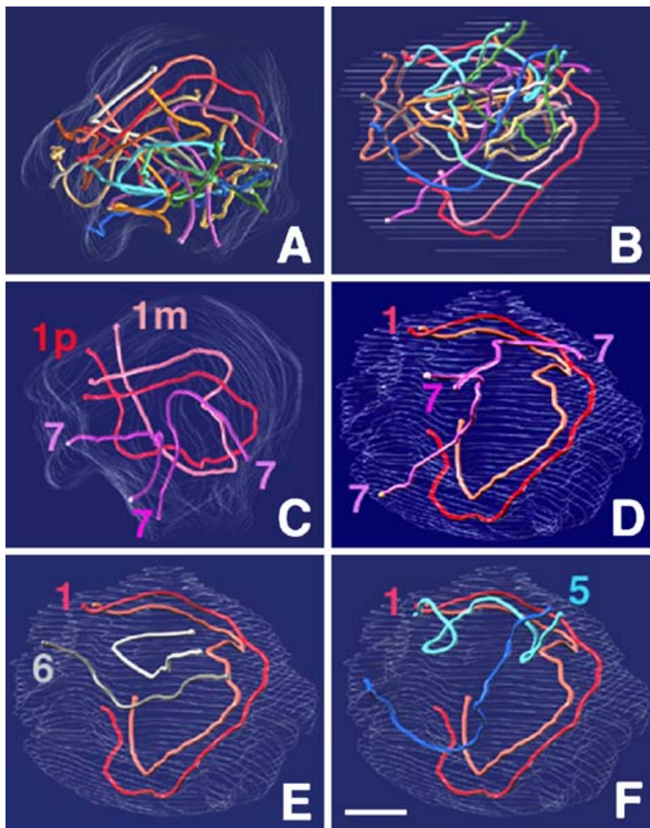

Fig. 4 Mid-leptotene nucleus. **a**, **b** Two different overviews of the reconstructed nucleus (from 65 EM serial sections/pictures). **c**, **d** Longer homologues 1 (*1m* and *1p* in *pink* and *red*) are completely co-aligned whereas the shorter homologues 7 (*violet*) show only segmental alignment (in **c**). In the second view after rotation of the nucleus, pair 7 (**d**) appears almost separated, underlining the interest of 3D representations. Homologues 6 (**e**) and 5 (**f**) are co-aligned at one telomere region (the other ends being widely separated). *Bar* represents 1 µm

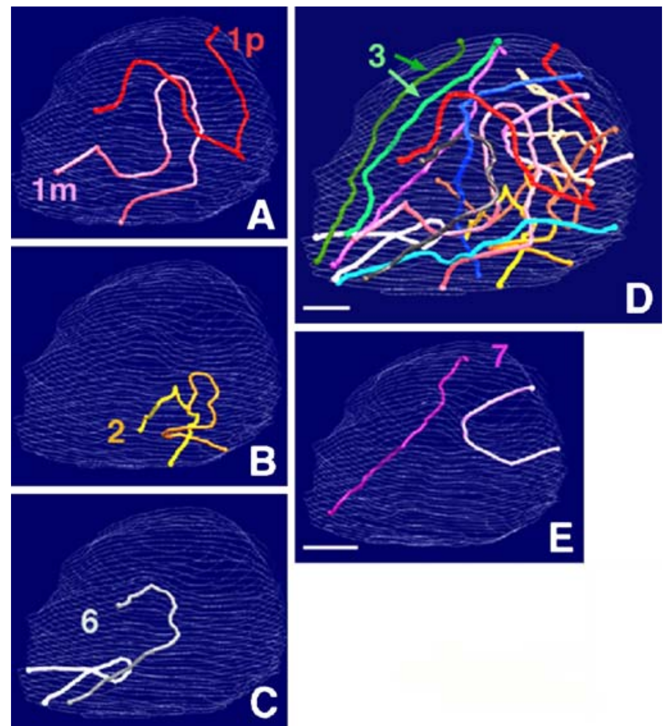

Fig. 5 Leptotene nucleus of Sordaria *spo11* null mutant (reconstructed from 56 EM serial sections/pictures). Homologues 1 (*1m* and *1p* in **a**), 2 (**b**), and 7 (**e**) are far apart. Homologues 3 are aligned (*green arrows* in **d**) and homologues 6 (**c**) show partial recognition at the telomere region. *Bar* represents 1 µm

and *C. elegans*, which form SCs in the absence of DSBs (above), might fail to set up conditions which couple SC formation to recombination, thus permitting uncoupled SC formation in the mutants (see discussion in Haber 1998). Second, in several organisms, the number and position of the SC recombination nodules that mark the sites at which crossovers are occurring closely correlate with the sites of SC initiation (see review in Carpenter 1988; Zickler and Kleckner 1999; Anderson and Stack 2005). A one-to-one correspondence between the frequencies of late nodules and the frequency of SC initiation is also observed: e.g., in the inverted region of a heterozygous inversion of maize (Maguire and Riess 1994) and in two *Sordaria* mutants (Zickler et al. 1992). Third, in budding yeast, one of the several proteins that coordinately promote the transition between DSBs and the crossover recombination products is Zip1, a major component of SC central region (Börner et al. 2004; Fung et al. 2004 and references therein). Two other proteins, Zip2 and Zip3, mark the sites at which Zip1/SC initiates. Both are required for SC polymerization along

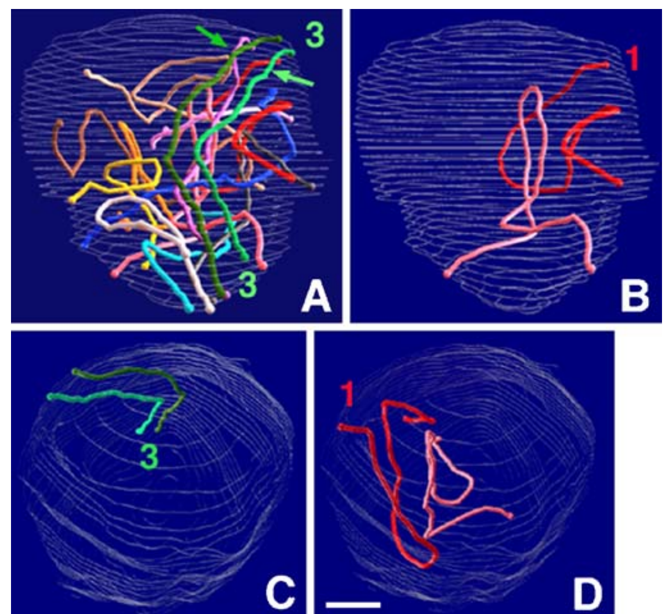

Fig. 6 Two successive views of the nucleus shown in **Fig. 5** confirm that homologues 3 are co-aligned (**a**, **c**), whereas homologues 1 are far apart (**b**, **d**). The three views show also that homologues 3 (in *green*) are located along the NE outside of the rest of the homologues. *Bar* represents 1 µm

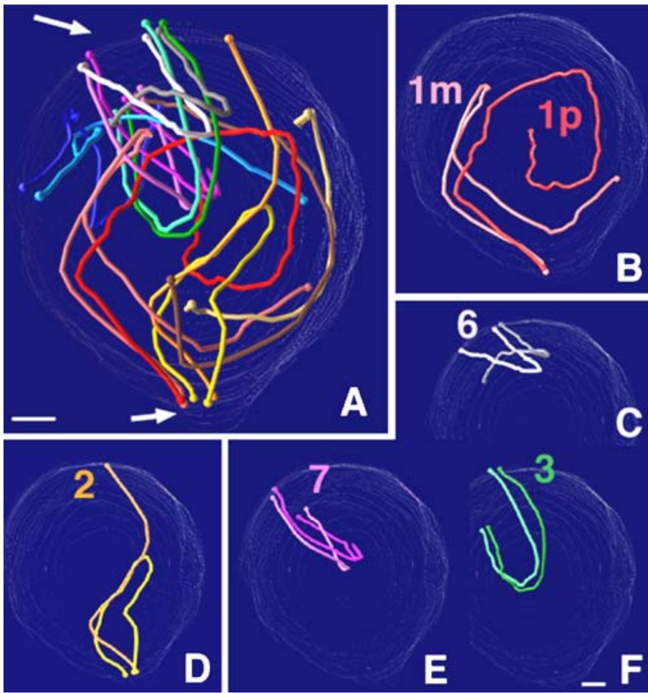

Fig. 7 Late leptotene nucleus with bouquet formation (reconstructed from 69 EM serial sections/pictures). **a** Overview of the nucleus. *Arrows* point to the two regions with clustered telomeres. Homologues 1 (*1m* and *1p* in **b**) and 2 (**d**) are co-aligned over half their length, whereas homologues 6 (**c**), 7 (**e**), and 3 (**f**) are completely aligned at 400 nm. *Bars* represent 1 μm

homologues, exhibit the same "interference pattern" as do crossovers (Fung et al. 2004), and vary in number in correlation with crossover levels (Rockmill et al. 2003; Henderson and Keeney 2004). In their absence, homologues align with each other at 400 nm and are connected at a few sites called axial associations (Agarwal and Roeder 2000; Fung et al. 2004). Both latter phenotypes are also seen in the *Sycp1* mutants of mouse (de Vries et al. 2005). In budding yeast, axial associations are inferred to be sites where synapsis would have occurred in wild-type meiosis (e.g., Rockmill et al. 2003; Fung et al. 2004).

Several questions regarding the homologue juxtaposition process remain open.

(1) How do homologues recognize one another in the recombination-independent pairing phase? Chromosome movements could provide the initial contacts by random collision (e.g., Fung et al. 1998). Several models include: direct DNA/DNA contacts; searching of duplex DNA by a single-stranded form or by specific DNA binding proteins; or, as now proven for gene silencing in plants and fungi, by sequence-specific RNA molecules (e.g., Bender 2004). As a 400-bp heterology is enough to trigger meiotic silencing (above), overall genome-checking mechanisms could help to discriminate between homology and heterology. Other models involve specific chromosome features like "pairing sequences" (below), nucleosome-free regions, proteins bound specifically at homologous sites, or RNA (see discussion in Zickler and Kleckner 1999).

(2) How do DSBs mediate alignment? In budding yeast, DSBs were shown to occur first in chromatin loops before their association with the chromosomal axes (Blat et al. 2002). Based on indications that axis association occurs either before or immediately after DSB formation (e.g., occurrence of Rad51 foci on axes at early stages of presynaptic alignment), Tesse et al. (2003) suggested that the axis-associated post-DSB recombination complex recognizes and captures the homologous sequence present in the chromatin loop of the homologous chromosome and then "reels in" the partner axis. As Rad51 and Dmc1 can promote efficient homologous interactions between kilobase-long DNA substrates (e.g., Sung et al. 2003), this ability could explain the role of Rad51 in presynaptic alignment of homologue axes.

(3) What holds homologues together when they are co-aligned at 400 nm over several microns? The "links" joining homologous axes at the presynaptic alignment distance (see examples in Albini and Jones 1987) could include structural components and/or increasing stability provided by the recombination intermediates Rad51/Dmc1/RPA/Msh4 that are seen associated to the links/association sites between the aligned homologues (e.g., Moens et al. 2002; Higgins et al. 2004; de Vries et al. 2005 and references therein).

(4) How can the finding described above that a single nucleated segment of SC cannot be extended outward in both directions along the entire length of the homologues be explained? Tesse et al. (2003) and Börner et al. (2004) suggested, in the context of emerging evidence that interference precedes SC formation, that the interference signal may license installation of SC within the affected regions. If so, SC would polymerize outward from its nucleation site only as far as the interference signal has spread.

Pairing centers and pairing maintenance in *Drosophila* and *C. elegans*

In both *Drosophila* female and *C. elegans*, the existence of "pairing centers" was suggested by the analysis of translocation heterozygotes. In *Drosophila* female (e.g., Hawley 1980; Hawley et al. 1992), heterozygous translocations suppress crossovers within intervals defined by specific boundary sites and not (or only mildly) in the adjacent areas. Boundary sites are mapped by determining which translocation suppresses crossovers in a given interval. Hawley (1980) showed that four such sites subdivide the chromosome X into three intervals independent from one another for crossovers. These early studies led to the proposal that the identified sites were involved in mediating pairing/synapsis of homologues as a prerequisite for recombination. Recent studies have shown that the reduction of exchanges in the translocation-defined regions

or in large paracentric inversions is not due to pairing/synapsis defects: homologues are not only normally paired in those crossover-suppressed regions but also mostly synapsed by SCs (Sherizen et al. 2005; Gong et al. 2005). Sherizen et al. (2005) further found that heterozygous translocations reduce gene conversion frequencies in parallel with crossover frequencies. This effect does not appear to result from an effect on DSB formation (Gong et al. 2005), implying that generalized effects at a post-DSB stage might be involved. The observed phenomena also imply the occurrence of communication along chromosomes, but it is not yet clear whether axial chromosome structure and/or the SC are involved (Sherizen et al. 2005; Gong et al. 2005).

In *C. elegans*, reciprocal translocations suppress recombination to one side of the breakpoint (see review in Zetka and Rose 1995). These and other studies led to the identification of a region near one end of each chromosome whose integrity is required for normal recombination throughout the chromosome length (e.g., Villeneuve 1994; Hillers and Villeneuve 2003). As in *Drosophila*, the “pairing centers” were suggested to establish the initial interactions between homologues and to promote homologous interactions independently of the SC. Recent studies show that the "pairing centers" play in fact two separable roles: stabilization of pairing indepedently of SC and initiation of SC polymerization from those centers, whatever their localization (MacQueen et al. 2005). Pairing centers are however not required for the occurence of pairing, synapsis or crossover along the X chromosomes (MacQueen et al. 2005; Martinez-Perez and Villeneuve, 2005). Moreover, the C2H2 Zinc-Finger protein HIM-8 localizes both to the genetically defined pairing center of the X chromosome and the nuclear envelope. This interesting finding provides the first example of a protein required for meiotic synapsis of a particular chromosome pair. However, binding of HIM-8 is not sufficient for synapsis of the X chromosomes: *him-8* mutants are defective in presynaptic alignment/pairing and SC formation (Phillips et al. 2005).

In both *Drosophila* female and *C. elegans*, however, pairing centers are not sufficient to stabilize pairing: mutants with severely compromised SC formation exhibit normal establishment of homologue pairing but then lose that relationship at the time that SC would normally have formed (MacQueen et al. 2002; Colaiacovo et al. 2003; Sherizen et al. 2005; Gong et al. 2005 and discussions therein). Moreover, in *Drosophila* male meiosis, even though recombination and SC do not occur, there is a tendency for increased individualization of both sister chromatids and homologues at the corresponding stage (“mid-G2”; Vazquez et al. 2002) in wild-type meiosis. These patterns might all correspond to tendencies for loss of sister and/or homologue juxtaposition at the leptotene/zygotene transition (Zickler and Kleckner 1999; Kleckner et al. 2004). While DSBs may occur after SC formation, their formation and repair are also, nevertheless, partially dependent on the SC components, indicating that SC (or its components) and recombination are mutually interdependent processes even in *Drosophila* female and *C. elegans* (Page and Hawley 2001; MacQueen et al. 2002; Jang et al. 2003; Colaiacovo et al. 2003; Couteau et al. 2004).

What is the role of the bouquet in homologous pairing and synapsis?

The bouquet is a specific well-conserved configuration of meiosis, which is formed at mid-prophase, concomitant with important events of homologue juxtaposition. Several features are common to all known bouquets: telomere regions are associated with the nuclear envelope, chromosome ends are grouped within a limited area, chromosomes move in and out of the bouquet, and, finally, formation of a bouquet requires neither DSBs nor the downstream processes of recombination and SC formation.

(1) Telomere regions are associated with the nuclear envelope. The association between chromosome ends and the nuclear envelope is an important component of the bouquet configuration (e.g., Liebe et al. 2004). Indeed, absence of the telomere protein Ndj1, involved in the processing of telomere repeats, de-localizes telomeres from NE and specifically inhibits bouquet formation in budding yeast (Trelles-Sticken et al. 2000; Joseph et al. 2005). Absence of the fission yeast telomere protein Taz1 or the mice telomerase similarly leads to defects in bouquet formation, pairing, and recombination (Cooper et al. 1998; Liu et al. 2004).

(2) At the bouquet stage, chromosome ends are grouped together within a limited area, generally facing the microtubule-organizing center (MTOC) in organisms with a clearly defined MTOC (e.g., Moens 1969; see review in Zickler and Kleckner 1998). In fission yeast, both telomere and spindle pole body integrity are necessary for bouquet formation (Cooper et al. 1997; Ding et al. 2004). The telomere protein Ndj1, interestingly, interacts with the spindle pole body component Msp3, which correlates with the bouquet defect of *ndj1* mutants (Uetz et al. 2000). Cytoplasmic microtubules are not required for yeasts and plants bouquet formation (Chikashige et al. 1994; Ding et al. 1998; Cowan and Cande 2002; Trelles-Sticken et al. 2005).

(3) Chromosomes move in and out of the bouquet at particular stages of meiosis, the leptotene/zygotene transition, and into early pachytene (as illustrated for *Sordaria* in Fig. 1d–l). Chromosome ends attach to the inner membrane at early leptotene but are still distributed throughout the nuclear volume (Figs. 1b, 2a, 3a, and 4a,b). At late leptotene, ends start to cluster into the bouquet either after chromosome alignment like in *Sordaria* (Fig. 1d,e) or coincidently with pairing like in maize (Bass et al. 1997). First, only one telomere region is seen clustered (Fig. 1d), then

telomeres group into sub-clusters (Fig. 7) before forming a loose bouquet (Fig. 1e) at the leptotene–zygotene transition stage. A relatively tight cluster persists throughout all of zygotene (Fig. 1f,g) and during early pachytene, after synapsis is completed (Fig. 1h,i). In middle pachytene, the telomere cluster disperses (Fig. 1j–l). As during its formation, bouquet release starts first from one telomere region of each pair (Fig. 1j) and, finally, all ends are dispersed (Fig. 1k,l). EM pictures show that chromosome ends remain attached at the NE until the end of pachytene (see review in Zickler and Kleckner 1998). This sequence of events implies that NE association and bouquet formation are two functionally distinct aspects of telomere behavior. Progression through these stages occurs more or less rapidly from one organism to the other. However, in all cases, the tight bouquet stage is relatively transient, e.g., 5–10% of all prophase nuclei in budding yeast and *Sordaria* (Trelles-Sticken et al. 2000; Storlazzi et al. 2003). Recent time-lapse analyses of living budding yeast meiocytes further reveal that chromosome ends move reversibly in and out of the bouquet configuration. Telomeres (visualized by Rap1-GFP) are rather immobile in vegetative or premeiotic cells and in cells arrested in meiotic S-phase. In contrast, they are highly mobile during meiosis from leptotene through pachytene, thus including the bouquet stage, which persists only ~30 s before telomeres move again out and in the cluster (Trelles-Sticken et al. 2005). At leptotene, telomeres first form mini-clusters as observed in *Sordaria* (Fig. 7), mammal cells (Rasmussen and Holm 1978; Bojko 1983), and plant meiocytes (Noguchi 2002; Carlton et al. 2003). Rapid chromosome movements, interestingly, continue well after the bouquet stage, when telomeres are dispersed over the entire nucleus at pachytene (Trelles-Sticken et al. 2005; White et al. 2004). Similar rapid chromosome movements were also observed in several other organisms (review in Zickler and Kleckner 1998). The bouquet is no longer formed when budding yeast cells are treated with latrunculin that prevents polymerization of G-actin; moreover, actin is also required for the pachytene telomere movement (Trelles-Sticken et al. 2005).

(4) Bouquet formation requires neither DSBs nor the downstream processes of recombination and SC formation (Trelles-Sticken et al. 1999; Storlazzi et al. 2003). In addition, bouquet forms in haploid meiosis and, thus, in the absence of a homologue (see review in Zickler and Kleckner 1998). However, when DSBs or DSB-dependent processes are absent, resolution of the bouquet is delayed; further exogenous DSBs restore timely release of the bouquet, indicating that exit from the bouquet is promoted by DSBs or some downstream recombination process (Trelles-Sticken et al. 1999; Storlazzi et al. 2003). Delay in bouquet exit was also observed in mutants defective in later stages of recombination, i.e., budding yeast *rad50S*, *C. elegans syp-1*, and mouse *Atm*−/− mutants (Trelles-Sticken et al. 1999; Scherthan et al. 2000; MacQueen et al. 2002). Therefore, it was suggested that the exit of bouquet is mediated by processes that sense progression of recombination after the DSB stage (Storlazzi et al. 2003).

The mechanistic basis for bouquet formation is not known. The bouquet configuration is often suggested to contribute to homologous chromosome pairing by bringing homologues in close proximity through their telomere grouping (Bass et al. 1997; Ding et al. 1998; Yamamoto et al. 1999; Scherthan 2001). An analogous argument has been made for the mid-prophase "clustering" stage in *C. elegans* (e.g., Martinez-Perez and Villeneuve 2005). However, even in fission yeast, where pairing and telomere clustering at SPBs are tightly coupled, wild-type pairing requires not only bouquet formation but also nuclear oscillation driven by both astral microtubules and the dynein heavy chain Dhc1 (Ding et al. 1998; Yamamoto et al. 1999). There is certainly a temporal link between bouquet formation and complete juxtaposition of homologues (e.g., MacQueen et al. 2002; Golubovskaya et al. 2002; Couteau et al. 2004). Nonetheless, bouquet formation and pairing/synapsis could be concomitant components of a general nuclear reorganization rather than being linked in a simple cause-and-effect relationship (for further discussion, see Zickler and Kleckner 1998). The cases of *Sordaria* and rye provide one argument against a role for the bouquet stage in early stages of homologue juxtaposition: in both cases, homologues are co-localized and co-aligned before grouping of telomeres (Fig. 1c,d; Zickler 1977; Noguchi 2002). One argument also used in favor of the bouquet as a pairing-mediator is that, in several cases, initiation of SC formation in the telomere regions is concomitant with bouquet formation. However, this phenomenon does not exclude the possibility that substantial levels of pairing were already achieved before bouquet formation (see examples in Zickler and Kleckner 1998; Scherthan 2001). Furthermore, in the absence of telomere/nuclear envelope attachments, not only bouquet formation but also pairing and synapsis are significantly delayed in both budding and fission yeast *ndj1/taz1* mutants (Trelles-Sticken et al. 2000; Ding et al. 2004 and references therein), which could imply a role for the bouquet in pairing/synapsis. On the other hand, pairing/synapsis is not abolished and events before bouquet formation (e.g., pre-leptotene redistribution of telomeres after meiotic induction and de-localization of telomeres towards the interior of the nucleus) are also affected, at least in a budding yeast *ndj1*null mutant (Trelles-Sticken et al. 2000).

We suggest that the bouquet could potentially play important roles not only in pairing, as usually suggested, but also in other aspects of prophase. (1) At leptotene/zygotene, rapid movements of telomeres and, thus, chromosomes may allow the resolution of the entanglements/

interlockings of chromosomes or bivalents often seen at that stage (Fig. 1g) especially in species with long chromosomes (see review in von Wettstein et al. 1984; Zickler and Kleckner 1998). (2) Clustering of chromosomes in a defined area of the nucleus may "rescue" the pairing of laggard homologues (e.g., von Wettstein et al. 1984). (3) The fact that chromosomes are bent in the bouquet configuration may facilitate faster transmission of a signal (e.g., RNA silencing) or checking for heterologies introduced during meiosis (e.g., transposons) along chromosomes as seen in MSUD (above). (4) Moving telomeres into the bouquet correspond also with an increase of the nuclear volume. Telomere movements could be necessary to take the chromosomes out of their pre-meiotic/early meiotic territories, while pachytene chromosome movements might be necessary to redefine new territories in preparation for chromatin condensation and/or pro-metaphase location before spindle attachment. (5) Entry into or exit from the bouquet could help to destabilize ectopic or homoeologous recombination interactions before stable association through SC (e.g., Niwa et al. 2000) or when regular SC formation is blocked by inappropriate interactions, respectively. In the latter case, as recombination-mutant phenotypes indicate that DSB-mediated control is exerted on exit of the bouquet and not on entry, exit from the bouquet could work as a checkpoint sensing the status of the chromosome or/and the recombination process before complete "locking-in" of homologues by the synaptonemal complex.

Conclusions

Now, what next? From the above description, certain areas of particular interest are apparent. There is almost no understanding of the mechanistic basis for recombination-independent homologue recognition and juxtaposition. With respect to recombination, a key challenge is to integrate recombination events at the DNA level into the higher-order organization of "the chromosome" from early recognition of homology to chiasma formation and maintenance. New insights into the early steps of recombination have opened new ways of thinking how it can fulfill its meiotic function but still have not explained how a chiasma is formed. We also must understand the reason of the chromosome movements; here, real-time fluorescence microscopy of entire chromosomes will be a powerful help. Finally, the models for chromosome recognition and clustering into the bouquet discussed here are still at a highly speculative stage, underlining our ignorance, will hopefully shape future thinking and provoke new investigations.

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