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REVIEW

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Chiasma formation: chromatin/axis interplay and the role(s) of the synaptonemal complex

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Abstract Meiotic recombination proceeds in biochemical complexes that are physically associated with underlying chromosome structural axes. In this study, we discuss the organizational basis for these axes, the timing and nature of recombinosome/axis organization with respect to the prophase program of DNA and to structural changes, and the possible significance of axis organization. Furthermore, we discuss implications and extensions of our recently proposed mechanical model for chiasma formation. Finally, we give a broader consideration to past and present models for the role of the synaptonemal complex.

Abbreviations L: leptotene · Z: zygotene · P: pachytene · EP: early pachytene · MP: middle pachytene · LP: late pachytene

Introduction

During meiosis, chromosomes remain in an organized, individualized state for an extended period of time. This same period includes recombination at the DNA level, which has the unique feature that many of its steps occur within biochemical complexes that are physically associated with underlying chromosome structural axes. The simple linear organization of meiotic prophase chromosomes, combined with the complex program of global and local events, provide a unique opportunity to study the nature and roles of basic chromosome structure and the functional interplay between that structure and chromatin status. Most importantly, the DNA events of meiosis provide a biochemical readout that is not available in studies of organized mitotic chromosomes. The current article brings together, and further elaborates upon, a number of ideas on these subjects previously published by our laboratory.

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The synaptonemal complex—50 years

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Meiotic prophase chromosome axes

Meiotic midprophase chromosomes are well-individualized linear entities whose organizational structure can be precisely described (Moens and Pearlman 1988; Zickler and Kleckner 1999). It will not be surprising if the basic structure described for these chromosomes is also present in mitotic prophase.

Co-oriented linear loop arrays

At the pachytene stage of meiosis, homologous chromosomes are joined via the synaptonemal complex (SC). Along each side of the proteinaceous SC, the chromatin of each chromatid is organized into a series of loops. The bases of these loops comprise a geometric axis, which, as elaborated by proteins, comprises a structural "axis" or "core." Furthermore, the axes of sister chromatids are stacked one above the other, perpendicular to the plane of the SC ribbon, tightly conjoined into a single morphological unit and with their chromatin loops emanating outward from the SC. Thus, within pachytene chromosomes, sister chromatid linear loop arrays are co-oriented.

Conserved loop density along pachytene axes

Detailed analysis of SC lengths, genome sizes, and/or loop lengths in spread preparations reveals that the density of loops along a pachytene axis is quite conserved across a wide variety of organisms, at ~20 loops per micron of axis length (Zickler and Kleckner 1999; Table 1 of Appendix). This feature points to the existence of some type of

organizational "loop base module," whose dimensions or spacing is evolutionarily conserved. Another implication of this regularity is that differences in total genome size among different organisms are accommodated by differences in loop size or total axis length (see also Moens and Pearlman 1988), not by variations in basic axial structure.

It is interesting to note that pachytene axis lengths can vary considerably within a given species, despite the fact that total genome size/DNA content is essentially constant (e.g., human male and female SC; reviewed in Kleckner et al. 2003). If loop densities are the same in all cases, differences in axis length in such situations should be accompanied by inversely correlated differences in loop size. This prediction is strikingly confirmed. Mutant mice lacking the axis component SMC1-beta exhibit half the SC/axis length and twice the loop size of wild-type mice (Revenkova et al. 2004); conversely, human female exhibits twice the SC/axis length of male and about half the loop size (Tease and Hulten 2004).

Axial substructure comprises dual loop modules

In a number of organisms, electron microscopic studies of SC lateral elements reveal regular striations that extend continuously across the two sister axes (review in Zickler and Kleckner 1999). This continuity implies that structural features along the two sister chromatid axes, i.e., their loop-base modules, must be "in register" as a "dual loop module." It is not known whether "paired" sister modules usually or necessarily form on homologous DNA regions or whether the two loops within a dual loop module sometimes or often contain different DNA sequences. Put another way, these considerations suggest that each homolog axis comprises a linear array of dual loop modules, with intermodule links both along and between sisters.

In accord with these two levels of organization, linkages along or between chromatids can be disrupted differentially: (1) there is a strong tendency for duality or splitting of sister axes along their lengths, particularly at certain stages (Dresser and Moses 1980; Zickler and Kleckner 1999) and (2) coordinate "fracturing" of conjoined axes also occurs in certain mutants (e.g., van Heemst et al. 1999).

Toward a detailed model for axis organization

The above and other observations can be used to construct a working model for axis organization.

1. Mitotic late-stage chromatids comprise arrays of chromatin loops organized along a molecularly definable axis. At the DNA level, this axis comprises coalescences of locally AT-rich regions referred to as an "AT queue" (Saitoh and Laemmli 1994). In yeast, meiosis-specific axis-associated proteins are bound preferentially to regularly spaced, locally AT-rich regions, suggesting that the meiotic prophase axis analogously comprises an AT queue (Blat et al. 2002).
2. There could be significant amounts of DNA within the central region of the SC, all along its length (Ortiz et al. 2002), implying that DNA may be protruding "below" the axis.
3. Two types of cytological studies suggest that axis-localized protein components occur in multiple horizontal "layers," with a "supra-axial meshwork" present above the ultrastructurally defined axis. First, when pachytene chromosomes are visualized by electron microscopy, a thin element along each edge that stains with heavy metals is revealed. This entity is called the "lateral element" of the SC, and when observed just before linking of homolog axes via SC central region components, it is called an "axial element." The precise nature of the stained entity varies with the staining method. It is unclear whether staining delineates epitopes for some particular protein (e.g., Anderson et al. 2005; Pelttari et al. 2001) or a clustering of epitopes from multiple components. In contrast, early immunoelectron microscopic visualizations reveal that two axis-associated proteins, Topoisomerase II along mouse SCs and Hop1 along yeast SCs, are localized "upward/outward" from the lateral element, rather than along the lateral element itself (Moens and Earnshaw 1989; Hollingsworth et al. 1990).

   Second, immunofluorescence visualizations of *Sordaria* chromosomes with the MPM2 antibody, which recognizes phosphoprotein epitopes perhaps of TopoII, reveal a thin line of staining in contrast to antiSpo76/Pds5 and Rec8, which reveal much thicker lines (van Heemst et al. 1999; D. Zickler, personal communication). Thus, chromosome axes may comprise not only the silver staining axis or core but a much more complex and bulky supra-axial meshwork of proteins bound to DNA at the bases of the loops (Zickler and Kleckner 1999).
4. SCs lengths often vary modestly but systematically over the different stages of pachytene; furthermore, SCs are prone to twisting (Zickler and Kleckner 1999). Both features suggest that the SC, while structurally robust, is also substantially elastic. Elasticity would presumably derive from DNA components along the two homolog axes, again suggesting that these axes are not simply continuous rigid rods but structures like the proposed complex meshwork assemblies of protein and DNA components.
5. In some organisms, axis length decreases dramatically, about twofold, concomitant with SC formation. One possible explanation is that the "loop base modules" along the axis remain fixed in number and simply become more tightly packed. If so, a decrease in length would be accomplished by "scrunching-up" of axis-associated DNA.

A model for the organization of meiotic prophase axes that incorporates the above features is shown in Fig. 1.

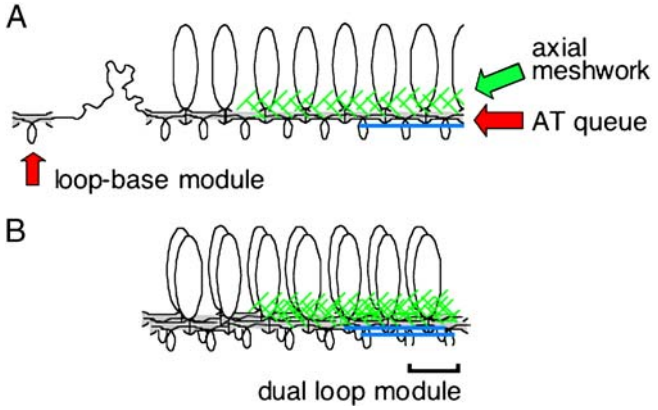

Fig. 1 Model for assembly and organization of linear loop axis array **(a)** and structure of co-oriented sister arrays characterized by in-phase "dual-loop modules"

Axis morphogenesis

Little is known about how chromosome axes assemble. However, several interesting issues are as follows:

1. How is the positioning of loop-base modules determined? The most obvious model for axis assembly would involve binding of loop-base modules to periodic sites along the chromatin, followed by the coming together of adjacent modules into a regularly spaced linear array. Chromatin loop size would thus be determined by the spacing between bound modules before assembly. Studies of chromatin loop size further imply that module positioning has to accommodate two important constraints. First, loop sizes are relatively constant and characteristic along the chromosomes of any given organism. Second, the same DNA sequence sometimes yields axes having different chromatin loop sizes according to the type of nucleus in which it occurs, as seen not only within a species (above), but also in artificial situations where DNA is moved into an entirely unrelated species (Zickler and Kleckner 1999). Neither of these constraints, and most particularly the second, is explained in a simple way by supposing that loop-base modules bind to specific DNA sequences. Thus, we have proposed that chromatin loop size, and therefore the positioning of loop-base modules along the DNA/chromatin fiber, is determined by chromatin fiber stiffness (persistence length) (Zickler and Kleckner 1999). For example, installation of one loop-base module might require a pairwise interaction with another module and, thus, (at least transient) formation of a chromatin loop; modules could then be packed in as densely as possible up to the point where chromatin fiber stiffness precluded further loop formation.
2. The presence of a sister is not essential for formation of a regular chromatid axis. In a mutant of *Coprinus*, where meiotic prophase occurs without a preceding round of DNA replication, single chromatids form axes that are morphologically normal, except that they are half the thickness of wild-type axes, and which go on to form SCs (Pukkila et al. 1995). Thus, at least in this case, axial organization and axis formation are independent of inter-sister connections.
3. What molecule(s) directly mediate formation of linear loop arrays? While a number of axis-associated proteins have been identified by immunocytological methods (e.g., Page and Hawley 2004), well-organized chromosomes occur in all of the corresponding mutants, implying that they do not comprise fundamental axis building blocks. Included among these mutants are those lacking the EM-defined axial/lateral element feature, implying that this entity is not required per se for development of axial chromosome structure (e.g., Pelttari et al. 2001; Zickler and Kleckner 1999). Based on the finding that the cohesin Rec8 localizes very early along prophase chromosome axes, the specific suggestion that cohesins comprise the fundamental axial building block has been made (Eijpe et al. 2003). However, it is difficult to reconcile a fundamental role for cohesins with the observation that axes can form in the absence of a sister chromatid (above). Furthermore, axis development is normal in *rec8* mutants of several organisms including mouse, *Arabidopsis* and *Sordaria* (Xu et al. 2005; Bannister et al. 2004; Bhatt et al. 1999; D.Zickler and A. Storlazzi, unpublished data). It is true that Rec8 is required for formation of an axial element in both budding and fission yeasts (Molnar et al. 2003; Klein et al. 1999). However, the existence of earlier structural organization has not been assessed in either case; moreover, in budding yeast, axial element formation occurs quite late, essentially concomitant with SC formation (Padmore et al. 1991), in accord with the possibility that earlier (basic) axis development is still normal in a *rec8* mutant. It begins to look as though molecules important for development of mitotic prophase chromosome axes should be prime candidates for basic axial building blocks, e.g., Topoisomerase II (e.g., Saitoh and Laemmli 1994; Gimenez-Abian et al. 1995) and shape-determining architectural proteins (Strick and Laemmli 1995).

Connections between homolog axes

Cytological studies have detected prominent linkages between homolog axes at three stages of meiosis.

1. The most prominent, of course, is the synaptonemal complex (Heyting 2005; Page and Hawley 2004).
2. Before SC formation, many organisms exhibit presynaptic co-alignment of homolog axes at a distance of ~0.4 μm. In favorable cases, this configuration is seen to be mediated by discrete interaxis "bridges" (Albini and Jones 1987). In some organisms, presynaptic alignment is a prominent discrete stage that occurs throughout the chromosomes before initiation of SC formation anywhere in the genome (e.g., Zickler

1977). In other organisms, bridge formation/alignment is usually observed in nuclei that, in other regions, have already begun to form SC, implying greater asynchrony of different events within individual nuclei. Presynaptic alignment and/or bridges have been reported in plants, animals, and fungi; but not, thus far, in the two best-studied invertebrates, *Drosophila melanogaster* and *Caenorhabditis elegans*. (Further discussion below).

3. Bridges between homolog axes have also been observed at a much later stage, after pachytene, at the sites of chiasmata on diplotene bivalents, e.g., in rat. These late bridges contain SCP2, SCP3, SMC1β, and SMC3, but not Rec8, in rat (Eijpe et al. 2003).

Crossing-over at the DNA and axis levels via linked parallel pathways

Three requirements of chiasma formation

Regular segregation of homologs to opposite poles at the first meiotic division is directed by the presence of a physical connection between the two segregating units, just as for segregation of sisters during mitosis. In most organisms, connections between homologs are provided by chiasmata, each of which corresponds to a reciprocal exchange of arms between one sister of each homolog. Correspondingly, programmed recombination at the DNA level is a prominent unique aspect of the meiotic program.

In addition, as chromatin is organized along structural axes from meiotic prophase onward, crossing over with the DNA/chromatin must be accompanied by an analogous process of exchange at the axis level, at the same sites as, and between, the same two chromatids as exchange at the chromatin/DNA level. The existence of such exchange is apparent from images of diplotene chromosome in which the axes are differentially stained (Fig. 2). The need for specialized axis-related events at sites of chiasmata is also apparent from the fact that Rec8 is present at diplotene between sister arms along their entire lengths, except that it is absent at chiasmata (Eijpe et al. 2003).

In summary, formation of a chiasma involves a coordinate set of local changes that include not only (1) exchange at the DNA/chromatin level but also (2) exchange at the axis level and (3) separation and differentiation of sister chromatids at both levels, all of which must occur at the appropriate corresponding positions along the involved elements (Blat et al. 2002).

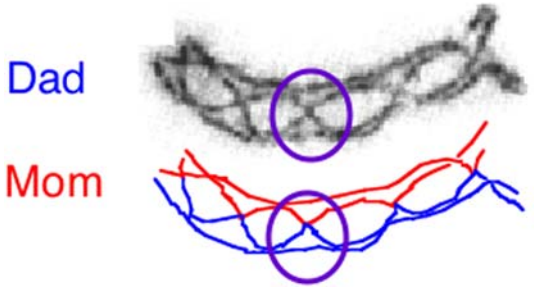

Fig. 2 Staining of diplotene bivalent axes reveals exchange at chiasmata. *Circle* highlights the fact that chiasmata involve not only exchange at the chromatin and axis levels but differentiation and separation of sister chromatids at both levels. (from Blat et al. 2002)

Recombinosome-axis association

A particularly striking feature of meiotic recombination is that the involved biochemical complexes, the "recombinosomes," are spatially associated with their underlying chromosome axes. This fact was first revealed by EM serial section studies, which identified prominent nodules and bars associated with the SC at pachytene, and by the accompanying appreciation that the number and distribution of these forms matches that of crossovers, as analyzed genetically, and/or chiasmata, as analyzed cytologically. A relationship between these "late" or "pachytene" nodules was further supported by the remarkable finding of Carpenter that DNA synthesis occurs at the sites of these so-called "late nodules" (von Wettstein et al. 1984; Carpenter 1975, 1981). This particular class of nodules is now known to correspond to immunostaining foci of Mlh1 and Mlh3, which show the same pattern as these late nodules (Barlow and Hulten 1998; Lipkin et al. 2002). Other types of recombination nodules and correlated immunostaining foci occur along SCs earlier in pachytene; moreover, axis-association of recombinosomes is also apparent at the presynaptic bridge stage (below).

In principle, there are two ways in which a spatial juxtaposition of recombining DNA sequences and chromosome axes could arise: (1) recombination might occur preferentially between DNA sequences that are located near the bases of their corresponding chromatin loops and (2) recombination might occur within sequences that are, organizationally, far from the bases of the loops, but with the corresponding recombinosomes becoming "tethered" to their underlying axes at some point in the process. Molecular studies have revealed that the second of these possibilities is in fact the case: recombination occurs within "tethered loop–axis complexes" (Blat et al. 2002; Fig. 3a).

What is the rationale for recombinosome/axis association?

Recombination occurs perfectly efficiently in situations where chromosome axes are absent, i.e., in prokaryotic organisms and during double-strand break (DSB) repair at early stages of the eukaryotic mitotic cycle. As a result, recombinosome–axis association is usually linked to special requirements of meiosis. Furthermore, most considerations suggest that axis association exists because it provides additional useful features to the recombination process. This feature characterizes many models for the role of the SC, as well as explanations for the pre-SC roles of axis components. However, we have proposed a different idea that recombinosome/axis association exists

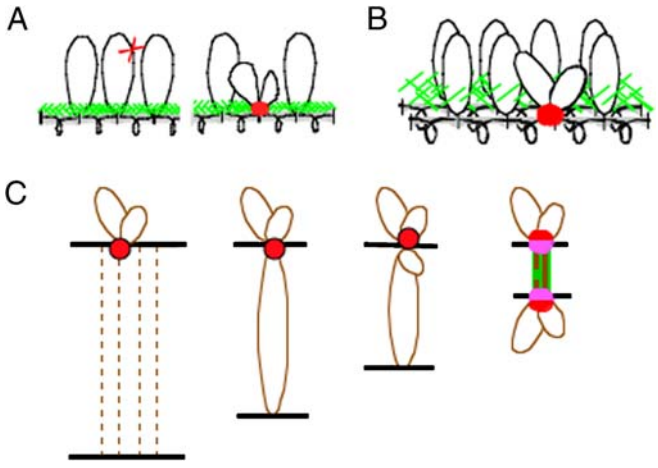

Fig. 3 Recombinosome–axis relationships. **a** Recombination occurs in tethered loop–axis complexes (Blat et al. 2002). **b** Association of a recombination complex to the base of its corresponding loop simultaneously marks the relevant position along the chromatin, along the axes, on the same chromatid at both levels puratively in a "three-way flaw" (Blat et al. 2002). **c** Reeling-in model for formation of interaxis bridges (Tessé et al. 2003; text)

due to the requirements of exchange at the structural axis level. Localization of the recombinosome to the base of its corresponding loop would target axis exchange such that it occurs at the appropriate position, specifically along the axis of the chromatid that is involved in exchange at the DNA level, and would permit temporal and functional coordination of relevant events at both levels (Fig. 3b).

It is now clear that recombination at the DNA level involves a progression of programmed changes that occupy most of prophase and that the corresponding recombinosomes are associated with chromosome axes at many or all of these stages (below). Correspondingly, the progression of local changes at the DNA level that lead to formation of a crossover should be accompanied by a correlated progression of local changes along the axes that lead to exchange at the axis level. This in turn leads to the idea of a "linked parallel progression of changes at the DNA and axis levels," with coordination of events at the two levels along one particular chromatid on each partner, mediated by direct physical association between recombination complexes and their underlying axes (Blat et al. 2002).

DNA events of recombination and their temporal and functional relationships to basic meiotic prophase chromosomal stages

Meiotic recombination is initiated via programmed DSBs mediated by the topoisomerase-like protein Spo11. Formation of a break is accompanied by specific resection of 5′ strand termini to give molecules with ~300 nt 3′ single-stranded tails (Keeney 2001; Neale et al. 2005). The majority of these DSBs appear to go on to interact with the homologous sequence on a homolog chromatid, rather than with the sister chromatid (Zickler and Kleckner 1999). However, only a small subset of these interactions ultimately yields a crossover. The remainder is resolved in such a way as to restore two intact DNA molecules but without accompanying exchange of flanking DNA sequences/chromosome arms. To put it in another way: "many are called, but few are chosen." More specifically, at a particular point in the recombination process, a subset of interactions is designated for maturation into crossover products, with exchange of flanking DNA/chromosome arms, while all remaining interactions are designated for simple resolution without crossing-over. Designation of certain recombinational interactions for eventual maturation into crossovers is a tightly controlled process, which results in specific spatial patterning of resulting crossovers/chiasmata (e.g., Kleckner et al. 2004).

It was long thought that crossover/noncrossover differentiation occurred at the very end of the recombination process, at the time of (double) Holliday junction resolution. However, recent studies have shown that this crucial differentiation step occurs much earlier, before stable, extensive interaction of DSB "tails" with a homologous partner DNA sequence (Hunter and Kleckner 2001; Börner et al. 2004; Bishop and Zickler 2004). The exact nature of the DNA species upon which crossover control operates has not been defined.

Once differentiation occurs, crossovers are formed via two long-lived intermediates: single-end invasions (SEIs) and double Holliday junctions (dHJs) (Hunter and Kleckner 2001; Börner et al. 2004; Allers and Lichten 2001). The pathway for formation of noncrossovers has not been elucidated, but synthesis-dependent strand annealing is a currently favored possibility (Allers and Lichten 2001).

Two secondary points regarding crossover/noncrossover differentiation are also noteworthy. First, synthesis-dependent strand annealing may also involve something like an SEI intermediate, albeit shorter lived and, thus, not detected in mutants where the crossover-specific pathway is abrogated (e.g., Börner et al. 2004). In that case, crossover/noncrossover differentiation of recombinosomes at the functional level may occur before appearance of any difference in the structures of involved DNA intermediates. Second, in some organisms, a subset of crossovers arise that do not obey the spatial patterning rules of the majority "specifically designated" crossovers (e.g., Copenhaver et al. 2002). These "extra" crossovers have been proposed to arise either as a minority species along the noncrossover pathway (N. Hunter in Börner et al. 2004) or via a third "branch" (Whitby 2005).

The DNA events of recombination occur in a specific, evolutionarily conserved, temporal relationship to the basic cytologically defined stages of meiosis in most, possibly

all, organisms (Figs. 4a,b and 5).[1] DSBs occur at leptotene, followed by bridge formation and presynaptic co-alignment. Crossover designation occurs at late leptotene, followed by nucleation and extension of SC. SEIs and dHJs occur during pachytene in the context of full-length SC. Finally, maturation of dHJs into crossovers during late pachytene. These relationships are known by direct analysis of DNA events in yeast (Padmore et al. 1991; Hunter and Kleckner 2001) and, for crossovers, in mouse (Guillon et al. 2005). Noncrossover products appear either at the same time as, or before, crossovers depending upon the exact situation (temperature and/or molecular construct) (Storlazzi et al. 1995; Börner et al. 2004; Allers and Lichten 2001). These same relationships also occur in many other organisms, as shown by analysis of immunostaining foci corresponding to recombination complexes. Because the length of the corresponding prophase period varies from hours to days to weeks according to the organism (Bennett 1977), this commonality is one of several indications that the progression of DNA events is governed by the overall meiotic program rather than by the rate at which biochemical steps can be completed.

Recombination and axis-related events are not only temporally correlated, but are directly functionally linked. (1) Formation of interaxis bridges is dependent upon formation of DSBs and thus appears to be mediated by the recombination process itself. Indeed, the only molecular components of these bridges known thus far are recombination proteins, notably RecA homologs and the single-strand binding protein RPA (e.g., Franklin et al. 1999; Tessé et al. 2003; Tarsounas et al. 1999; Plug et al. 1997; Moens et al. 2002; de Vries et al. 2005; Fig. 5). It remains to be determined whether these recombination complexes comprise the bridges or whether they are layered upon structural components. Given the existence of tethered loop–axis complexes (above), it has been proposed that a DSB present in a recombination complex associated with one homolog axis would identify the corresponding sequence in a homolog partner, thus triggering the "reeling-in" of the partner axis (Tessé et al. 2003; Fig. 3c). (2) Installation of SC involves specific nucleation followed by polymerization outwards from nucleation sites. Nucleation occurs preferentially at the sites of recombinational interactions that have just been designated to become crossovers (Keeney 2001). More specifically, it appears that SC installation is nucleated specifically at sites of crossover-designated interaxis bridges, concomitant with their disappearance (for discussion, see Börner et al. 2004 below; Fig. 5).

In both of these phenomena, recombination-related processes are determinative of structural progression. Other likely related phenomena were also described.

To cite a few early examples (Alani et al. 1990; Bishop et al. 1992): in yeast, a *rad50* null mutation eliminates DSB formation and also eliminates formation of axial elements, as well as SC; a *rad50S* mutation permits DSB formation, but eliminates resection at the ends of the breaks and ensuing interaction with partner DNA permits axial element formation and only a negligible amount of SC; and a *dmc1* null mutation that permits DSB formation and resection but blocks immediately following steps permits axial element formation and substantial (albeit significantly delayed) SC formation.

It is also clear that, oppositely, axial structure is important for recombination (below).

Linked parallel pathways of DNA and axis changes

The model proposed above envisions that recombinosome/axis association targets axis changes to appropriate sites and permits their functional integration with events at the DNA level. In accord with the proposed targeting role, recombinosome/axis association is established very early in the chiasma formation pathway. It is clearly present by the time of the "bridge stage" and even earlier, during bridge formation; for example, in *Sordaria*, Rad51 foci can be found on or between progressively juxtaposing homolog axes (Tessé et al. 2003). Additionally, prominent nodules can be observed in leptotene along chromosome axes that are not yet in detectable proximity to their homologs (e.g., Zickler and Kleckner 1999). These structures might well represent DSB-containing recombination complexes that are associated with their underlying homolog axis even before the DSB recognizes a homolog partner.

However, it is as yet unclear whether recombination complexes are axis-associated even before recombination begins, i.e., before DSB formation, or whether this association is established immediately after a break has occurred. Mitotic DSB repair occurs in the context of chromosome structure proteins, as shown by DNA repair defects in mutants lacking cohesin or the cohesin-associated axis component Spo76/Pds5. Recent molecular studies show that at least one cohesin accumulates in a large domain around the site of the break (Unal et al. 2004). It has been suggested, by analogy, that the programmed DSBs of meiosis occur outside of the context of chromosome axes and then migrate to the chromosome axes thereafter (van Heemst and Heyting 2000). Consistent with this possibility, in *Sordaria*, where axes are uniquely discernible from very early leptotene on, Spo11 does not exhibit obvious spatial association with chromosome axes (Storlazzi et al. 2003). On the other hand, in yeast, deletion of any of three known meiosis-specific axis components (Red1, Hop1, or Rec8) alters the efficiency and/or

[1] It has been proposed that the relationship of DNA events to cytological stages might be different in Drosophila from that described above as the general case. Specifically, it has been suggested that DSBs occur after SC formation (McKim et al. 2002). The primary direct evidence for this view is that gamma-H2AX foci, which mark the sites of both mitotic and meiotic DSBs, can be observed only at pachytene. However, this finding would imply only that SCs form very rapidly after DSB formation, e.g., with little or no "bridge stage." Alternatively, gamma-H2AX foci have recently been reported also to occur during the pachytene stage in grasshopper (Viera et al. 2004) as a second wave, separate from the late leptotene wave. This raises the additional possibility that, for some reason, only the later foci are detected in Drosophila, thus leading to mistiming of DSB formation.

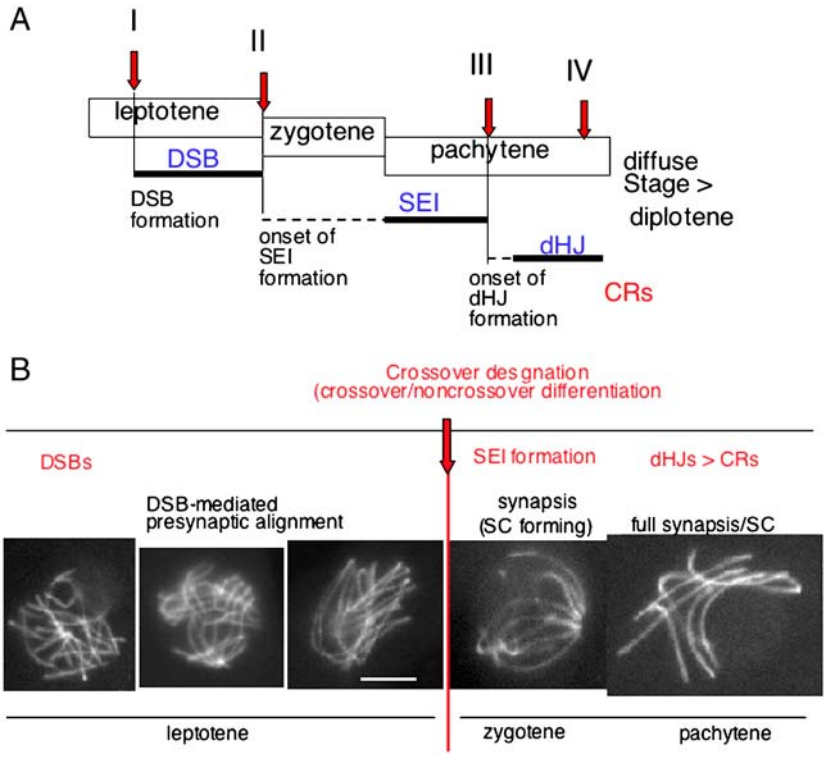

Fig. 4 Relationship between DNA events of crossover formation and chromosome morphogenetic changes. **a**. Four DNA transitions. **b**. Events relative to chromosomal stages depicted by staining of Sordaria chromosome axes by Spo76-GFP (images courtesy of D. Zickler)

positions of DSBs (e.g., Blat et al. 2002; Glynn et al. 2004; A. Jordan and N.K. unpublished data), implying some type of participation of these components in break formation. Similarly, in *S. pombe*, mutants lacking Rec8 exhibit severe DSB defects (Ellermeier and Smith 2005). Furthermore, Spo11 is also found to associate with chromosome axes in many organisms at post-DSB stages, implying that there is the potential for such association before DSB formation.

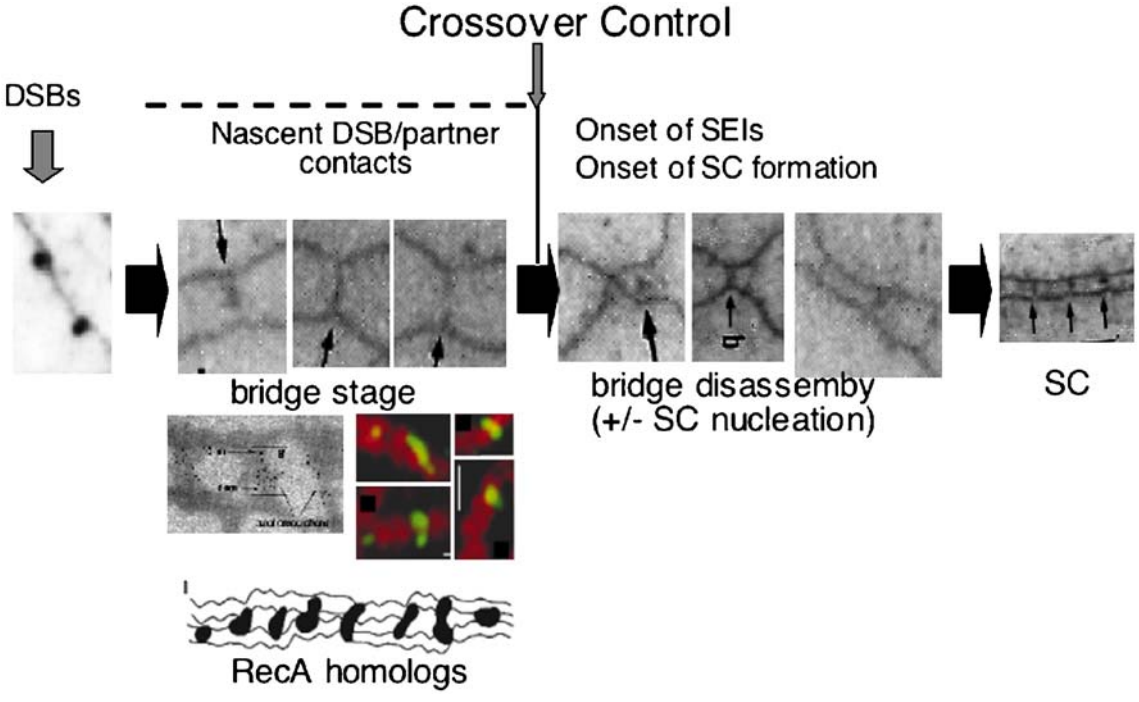

Fig. 5 Axis association of recombinosomes and temporal relationships between DNA events and axis changes. Association established before or immediately after DSB formation, leading to nodules associated with individual homolog axes. Next stage involves interaxis bridges that include RecA homologs. Finally, axes are associated via SC, with associated nodules that represent both pre-crossover and pre-noncrossover recombinational interactions. Images are from Albini and Jones 1987 except far left (Stack and Anderson 1986) and RecA homologs (from Franklin et al. 1999 and Tarsounas et al. 1999)

Answering this question is further complicated by the fact that DSB formation might, in some situations, be contemporaneous with axis development, thus opening the way to even more complex functional interrelationships.

Precisely what type of axis changes might be involved in chiasma formation and when might they occur? It seems probable that the pathway of local axis changes mirrors the pathway of DNA events. DSB-mediated bridges appear to represent essentially all initiated recombinational interactions. Thus, recombinosome-axis associations before this transition should be involved in setting up the bridge stage. Notably, this process will involve concomitant targeting of appropriate axis sites on the partner axis, as well as on the initiating (DSB-associated) axis. Crossover designation then occurs at a specific subset of bridge ensembles, concomitantly triggering SC nucleation, at the leptotene/zygotene transition (above). It is therefore expected that the first crossover-specific change at the axis level should also occur at this stage. What could this change be? Clearly, for axis exchange to occur, a "hole" must be created in each of the two involved axes; perhaps, this is the event that occurs during crossover designation, with formation of the SC helping to stabilize axial structure across these holes. This model could explain why there is a pronounced tendency for axes to be destabilized in a recombination-dependent manner in certain mutant conditions, specifically at this stage (e.g., van Heemst et al. 1999; Storlazzi et al. 2003). It is also interesting that crossover-designated recombination complexes are more tightly associated with newly formed SC than are noncrossover complexes (Sherman et al. 1992), suggesting that recombinosome association may also provide extra stability around these "sensitized sites." At the DNA level, finalization of crossing over is well-separated functionally and in time from crossover designation. The same would be true of axis exchange. Given that crossover designation results in creation of axial holes, actual exchange of the interrupted axes might then occur during pachytene, concomitant with late stages of crossover formation at the DNA level. We have proposed that this exchange is mediated by twisting of the SC (Börner et al. 2004; below).

How and why meiotic prophase might have evolved from the latter stages of the mitotic program

It is becoming increasingly apparent that proteins normally involved in regulating post-prophase stages of the mitotic cell cycle are playing key roles in meiotic prophase, e.g., polo kinase (Clyne et al. 2003). These molecular findings are anticipated by appreciation of several cytological relationships (Kleckner et al. 2004). First, both meiotic prophase and mitotic prometaphase can involve interaxis bridges between homologs in the former case (above) and between sisters in the latter (Gimenez-Abian et al. 1995). Second, the *Sordariaspo76-1* mutation confers analogous tendencies for sister separation and chromatin diffuseness at meiotic late-leptotene and at mitotic prometaphase (van Heemst et al. 1999). Third, meiotic prophase involves global cycles of histone H3 phosphorylation, a prominent hallmark of mitotic prometaphase (Kleckner et al. 2004). Finally, three cycles of global chromatin expansion and contraction can be identified in meiotic prophase and suggestively correlated with three corresponding cycles of the mitotic program that encompass metaphase, pre-anaphase/anaphase, and telophase/G1 (Kleckner et al. 2004). Taken together, these findings suggest that meiotic prophase has evolved directly from the latter stages of the mitotic program.

Why should this be the case? The key role of meiotic prophase is to produce chiasmata. Production of chiasmata involves local changes at the DNA level, and these appear to have evolved from the mitotic DNA DSB repair process. However, during meiosis, these DNA changes are accompanied by corresponding local changes in chromosome structure, along chromosome axes and between sister chromatids, and changes in these structural components are a hallmark of the latter stages of the mitotic cell cycle. To put it another way, during meiotic prophase, structural changes that would normally occur globally all along the chromosomes, e.g., sister separation and axis coiling, are constrained from occurring by robust axial structures and instead occur locally, targeted specifically to the sites of recombination reactions via tethered loop–axis complexes. Thus, evolution of the meiotic prophase program can be envisioned, coupling of the local events of DNA repair to global chromosome structure changes of mitotic prometaphase and later stages and, concomitantly, the corresponding cell cycle regulatory mechanisms. The mechanical model for chiasma formation described below is also in full accord with this possibility, with changes at the structural level playing a key role in triggering changes and progression at the DNA level.

A mechanical model for chiasma formation

Crossover interference implies communication along chromosomes

In most organisms, the distribution of crossovers/chiasmata along and between meiotic chromosomes is strikingly nonrandom. Three features are particularly striking (e.g., Kleckner et al. 2004).

First, along a particular chromosome, events occur at different positions in different meiotic nuclei, implying a stochastic element to the designation of crossover sites.

Second, every pair of homologs (every "bivalent") acquires at least one crossover/chiasma, in accord with an essential role in homolog segregation. Moreover, this requirement is not met by the occurrence of a very large number of randomly distributed crossovers sufficient to ensure that the probability of zero events is very small: the average number of crossovers per bivalent is small, usually two or a few, and sometimes one and only one.

Third, when two or more crossovers are present, they tend not to occur near one another, a phenomenon known as "interference." The magnitude of this effect is maximal

at short intercrossover/chiasma distances and decreases progressively with increasing inter-event distance.

The entire phenomenon of crossover designation can be viewed as follows. Initially, a first undifferentiated precursor interaction is designated for eventual maturation into a crossover/chiasma. This first event sets up interference, which disfavors the occurrence of additional crossover designation events nearby. As a result, any second crossover designation event will tend to occur away from the first. More generally, as subsequent designation events occur, they will tend to fill in the holes between the sites of prior events. The overall result of these effects is a tendency for crossovers/chiasmata to be relatively evenly spaced along the chromosomes.

The phenomenon of interference is of particular interest because it implies the existence of communication along the chromosomes. The basis for this communication is unknown. However, it is important that the patterning exhibited by meiotic crossovers/chiasmata is also observed for a number of basic chromosomal features, e.g., replication origin firings, nucleosomes, and chromosome coils (Kleckner et al. 2004). Thus, analysis of meiotic crossover/chiasma distributions could potentially shed light on many types of fundamental chromosomal phenomena.

A mechanical model for crossover designation and interference

We have proposed that crossover designation and accompanying interference occur by a mechanism that involves imposition and relief of mechanical forces (stresses) along the chromosome axes (Kleckner et al. 2004). Involvement of mechanical forces is an attractive explanation for several reasons, as discussed in detail below. Most importantly, such a mechanism can automatically explain interference because, in a physical system, communication can occur automatically via the redistribution of mechanical stress or stress relief.

As mentioned above, it appears that crossover designation and accompanying patterning of crossover sites occur at the "bridge stage." A specific physical model for the basic events of this process could be as follows (Fig. 6): mechanical compression (pushing) stress arises all along the length of the axes. Interaxis bridges, being discontinuities, comprise weak points or "flaws" along the axes. Thus, as the level of pushing force rises, one end of some bridge will eventually give way, with concomitant buckling of the axis at the corresponding point. This effect, in turn, will place the affected bridge ensemble in a new configuration. And by hypothesis, that configuration would commit the corresponding recombinational interaction to the crossover fate.

This model readily accommodates the basic features of crossover control as described above. Crossover designation events will occur at different positions along a given chromosome in different meiotic nuclei according to which particular sites are affected by stress forces. Occurrence of at least one crossover along each homolog is easily achieved by ensuring that the imposed stress reaches a suitably high level. And most importantly, this model automatically explains crossover interference as follows. (1) Buckling of a bridge will result in local relaxation (relief of compression stress) at the site of the buckle. (2) Disassembly of the bridge will then result in a corresponding buckling and local relaxation on the partner axis. (3) Given the elastic nature of chromosomes, local relief of stress will automatically tend to redistribute, spreading outward along the two axes from the two nucleation sites. (4) The result of this spreading relief of stress is a corresponding decrease to the probability that additional (stress promoted) crossover designation events will occur within the affected region.

A common example of stress redistribution occurs when a rubber band is pulled and then cut. The rubber band relaxes not only at the site of the cut, but all along its length. In our model, the imposed stress is tensile ("pulling") rather than compressive ("pushing"), but the principle is the same. It is also important in our case that relaxation and relief of stress will not extend along the entire length of the chromosome but instead will tend to be absorbed by other ("stressed") components as it spreads,

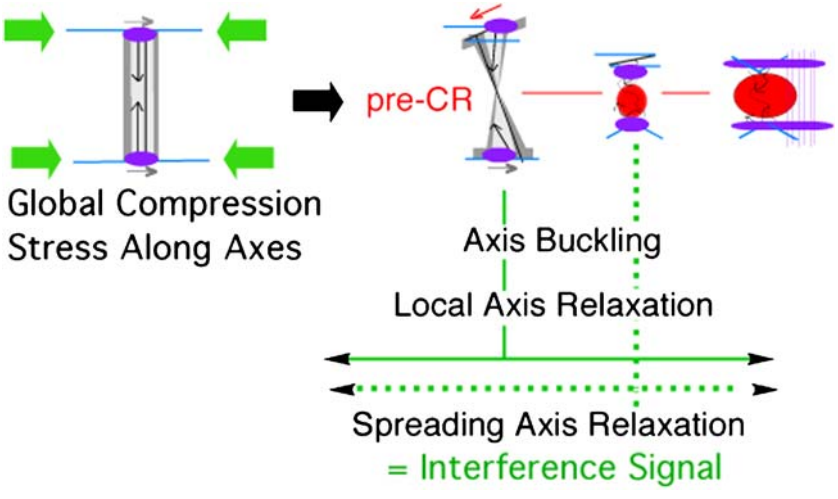

Fig. 6 Mechanical model for crossover designation and interference via local and spreading axis relaxation. SC nucleation and spreading (not shown) would occur as secondary effects. (Börner et al. 2004)

thus decreasing in intensity with distance. The effect is a graded spatial domain of stress relief whose dimensions define the magnitude and extent of crossover interference.

One prediction of this model is that interference would not involve polymerization of the synaptonemal complex. This prediction has recently been verified (below). This model further predicts that the axes of the homologs will behave asymmetrically, because one axis is affected before the other. Exactly such asymmetry has been observed in *Sordaria humana*. At pachytene, axial bulges are observed specifically in the vicinity of crossover-correlated recombination nodules and specifically on only one homolog axis (Zickler and Sage 1981). Finally, this model can even accommodate the requirements of a mathematical model, which proposes that adjacent crossover interactions are separated by a fixed, integral number of noncrossover interactions (Lande and Stahl 1993). This model is interpreted to mean that interference mechanism "counts" recombinational interactions; the basic point can be restated more formally to say that the distance along the chromosome over which interference is "felt" is influenced or determined by the number of "undifferentiated" ensembles encountered by the spreading interference signal. Our stress model for interference readily explains such an effect: as stress relief spreads along the chromosome axes, there will be a tendency for it to be absorbed differentially at the positions of encountered bridge ensembles because these are especially stressed positions. Correspondingly, the effective stress relief distance could be influenced, or even specifically defined, by the number of undifferentiated bridge ensembles encountered.

Source of the force?

How would compression stress arise along chromosome axes? We have proposed that stresses are created by chromatin expansion. It is normally envisioned that chromatin decompaction is a passive disassembly process. We suggest instead that when chromatin decompacts into a large volume that expansion occurs in such a way as to exert a force; that expansion force would push on any constraining components, which would push back. These diverse compression stresses would then perform chromosomal work (Kleckner et al. 2004). For the particular case of late leptotene chromosome axes, compression stress would be generated because the presence of the axis would constrain free expansion of the chromatin loops of the co-oriented linear loop arrays. Adjacent chromatin loops would push against one another, initially causing extension and distension along the axis. But as the axis reaches its maximum degree of extension, chromatin will tend to expand differentially in the "tops" of the loops where there is more "room" as compared to along the axis. As a result, the axis will tend to bend, with outward forces along the tops of the loops (generating tension) and opposing inward pushing forces along the axes (generating compression) (Fig. 7). (In fact, in addition to compression stresses, chromatin expansion forces are predicted to result in a tendency for axis twisting, which will place adjacent loops out of phase (Fig. 7). Both types of forces could be focused coordinately, specifically at the flaws created by bridges, thus promoting bridge buckling.) Buckling of the axis would alleviate axial compression stress, and this alleviation would then spread outward for some distance along the axis, disfavoring the occurrence of additional stress-promoted crossovers nearby, to a decreasing extent with increasing distance.

In accord with this model, with respect to meiotic crossover control, it has been shown that there is a tendency for global chromatin expansion specifically at late leptotene, exactly at the time of crossover designation and interference, followed by a tendency for global chromatin compaction during zygotene (Fig. 8).

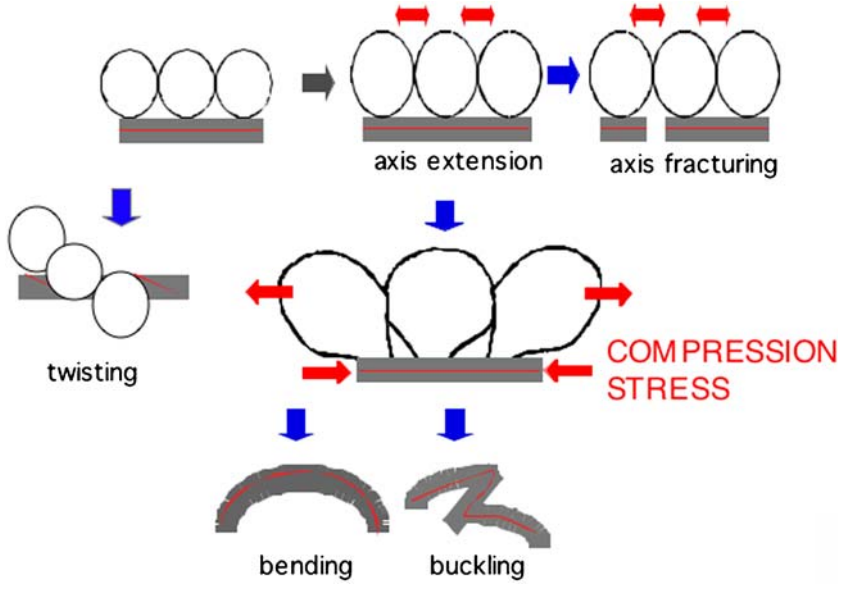

Fig. 7 Predicted effects of chromatin expansion forces arising within a linear loop axis array (from Kleckner et al. 2004)

Generalization of the model

In the model described above, global chromatin expansion creates stresses along the chromosome axes; these stresses are focused specifically at the sites of axis-associated recombination complexes because they comprise the weakest points along the axes. When a weak point (flaw) gives way, it does so in a programmed way so as to produce the desired effects. An analogous sequence of events could, of course, occur at any step of the recombination reaction.

An even more general description of chiasma formation can be formulated by considering components other than chromosome axes. Chromatin expansion could promote local changes at all three of the levels required for chiasma formation (see above, Kleckner et al. 2004). (1) Chromatin expansion could also create compression stresses along the DNA/chromatin fiber that could promote buckling or local denaturation of the DNA duplex and, thus, important transitions at the DNA level. (2) Compression stress along chromosome axes can promote a variety of different types of axis changes beyond the buckling described above. (3) Expanding sister chromatin masses would tend to push one another apart, with a resulting tendency for separation both within the chromatin and along the two underlying chromatid axes. Furthermore, all three types of stresses should be focused specifically to the same positions along the corresponding components via axis-associated recombination complexes: in this context, these ensembles comprise "three-way flaws," which will target stress-promoted changes to the appropriate corresponding positions at all three levels (Fig. 3b). Moreover, as stress-promoted changes begin to occur, coordination of changes among components involved at the three different levels can occur via direct mechanical linkage.

Such a scenario could be involved in any or all steps along the chiasma formation pathway. In each case, global chromatin expansion would impose stresses within the chromatin, along chromosome axes and between sister chromatids at both levels, and those stresses would be targeted and transduced into appropriate local changes at all three levels via axis-associated recombinosomes. Moreover, redistribution of stress relief along the chromosomes could occur not only along chromosome axes, but also along the chromatin or along inter-sister boundaries, and not only at the leptotene/zygotene transition, but also at earlier stages, e.g., during DSB-formation.

In accord with these ideas, meiotic prophase involves several different periods of global chromatin expansion; each such period is accompanied by tendencies for sister separation, as seen at the chromatin or axis levels, and by tendencies for chromosome destabilization (e.g., Fig. 8). Each of the important and known transitions of recombi-

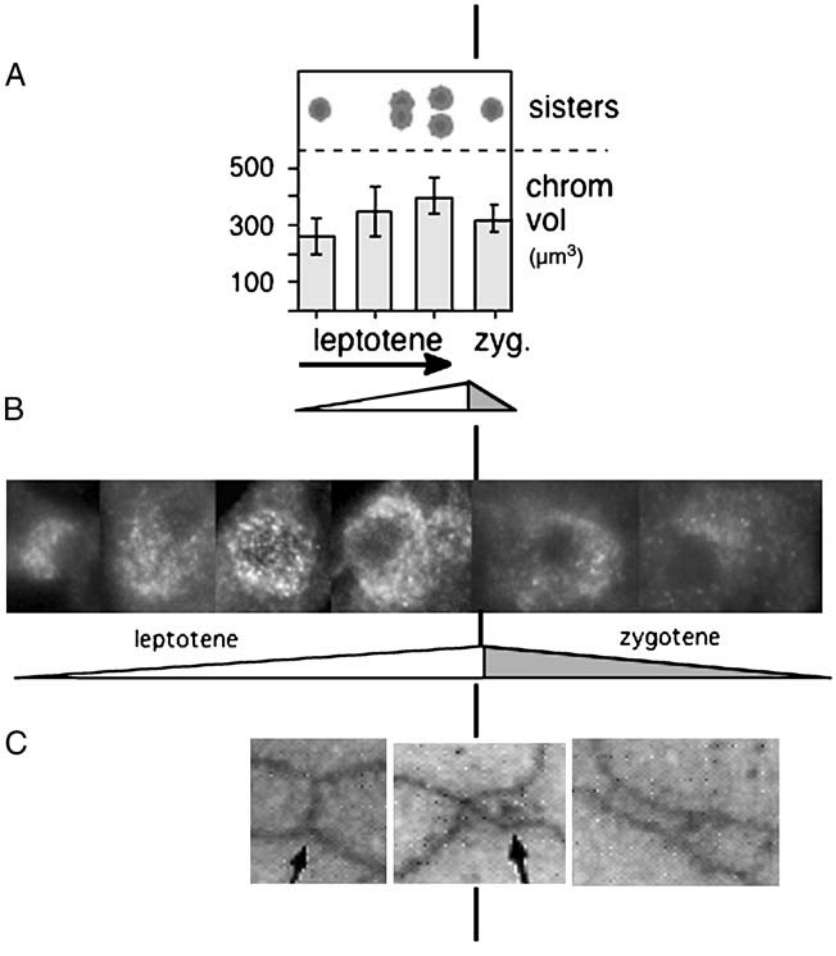

Fig. 8 Events of the leptotene/zygotene transition. **a** Chromatin volume increases and decreases with concomitant individualization and reconjunction of sister chromatid chromatin (Dawe et al. 1994). **b** Progressive increase and decrease in histone H3 phosphorylation in Sordaria (D. Zickler in Kleckner et al. 2004). **c** Disappearance of bridges with concomitant nucleation of SC (Albini and Jones 1987)

nation at the DNA level occurs during one of these expansion periods (Kleckner et al. 2004; Zickler and Kleckner 1999; Dawe et al. 1994; van Heemst et al. 1999). The crossover control transition modeled above occurs at leptotene/zygotene. A model for a later stress-mediated transition at pachytene is described below. It would not be difficult to construct a model for stress-mediated DSB formation, with or without axis-association of recombination complexes.

Complexities of the leptotene/zygotene transition viewed through the lens of the stress hypothesis

Crossover designation and interference are explained "in principle" by imposition of stress plus local relief of stress and redistribution of that stress relief along the chromosome axes. But this is just the tip of the iceberg with regard to the multiple requirements and events of this period, and new ways of looking at these events emerge by considering additional specific predictions of the stress hypothesis.

Chiasma designation at the axis level Commitment to crossing over at the DNA level should be accompanied by commitment to crossing over at the axis level, which, as suggested above, might involve creation of axial holes at the appropriate positions along the two involved chromatids. It is easy to envision how mechanically promoted axis buckling and ensuing elimination of interaxis bridges might promote creation of such holes, i.e., at the "bridge abutments."

Axis extension/distension and relaxation Chromatin expansion should first cause the homolog axes to become more extended (distended) as a simple way of alleviating pushing forces between adjacent loops; then, once the axis reaches its extension limit, compression stress begins to arise (Fig. 7). If axis relaxation occurs locally (as the result of crossover designation) or globally by redistribution of stress relief (i.e., the "interference signal"), expansion stresses will be alleviated and axes will become shorter and floppier. Axis extension and relaxation could play crucial roles in several aspects of the crossover control transition beyond crossover designation and interference in the general sense, as described in the next two sections.

Regulated interplay between progression and fate Crossover/noncrossover differentiation has several requirements not discussed above. First, regulated differentiation of pre-chiasma interactions into pre-crossover and pre-noncrossover types requires that the chiasma formation process be arrested specifically at the decision point so that differentiation can be imposed. Arrest presumably involves some feature of the bridge ensemble while progression is presumably licensed by bridge disassembly. Second, by the scenario, an interaction is specified for maturation into a noncrossover simply by the "absence of crossover designation." Taken together, these requirements suggest that bridge ensembles may all arrest in a pre-noncrossover mode. Specific programmed events then designate some ensembles to be crossovers/chiasmata, concomitantly triggering bridge disassembly, and thus licensing progression toward that fate. To ensure that "leftover" ensembles proceed to the noncrossover fate, it would be necessary only to release the progression block, i.e., to eliminate the corresponding bridges. How are bridge ensembles arrested and what promotes the disassembly of bridges that are not crossover/chiasma designated? We suggest that extension/distension of axes is required to stabilize and arrest bridge ensembles at the decision point. If so, local stress relief will destabilize bridges at sites of crossover designation while the spreading stress relief signal that mediates interference will concomitantly trigger destabilization of encountered nondesignated bridges, thus sending their interactions toward the noncrossover fate. Furthermore, any bridges that are too far away from a crossover site to be affected by the interference signal will be disassembled automatically during global chromatin contraction and accompanying global relief of stress at zygotene (Fig. 8).

SC formation Crossover designation is accompanied by nucleation and polymerization of SC. These processes clearly require elimination of bridges, which hold homolog axes apart at a too far distance. We further propose that SC installation requires relaxation of homolog axes, e.g., to permit stabilizing contacts between adjacent central region transverse filament or central element subunits. By the combination of both effects, SC would initially be installed specifically at crossover designated sites and then would spread outward, following and responding to the interference signal, with installation in any leftover regions during global relaxation at zygotene. Involvement of axis relaxation in SC formation may also help to explain certain other phenomena. For example, SC is installed promiscuously at late pachytene in several organisms (Zickler and Kleckner 1999) and this is a period of global chromosome contraction (Kleckner et al. 2004). Moreover, no late leptotene bridges are present at this stage. Thus, the only requirement for SC formation should be close juxtaposition of two axes, either by chance or by folding back of a single axis upon itself, as is commonly observed. In support of such a possibility, homolog axes are more "floppy" at the end of pachytene (Kleckner et al. 2004). Also, in several mutants where SC is installed very late in an aberrantly prolonged prophase, the resulting chromosomes have a marked "wiggly" appearance (e.g., Börner et al. 2004).

The above considerations highlight the fact that, for organisms that use the "canonical" program, initiation of recombination (plus, by hypothesis, appropriate global chromatin status) sets up conditions that prevent SC formation. This makes it possible for additional features to ensure that the structure is installed specifically at the sites of pre-chiasmata, with appropriate timing.

Given this situation, it might be expected that SC would form promiscuously in the absence of recombination initiation, analogously to promiscuous SC formation at late pachytene in other cases. Indeed, this is exactly the

case in *Drosophila* and *C. elegans*; however, in most organisms, abrogation of DSB formation severely compromises SC formation (for discussion, see Page and Hawley 2004; Pawlowski and Cande 2005). The latter phenotype may suggest that, in most cases, recombination-related features play essential positive roles in SC formation, as well as negative roles. For example, SC installation involves functional interplay between close local juxtaposition between homolog axes and relaxation/contraction along homolog axes; in *Drosophila* and *C. elegans*, axis relaxation may be strong enough that fortuitous contacts between homolog axes suffice to nucleate SC. In other organisms, in contrast, features that are related to programmed bridge disassembly might be required. In certain cases, homolog axes appear to come together and literally touch one another at sites of SC nucleation (Albini and Jones 1987; Zickler and Kleckner 1999), perhaps because of axis buckling and/or via contraction of RecA homolog/DNA filaments (Franklin et al. 1999). In any case, the unique behavior of *Drosophila* and *C. elegans* is often interpreted to mean that the two organisms have significantly different programs during wild-type meiosis. The above considerations suggest that this need not be the case. Subtle differences in the relative contributions of different components to SC formation and resulting differences in the effects of a DSB-minus mutation could be sufficient.

Implications of the stress hypothesis for pachytene events, recombination nodules and synaptonemal complexes

Our proposal suggests new ways of looking at the pachytene stage and the roles of the SC and (late) recombination nodules (RN). Perhaps these structures are so prominent because they primarily play structural roles. In the context of our proposed model, the SC might act as a transducer of mechanical stress forces, while late recombination nodules might target those effects to the appropriate sites, as discussed above. Additionally, the structural prominence of recombination nodules might reflect the fact that a robust physical entity is required to appropriately constrain and transduce global stress forces.

We have proposed that global chromatin expansion during the pachytene stage exerts its effects along chromosome axes by causing twisting of the SC (Börner et al. 2004). Twisting will be specifically promoted because it places the loops along each axis "out of phase" with one another, giving them more room to expand and thus alleviating chromatin expansional stress forces (Fig. 7). Moreover, twists are a common feature of pachytene chromosomes. Twisting forces, as well as other predicted effects such as tendencies for bending or buckling, will naturally be focused to the sites of late recombination nodules, thereby promoting the appropriate local effects at those sites.

Exactly what local effects are appropriate to this stage? At the DNA level, the promoted step(s) could be the SEI-to-dHJ transition and/or, perhaps more likely, the resolution of dHJs to crossovers. At the structural level, axis exchange must be finalized, via disruption of old linkages and formation of new ones. In this context, the SC could serve two key roles. First, SC twisting is precisely the motion required to promote axis exchange; thus, the SC might specifically direct this process (see Börner et al. 2004). Second, the SC might provide an external "clamp," stabilizing homolog associations via links in regions flanking the exchange point while promoted changes are in progress.

Domanial chromatin/axis interplay in mitotic and meiotic cells

The above discussion has focused primarily on local effects of chromatin expansion forces. There are two sets of observations that point to functional interactions between chromatin status and axis status, which could be explained by global effects of chromatin expansion/contraction status. Mammalian chromosomes are composed of locally GC-rich and AT-rich isochore domains whose differential staining leads to their designation as R-bands and G-bands, respectively; yeast chromosomes are similarly organized (for discussion, see Blat et al. 2002). In both cases, chromatin in GC-rich regions is more expanded relative to chromatin in AT-rich regions (Gilbert et al. 2004; J. Dekker, personal communication). In yeast meiotic chromosomes, the axis-associated protein Red1 is more abundant along axes in R bands than in G bands (Blat et al. 2002), and in mammalian chromosomes, chromosome axes are straight in R bands and coiled in G bands (Saitoh and Laemmli 1994). These features both point to some type of communication between domanial chromatin structure and axis status in which chromatin expansion status influences axis status. In the context of our stress hypothesis, increased Red1 loading might be explained by a tendency for wider (expansion promoted) separation between sister axes in R-band regions. Axis coiling is predicted to be promoted by chromatin expansion, the opposite of the correlation observed. On the other hand, effects along and between sister axes will be mechanically linked. Thus, if chromatin expansion forces differentially promote sister separation in R-band regions, stress forces might be differentially alleviated in those domains. With the result, there would be a lesser tendency for (stress promoted) coiling in *C. elegans* and *Drosophila* but less so in budding yeast or *Coprinus*.

What are the roles of the SC?

This issue can be subdivided into two related questions. First, what does the SC actually do for the meiotic program as it currently exists? Second, what was the evolutionary driving force that has resulted in the appearance of this structure?

Historical background

From the time of its discovery, the SC was known to mediate the intimate synapsis of homolog axes at pachytene. The discovery of SC-associated recombination nodules then triggered considerations of possible roles of the SC for crossover formation.

One idea of this type suggested that the SC might bring chromosomes together to make recombination possible. However, one early (and prescient) challenge to this idea was provided by was Maguire, whose studies of maize inversion heterozygotes led her to the conclusion that nucleation of SC formation is a consequence of, or very tightly linked in parallel with, what would now be called crossover site designation (Maguire 1966; Maguire 1972). A second challenge was raised by von Wettstein and Stern, whose preselection hypothesis considered that, because only a small fraction of DNA is located along/within the SC, there had to be some determinant that ensured that "corresponding regions on the homologs" were present there (Stern et al. 1975). The general underlying concern was the existence of a "chicken and egg" problem: if the SC mediated synapsis to permit recombination, how was it determined that the SC would form specifically between homologs? In any case, subsequent studies have shown that formation of SC is not the primary mechanism for bringing homologs together. Rather, it appears to be the very last in a series of steps (above). In fact, the functional relationship between recombination and SC formation turns out to be just the opposite from that originally proposed: in most situations, recombination is needed to bring homologs together so as to permit SC formation, rather than the other way around.

A second type of early model proposed that the SC is used for physical support of the recombination machinery (e.g., Moses 1969; von Wettstein et al. 1984; Egel 1995). This possibility is still viable. However, there remains the question of why such support would be necessary for meiotic recombination when it is not required for recombinational repair of double-strand breaks in mitotically dividing cells or for recombination in prokaryotes. One might invoke the possibility that chromosome movements during meiosis impose special requirements for physical stabilization; on the other hand, some of the most violent motions known occur in *S.pombe* (e.g., Yamamoto et al. 2001), where no SC is formed. Thus, it seems as if there must be more going on than physical stabilization.

A third set of proposed models invoked a role for the SC in crossover interference. The first such model emerged from Maguire's findings (above) and implied that SC polymerization might be used to mediate interference (Egel 1995; Maguire 1988). This model also regained prominence more recently for other reasons (Sym and Roeder 1994). A second early model assumed that crossover designation occurred during pachytene and envisioned the use of SC as static structure, i.e., a railroad track along which the interference signal would travel (King and Mortimer 1990; Stack and Anderson 1986; Carpenter 1987). Recent studies suggest, however, that neither of these models is correct. Instead, crossover interference occurs before and is independent of the SC, as seen in both in yeast and *Arabidopsis* (Börner et al. 2004; Heyting 2005; Bishop and Zickler 2004; Fung et al. 2004; Higgins et al. 2005), leading (e.g.) to the model described above.

Roles for SC in recombination

Mutants specifically lacking an SC central region protein have now been described in several organisms. Formation of crossovers is defective in all cases. However, the magnitude of the observed defects can vary widely among different situations. At one extreme, severe reductions in crossovers are observed, e.g., in *C. elegans syp1* mutants (MacQueen et al. 2002) and in yeast *zip1* mutants at high temperature and/or the BR strain background (Börner et al. 2004). At the other extreme, an *Arabidopsiszyp1* mutant exhibits 80% of the wild-type level of crossovers (as well as normal interference) (Higgins et al. 2005). These defects likely reflect roles for SC components at the time of SC nucleation at leptotene/zygotene (before SC polymerization). Defects at this stage are apparent in SC central region defective mutants of *Saccharomyces cerevisiae*, *C. elegans*, mouse, and *Arabidopsis* as evidenced by (1) delayed turnover of immunostaining foci corresponding to DSBs (of RecA homologs, RPA and/or Msh4/5) and (2) in yeast, at the DNA level, defective progression out of the DSB stage (de Vries et al. 2005; other references above). The severity of the recombinational progression block at this stage varies considerably from organism to organism and in yeast, according to both temperature and strain background, approximately in relation to the severity in the reduction of crossovers, suggesting that the block at this stage is primarily responsible for the observed mutant phenotypes.

Given these early defects, it is not possible to assess roles for the SC at later stages. However, because late steps in formation of crossovers occur during pachytene and prominent crossover-correlated recombination complexes occur along pachytene SCs, a role for the SC in these events seems probable (e.g., above).

Mutants specifically lacking SC central regions are also known in *Drosophila*; in this case, crossover formation is defective but the reasons as to why and at what stage(s) are not yet clear (e.g, Anderson et al. 2005).

Additional interesting possibilities for roles of the SC in recombination are raised by the properties of an *Arabidopsis* mutant lacking SC central region components, This mutant exhibits only very modest reduction in crossovers (which exhibit interference) (Higgins et al. 2005). In contrast, the major effect of the mutant is the occurrence of high levels of ectopic recombination. Although such an effect has not been reported in other organisms, the very high levels of homoeologous sequences in *Arabidopsis* may make it an exceptionally sensitive system for detecting such interactions, which are observable by basic cytological characterization of chromosomes.

This phenotype could reflect a role of the SC either in precluding the identification of ectopic partners at the DSB stage or in rejecting nascent associations before they are finalized. The latter seems more probable because (1) DSB/partner associations arise before SC formation (above); (2) global rejection of homoeologous associations is known to occur in allohexaploid wheat immediately after formation of SC between various pairs of appropriate and inappropriate partners (reviewed in Zickler and Kleckner 1999); and (3) the DNA mismatch repair machinery is known to specifically preclude conversion of homoeologous interactions into crossovers via a mechanism that requires formation of significant heteroduplex DNA (Hunter et al. 1996), and such DNA normally arises during meiosis at the leptotene/zygotene transition, concomitant with SC nucleation at the corresponding sites (Hunter and Kleckner 2001). Thus, one specific interpretation of the *Arabidopsis* findings is that SC central region components (e.g., patches of SC) are required for biochemical rejection of homoeologous interactions after the onset of stable strand invasion.

Roles for the SC beyond DNA recombination?

Axis exchange? As described above, we have suggested that the SC might be needed to promote axis exchange and/or or to stabilize homolog associations while that process is in progress (Börner et al. 2004). On the other hand, if the SC has such a role, how does one explain the case of *S. pombe* and *Aspergillus*, where large numbers of unregulated crossovers occur and homologs successfully disjoin without benefit of an SC (Molnar et al. 2003; Egel-Mitani et al. 1982)? The obvious response is that, in these cases, some other mechanisms come into play.

Sister cohesion? Maguire proposed that the SC per se was important for maintaining sister chromatid connections (Maguire 1995). This model was based on the finding that certain mutants make SC (albeit sometimes slightly abnormal in structure), but then after dissolution of the SC, exhibit defects in the number of chiasmata plus apparent exchange of sister chromatid pairing partners as indicated by the positions of homolog-specific heterochromatic knobs. In support of this possibility, (1) a yeast *zip1* mutant does show low levels of sister segregation at meiosis I in some conditions (N. Hunter and G.V. Börner, personal communication), as does a *Drosophila* mutant defective in SC assembly (Manheim and McKim 2003), and (2) cytological observations point to a tendency for sister axis separation along SCs at midpachtyene (Zickler and Kleckner 1999).

Sensing of interlocks? It has been proposed that SC polymerization might be important in sensing interchromosomal entanglements and triggering an appropriate response (Kleckner et al. 1991), e.g., resolution (Rasmussen 1986) or, more probably, arrest of the meiotic program (Bishop et al. 1992). Two findings that might fit with such a role are: (1) occurrence of high levels of multivalents in *Arabidopsis* mutants lacking SC (Higgins et al. 2005) and (2) occurrence of frequent ring bivalent interlocks in grasshopper in correlation with absence of SC in these bivalents might be attributable to absence of such a role. On the other hand, G.H. Jones (personal communication) points out that a number of organisms exhibit highly localized SC formation around crossover sites on bivalents that have only a single chiasma, a situation in which sensing of interlocking at zygotene/pachytene is clearly not an issue.

Silencing? A recent report shows that chromatin is silenced specifically at the junctions of translocation heterozygotes where SC does not form, suggesting a possible role for the SC in that process (Turner et al. 2005; Schimenti 2005). However, such effects could also be explained as yet another consequence of the failure of DSBs to progress via a normal interaction with a partner.

Evolutionary rationale for existence of the SC

The above considerations still leave unanswered the basic question of why the SC has evolved as a very common feature of the meiotic program.

The fact that two fungi, *S. pombe* and *Aspergillus*, lack both SCs and regulated crossover formation (above) remains intriguing. The association of these two phenotypes has long been attributed to the fact that the SC is required for interference, but this explanation is no longer tenable. An alternative possibility is exactly the opposite: the SC might be required only in organisms that have acquired regulated crossover designation, e.g., for "neatness" of chromosomes that are only connected by a few recombination-related linkages during pachytene (Kleckner 1996). Such a relationship might be favored by the fact that crossover designation occurs earlier in prophase than SC formation, as if the first feature had promoted evolution of the second. Of course, we would then be left to explain the evolutionary value of regulated crossover positioning, but this remarkable feature requires an explanation in any case. Here is one idea: perhaps interference is valuable because it ensures that large chunks of chromosomes tend to remain associated even when recombination occurs, thus mitigating the tendency for crossing over to eliminate "good combinations" of genes.

Suffice to say that, like so many other aspects of meiosis, these issues provide fertile ground for future thought and experimentation.

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Appendix

Table 1 Calculation of loop densities along pachytene SCs, Denise Zickler, Ruth Padmore and Nancy Kleckner

A. organism	B. loop length in μm	C. B-DNA/ μm loop	D. haploid DNA in Mb	E. haploid B-DNA μm	F. loops/ haploid genome	G. SC length in μm	H. loop density	J. references Cols. B,D,G
S. cerevisiae	1	7	14	4760	680	28	24	1, 2, 3
	0.8-1.2	5.6-8.4	14.9	5066	724	25.5-31.7	28-23; 32-25; 33-25; 34-20,3	1, 4, 5
			14	4760	680	22.4-33.1	27-23; 30-20.5; 31-23.4; 32-19	--,2, 6
			15	5100	729	21.8-29	mean: 25.9-24.3	--. 5, 1
						21.2-35.7		--,--,7
S. pombe	1	7	14	4760	680	34	20	8, 2, 8
	0.5-0.6	3.5-4.4	13.8	4692	1082-1117	33.3-38	28-32	8, 9, 8
N. crassa	1.6	11	46	15640	1422	47	30	10, 2, 10
	1.5-1.6	10.5-11.2	43	14620	1392-1554	29-45	48-31; 31.6-29.6 (1392)	10, 9, 10
			48	16320	1329-1484	44-47	51-33; 33.8-31.7 (1489)	--, 10, 11
						29.5-34;5	23	--,--, 12
			34	11500	1051			--, 2--
Coprinus	1	7	36	12240	1749	70	25	13, 14, 13
	1-1.5	7-10.5	37	12580	1797	36-45	49.9-40	15, 16, 17
					1166-1198		32.4-25.9	
Zea mays	8-10	300	5000	1700 000	5667	353	16	18, 19, 18
		240-300	4053-4200	1 394 000	5800-3560	110-443 mean 353	353: 13.2-16.4 (ref 20)-- 8.1-10.1 (ref 19)	--, 20, 21
			2291-2735	854 400	4647-2848	332-591		--, 19, 18
						219-633 mean 420		--, --, 22
			7500	2 550 000	10625-8500		420: 11-13.8 (ref 20) -- 6.7-8.5 (ref 19)	--, 5, --
							20.2-25.3--24-30 (ref 5)	
Lycopersicum aesculatum	6	180	2000	680 000	3778	207	18	23, 19, 23
	5.25-6.75	158-202	869-1061	295 460	1927-2125	153-296 mean 207	207: 9.1-10.3	4, 20, 4
			907-1000	308 380	1713-1888	194-279 mean 234	234: 7.3-9.1	--, 19, 20
				340 000			extremes: 6.5-13.8	
Bombyx mori	0.83	25	500	170 000	6800	260	26	24, 2, 25
male	0.6	18			9444	L=180; Z=198; P=258; LP=347	L=52; Z=47.6; P=36.6; LP=27.2	26, --, 26
female						203-220	46.5-43	--, --, 27
Mus musculus	5.57-7	165-210	3232	1 098 880	6660-6182	156	36.3-42.7; 41.1-48.5	1, 28, 29
	6	180	3000	1 020 000	6105-5667	138-199	48-33.4	30, 5, 30
						144-192	41-28.5	--, --, 31

Table 1 (continued)

A. organism	B. loop length in μm	C. B-DNA/ μm loop	D. haploid DNA in Mb	E. haploid B-DNA μm	F. loops/ haploid genome	G. SC length in μm	H. loop density	J. references Cols. B,D,G
Home sapiens	9.6	288	3000	1 020 000	3542			32, 5, 32
male	4.7					Z=321; EP=207; P=231;	15.3; 17; 15.3	--, --, 34
female	3.1					EP=533; MP=458; LP=519	6.6; 7.7; 6.8	--, --, 34

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