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Review

Meiosis: How could it work?

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ABSTRACT **A physical connection between homologs is required for reductional segregation at the first division of meiosis. This connection is usually provided by one or a few well-spaced crossovers. A speculative overview of processes leading to formation of these crossovers is presented.**

Meiosis is essential for sexual reproduction. During gamete formation the cellular chromosome complement is reduced by half; union of two gametes to form a zygote thus restores the normal chromosome complement rather than doubling it. Halving of the chromosome complement is accomplished during meiosis because a single round of DNA replication is followed by two successive rounds of chromosome segregation.

A diploid cell contains two versions of each chromosome, "homologs." In a mitotic cell cycle, DNA replication and chromosome compaction are followed by equational segregation of sister chromatids, and the starting genetic situation is restored (Fig. 1). In meiosis, chromosomes replicate and compact, then at the first division, homologs move to opposite poles ("reductional segregation"); and at the second division, sister chromatids move to opposite poles as during mitosis. Meiosis thus yields four haploid products (Fig. 1).

During mitosis, connected sister chromatids are aligned between two poles via sets of microtubules that exert opposing poleward forces; when sister chromatid connections lapse, sisters move to opposite poles. Given a physical connection between homologs and suitably modified kinetochores, the same processes are used for alignment and segregation of homologs to opposite poles at meiosis I (1).

In most organisms, the requisite physical connection is provided by crossover(s), in combination with connections between sister chromatids along their lengths. In condensed late prophase chromosomes, the interstitial interhomolog connections corresponding to crossovers are seen cytologically as "chiasmata" (refs. 2 and 3; see Fig. 6*D*).

At anaphase of meiosis I, the physical connection between homologs is abrogated by dissolution of connections between sister chromatid arms. Sister connections at the centromere remain; each sister pair is thereby aligned on the meiosis II spindle. When centric connections lapse at anaphase II, individual chromatids segregate.

Over the past decade, many laboratories have provided new cytological, genetic and molecular insights into the meiotic process. This article attempts to integrate new and old information in a framework provided by temporal analysis of meiotic prophase chromosome metabolism in yeast (Fig. 2).

Events of Meiotic Prophase

Intersister connections are established very early, likely during S phase, as in mitotic cells (10–13, 98). Homologs identify one another and recombine during the first part of prophase, with chromosomes in a relatively extended state. Higher order compaction occurs later, after interhomolog interactions are complete.

Events Along Each Sister Pair. In early prophase, each pair of sister chromatids forms a single linear array of loops connected at their bases by a bulky structural axis (11, 14, 15). One sliver of this axis is the silver-staining "axial element." Ultimately, the chromatin loops of both sisters come to lie on the same side of this axis (11).

A conjoined sister/sister axis and asymmetric positioning of chromatin loops with respect to that axis are both unique to meiosis. In mitotic cells, sister chromatid axes are distinct, though closely juxtaposed, and chromatin loops flank the two axes (16).

The conjoined sister–sister axis may facilitate interhomolog interactions by reducing a "four-body" problem to a "two-body" problem. Asymmetric chromatin disposition likely also facilitate the interhomolog interaction process since interhomolog connections ultimately form between the two structural axes (below). Both features likely also promote development of an axis-associated kinetochore shared by both chromatids (e.g., refs. 17 and 18).

Interhomolog Interactions. Homologs interact during and/or after development of the axial linear loop configuration.

Homolog pairing. Homologs seem to come together in two conceptually distinct stages: "colocalization" of the pair into a joint spatial domain followed by their "coalignment" (11, 19, 98). Such a progression is sometimes observable in the configurations of axial elements (refs. 19–21; Figs. 3 and 4).

These two stages need not be entirely distinct temporally. They are proposed to occur via a progression of interstitial interactions that are initially unstable and become increasingly stable as the process progresses (10, 19). Inappropriate juxtapositions and entanglements could thus be eliminated early, before becoming permanent.

Multiple interstitial pairing interactions are revealed by fluorescence *in situ* hybridization (FISH) analysis of spread yeast chromosomes with small locus-specific probes (10). These interactions are proposed to be guided by direct DNA–DNA contacts between intact duplexes with homology searching facilitated by appropriate proteins (10, 19, 22). No known RecA homologs are involved, however (ref. 10 and unpublished data). DNA–DNA contacts would be stabilized by other types of interactions.

Recombination. Biochemical stages of the yeast recombination reaction are revealed by physical analysis of meiotic DNA (Fig. 2; refs. 23–25). Recombination is initiated by meiosis-specific double-strand breaks (DSBs). 5′ strand termini are rapidly resected, leaving 3′ single-stranded tails suitable for strand invasion and polymerase extension. DSBs are then converted to double Holliday junctions, which are the first chemically stable connections between homologs at the DNA level. Finally, crossover and noncrossover products appear. In a noncrossover, also known as "gene conversion" or "patch," a local DNA interaction is resolved without concomitant exchange of flanking chromosome arms. Crossovers and noncrossovers seem both to arise via double Holliday junctions (25). Appearance of products is preceded by appearance of experimentally detectable heteroduplex DNA (7, 9), which likely signals the onset of Holliday junction resolution (25).

Cytological evidence. Multiple interstitial interhomolog connections were first observed cytologically (for *Allium*, see Fig. 4; refs. 21, 26, and 27). The axial elements of homologs come together especially closely at periodic "association sites." These sites are marked with bidentate connector structures or nodules or both. Association sites are proposed to be involved in pairing and/or recombination, with nodules, at least, representing ongoing recombinational interactions (11, 21, 28).

At association sites, homolog axes are ≥ 0.4 μm apart (Fig. 4). Thus, any DNA segments participating in pairing or recombination must be held closely to their adjoining structural axes.

Synaptonemal complex. Structural axes next become connected along their lengths via a highly ordered structure, the synaptonemal complex (SC; Fig. 5; refs. 11, 30, and 31). A nucleus with a full SC complement is at "pachytene."

Late recombination nodules. Recombinational interactions that mature as crossovers are marked by specific "pachytene" recombination nodules that sit atop the central region of the SC (11, 28, 32). Thus, mature/maturing crossovers are juxtaposed to the homolog axes. Noncrossovers have no cytological correlate after midpachytene.

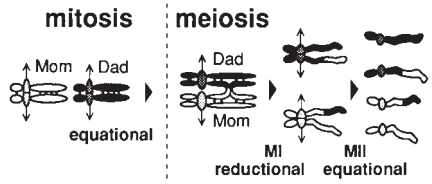

FIG. 1. Mitosis and meiosis.

Temporal relationships. The relative timing of pairing, recombination, and cytological events in yeast is shown in Fig. 2.

Homologs are paired via multiple interstitial interactions in premeiotic cells and likely also in vegetative cells (ref. 10 and unpublished data). Intimate interstitial interactions and initiation of recombination are lost during meiotic S phase, though homologs may remain substantially colocalized; interactions then reappear, independent of and probably prior to DSBs and initiation of recombination (10, 22, 33–35, 99, 102). In other organisms, chromosome pairing may either precede meiosis or follow meiotic S phase (19).

DSB formation precedes initiation of SC polymerization, which is approximately concomitant with double Holliday junction formation. By analogy, coaligned *Allium* chromosomes should be at the DSB stage and/or just beyond. Crossovers and noncrossovers appear at the end of pachytene, immediately before or concomitant with (but not dependent upon) SC disappearance (refs. 4, 36 and 44).

After Pachytene. Meiotic chromosomes now return progressively to a nonmeiotic state (Fig. 6).

The SC disassembles. Concomitantly, or shortly thereafter, axial elements are lost and the homolog axes reorganize (38, 39). Then higher order axial compaction occurs in progressive stages, which are dramatic in organisms with long chromosomes (Fig. 6).

After reorganization of the homolog axes, individualized sister chromatid axes appear in a close parallel array (Fig. 6*E*; ref. 37). Sister chromatin loops remain on the same side of the pair of axes, however, presumably side-by-side (37, 40). This arrangement continues to promote coordinate behavior of sister chromatids—i.e., coordinate coiling, and convergence of homolog axes at the points of interstitial interactions (Fig. 6*E*); reductional kinetochore behavior is also promoted (17, 37).

Evolutionarily, side-by-side juxtaposition of sister axes would be sufficient to promote reductional segregation: sister kinetochores would thus be cooriented and therefore would tend to become connected to the same pole even without any sophisticated comorphogenesis (refs. 1 and 37).

Finally, fully condensed homologs become aligned in the metaphase I spindle and undergo the anaphase I transition. At this point, sister chromatid axes separate along the arms of the chromosomes; each arm becomes embedded centrally within its own chromatin as at mitotic anaphase (Fig. 6*F*). At the centromere, in contrast, sister

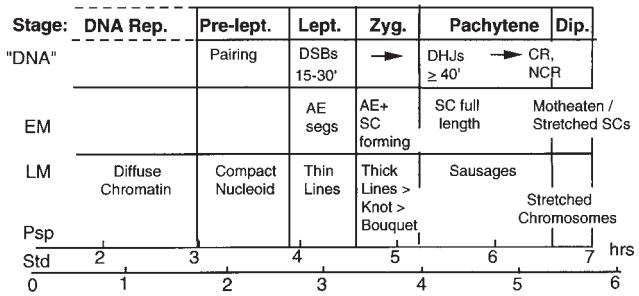

FIG. 2. Time course of meiosis in *Saccharomyces cerevisiae*. "DNA" events include interstitial pairing interactions and recombination; EM and LM events are those detected by electron and light microscopy. DNA replication (Rep.), LM and EM events, and DSBs and crossovers (CRs) at one locus have been examined in parallel in a single sporulating culture (4, 5). Timing for other DNA events are relative to DSBs and CRs examined in parallel (6–9). Two time scales reflect two different culture conditions; Psp, cells spheroplasted prior to meiosis; Std, standard conditions. Lept., Leptotene; Zyg., Zygotene; Dip, Diplotene; DHJs, double Holliday junctions; NCR, noncrossover; AE, axial element.

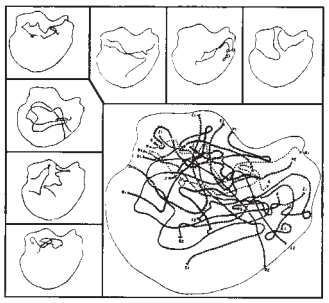

FIG. 3. Colocalization of homologs in *Sordaria* (19, 20). Two-dimensional projection of an early leptotene nucleus containing seven pairs of homolog axes; *Insets* are individual homolog pairs in the same nucleus.

axes stay together, though perhaps with some degree of dissociation, until anaphase II (41, 42).

Two aspects of these late stages are notable. First, the DNA segments involved in crossover interactions seem to be held relatively closely to the axes of their respective chromatids even at anaphase I: at that stage, the two separated nonsister axis arms converge at presumptive points of crossovers (Fig. 6*F*; refs. 37, 43, and 40).

Second, intersister connections lapse in the same two phases in both mitosis and meiosis: arm connections first and then centric connections. The only difference is whether the two phases occur in succession during a single round of division or in two successive rounds. Thus, intersister interactions may be molecularly the same in meiotic and mitotic cells, with meiosis-specific features simply exaggerating the persistence of centric cohesion (13, 45).

Interhomolog Interactions: A Closer Approach

A Single Progression of Interhomolog Interactions? Available data fits comfortably with the idea that interstitial pairing interactions and recombinational interactions occur in succession at the same positions along each pair of interacting homologs and that these positions correspond to cytologically observed interaxis association sites (8, 10, 22, 35, 46, 47, 100, 102).

If correct, this scenario raises two questions. How do FISH-detected pairing interactions and DSB recombination complexes come to occur at the same particular positions along each individual pair of interacting homologs? And how do these interactions come to be axis-associated? Several constraints pertain.

- Pairing interactions and DSBs each can occur at many different potential sites along a chromosome, with only a subset of those sites used in each meiotic nucleus (e.g., refs. 10 and 48–50).
- Pairing and DSB formation are substantially independent of one another: pairing occurs prior to and independent of DSBs (above), and DSBs form, though not exactly normally, on a chromosome lacking a perfect homolog (35, 51, 52, 102).
- DSBs occur in regions where DNA is in an especially accessible chromatin configuration (48, 49, 53). But the positions of strong DSB hot spots can be affected by genetic alterations as far away as 30 kb (T.-C. Wu and M. Lichten, personal communication).

The following model begins to account for these observations.

Structurally differentiated sites with potential for pairing tend to develop near the bases of chromatin loops as an automatic conse-

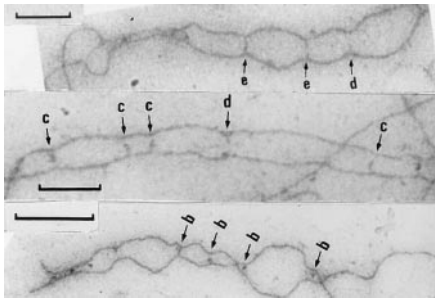

FIG. 4. Coaligned homolog axes in *Allium cepa* exhibiting periodic association sites (21). Arrows indicate (*i*) "early" recombination nodules either with association sites (b) or midway between axial cores in close alignment (c) and (*ii*) bidentate structures at matching sites (d) or bridging the space between two converging axial cores (e). (Bars: top, middle: 1 μm, bottom: 5 μm.)

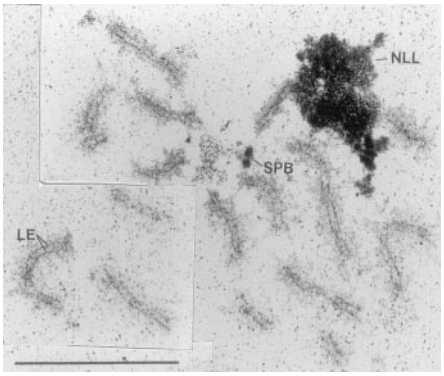

FIG. 5. Pachytene nucleus of *S. cerevisiae* (29). Sixteen chromosomes, each with two lateral elements (LE), full SC with central elements occasionally visible, and chromatin loops, partially extracted and seen as "fuzz"; also seen are the nucleolus (NLL) and duplicated spindle pole bodies (SPB). (Bar = 3 μm.)

quence of axial structure development. On a given chromosome, the specific positions at which such sites occur are selected from among a larger subset and vary from nucleus to nucleus. If a homolog is present, pairing contacts occur in regions where DNA is accessible; these interactions feed back upon the development process, promoting more specialized differentiation of certain favored sites, ensuring that pairing sites develop at the same positions on both homologs, and at the same time promoting colocalization/coalignment. Concomitantly, but independently, machineries for meiotic DSBs assemble preferentially at/near these sites via common underlying components. A few DSBs might occur at other accessible regions, however, provoking "rogue" recombination events.

[One protein that could act at the interface amongst pairing, recombination, and development of axial chromosome structure is Rad50. A *rad50* null mutant is defective in all three processes (10, 54). Rad50 likely occurs at interstitial sites, is an ATP-dependent DNA binding protein, and is related to SMC proteins, implicated in chromosome compaction (55–57).]

Crossover Control. Meiotic recombination is subject to important biological controls (3, 11, 28, 58). In particular, along any pair of homologs ("bivalent"), crossovers seem to be minimal in number and are maximally spaced as follows.

- Each bivalent usually acquires only one or two crossovers.
- Despite the low average number of crossovers per bivalent, the probability that a pair of homologs will acquire zero crossovers is very low, often $<0.1\%$.
- Crossovers tend not to occur very close together—i.e., they "interfere" with one another. Interference is stronger over shorter distances and weaker zero or even negative over longer distances.

These and other manifestations of crossover nonrandomness are coordinately disrupted in certain mutants and thus may reflect a single "crossover control" process (28).

The total number of recombinational interactions along a chromosome is apparently large, with a carefully selected few maturing as crossovers and the remaining majority likely maturing into noncrossovers. Noncrossovers exceed crossovers by factors of 2 in *Neurospora* (59), 4 in *Drosophila* (60), and 30–40 for *Allium* early nodules (G. H. Jones, personal communication).

Crossover control may thus act upon undifferentiated recombinational interactions to determine their fates (28, 61, 62). Cytological and physical DNA data taken together suggest that this crossover/noncrossover "decision" may be made relatively early in the recombination reaction.

- Crossover control has clearly been implemented by midpachytene: crossover recombination nodules exhibit an "interference" distribution (11, 28, 32).
- *Sordaria* and tomato exhibit a 1:1 correspondence between crossover nodules and SC initiation sites (63, 64). If exact, this correspondence excludes the possibility that crossovers are designated after SC is fully formed.
- In several organisms, axis-associated nodules undergo changes in number, stability, and/or distribution concomitant with SC formation (11, 63, 65), again marking this as the latest possible time for crossover/noncrossover differentiation.
- In yeast, SC formation and double Holliday junction formation are approximately concomitant, while Holliday junction resolution does not occur until the end of pachytene (above). Thus, crossover control should be implemented at or before Holliday junction formation, not at the time of resolution. If so, there should be two functionally distinct types of double Holliday junctions differing in detailed chemical structure (e.g., braided vs. unbraided; ref. 25) or, more likely, in protein/chromosomal context (e.g., ref. 66). (Or, noncrossovers might occur via a non-Holliday junction intermediate.) Furthermore, specific features must "enforce" the crossover interference decision from the time it is made until mature products appear. (96)

Minimization. If pairing and recombination are both considered, meiotic prophase chromosomes can be considered to undergo a "minimization" process: the number of interstitial interactions is reduced from the many pairing interactions present at early stages to the few maximally spaced crossovers present at and after the end of pachytene.

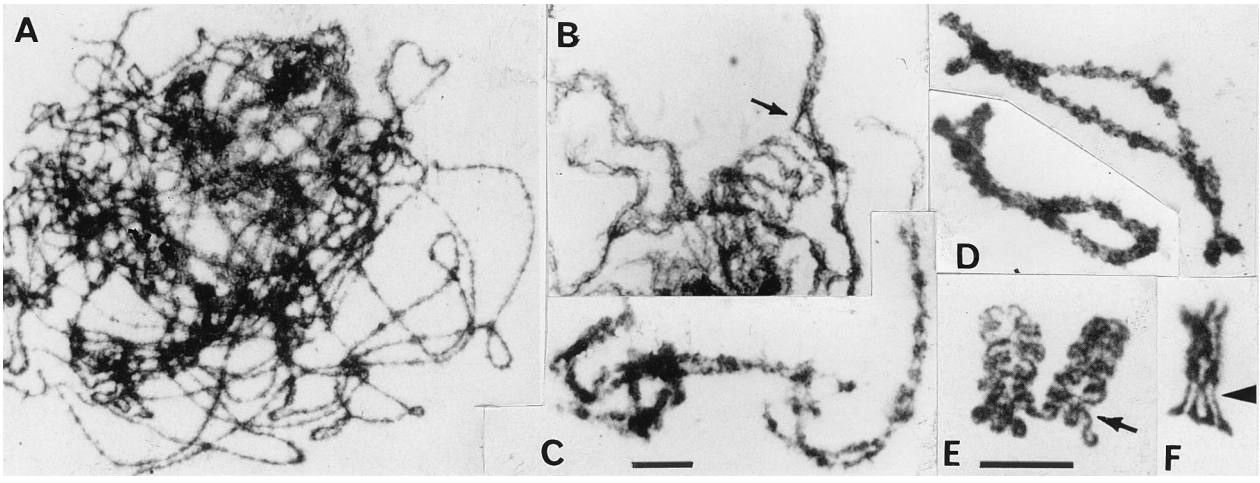

FIG. 6. Meiotic chromosomes of lily at and after pachytene (37). (*A*) Pachytene. (*B*) Portion of a diffuse diplotene nucleus with structural axes undergoing reorganization. (*C*) Portion of a nucleus just after *B* with onset of axial compaction. (*D*) Two bivalents at diplotene; chiasmata are points of convergence. (*E*) Two bivalents at diakinesis. Pairs of sister cores coil coordinately; gyres of two homologs are of opposite helical handedness and are connected at sites of presumptive chiasmata (arrow). (*F*) One bivalent at anaphase I. Sister chromatid cores lie far apart in preparation for separation; chiasma is visible at the point where the sister cores of the two homologs intersect (arrowhead). *E* and *F* are at higher magnification than *A–D*. (Bars in *C* and *E* = 5 μm.)

Crossover Connections Constrain Compaction. Images of postpachytene meiotic chromosomes suggest that crossover connections between homologs severely constrain higher order axial compaction (Figs. 6 and 7).

- At diplotene, homologs tend to be as far apart as possible except at the positions of chiasmata; moreover, in each interchiasma region, the plane of the homolog pair is perpendicular to the planes in adjacent interchiasma regions. Steric constraints due to convergence of "fat chromosomes" at chiasmata, chromosome stiffness, and/or "repulsion" forces could be responsible (e.g., refs. 68 and 69).
- At diakinesis, homologs coil into higher order gyres. Moreover, in general, the gyres of the two homologs are phased with one another and are of opposite helical handedness (discussed in refs. 37 and 70). These latter features must arise during compaction, enforced by the presence of previously established interhomolog crossovers.
- At anaphase I, highly condensed chromatid cores converge at chiasmata; large numbers of such convergences could preclude maximal compaction.

These descriptions suggest that strong physical forces may be exerted on crossover connections during compaction. Tight juxtaposition of crossover segments to their structural axes could thus be essential to ensure that interhomolog connections are not ripped apart by compaction forces. If so, positioning of recombinational interactions between the homolog axes in early prophase, which establishes this juxtaposition, is crucial for eventual crossover function.

Also, intuitively, maximal compaction should be favored if crossovers are minimum in number and maximally spaced. Thus, the arrangement of crossovers that least constrains axial compaction is precisely the arrangement conferred by crossover control. It is tempting to consider a mechanistic relationship between axial compaction and crossover control.

Interhomolog Interactions Are Monitored by the Meiotic Cell Cycle. In yeast, defects in prophase chromosome metabolism can trigger regulatory arrest prior to spindle formation (27, 71, 72). Meiotic cells appear to monitor the recombination process rather specifically, via a checkpoint mechanism, which senses the status of the interhomolog recombination complex (44, 71), and involves components of the mitotic DNA damage checkpoint system (103).

Also, in *Drosophila*, programmed arrest of female meiosis at the metaphase I–anaphase I transition requires interhomolog crossovers (73).

Are Meiotic Interhomolog Interactions Derived from Interstitial Intersister Interactions in Mitotic Cells?

Analogies Between Intersister and Interhomolog Interactions. The nature of the fundamental cell cycle-regulated connections between sister chromatids in mitotic cells is unknown. A reasonable case can be made, however, that (*i*) these connections are provided by axis-associated interstitial interactions (rather than associations between the axes *per se* or interactions confined to the periphery of chromatin loops), and (*ii*) a fundamental evolutionary and mechanistic relationship exists between these intersister connections and axis-associated interstitial interhomolog connections of meiotic cells.

- Boy de la Tour and Laemmli found a striking analogy between interstitial intersister connections in mitotic chromosomes and crossover connections between meiotic homologs: sister chromatids are coiled with opposite helical handedness as are meiotic homologs (ref. 70; Fig. 8). It was recognized that this arrangement permits regular interactions between sister chromatids at homologous positions (Fig. 8). By implication, axis-associated intersister connections established earlier would constrain the higher order coiling of sister chromatids in late mitotic prophase just as preexisting crossovers constrain higher order coiling of meiotic chromosomes.

FIG. 7. Largest chromosome of *Stenobothrus parallelus* [ref. 67 (pp. 96–97)]. Left to right: middle diplotene, late diplotene, diakinesis, metaphase I, and anaphase I. Reprinted from Darlington, 1937.

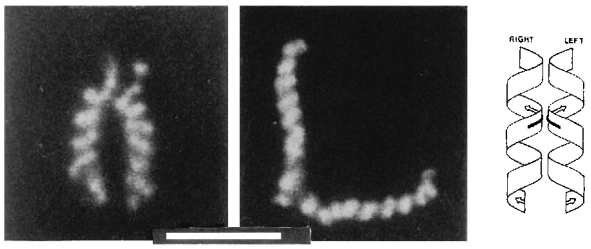

FIG. 8. Mitotic sister chromatids are coiled with opposite helical handedness (70). (*Left* and *Center*) Optical sections through a chromosome immunostained with anti-topoisomerase II antibodies: top section (*Left*) and bottom section (*Center*). (*Right*) Two helical ribbons related by mirror symmetry; arrows indicate the positions of homologous regions along the ribbons, which approach each other in space at each helical turn. (Bar = 5 μm.)

- Silver staining of mitotic prophase chromosomes reveals linear sister chromatid cores joined via transverse connectors (16)—features reminiscent of coaligned meiotic homologs and again suggestive of axis-associated interstitial connections.
- In mitotic cells, sister axes are closely juxtaposed prior to anaphase (e.g., ref. 16). Thus, any interstitial intersister connections must be held closely to and lie between individual chromosome axes, as for meiotic interhomolog connections.
- Successive segments of mitotic chromosomes tend to occur perpendicular to one another (68), perhaps a few constraining interconnections exist as in meiotic chromosomes (Fig. 7).

Further Plausible Analogies.

- Since intersister connections are likely established when chromosomes are extended (i.e., during S phase), multiple interstitial interactions should be required to keep sister chromatids coaligned at this point just as for homologs in early meiotic prophase.
- Irrespective of their precise molecular nature, interstitial intersister interactions might well form between homologous DNA segments, guided by weak DNA–DNA interactions between intact duplexes. Since sister chromatids are automatically "colocalized" as they form, small contributions from DNA–DNA contacts might suffice.
- Given the above considerations, mitotic intersister pairing interactions should also require a "minimization" process, dictated by the requirements of axial compaction and analogous in functional consequence to the minimization of interhomolog connections in meiotic chromosomes.

Chromomeres. Meiotic and mitotic chromosomes viewed by light microscopy exhibit periodic thickenings that occur at matching positions along a pair of homologs (Fig. 9). It would be interesting to know whether interstitial interchromosomal interactions occur in some particular relationship to these thickenings within or, perhaps more likely, between them.

Meiotic Interhomolog Interactions: A Prospective View

Development of Pairing Interactions and DSB Complexes. If meiotic interhomolog interactions are functionally related to mitotic intersister interactions, the scenario for development of axis-associated interhomolog interaction sites described above now seems quite natural: sites having the potential for interhomolog interactions will tend to develop automatically at the bases of loops along the homolog axis irrespective of the presence or absence of a homolog because axis-associated intersister interactions normally develop in this way. This basic situation can then be modified by meiosis-specific

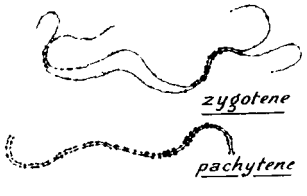

FIG. 9. Chromomeres in spread meiotic chromosomes at zygotene and pachytene [ref. 67 (p. 88)]. Reprinted from Darlington, 1937.

differentiation of developing sites, including influences from pairing interactions.

Furthermore, recruitment of the DSB/recombination machinery to developing pairing sites via underlying components would set the stage for a transition from "mitotic" pairing contacts involving intact chromosomes to the "meiosis-specific" interhomolog connections provided by DNA crossovers.

Crossover Control. It is suggested above that meiotic crossover control might be mechanistically related to axial chromosome compaction. But this idea involves a paradox: crossover interference is imposed on meiotic chromosomes long before higher order compaction. In fact, at the relevant time, chromosome axes are actually restrained from further compaction by axial structures.

No such constraints apply to mitotic chromosomes, however. In that case, the analogous minimization process could be driven by axial compaction *per se*. Perhaps, then, mitotic axial compaction forces have been adapted to drive meiotic crossover control. Indeed, since mitotic chromosomes likely compact progressively throughout prophase (16), the earliest phases could occur at points roughly analogous to early/mid-meiotic prophase.

Projection of a mitotic minimization process onto meiotic chromosomes is particularly easy to envision if meiotic recombination occurs at sites that correspond to intersister interaction sites in mitotic cells. Furthermore, the meiotic minimization process should coordinately affect pairing interactions and recombinational interactions, coordinately destabilizing the former and provoking differentiation of the latter into two forms.

Mechanistically, the relevant minimization force could be provided by changes at the interface between the axis and the associated chromatin at the bases of loops (e.g., chromatin compaction). Such forces are presumptively involved in chromosome compaction in mitotic cells, and DNA segments engaged in pairing/recombination will be responsive to such forces because they are directly continuous with the nearby axis-associated chromatin.

Two observations support this notion. First, maize chromosomes undergo a dramatic change in overall chromatin configuration just before SC formation (74), a point at which crossover control could be imposed (below). Second, the *Drosophila mei4l* gene is required for both crossover interference and chromatin compaction (75, 76).

In fact, during meiosis, effects of the minimization force might be stored in the chromosomes and used to promote compaction later, after restraining structures are gone.

A Model for Meiotic Crossover Control. From these and other considerations we have developed general and specific models for meiotic crossover control (N.K., J. Hutchinson, and G. H. Jones, unpublished data) as follows.

Crossover control is considered to occur via the imposition and relief of stress. Undifferentiated recombinational interactions along a pair of homologs are all placed under stress; also, each interaction has an intrinsic sensitivity to that stress; all interactions are therefore "activated", though to different degrees. On a given homolog pair, the individual interaction with the highest activation level then "goes critical" and is committed to becoming a crossover. In addition, stress is relieved in the immediate vicinity of that interaction and, to a progressively decreasing extent, nearby. Within the affected region, the formation of additional crossovers is disfavored. This would be crossover interference. If additional interaction(s) subsequently go critical, their numbers and positions would be influenced by preceding event(s).

This model can also explain why every bivalent acquires at least one crossover. Since stress is imposed on a "per bivalent" basis, the levels of stress and/or sensitivity to stress can be set high enough to ensure that every bivalent undergoes at least one crossover activation.

We further suggest that tension is imposed along each individual homolog axis via differential compaction of axis-associated chromatin against resistance imposed by the structural axes. If the axes are elastic, such tension will be manifested as stress (e.g., "pulling") at the sites of recombinational interactions. Relief of stress can then be achieved by release of chromatin/axis connections at the site of an activated interaction. In addition, by virtue of axis elasticity, stress relief is automatically transmitted outward along the axes in both directions, diminishing progressively with distance.

This model accounts quantitatively and qualitatively for diverse aspects of crossover control and for detailed phenotypes of certain interference-defective mutants. (e.g., ref. 96)

This specific mechanism differs from most previous considerations (e.g., refs. 61, 77, and 78) in that the SC need not be involved. Here, information is transmitted along the individual homolog axes with the behavior of the two homologs coupled via their shared interstitial interhomolog interactions. As far as we know, all observations cited as evidence for involvement of the SC in interference are also accounted for by this new model (96, see below).

Is Each Major Stage of Meiotic Recombination Triggered by a Cell Cycle Regulatory Event? It seems possible that coordination between the interhomolog interaction process and other cellular events of prophase is maintained by communication in both directions, with information flowing not only from the chromosomes to the cell cycle (above) but also from the cell cycle to the recombination process.

The yeast meiotic recombination reaction pauses significantly at two stages: the DSB stage and the double Holliday junction stage (Fig. 2). Progression of the reaction into and out of these stages could be determined by biochemical events alone. But it is intriguing to consider that the reaction might be driven instead by cell-wide regulatory processes. If so, temporal analysis reveals particular breakpoints because the reaction pauses at particular biochemical stages awaiting the next cell cycle signal. Initiation of Holliday junction formation, onset of Holliday junction resolution, and/or DSB formation itself, might be triggered by such a signal.

This idea could explain why the stages of meiotic recombination at the DNA level correlate remarkably directly with the classical stages of meiosis defined by light and electron microscopic analysis, which monitor overall chromosome configuration and axial element/SC status, respectively (Fig. 2).

Coupling of recombination to the cell cycle would make the interhomolog interaction process responsive to other aspects of cellular physiology. Furthermore, nucleus-wide signals would keep the entire chromosome complement more or less "in sync." These features are attractive given that early–mid prophase takes several hours in yeast but up to several days in other organisms (79).

This idea also fits with the notion that a change in the axis/chromatin interface drives meiotic crossover control; any such transition would presumably be triggered by a cell cycle regulatory signal. Indeed, the *mei4l* gene, required for interference, encodes a major cell cycle regulatory kinase (76). More generally, all three transitions in the recombination reaction might be triggered by analogous, but slightly different, changes in the axis/chromatin interface.

Cell cycle control may be imposed at particular points for mechanistic reasons, but biological reasons also can be imagined:

- DSB formation initiates chemical disruption of the chromosomes and thus should be carefully considered.
- The second transition, when a DSB begins to invade an intact duplex, is a biologically attractive candidate for the point at which minimization is imposed. If the meiotic minimization process destabilizes interhomolog pairing interactions as well as direct crossover control, implementation at this point would mean that weakened pairing interactions would be replaced by nascent DNA connections at the critical stage. Given the importance of minimization, the relevant step should be under cell cycle control.
- If Holliday junction resolution were under regulatory control, the final events of recombination could be blocked until other critical processes are complete.

The SC As a Scaffold for Interstitial Interhomolog Interactions. The SC is a cytologically prominent and evolutionarily conserved structure. Its function is unknown (11, 30, 31, 77, 80).

It seems possible that the SC serves as a scaffold for the meiotic interstitial interaction process (Fig. 10 *Left*). Three roles can be envisioned: (*i*) Locally, to shepherd the transition of interstitial interhomolog connections from mitotic-like pairing interactions to meiosis-specific DNA-based crossovers; (*ii*) Globally, as the number of physically effective interstitial interactions is reduced to one or two, to keep pairs of homologs coaligned along their lengths in their extended form, prior to the onset of compaction, while recombination and other cellular processes are completed; (*iii*) To promote the eviction of noncrossover interactions from their association with the

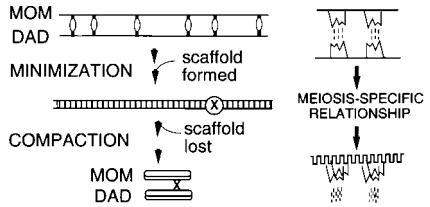

FIG. 10. Speculations. (*Left*) The SC as a meiosis-specific scaffold for interhomolog interactions (see text). (*Right*) Conversion of mitotic interstitial intersister interaction sites for interhomolog interactions in meiotic cells via the meiosis-specific relationship between sister chromatid axes. If necessary, intersister coalignment could be maintained by the conjoined sister–sister axis.

homolog axes while simultaneously replacing them functionally (for a related idea, see ref. 61).

A possible sequence of events is thus as follows: crossover control is imposed at the DSB stage; in consequence, pairing interactions are weakened and strand invasion and SC formation are initiated. As strand exchange proceeds, intermediates destined to become crossovers retain their firm axis associations; for those destined to become noncrossovers, such associations are weakened or lost; SC polymerization continues. Eventually, after crossing over and other cellular events are complete, SC disassembles.

The Existence of Minimization Should Dictate Existence of the SC. If the SC has the scaffolding function proposed, it should be unnecessary in the absence of a minimization process.

In such a situation, interhomolog pairing interactions would remain intact and could be eliminated during higher order compaction. Moreover, if the DSBs present at early stages were matured randomly into axis-associated crossovers and noncrossovers, each pair of homologs would be connected at many positions by crossovers. Multiple crossover interconnections would impede axial compaction but might be tolerated as an intermediate evolutionary stage or as a final condition in organisms whose chromosomes need not compact extensively after midprophase (e.g., ref. 81).

If it is only the minimization of meiotic interhomolog connections that imposes the requirement for an SC, the crossover control process should provoke SC formation mechanistically and evolutionarily. Several observations relating the SC and crossover interference are thus explained as follows.

- Certain fungi lack both features (77). This correlation is often cited as evidence that the SC is required for interference. But it could as easily mean that these organisms either never acquired or acquired and then lost interference; as a result, SC either never evolved or became vestigial and was lost. Correspondingly, chromosomes of these organisms may not undergo much axial compaction beyond linear loop configuration (81).
- Zip1 protein is a yeast SC central region component and is required for crossover interference (27, 82). A *zip1* mutant nonetheless exhibits high levels of viability among its meiotic products (82), as this model could predict.
- A 1:1 correspondence between crossing over and SC initiation sites (above) would be natural (but not obligatory).
- Zip1 is not just an SC component; it is also required for normal meiotic recombination (82, 96). Moreover, the role of Zip1 in recombination does not require SC polymerization along the chromosomes, and it is proposed that Zip1 acts prior to initiation of bulk SC formation as part of the crossover control process (96). This molecule could have evolved to be the molecular coupler of the two processes, first promoting recombination as part of the minimization process and then provoking full SC polymerization along the chromosomes.
- This scenario rationalizes observations suggesting that progression of the recombination process provokes SC formation rather than vice versa (46, 83, 84).
- The notion of SC as a scaffold for conversion of pairing interactions to crossovers explains situations in which SC is discontinuous but each patch of SC either includes a recombination nodule or occurs specifically at positions where chiasmata are subsequently seen (11, 78, 85).

Intersister Interactions: A Prospective View

Converting Intersister Interactions into Interhomolog Interactions. Intersister interaction sites of mitotically cycling cells could become interhomolog interaction sites almost automatically by acquiring the meiosis-specific relationship between sister chromatids.

Sites on sister chromatids that interact "face-to-face" in mitotic cells would now effectively occur "side-by-side" in meiotic prophase chromosomes (Fig. 10 *Right*), with chromatin out of the way, ready to mediate interactions with another homolog. Concomitantly, sites on sisters would be precluded from interacting with one another. And if pairing and recombination occur at the same sites, this scenario could help explain why meiotic recombination occurs preferentially between homologs as compared to between sisters (reviewed in ref. 6; 97) while mitotic recombinational repair exhibits the opposite bias (86).

Coordinating Intersister and Interhomolog Interactions During Meiosis. During meiosis, intersister and interhomolog interactions both occur. One observation might be evidence that the two types of interactions are related: insertion of a yeast meiotic DSB/recombination hot spot into a human DNA yeast artificial chromosome promotes high levels of meiotic recombination and also dramatically improves cosegregation of sister chromatids at meiosis I (87); perhaps effective intersister connections also form frequently within the insert.

If meiotic intersister and interhomolog interactions do involve functionally related sites, how are the two processes coordinated? Perhaps interstitial intersister connections are modified so that they can be used simultaneously for interhomolog interactions. Alternatively, the two types of interactions might use different subsets of a common set of potential sites. Or, finally, a more intriguing possibility is that exactly the same sites are used for both types of interactions. If so, intersister interactions would develop at a subset of potential sites, be converted to interhomolog interaction sites during midprophase, and then be converted back into intersister interaction sites at the end of pachytene for eventual use at meiosis I.

In the last, most extreme, scenario, sister chromatids would presumably be held together during the intermediate period exclusively via the conjoined sister–sister axis. (If other features help hold sister chromatid chromatin to one side of this axis, they might also contribute.) Thus, these midprophase-specific structures would constitute a scaffolding for interstitial intersister interactions (Fig. 10 *Right*). The same could well be true even in the less extreme scenarios, however, with the need for scaffolding dictated in a general way by the need to modify intersister interactions for use in the interhomolog interaction process.

The notion that interstitial intersister connections are severely weakened or absent during midprophase is uncomfortable at first but could have several advantages.

- It predicts the existence of mutants in which intersister connections are converted for use as interhomolog interactions as usual but then never restored. Desynaptic mutants of maize fit this description: crossing over occurs in these mutants; but by diakinesis, sister chromatids that can be seen to have undergone recombination often are no longer connected to a partner chromatid, or else have reassorted into nonsister combinations (80) held together presumptively by secondary features (e.g., adventitious topological catenations; see below). Such mutants are difficult to understand if mitotic-like sister connections persist intact throughout meiosis from S phase to metaphase I. Moreover, desynaptic mutants exhibit aberrant SC, as could be expected if the homolog axes are aberrant.
- A defect in restoration of robust intersister interactions might result from (or be accompanied by) a defect in interhomolog interactions. This scenario could account for an entire set of meiosis-specific mutations that affect crossover formation only modestly but grossly affect the ability of those crossovers to ensure homolog segregation (2). (The latter defect is that predicted from an absence of effective intersister connections at metaphase I.) Also, one such mutation, *red1* in yeast, is known to affect a prominent structural component of meiotic chromosome axes (24).
- *spo76* mutants of *Sordaria* exhibit defects expected for loss of the scaffolding function: split lateral elements at lepotene and loss of sister chromatid cohesion at diplotene (88).

Additional observations support the general notion that axial structure is important for maintaining intersister connections as follows. In *Triatoma infestans*, autosomes segregate reductionally at meiosis I, while sex chromosomes segregate equationally; autosomes have axial elements and SC, while sex chromosomes have neither feature (39). Conversely, reductional segregation is observed in several situations, including normal sex chromosomes, where axial elements are present but SC is absent (27, 39, 81). Finally, the *rec8* gene of *S. pombe* disrupts linear element formation and sister chromatid cohesion (101).

The interconversion scenario does raise the question of how intersister and interhomolog interactions are coordinated in mitotically cycling cells in organisms where both occur. Interhomolog pairing might occur only in G_1 when no sister is present; or incompatibility might be a meiosis-specific differentiation.

Adventitious Topological Catenations Between Sister Chromatids? At mitotic anaphase and anaphase I of meiosis, sister chromatid chromatin masses lie side by side (Fig. 6*F*). Perhaps this association is maintained by adventitious topological catenations between loops in the peripheral chromatin. Such catenations could arise via undirected topoisomerase II (TopoII)-mediated interloop strand passage events. Any such catenations should be eliminated automatically when sister chromatids move to opposite poles because poleward forces would impose directionality on the TopoII reaction.

Meiotic chromosomes provide special opportunities for detecting intersister connections whose elimination is dependent upon opposing poleward forces.

- In maize, an acentric fragment resulting from a three-strand double crossover in a paracentric inversion heterozygote tends to remain attached to its sister after anaphase I (80). Necessarily, any connections between such a fragment and its sister are not under tension from spindle fiber forces. Thus, lagging associations could be due to topological connections.
- More generally, for any given pair of sister chromatids, intersister connections that lie between the centromere and the most proximal crossover on either side are not under tension until meiosis II: within these regions, sisters move as a unit during the reductional division. Thus, topological connections might remain within such regions. In accord with this expectation, sister chromatids in lily chromosomes run parallel to each other near the centromere during early anaphase I, occasionally with intersister fibers visible (ref. 37; S. M. Stack, personal communication).

By anaphase of mitosis or anaphase I of meiosis, cell cycle-regulated sister chromatid connections should have lapsed. Thus, any topological connections remaining at those points might be entirely adventitous and unrelated to the intersister connections that maintain sister chromatid association through earlier stages (e.g., ref. 89).

The speculations above are united by general idea that meiotic interhomolog interactions evolved from mitotic intersister interactions. More specifically, interacting pairs of chromosomes, mitotic or meiotic, are connected by multiple interstitial interactions held closely to, and between, the corresponding structural axes; and the progression of events at those points of interstitial connection is influenced by transitions that occur along the interface between the structural axes and the axis-associated chromatin.

In this context, mitotic intersister interactions must be adapted to the meiotic interhomolog interaction process. This adaptation would include conversion of intersister interaction sites into interhomolog interaction sites, use of those sites for homolog pairing, recruitment of a DSB/recombination to those sites and functional coupling of the recombination process to changes along the axis-chromatin interface. Most particularly, crossover/noncrossover differentiation would be coupled to the underlying mitotically-derived minimization process.

The two major meiosis-specific structures of mid-prophase chromosomes, the conjoined sister-sister axis and the SC, could correspondingly be responsible for shepherding the two major transitions required by this scenario: the conversion(s) of intersister interaction sites to interhomolog interaction sites (and back), and the conversion of mitotic-like pairing interactions into DNA-based-crossovers. The two structures might thus both be scaffolds, for interstitial intersister interactions and for interstitial interhomolog interactions respectively.

Evolution of Meiosis from a Mitotic Cell Cycle

Given the considerations above, a plausible scenario for evolution of meiosis can be formulated.

Crossovers Before Two Divisions. Meiosis differs from a diploid mitotic cell cycle in two fundamental respects: the presence of crossovers and the occurrence of two successive rounds of chromosome segregation. Mechanistic considerations suggest that during evolution of meiosis, crossovers appeared first.

Sequential reductional and equational divisions in the absence of crossing over should be disastrous: homologs would segregate randomly at the first division. But the presence of crossovers during a mitotic cell cycle would be relatively inocuous. Crossovers would create interhomolog connections, as during meiosis, but those connections would not impede sister chromatid separation because they would disappear when intersister connections lapse.

How Did It Start? If crossovers appeared first, where did they come from? A likely source is recombinational repair of DNA damage. Diploidy could have arisen in response to the need for repair of damage via recombination off of a homolog (e.g., ref. 90). A newly evolved diploid cell population would thus be faced automatically with unscheduled interhomolog crossovers.

A Modified Single-Division Cell Cycle as an Intermediate Stage. Secondary deleterious effects of unscheduled interhomolog crossovers could provoke evolution of new, meiotic-like, features in the context of a single-division cell cycle.

Any crossover that occurs between the centromere and the nearest intersister connection would disrupt the regular tension-mediated orientation of sister chromatids between spindle poles. And if crossing over occurred between extended, randomly arranged homologs, interchromosomal entanglements would be trapped that also could cause malorientation at prometaphase (83).

The first problem would be eliminated if kinetochores directed reductional rather than equational segregation. The second problem would be ameliorated by homolog colocalization. By the considerations above, both problems would be solved if a mitotically dividing diploid organism acquired the meiosis-specific "side-by-side" relationship between sister chromatids.

The evolving cellular program would not yet be mechanistically stable, however. If kinetochores provoke exclusively reductional segregation, pairs of homologs not connected by a crossover would segregate inappropriately; and the number of crossovers provoked by DNA damage would likely be too few to ensure connection of every homolog pair in every cell cycle. Two solutions are possible.

- A single-division cell cycle would always yield rational segregation of genetic material if connected homologs always underwent reductional segregation while unconnected homologs always gave equational segregation (91). In fact, several yeast mutations confer single-division meiosis in which chromosomes undergo a mixture of reductional and equational segregation (91). A "mixed meiosis" condition could have been an intermediate stage in evolution of full meiosis as revealed by loss of recently added refinement functions.

This situation could be achieved mechanistically if persistence of the reductional kinetochore configuration were dependent upon tension between the microtubule assembly and the centromere/kinetochore, which in turn requires that homologs be connected. On unconnected homologs, kinetochores would degenerate into an equational form. This model explains other findings in ref. 91.

- Additional interchromosomal connections might evolve by acquisition of a nonspecific endogenous nuclease—i.e., by creation of "more damage." For increased effectiveness, nuclease action might be targeted preferentially to sites of axis-associated intersister interactions, which will likely tend to be especially accessible (92); saturation of such sites could provide crossovers to all chromosomes with a minimum number of extraneous lesions.

Two-Division Meiosis. A full two-division meiosis could now evolve. The occurrence of two divisions without an intervening round of DNA replication is usually ascribed to a minor dip in the level of maturation promoting factor (MPF) after the first division (93, 94). Also, one mutation that confers single-division meiosis in yeast, *spo13*, might affect a protein that directly modulates MPF

(94). Furthermore, since DSBs can block cell cycle progression by blocking MPF activation (93), an MPF dip might have evolved as a poorly controlled attempt to overcome such a block.

The other requirement of two-division meiosis, that centric intersister connections remain through the first division, might also be provided by the meiosis-specific relationship between sister chromatids. Synergy between meiosis-specific features and the intrinsic mitotic tendency for delayed centric region separation could suffice.

At some point, DSB-promoted recombination would become coupled to the mitotic-like minimization process. And only then, in response to the need for a scaffolding function, would the SC evolve.

Finally, some organisms alternate meiosis with a stable mitotic diploid phase. By the ideas above, the latter phase would have to arise via reversion to a standard cell cycle, including pure equational segregation. Complexities might become dispensible if DNA damage disappeared from the environment. Alternatively, cells might learn to cope with damage in another way, by channeling recombinational repair preferentially into a noncrossover mode (95).

Motivations. Survival of meiosis over evolutionary time requires long-term selective forces—e.g., advantages of sexual reproduction. The need to ameliorate modest mechanistic problems that arose in diploid cells exposed to high levels of DNA damage could have provided short-term advantages to relevant processes, thus providing time for longer term advantages to come into play.

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1. Nicklas, R. B. (1977) *Philos. Trans. R. Soc. London B* **277,** 267–276.
2. Carpenter, A. T. C. (1994) *Cell* **77,** 957–962.
3. Jones, G. H. (1987) in *Meiosis*, ed. Moens, P. B. (Academic, NY), pp. 213–244.
4. Padmore, R., Cao, L. & Kleckner, N. (1991) *Cell* **66,** 1239–1256.
5. Padmore, R. P. (1993) Thesis, (Harvard Univ., Cambridge, MA).
6. Schwacha, A. & Kleckner, N. (1994) *Cell* **76,** 51–63.
7. Goyon, C. & Lichten, M. (1993) *Mol. Cell. Biol.* **13,** 373–382.
8. Storlazzi, A., Xu, L., Cao, L. & Kleckner, N. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 8512–8516.
9. Nag, D. K. & Petes, T. (1990) *Mol. Cell. Biol.* **13,** 2324–2331.
10. Weiner, B. & Kleckner, N. (1994) *Cell* **77,** 977–991.
11. von Wettstein, D., Rasmussen, S. W. & Holm, P. B. (1984) *Annu. Rev. Genet.* **18,** 331–413.
12. Guacci, V., Hogan, E. & Koshland, D. (1994) *J. Cell Biol.* **125,** 517–530.
13. Miyazaki, W. Y. & Orr-Weaver, T. L. (1994) *Annu. Rev. Genet.* **28,** 167–187.
14. Rattner, D., Goldsmith, J. B. & Hamkalo, M. R. (1981) *Chromosoma* **82,** 341–351.
15. Moens, P. B. & Perlman, R. E. (1988) *BioEssays* **9,** 151–153.
16. Gimenez-Abian, J. F., Clarke, D. J., Mullinger, A. M., Downes, C. S. & Johnson, R. T. (1995) *J. Cell Biol.* **131,** 7–17.
17. Rufas, J. S., Mazzella, C., Suja, J. A. & Gardia de la Vega, C. (1989) *Eur. J. Cell Biol.* **48,** 220–226.
18. Goldstein, L. S. B. (1981) *Cell* **25,** 591–602.
19. Kleckner, N. & Weiner, B. (1993) *Cold Spring Harbor Symp. Quant. Biol.* **58,** 553–565.
20. Zickler, D. (1977) *Chromosoma* **61,** 289–316.
21. Albini, S. M. & Jones, G. H. (1987) *Chromosoma* **95,** 324–338.
22. Kleckner, N. (1996) *Harvey Soc. Lect.*, in press.
23. Goldman, A. & Lichten, M. (1996) *Annu. Rev. Genet.*, in press.
24. Roeder, G. S. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 10450–10456.
25. Schwacha, A. & Kleckner, N. (1995) *Cell* **83,** 783–791.
26. Anderson, L. K. & Stack, S. (1988) *Chromosoma* **97,** 96–100.
27. Sym, M., Engebrecht, J. & Roeder, G. S. (1993) *Cell* **72,** 365–378.
28. Carpenter, A. T. C. (1988) in *Genetic Recombination*, eds. Kucherlapati, R. & Smith, G. R. (Am. Soc. Microbiol., Washington, DC), pp. 529–548.
29. Dresser, M. & Giroux, C. N. (1988) *J. Cell Biol.* **106,** 567–573.
30. Moses, M. J. (1968) *Annu. Rev. Genet.* **2,** 363–412.
31. Schmekel, K. & Daneholt, B. (1995) *Trends Cell Biol.* **5,** 239–242.
32. Stack, S. & Anderson, L. K. (1986) *Chromosoma* **94,** 253–258.
33. Loidl, J., Klein, F. & Scherthan, H. (1994) *J. Cell Biol.* **125,** 1191–1200.
34. Nag, D. K., Scherthan, H., Rockmill, B., Bhargava, J. & Roeder, G. S. (1995) *Genetics* **141,** 75–86.
35. Xu, L. & Kleckner, N. (1995) *EMBO J.* **14,** 5115–5128.
36. Henderson, S. A. (1970) *Annu. Rev. Genet.* **4,** 295–324.
37. Stack, S. M. (1991) *Genome* **34,** 900–908.
38. Heyting, C. & Dietrich, A. J. J. (1992) *Cell Biol. Int. Rep.* **16,** 749–760.
39. Solari, A. J. (1981) in *International Cell Biology, 1980–1981*, ed. Schweiger, H. G. (Springer, New York), pp. 178–186.
40. Rufas, J. S., Gimenez-Albian, J., Suja, J. A. & Garcia De La Vega, C. (1987) *Genome* **29,** 706–712.
41. Solari, A. J. & Tandler, C. J. (1991) *Genome* **34,** 888–894.
42. Orr-Weaver, T. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 10443–10449.
43. Moens, P. B. & Spyropoulos, B. (1995) *Chromosoma* **104,** 175–182.
44. Xu, L. & Kleckner, N. (1996) *Genes Dev.*, in press.
45. Suja, J. A., Antonio, C. & Rufas, J. S. (1992) *Chromosoma* **101,** 493–501.
46. Rockmill, B., Sym, M., Scherthan, H. & Roeder, G. S. (1995) *Genes Dev.* **9,** 2684–2695.
47. Goldway, M., Sherman, A., Zenvirth, D., Arbel, T. & Simchen, G. (1993) *Genetics* **133,** 159–169.
48. Wu, T.-C. & Lichten, M. (1994) *Science* **263,** 515–518.
49. Ohta, K., Shibata, T. & Nicolas, A. (1994) *EMBO J.* **13,** 5754–5763.
50. Zenvirth, D., Arbel, T., Sherman, A., Goldway, M., Klein, S. & Simchen, G. (1992) *EMBO J.* **11,** 3441–3447.
51. de Massy, B., Baudat, F. & Nicolas, A. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 11929–11933.
52. Gilbertson, L. A. & Stahl, F. W. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 11934–11937.
53. Fan, Q. & Petes, T. D. (1996) *Mol. Cell Biol.*, in press.
54. Alani, E., Padmore, R. & Kleckner, N. (1990) *Cell* **61,** 1089–1101.
55. Raymond, W. & Kleckner, N. (1993) *Nucleic Acids Res.* **21,** 3851–3856.
56. Johzuka, K. & Ogawa, H. (1995) *Genetics* **139,** 1521–1532.
57. Sharples, G. J. & Leach, D. R. F. (1995) *Mol. Microbiol.* **17,** 1215–1220.
58. Jones, G. H. (1984) *Symp. Soc. Exp. Biol.* **38,** 293–320.
59. Perkins, D. D., Lande, R. & Stahl, F. W. (1993) *Genetics* **133,** 690–691.
60. Hilliker, A. J., Clark, S. H. & Chovnick, A. (1993) *Genetics* **129,** 779–781.
61. King, J. S. & Mortimer, R. K. (1990) *Genetics* **126,** 1127–1138.
62. Lande, R. & Stahl, F. W. (1993) *Cold Spring Harbor Symp. Quant. Biol.* **58,** 543–552.
63. Zickler, D., Moreau, P. J., Huynh, A. D. & Slezec, A. M. (1992) *Genetics* **132,** 135–148.
64. Havekas, F. W. J., de Jong, J. H., Heyting, C. & Ramanna, M. S. (1994) *Chromosome Res.* **2,** 315–325.
65. Sherman, J. D., Herickhoff, L. A. & Stack, S. M. (1992) *Genome* **35,** 907–915.
66. Szostak, J. W., Orr-Weaver, T. L., Rothstein, R. J. & Stahl, F. W. (1983) *Cell* **33,** 25–35.
67. Darlington, C. D. (1937) *Recent Advances in Cytology* (Blakiston, Philadelphia), 2nd Ed.
68. Molè-Bajer, J. (1958) *Chromosoma* **9,** 332–358.
69. Douglas, L. T. (1970) *Genetica* **41,** 231–256.
70. Boy de la Tour, E. & Laemmli, U. K. (1988) *Cell* **55,** 937–944.
71. Bishop, D. K., Park, D., Xu, L. & Kleckner, N. (1992) *Cell* **69,** 439–456.
72. Rose, D. & Holm, C. (1993) *Mol. Cell Biol.* **13,** 3445–3455.
73. Jang, J. K., Messina, L., Erdman, M. B., Arbel, T. & Hawley, R. S. (1995) *Science* **268,** 1917–1919.
74. Dawe, R. K., Sedat, J. W., Agard, D. A. & Cande, W. Z. (1994) *Cell* **76,** 901–912.
75. Carpenter, A. T. C. (1979) *Chromosoma* **75,** 259–292.
76. Hari, K. L., Santerre, A., Sekelsky, J. J., McKim, K. S., Boyd, J. B. & Hawley, R. S. (1995) *Cell* **82,** 815–821.
77. Egel, R. (1995) *Trends Genet.* **11,** 206–208.
78. Jones, G. H. & Tease, C. (1981) *Chromosomes Today* **7,** 114–125.
79. Bennett M. D. (1977) *Philos. Trans. R. Soc. London B* **277,** 201–226.
80. Maguire, M. P. (1995) *J. Hered.* **86,** 330–340.
81. Kohli, J. & Bahler, J. (1994) *Experientia* **50,** 296–306.
82. Sym, M. & Roeder, G. S. (1994) *Cell* **79,** 283–292.
83. Kleckner, N., Padmore, R. & Bishop, D. (1991) *Cold Spring Harbor Symp. Quant. Biol.* **56,** 729–743.
84. Roeder, G. S. (1990) *Trends Genet.* **6,** 385–389.
85. Solari, A. J. (1988) *Genetica* **77,** 149–158.
86. Kadyk, L. C. & Hartwell, L. H. (1992) *Genetics* **132,** 387–402.
87. Sears, D. D., Hieter, P. & Simchen, G. (1994) *Genetics* **138,** 1055–1065.
88. Moreau, P. J. F., Zickler, D. & Leblon, G. (1985) *Mol. Gen. Genet.* **198,** 189–197.
89. Holm, C. (1994) *Cell* **77,** 955–957.
90. Maynard Smith, J. & Szathmary, E. (1995) *The Major Transitions in Evolution* (Freeman, New York).
91. Simchen, G. & Hugerat, Y. (1991) *BioEssays* **15,** 1–8.
92. Gross, D. S. & Garrard, W. T. (1988) *Annu. Rev. Biochem.* **57,** 159–197.
93. Murray, A. & Hunt, T. (1993) *The Cell Cycle* (Freeman, New York).
94. McCarroll, R. M. & Esposito, R. E. (1994) *Genetics* **138,** 47–60.
95. Worth, L., Jr., Clark, S., Radman, M. & Modrich, P. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 3238–3241.
96. Storlazzi, A., Xu, L., Schwacha, A. & Kleckner, N. (1996) *Proc. Natl. Acad. Sci. USA*, in press.
97. Petes, T. & Pukilla, P. (1995) *Advances in Genetics* **33,** 41–62.
98. Scherthan, H., Bähler, J. & Kohli, J. (1994) *J. Cell. Biol.* **127,** 273–285.
99. Goldman, A. S. H. & Lichten, M. (1996) *Genetics*, in press.
100. Keeney, S. & Kleckner, N. (1996) *Genes Cells* **1,** 475–489.
101. Molnar, M. Bähler, J., Sipiczki, M. & Kohli, J. (1995) *Genetics* **141,** 61–73.
102. Rocco, V. & Nicolas, A. (1996) *Genes Cells*, in press.
103. Lydall, D., Nikolsky, Y., Bishop, D. K. & Weinert, T. (1996) *Nature (London)*, in press.