Homologous Pairing Is Reduced But Not Abolished in Asynaptic Mutants of Yeast

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Abstract. In situ hybridization was used to examine chromosome behavior at meiotic prophase in the *rad50S*, *hop1*, *rad50*, and *spo11* mutants of *Saccharomyces cerevisiae*, which are defective in chromosome synapsis and meiotic recombination. Painting of chromosomes I and III revealed that chromosome condensation and pairing are reduced in these mutants. However, there is some residual pairing in meiosis, suggesting that homologue recognition is independent of synaptonemal complex formation and recombination. Association of homologues was observed in the *rad50*, *rad50S*, and *spo11* mutants, which are defective in the formation or processing of meiotic double-strand breaks. This indicates that double-strand breaks are not an essential component of the meiotic homology searching mechanism or that there exist additional or alternative mechanisms for locating homologues.

In sexually reproducing eukaryotes, homologous chromosomes separate from each other during meiosis to produce haploid gametes. During meiotic prophase, homologous chromosomes locate each other and associate as a precondition for their disjunction at the first meiotic division. The mechanisms by which homologous chromosomes are recognized are largely unknown, but it is likely that the matching of DNA base sequences at corresponding chromosomal sites is involved. Early in the pairing process, homologous chromosomes become arranged in parallel at some distance from each other (presynaptic alignment; for review see Loidl, 1990). Later, chromosomes develop proteinaceous axial elements along their lengths that then become connected by transverse filaments to form the synaptonemal complex (SC)[1]. At around this time, crossing over takes place. There is evidence that early steps in meiotic recombination are initiated before synapsis (Padmore et al., 1991), but in most organisms, the formation of mature SC is a precondition for chiasma formation and the orderly segregation of chromosomes during the first meiotic division (von Wettstein et al., 1984).

A number of mutants of *Saccharomyces cerevisiae* show combined defects in the formation of double-strand breaks (DSBs), reciprocal recombination, and SC formation. It is unclear how these phenotypes are related. It has been suggested that homology recognition, chromosome synapsis, and the initiation of recombination depend on DSBs (Alani et al., 1990; Sun et al., 1991). Meiotic DSBs are processed to produce single-stranded tails with 3′ overhangs (Sun et al., 1991; Bishop et al., 1992); these single strands could search for homology by invading duplex DNA. Alternatively, DSBs may be recombination intermediates that are formed only at sites where homologous contacts have already been established (Goyon and Lichten, 1993; Hawley and Arbel, 1993).

Meiotic chromosome pairing is monitored conventionally by examination of the SC in silver-stained spread chromosome preparations. In mutants that fail to make SC or at stages in meiosis before SC formation, fluorescence in situ hybridization (FISH) can be used to visualize the behavior of meiotic chromosomes. Previously, FISH with chromosome-specific probes has been applied to studies of the relative positioning of homologous chromosomes in interphase nuclei (see Lichter et al., 1991). Using FISH, the onset of somatic pairing in *Drosophila* embryos at the beginning of nuclear cycle 14 was observed (Hiraoka et al., 1993), and presynaptic homologous alignment in yeast meiosis was demonstrated (Scherthan et al., 1992; Loidl, 1993). The delineation of whole chromosomes, or large parts thereof, by FISH with pooled contiguous DNA probes (chromosome painting) has the advantage that it reveals not only the relative positions of chromosomes, but also their degree of condensation.

We have used chromosome painting to study various yeast mutants (*rad50S*, *hop1*, *rad50*, and *spo11*) known to be deficient in meiotic recombination, DSB formation, and SC formation for their ability to undergo meiotic chromosome condensation and homologous pairing. To determine whether pairing capability is correlated with the extent of axial element and/or SC formation as seen in silver-stained prepara-

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1. *Abbreviations used in this paper*: DSB, double-strand breaks; FISH, fluorescence in situ hybridization; SC, synaptonemal complex.

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tions, we compared pairing and axial element/SC development at different times during sporulation and in different mutants.

Mutations can be variably expressed in different genetic backgrounds, and deviating results are often obtained in different laboratories because of variations in experimental protocols. In particular, variations in cytological preparation techniques may influence the preservation of SC-related structures, especially in mutants in which they may be unstable. By studying mutants that share an identical genetic background and by using identical preparation procedures, we have obtained comparable data on chromatin condensation, chromosome pairing, and the development of meiosis-specific structures in the wild-type and various mutants.

Materials and Methods

Source and Construction of Strains

All strains used are isogenic to SK1 (Table I). We used the haploid strains SK1a and SK1α to disrupt meiotic genes. Mutations were introduced by one-step gene replacement (Rothstein, 1983), and correct replacement was verified by Southern blotting (results not shown). The *rad50::hisG* deletion was verified by testing for radiation sensitivity at an x-ray dose of 12 krad. All mutant strains constructed display dramatically lowered spore viability in agreement with their severe meiotic defects.

The *rad50::hisG* deletion was constructed using deletion plasmid pNKY83 (Alani et al., 1990). In the first step, the entire coding region of the *RAD50* gene was replaced by a *hisG::URA3::hisG* fragment (Alani et al., 1987). In the second step, a derivative was selected that had lost the *URA3* marker by homologous recombination between the two adjacent *hisG* fragments. The deletion was introduced into SK1a and SK1α independently, and the two derivatives were mated to yield SK1 *rad50.* The *hop1::LEU2* disruption was constructed using plasmid pNH37-2 (Hollingsworth et al., 1990), which inserts a *LEU2* fragment in the BamH1 site of the *HOP1* gene. Two haploid SK1 strains were transformed independently, and the transformants were mated to give SK1 *hop1. hop1::LEU2* probably represents a null mutation, since no Hop1 protein could be detected in pachytene by immunostaining using a polyclonal α-Hop1 antibody (data not shown). The *spo11::LEU2* disruption was constructed using plasmid p(*spo11*)35 (gift from R. E. Esposito, Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL). The *SPO11* gene was disrupted by inserting the 1.1-kb *URA3* EcoR1 fragment into the EcoR1 site of *SPO11.* Strains carrying this disruption homozygously do not initiate meiotic recombination at all. Only SK1a was disrupted. The resulting SK1a *spo11::LEU2* was crossed to SK1 *HO* wild type. 50% of the segregants were *HO* and therefore sporulated, and 50% out of these produced dead spores because of the *spo11::LEU2* allele. One diploid segregant was chosen as SK1 *spo11.*

Growth, Sporulation, and Chromosome Spreading

Yeast strains were grown as reported previously (Loidl et al., 1991). Cells were grown in presporulation medium to a density of $\sim 2 \times 10^7$ cells/ml, and cell suspensions were stored at 0°C overnight. On the next day, cells were sporulated in 2% potassium acetate. In wild-type SK1, a time course was performed to determine the time of maximal SC formation (Table II). It was found that it occurs at ~5 h in sporulation, which is in agreement with an earlier study (Scherthan et al., 1992). In the mutants, development of SCs and SC-related structures was checked in silver-stained preparations after 5 h sporulation (when SC formation is at a maximum in the wild type) and after 7 h to see if SC development is retarded in the mutants. In *hop1*, *rad50*, and *spo11* no increase in SC related structures was found, whereas in *rad50S*, SC precursors and mature SCs were more abundant after 7 h. In situ hybridization experiments for the determination of homologous associations were performed at the stage when SC formation was at a maximum (i.e., after 5 h), in *hop1*, *rad50*, and *spo11* and after 5 h and 7 h in *rad50S*. Whole mount spreads of meiocytes were prepared as reported previously (Loidl et al., 1991), both for light microscopic and electron microscopic investigation of SCs and for FISH.

DNA Probes and Labeling

Complex DNA probes for yeast chromosomes I and III were generated as follows. Lambda clones D39c, K3c, G4a, and F58f (Steensma et al., 1987) were combined in approximately equimolar amounts to give a probe pool for chromosome I. The pool included sequences from the centromere and adjacent sequences covering 60 kb of this 230-kb chromosome. A probe pool for chromosome III was obtained by combining YIp5 plasmid clones E5FR, J10A, G2F, M5G, C1G, C2G, D12B, J11D, K3B, and I2B (Newlon et al., 1991) in equimolar amounts. This complex probe contained 120 kb spanning ~185 kb of this 340-kb chromosome.

1 μg of the chromosome I probe pool was labeled with digoxigenin-dUTP (Boehringer-Mannheim Corp., Indianapolis, IN) and 1 μg of the chromosome III probe pool with biotin-14-dATP (Life Technologies, Inc., Grand Island, NY) using a nick translation kit (Life Technologies, Inc.), according to the instructions of the supplier. Probes were then ethanol precipitated and dissolved in 25 μl of hybridization mixture (2× SSC, 50% formamide, 10% dextran sulfate, 1 μg/μl salmon testis DNA). Probes were kept at −20°C until use.

Chromosome Painting

FISH to spreads of meiotic chromosomes was performed as described in detail by Scherthan et al. (1992, 1993). In brief, slides were incubated in 4× SSC, 0.1% Tween 20 at 37°C for 2 h. After a brief wash in distilled H_2O, the preparations were submerged in 70% formamide/2× SSC for 3 min at 75°C to denature chromosomal DNA. Digoxigenin- and biotin-

Table I. List of Strains

Name	Genotype	Constructed
SK1 Wild type	*MATa HO* / *MATα HO*	Kane and Roth (1974)
SK1a	*MATa ho::LYS2 leu2::hisG his4X ura3 lys2*	N. Kleckner
SK1α	*MATα ho::LYS2 leu2::hisG his4B ura3 lys2*	N. Kleckner
SK1 *rad50*	*MATa leu2::hisG his4X rad50::hisG ura3* / *Matα leu2::hisG his4B rad50::hisG ura3*	This work
SK1 *hop1*	*MATa leu2::hisG his4X hop1::LEU2 ura3* / *MATα leu2::hisG his4B hop1::LEU2 ura3*	This work
SK1 *spo11*	*MATa leu2::hisG his4X spo11::URA3 HO ura3* / *MATα leu2::hisG his4X spo11::URA3 HO ura3*	This work
SK1 *rad50S*	*MATa rad50-K181-URA3 ho::LYS2 ura3 lys2* / *MATα rad50-K181-URA3 ho::LYS2 ura3 lys2*	Alani et al. (1990)

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Table II. Chromosome Pairing, Condensation, and SC Formation at Different Times in Meiosis in Wild Type

Time in sporulation		0 h	1 h	2 h	3 h	4 h	5 h
FISH preparations:	Homologous associations* in nuclei with compact signals	49%	43%	30%	37%	94%	98%
	Heterologous associations‡ in nuclei with compact signals	15%	15%	12%	6%	3%	3%
	n (FISH signal pairs)	286	198	242	262	236	226
	Nuclei with compact signals	15%	3%	6%	24%	66%	75%
	n (nuclei)	150	270	215	177	170	244
Silver-stained preparations:	Unstructured nuclei	100%	100%	98%	79%	10%	7%
	Axial elements and SC fragments	0%	0%	2%	19%	35%	32%
	complete (or nearly complete) SCs	0%	0%	<1%§	2%	55%	61%
	n (nuclei)	500	500	203	426	282	223

n, Sample size.
* Two chromosome I (red) or two chromosome III (green) compact FISH signals associated.
‡ Chromosome I and chromosome III compact FISH signals associated with each other.
§ Estimated value. On a slide with several thousands of nuclei, two nuclei with complete SCs were detected.

labeled chromosome I and chromosome III probes were denatured for 5 min at 95°C and applied to the preparation under a coverslip. Hybridization was performed for 36 h at 37°C. Preparations were then washed 3×, 5 min in 0.02× SSC, and transferred to bicarbonate/Tween buffer (0.15 M $NaHCO_3$, 0.1% Tween 20). Biotinylated chromosome III probe was detected using avidin-FITC (Sigma Immunochemicals, St. Louis, MO) (Pinkel et al., 1988) and the digoxigenin-labeled chromosome I probe was detected with tetramethylrhodamine isothiocyanate-conjugated secondary and tertiary antibodies (Sigma Immunochemicals) to a primary mouse antidigoxigenin antibody (Boehringer-Mannheim Corp.) (Scherthan et al., 1992). FISH with the compound probe for chromosome I produced, in addition to the major signals, a pair of dots that we assume to represent cross-hybridization with a site on a different chromosome. Only the major signals were used for evaluation of spatial relationships.

Microscopy

Preparations were mounted in antibleach medium (Vectashield; Vector Laboratories, Burlingame, CA) supplemented with 0.5 μg/ml 4'6-diamidino-2-phenylindole for staining the chromatin background. Preparations were examined using a fluorescence microscope (Axioskop; Carl Zeiss) equipped with a dual bandpass filter for simultaneous excitation and detection of red (tetramethylrhodamine isthiocyanate) and green (FITC) fluorescence. Micrographs were taken on color slide film (Ektachrome 400; Kodak Corp., Rochester, NY).

Evaluation of Homologous Pairing

The frequency of associations between FISH signals in spread preparations is influenced by the relationship between signal size and the area covered by the spread nucleus. In other words, in smaller or weakly stained nuclei, the chance of merely accidental associations of signals is expected to be higher. To account for this variable, we used the frequency of heterologously associated signals to estimate the fraction of accidentally homologous associations. In the following derivations, two signal pairs are indicated by a and b.

List of variables:

- x_a fraction of true homologous associations between the signals of pair a
- y_a, (y_b) fraction of observed homologous associations between signals of pair a (pair b)
- p_a, (p_b) probability for merely accidental associations between signals of pair a (pair b)
- p_c probability for accidental associations between heterologous signals
- z_b average number of separate signals from pair b per nucleus.

The relation between true and observed associations is

$$x_a = y_a - p_a (1 - x_a).$$

A signal of an unassociated pair a has either one homologous and two heterologous partners for accidental association ($p_a = p_c/2$), or one homologous and one heterologous partner ($p_a = p_c$) if signal pair b is fused. Therefore, on the average

$$p_a = p_c/z_b, \text{ where}$$
$$z_b = y_b + 2(1 - y_b). \text{ It follows that}$$
$$x_a = \frac{y_a - p_a}{1 - p_a}, \text{ where } p_a = \frac{p_c}{2 - y_b}.$$

Results

Measurements of Chromosome Pairing and Compaction by FISH

Spread nuclei were painted with probes for chromosomes I and III, which were detected as red and green signals, respectively. Two spots of the same color indicate that homologous chromosomes are unpaired, whereas a single spot indicates homologous pairing (Fig. 1). A subset of nuclei showed patterns of dispersed dotlike signals that allowed no clear decision about the spatial relationships among chromosomes (Fig. 1 *b*). Only nuclei showing clear and compact hybridization signals for both chromosomes I and III were selected for evaluation. It is likely that signal compaction reflects chromosome condensation, which reaches a maximum at pachytene in *S. cerevisiae* (Dresser and Giroux, 1988). In the wild type, FISH signals were either dot- or hyphen-shaped and continuous except for occasional visible gaps in the chromosome III signal, representing regions not covered by clones of the compound probe. In the mutants, highly compacted signals were less frequent. Nevertheless, many nuclei displayed string-shaped signals with a traceable course, and hence, these nuclei were appropriate for analysis.

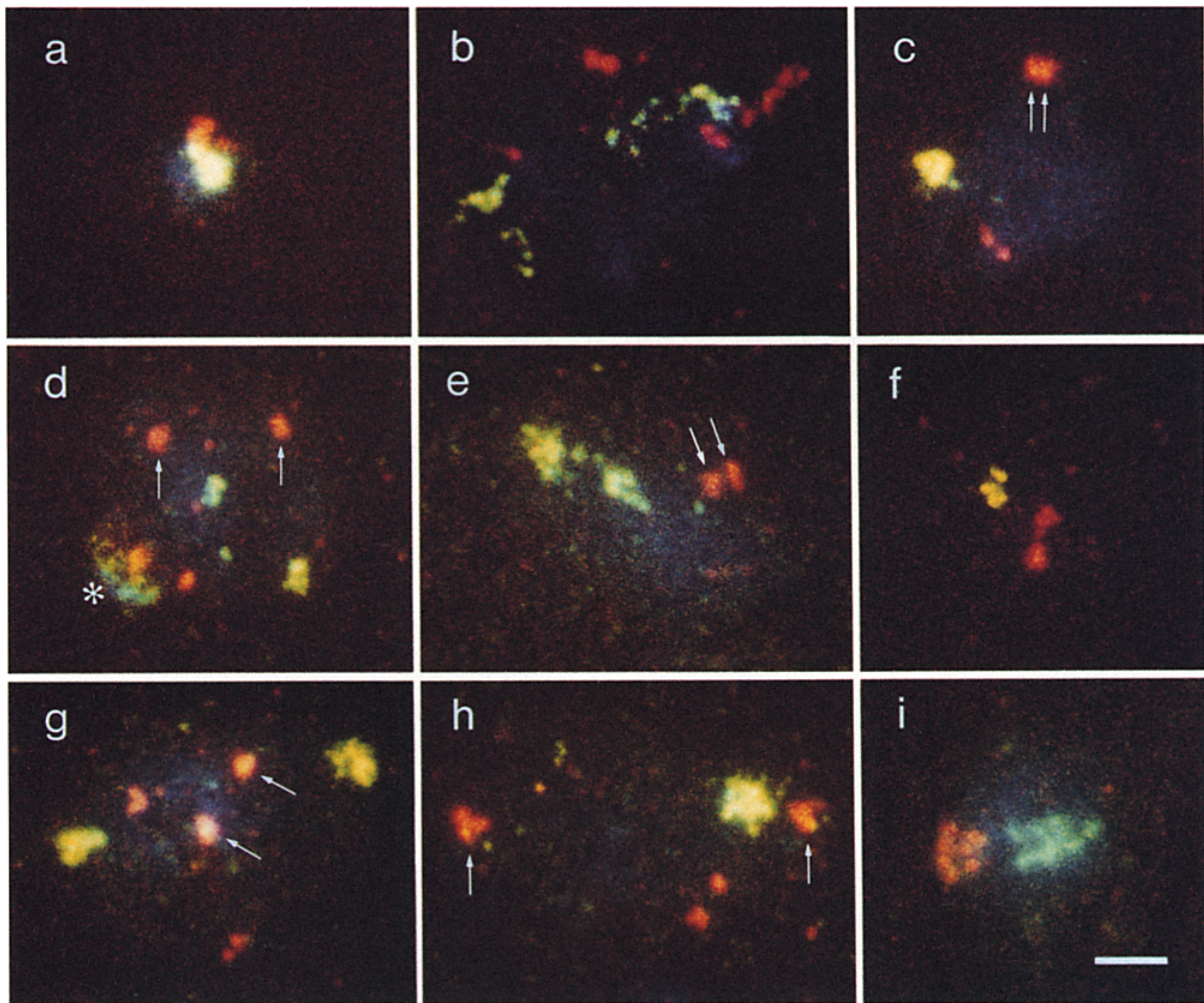

Figure 1. FISH with probes delineating parts of yeast chromosomes I (*red*) and III (*yellow-green*) of meiotic nuclei. Sometimes, two small red spots can be seen in addition to the main signals; these result from cross-hybridization of the chromosome I probe with part of a different chromosome. Where this is the case, the main signals are denoted by arrows. The blue background color in some of the nuclei results from staining with the DNA specific dye DAPI. The fusion or association of fluorescence signals of the same color indicates pairing of the corresponding chromosomes. (*a–c*) Wild type. (*a*) At time point zero of sporulation, there are still many mitoses where homologous and nonhomologous chromosomes are strongly condensed and clustered. (*b*) In G_0 and early meiotic stages, chromosomes are decondensed and reveal a dispersed FISH pattern. (*c*) Nucleus after 5 h in sporulation, with both chromosomes I and III associated. (*d–i*) Nuclei of mutants with compact signals after 5 h (*rad50S* 7 h) in sporulation. (*d*) *spo11*, all chromosomes separate. *Unspecifically labeled nucleolus. (*e*) *rad50S*, chromosomes I associated. (*f*) *hop1*, both chromosomes associated. (*g–i*) Nuclei of the *rad50* mutant with all chromosomes separate (*g*), chromosomes III associated (*h*), and both chromosomes associated (*i*). Bar, 2 μm.

The ability of different mutants to undergo homologue association (and hence homology recognition) was determined by assessing the frequency of associated vs nonassociated homologous FISH signal pairs (Tables II and III). We classified as associated those signals that were completely fused or touching each other (Fig. 1). In some nuclei, associations between nonhomologous signals were found. This suggests that accidental associations may contribute to the observed homologous associations. We inferred the frequency of accidental homologous associations from the frequency of heterologous associations as explained in the Materials and Methods. These accidental associations were then subtracted from the observed homologous associations to obtain an estimate of truly homologous interactions (Table III).

As indicated above, the frequency of homologous associations was determined only in nuclei with compact FISH signals. Nuclei with dispersed signals probably represent premeiotic interphase cells or cells at early stages in meiosis. Although the frequency of homologous associations could not be evaluated quantitatively in these nuclei, homologue pairing appeared to be rare. Throughout the remainder of this paper, it is assumed that meiotic homologous pairing does not occur before the onset of meiotic chromatin condensation. Since only a subset of nuclei display compact FISH signals, the frequency of homologous associations among to-

Table III. Chromosome Pairing, Condensation, and SC Formation in Wild Type and Meiotic Mutants

Time in sporulation		Wild type 5 h	*rad50S* 7 h	*hop1* 5 h	*rad50* 5 h	*spo11* 5 h
FISH preparations*:	Homologous associations‡ in nuclei with compact signals	95%	47%	57%	32%	26%
	Heterologous associations‡ in nuclei with compact signals	7%	5%	6%	7%	8%
	n (FISH signal pairs)	588	712	470	476	472
	Estimate of true pairing§ in nuclei with compact signals	95%	45%	55%	29%	22%
	Pairing in nuclei with compact signals relative to wild type	100%	47%	58%	30%	23%
	Nuclei with condensed chromosomes‖	73%	53%	39%	25%	34%
	Pairing in total nuclei¶	69%	25%	22%	8%	9%
	Pairing in total nuclei relative to wild type	100%	36%	32%	12%	13%
Silver-stained preparations:	Unstructured nuclei	26%	39%	63%	77%	50%
	Axial elements and SC fragments**	15%	54%	37%	23%	50%
	Complete (or nearly complete) SCs	59%	7%	0%	0%	0%
	n (nuclei)	686	224	152	167	176

n, Sample size.
* Values are averaged from three experiments each. Only nuclei with signals for both chromosomes I and III were used for evaluation and data for chromosomes I and III were pooled.
‡ The difference between total FISH signals and those involved in homologous and heterologous associations does not equal the frequency of single signals because signals that were part of clusters involving homologously and heterologously associated signals were included in both categories. Therefore, homologous plus heterologous associations amount to >100% in the wild type.
§ An estimate of truly homologously interacting signals is derived from the incidence of heterologously associated (red-green) signals. For the calculation of corrected values, see Materials and Methods.
‖ Identified by compact FISH signals.
¶ Under the assumption that pairing occurs only in nuclei with condensed chromosomes.
** In the wild type and in *rad50S*, SC precursors included axial elements and SC fragments; in *hop1* and *rad50*, only axial elements were found. In *spo11*, a few nuclei (<1%) with very short presumptive SC fragments were observed in one experiment.

tal nuclei is always less than the frequency among nuclei with condensed chromatin (Table III).

It should be noted that the pairing behavior of chromosomal subregions as delineated by the hybridization probes cannot be taken as an absolute measure of the extent of pairing because homologous contacts outside the highlighted regions would escape detection. However, pairing of chromosomal subregions can be used to compare the pairing capacity of mutants relative to wild type. Since the efficiency of sporulation was found to vary considerably in different experiments, each experiment was performed several times. In Table III, the values from the three experiments with the highest incidences of homologous associations for the wild type and mutants are pooled.

Pairing in Wild Type

A meiotic time course experiment was carried out to determine the period of maximal SC development, chromosome condensation, and pairing in wild-type SK1. The frequencies of nuclei with compact signals detected by FISH and with SC or SC-related structures observable in silver-stained preparations were compared at 0–5 h after introduction into sporulation medium (Table II). Nuclei with SCs appeared after 3 h of sporulation, and their increase over time paralleled the increase in nuclei with compact FISH signals. Thus, signal compaction at meiosis is correlated with the development of SCs, and we assume that nuclei with disperse signals are at earlier stages of prophase. However, at 0 h of sporulation, a considerable portion of nuclei displayed compact FISH signals, whereas no axial elements or SCs were present (Table II). After 1 and 2 h in sporulation, which is probably during premeiotic interphase, their number decreased and later increased again with the onset of SC formation. We tentatively assume that nuclei with compact signals that appear at 0 h in sporulation contain condensed mitotic chromosomes (see below).

In nuclei containing condensed chromatin, 98% of FISH signal pairs were homologously associated after 5 h of sporulation in one experiment (Table II). Repetitions revealed some variability and produced values of 94, 93, and 88%. Table III shows the average from the three experiments with the highest values (95%). Since 73% of nuclei showed chromatin condensation and compact signals, the frequency of chromosomes I and III involved in meiotic homologous associations is estimated as 69% of total nuclei (Table III). Consistent with the high incidence of pairing in the wild-type, mature SCs were found in ~60% of nuclei (Tables II and III; Table III shows an average from several experiments). The lower incidence of SCs than of homologously associated chromosomes can be explained by the fact that homologous alignment precedes the development of SCs (see

Scherthan et al., 1992). In addition, associations may persist into diplotene after SCs become degraded.

Surprisingly, ~50% of homologous FISH signals in nuclei with compact signals are associated at the time of introduction into sporulation medium (Table II). Also, the incidence of associations between heterologous FISH signals is much higher early in sporulation than after 5 h (Table II; Fig. 1 *a*). This suggests that at least some of the contacts between homologues before meiotic pairing may be unspecific and caused by the dense packaging of chromosomes in these nuclei. Since we assume that nuclei with compact signals are at mitotic division stage (see above), chromosome clustering could result from their attachment to the mitotic spindle. Since the frequency of nuclei with compact FISH signals is 15%, the incidence of associations may be only 7% of total nuclei.

Chromosome Pairing, Chromatin Condensation, and SC Assembly in Meiotic Mutants

All of the mutants examined showed axial element formation to various degrees, but none displayed wild-type levels of SC formation (Fig. 2). Wild-type SK1 attained maximal chromosome pairing and SC development after 4.5-5 h in sporulation medium (Table II; Scherthan et al., 1992). In all of the mutants except *rad50S*, axial element development reached a maximum at ~5 h sporulation. In *rad50S*, maximum development of SC-related structures was highest only after 7 h (see Materials and Methods).

The *rad50S* mutant is unable to form spores and this defect is not suppressed by *spo13*, suggesting that this mutant progresses through meiotic prophase to a point where it cannot be rescued by bypassing the first division (Alani et al., 1990). The *rad50S* mutant makes meiotic DSBs, but it is unable to produce single-stranded 3′ overhangs by 5′ strand resection (Sun et al., 1991; Bishop et al, 1992). Since single strands have been attributed a role in homology searching by several authors, it was interesting to see if this defect has consequences on homologous chromosome pairing. Indeed, after 5 h sporulation, homologous association of FISH signals was observed in only 27, 26, and 23% of nuclei with condensed chromatin in three experiments. However, after 7 h, homologous association reached 50, 49, and 41% (Table III shows the average of 47%). Since the frequency of nuclei with condensed chromosomes was 53%, homologues were associated in 25% of total nuclei. Thus, pairing is 36% of the wild-type level (Table III).

Alani et al. (1990) reported the formation of axial elements and only occasional short stretches of SCs in *rad50S*. In contrast, we observed fairly extensive SC formation that was nearly complete in ~7% of cells after 7 h (Fig. 2 *c* and Table III). This might be caused by more gentle conditions of preparation that may preserve the less stable SC formed by this mutant. Apparently, pairing and SC formation progress slower than in wild type and reach lower final levels.

The *HOP1* gene has been shown to encode a component of meiotic chromosomes. Homologous meiotic recombination is reduced to ~10% of wild type level in the *hop1-1* mutant (Hollingsworth and Byers, 1989), whereas it is as low as 1% in the null mutant (Rockmill and Roeder, 1990). After 5 h of sporulation, we found chromosome condensation in 39% of nuclei of the *hop1* mutant (Table III). In three different FISH experiments, condensed homologous chromosomes were associated at frequencies of 60, 57, and 56%, (mean: 57%; Table III), indicating that Hop1 protein is not essential for homology recognition. In total nuclei (including those with dispersed FISH signals), the frequency of pairing amounts to 22%, which leads to an estimate of the pairing frequency in the *hop1* mutant as 32% of wild type (Table III).

The Hop1 protein has been localized to the axes of pachytene chromosomes (Klein, F., and B. Byers, manuscript in preparation), suggesting that it plays a structural role in the axial elements of mature SCs. However, it is not essential for axial element formation per se, since in the *hop1* mutant, we found axial elements and axial element fragments in 37% of nuclei in spreads made after 5 h of sporulation (Fig. 2 *d*). Nuclei with SCs were not present (Table III).

rad50 null mutants are defective in all types of genetic exchange and are unable to form SCs (see Alani et al., 1990). DSBs are eliminated at a strong DSB site at the *HIS4-LEU2* locus (Cao et al., 1990), and it is presumed that there is a general inhibition of DSBs at all loci. The occurrence of short axial elements in *rad50* nuclei was reported by Alani et al. (1990). Consistent with this finding, we observed short axial element fragments in 23% of nuclei (Table III; Fig. 2 *e*).

In the *rad50* mutant, only 25% of nuclei showed compact FISH signals (Table III). In these nuclei, 32% (mean of 34, 30, and 29) of the signals were homologously associated (Table III). The frequency of homologously associated signals relative to total nuclei was 8%, which corresponds to 12% of wild type (Table III).

Null mutants in the *SPO11* gene fail to undergo meiotic recombination (Klapholz et al., 1985; Giroux, C., personal communication) and DSB formation (Cao et al., 1990), and they are devoid of SCs (Giroux et al., 1989). In the *spo11* strain used in the present investigation, we found axial elements, some of considerable length, in 52% of nuclei after 5 h sporulation (Fig. 2 *f*; Table III). In one experiment, *spo11* showed very short segments of what might be SC (Fig. 2 *g*).

In the *spo11* mutant, 26% (mean of 29, 26, and 25) of condensed FISH signal pairs were homologously associated (Table III). Since condensed signals were found in 34% of nuclei, 9% of total nuclei display homologous associations and pairing in *spo11* is 13% relative to wild type (Table III).

In all mutants, the frequency of nuclei with both signal pairs associated was higher than expected from the overall frequency of homologous associations, under the assumption that pairing of chromosome pairs I and III occurs independently (Table IV). In this case, frequencies of nuclei with 0, 1, and 2 pairs associated would follow a binomial distribution. Deviation from the expected frequencies suggests that pairing is concerted within nuclei, whereas pairing levels vary between nuclei.

Polycomplexes in the Mutants

Polycomplexes are thought to be aggregates of SC components devoid of chromatin, and they have been observed in a wide variety of organisms. In wild-type yeast, we found polycomplexes only sporadically after 5 h sporulation. However, polycomplexes were present in ~27% of nuclei after prolonged sporulation (7 h) (Alversammer, 1993), supporting the view that they are products of SC decomposition (see Goldstein, 1987). In the mutants, polycomplexes were frequent. In *rad50S*, we found them in 68% of the nuclei with

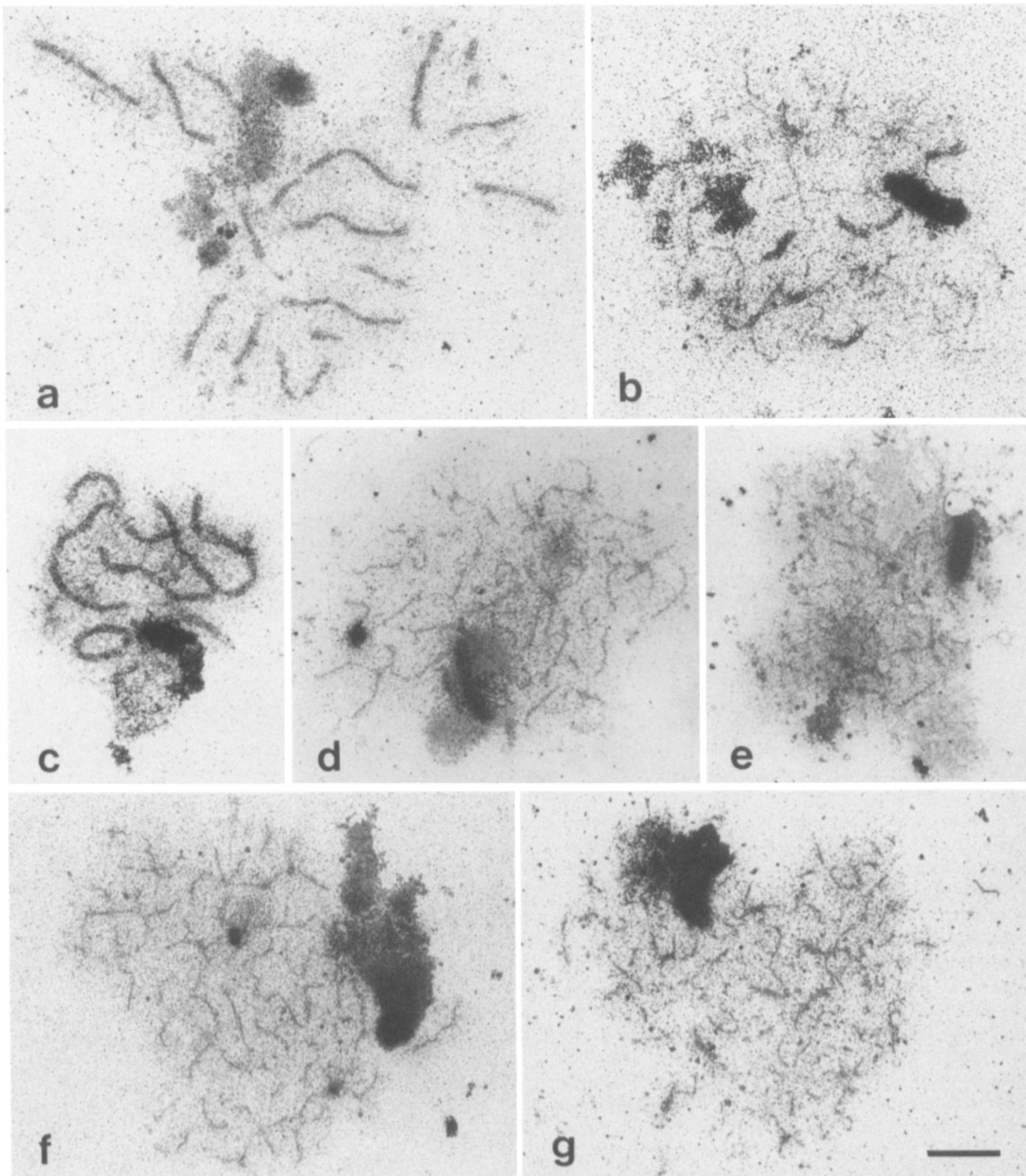

Figure 2. Electron micrographs of spread silver-stained nuclei at pachytene or corresponding stages in SK1 wild type and meiotic mutants. The pictures are typical of the maximally attainable development of axial elements and SCs. (*a*) Pachytene in wild type. (*b*) *rad50S* shows extensive axial elements and SC fragments. (*c*) In 7% of *rad50S* nuclei, synapsis was virtually complete with no unsynapsed axial elements. (*d*) Extensive axial element development in *hop1*. (*e*) Only a few short axial element fragments are found in *rad50*. (*f*) Axial elements in *spo11*. In one experiment, some pairing of axial elements was observed (*g*). Polycomplexes are shown in *b–e* and *g* as electron-dense oblong structures. Bar, 2 μm.

Table IV. Frequencies of Nuclei with Both (I and III), One, and No Chromosome Paired

	Wild type	*rad50S*	*hop1*	*rad50*	*spo11*
			Percent observed (expected*)		
Both chromosome pairs associated	94	39 (22)	47 (32)	23 (10)	19 (7)
One chromosome pair associated	2	16 (50)	20 (50)	18 (44)	14 (38)
Both pairs separate or nonhomologously associated	4	45 (28)	33 (18)	59 (46)	67 (55)

* Expected values for the distribution of nuclei with 2, 1, and 0 chromosome pairs homologously associated, if pairing of chromosomes I and III was independent.

SCs or SC precursors after 7 h of sporulation (Fig. 2, *b* and *c*). In *hop1*, *rad50*, and *spo11*, polycomplexes were present in 9, 14, and 55% of nuclei, respectively, after 5 h of sporulation (Fig. 2, *d–g*). The abundance of polycomplexes in the mutants suggests they may represent aggregates of excess SC components that are not properly used for SC assembly (compare Gillies, 1984).

Discussion

Premeiotic Homologous Contacts

At 0 h of sporulation we found a high incidence of homologous associations in wild-type nuclei (Table II). They occur not only after growth in presporulation medium, but also during logarithmic growth in rich medium (unpublished). Because of this, and because SC and SC-related structures are missing, these associations do not result from meiotic pairing. Similarly, Kleckner and Weiner (1994) reported unexpectedly high rates of homologous associations in vegetative nuclei. Since we observed homologous associations in nuclei with compact FISH signals, which are likely to be in mitotic metaphase (see Results), it is possible that the associations result from the unspecific aggregation of chromosomes at the metaphase plate. This interpretation is supported by the fact that also associations of all four FISH signals are frequent in these nuclei (Fig. 1 *a*; Table II). The preference for the association of homologous chromosomes could result from merely mechanic disposition of chromosomes, such as size-dependent arrangement in the metaphase plate (e.g., Mosgöller et al., 1991), rather than specific interactions ("vegetative pairing"). From the observation that allelic and ectopic copies of LEU2 recombine at similar rates in vegetative cells, Lichten and Haber (1989) concluded that parental homologues interact with each other no more frequently than do nonhomologous chromosomes in yeast. This, too, is evidence against vegetative pairing.

Preliminary results indicated that also in the mutants there is association of homologous signals in ~50% of those vegetative nuclei that showed compact FISH signals. Again, the incidence of heterologous associations is relatively high (~15%), which supports our interpretation that the association of homologous signals results from general clustering. Although the existence of somatic or vegetative homologous pairing has been claimed to occur in several organisms, evidence for it is only circumstantial, except for dipterans, and the issue has remained controversial (for review see Hilliker and Appels, 1989). If the vegetative arrangement of the homologues described here should turn out to be specific and stable, it could possibly contribute to the residual pairing in meiotic nuclei in some of the mutants.

Homologous Chromosomes Can Pair in Asynaptic Mutants

Compared to FISH with single-sequence probes, chromosome painting with compound chromosome-specific DNA probes has the advantage that it reveals not only the relative positions of homologues, but also the degree of chromosome compaction. Painting probes produce disperse hybridization patterns in presumptive interphase nuclei with decondensed chromatin (Fig. 1 *b*) and compact FISH signals in meiotic prophase nuclei. In the wild type, chromatin condensation during meiotic prophase takes place concomitantly with homologous alignment and synapsis (and may be causally related to these events) (Scherthan et al., 1992). As shown in Table II, the increase in nuclei with compact signals during meiotic prophase parallels the development of axial elements and SCs.

Evaluation of pairing in condensed nuclei revealed substantial homologue pairing even in meiotic mutants (Fig. 1; Table III). However, since meiotic chromosome condensation in the mutants is reduced relative to the wild type, the pairing efficiency in total nuclei is considerably reduced relative to wild type (Table III). In the case of the *rad50* and *spo11* mutants, a weak pairing capability could be clearly established only by selecting for nuclei with compact FISH signals.

In the mutant strains, except *rad50S*, the formation of SCs is virtually completely inhibited (Fig. 2). The mutants are nevertheless capable of some degree of homologous chromosome pairing, supporting the notion that homologue recognition is independent of the presence of mature SC (e.g., Loidl, 1990; Roeder, 1990; Scherthan et al., 1992). Axial elements or fragments thereof were formed by all mutants to various extents (Table III). However, the association of homologous chromosomes as seen in the FISH preparations was not accompanied by the alignment of axial elements in silver-stained preparations. Thus, during the homology search, interactions may take place between segments of chromatin loops that are distant from the axial elements of the chromosomes. So far, no mutant capable of wild-tye levels of interchromosomal meiotic recombination and/or chromosome pairing has been described in which axial elements are completely absent. Therefore, it cannot be excluded that localized formation of axial elements is a requirement for pairing and meiotic levels of recombination. Also, *Schizosaccharomyces pombe*, which performs regular meiosis in the ab-

sence of mature SC, displays structures that strongly resemble axial element fragments (Bähler et al., 1993). This suggests that a scaffold, as constituted by the axial element, may impose a looped configuration on the chromatin that facilitates DNA interactions for homology recognition and recombination (Loidl, 1993).

Is Pairing an All-Or-Nothing Process?

At early time points in sporulation in the wild type, when the overall frequency of pairing is low, nuclei with both chromosomes I and III paired outnumber those with only one chromosome paired (not shown). This indicates that chromosomes within the same nucleus pair fairly synchronously, whereas different nuclei are less synchronized. The same is true for the mutants, where pairing is slowed down or reduced. Nuclei with both chromosomes homologously paired are strongly overrepresented compared to a random distribution (Table IV). If we assume that each chromosome pair associates with a certain probability and different pairs associate independently from each other, then the number of nuclei with 0, 1, or 2 paired signals should follow a binomial distribution. The observed distributions differ significantly from such a random distribution ($P_{(\chi^2)} < 0.05$) in that nuclei with only one pair associated are strongly underrepresented. Therefore, we reject the random model and suggest two possible explanations for the observed all-or-nothing process. One is a cooperative effect between pairing chromosomes so that the presence of paired chromosomes increases the chance for remaining chromosomes to associate with their partners. It is conceivable that with the number of paired chromosomes increasing, the choice for the remaining ones and hence their expenditure on homologue search is reduced (see Loidl and Länger, 1993). Alternatively, it is possible that in mutant cultures only few cells can provide the threshold concentration of a factor necessary for pairing. In these cells, pairing would be entered and largely completed, whereas in the others it would fail completely.

Since nuclei with only one of the two chromosome pairs associated were rare, it was impossible to determine whether there is a tendency for a particular chromosome to precede the other with homologous association. It has been reported from grasshoppers that smaller chromosomes commence (and finish) pairing earlier than larger ones (Jones and Croft, 1986; Santos et al., 1993). This remains to be tested by painting yeast chromosomes differing in size more than chromosomes I and III.

DSBs Are Not Essential for Homology Searching

The *rad50S* mutation confers a defect in the processing of meiotic DSBs (Cao et al., 1990). Normally, single strands are produced at the site of DSBs, and these may invade double-stranded DNA to form heteroduplex (Sun et al., 1991). Such heteroduplexes are probably intermediates in meiotic recombination, but they have also been postulated to play a role in homology testing (Smithies and Powers, 1986; Carpenter, 1987). Our observation that in the *rad50S* strain chromosomes pair at ~50% of wild-type level demonstrates that the processing of DSBs is not essential for homology recognition. Previously, rare observations of apparently homologously aligned axial elements were taken as a suggestion that *rad50S* might be able to perform a homology search (Kleckner et al., 1991).

In the *rad50* and *spo11* mutants, which lack a detectable amount of meiotic DSBs, a residual capacity for homology recognition does exist. Provided that the absence of DSBs at the loci investigated is accompanied by a loss throughout the whole genome, this result indicates that DSBs are not a constituent of the homology searching mechanism. It has been reported that stable heteroduplexes, which are formed probably when the 3′ single strands formed at DSBs invade duplex DNA, arise late in meiosis (Goyon and Lichten, 1993). These observations provide additional evidence that the matching of base sequences in heteroduplex DNA is unlikely to constitute the mechanism for primary homology recognition. Instead, primary homology recognition might involve transient, weak interactions between intact DNA molecules (Camerini-Otero and Hsieh, 1993; Goyon and Lichten, 1993). It is conceivable that two rounds of homology recognition take place during meiosis; one to effect general alignment of homologous chromosomes or extended chromosome regions, and another to guarantee precise sequence matching at the site where crossovers are to occur (Stern and Hotta, 1987; see also Loidl, 1990, 1991). The reduced homologous pairing observed in the DSB-deficient mutants could then be explained by the transient nature of the early weak contacts, since they are not enforced by subsequent heteroduplex formation and SC assembly. Alternatively, it is possible that DSBs function in homology searching, but that there exist independent pathways that account for the residual pairing capacity found in the mutants.

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References

Alani, E., L. Cao, and N. Kleckner. 1987. A method for gene disruption that allows repeated use of *URA3* selection in the construction of multiply disrupted yeast strains. *Genetics.* 116:541–545.

Alani, E., R. Padmore, and N. Kleckner. 1990. Analysis of wild-type and *rad50* mutants of yeast suggests an intimate relationship between meiotic chromosome synapsis and recombination. *Cell.* 61:419–436.

Alversammer, G. 1993. Cytologische Charakterisierung verschiedener Meiose-defizienter Hefemutanten (*Saccharomyces cerevisiae*). M.Sc thesis. University of Vienna. 58 pp.

Bähler, J., T. Wyler, J. Loidl, and J. Kohli. 1993. Unusual nuclear structures in meiotic prophase of fission yeast: a cytological analysis. *J. Cell Biol.* 121:241–256.

Bishop, D. K., D. Park, L. Xu, and N. Kleckner. 1992. *DMC1*: a meiosis-specific yeast homolog of *E. coli recA* required for recombination, synaptonemal complex formation, and cell cycle progression. *Cell.* 69:439–456.

Camerini-Otero, R. D., and P. Hsieh. 1993. Parallel DNA triplexes, homologous recombination, and other homology-dependent DNA interactions. *Cell.* 73:217–223.

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

Carpenter, A. T. C. 1987. Gene conversion, recombination nodules, and the initiation of meiotic synapsis. *BioEssays.* 6:232–236.

Dresser, M. E., and C. N. Giroux. 1988. Meiotic chromosome behavior in spread preparations of yeast. *J. Cell Biol.* 106:567–573.

Gillies, C. B. 1984. The synaptonemal complex in higher plants. *CRC Crit. Rev. Plant Sci.* 2/2:81–116.

Giroux, C. N., M. E. Dresser, and H. F. Tiano. 1989. Genetic control of chromosome synapsis in yeast meiosis. *Genome.* 31:88–94.

Goldstein, P. 1987. Multiple synaptonemal complexes (polycomplexes): origin, structure, and function. *Cell Biol. Int. Rep.* 11:759–796.

Goyon, C., and M. Lichten. 1993. Timing of molecular events in meiosis in *Saccharomyces cerevisiae*: stable heteroduplex DNA is formed late in meiotic prophase. *Mol. Cell. Biol.* 13:373–382.

Hawley, R. S., and T. Arbel. 1993. Yeast genetics and the fall of the classical view of meiosis. *Cell.* 72:301–303.

Hilliker, A. J., and R. Appels. 1989. The arrangement of interphase chromosomes: structural and functional aspects. *Exp. Cell Res.* 185:297–318.

Hiraoka, Y., A. F. Dernburg, S. J. Parmelee, M. C. Rykowski, D. A. Agard, and J. W. Sedat. 1993. The onset of homologous chromosome pairing during *Drosophila melanogaster* embryogenesis. *J. Cell Biol.* 120:591–600.

Hollingsworth, N. M., and B. Byers. 1989. *HOP1*: a yeast meiotic pairing gene. *Genetics.* 121:445–462.

Hollingsworth, N. M., L. Goetsch, and B. Byers. 1990. The *HOP1* gene encodes a meiosis-specific component of yeast chromosomes. *Cell.* 61:73–84.

Jones, G. H., and J. A. Croft. 1986. Surface spreading of synaptonemal complexes in locusts. II. Zygotene pairing behaviour. *Chromosoma (Berl.).* 93:489–495.

Kane, S., and R. Roth. 1974. Carbohydrate metabolism during ascospore development in yeast. *J. Bacteriol.* 118:8–14.

Klapholz, S., C. S. Waddell, and R. E. Esposito. 1985. The role of the *SPO11* gene in meiotic recombination in yeast. *Genetics.* 110:187–216.

Kleckner, N., R. Padmore, and D. K. Bishop. 1991. Meiotic chromosome metabolism: one view. *Cold Spring Harbor Symp. Quant. Biol.* 56:729–743.

Kleckner, N., and B. M. Weiner. 1994. Potential advantages of unstable interactions for pairing of chromosomes in meiotic, somatic, and premeiotic cells. *Cold Spring Harbor Symp. Quant. Biol.* In press.

Lichten, M., and J. E. Haber. 1989. Position effects in ectopic and allelic mitotic recombination in *Saccharomyces cerevisiae. Genetics.* 123:261–268.

Lichter, P., A. L. Boyle, T. Cremer, and D. C. Ward. 1991. Analysis of genes and chromosomes by nonisotopic in situ hybridization. *Genet. Anal. Techn. Appl.* 8:24–35.

Loidl, J. 1990. The initiation of meiotic chromosome pairing: the cytological view. *Genome.* 33:759–778.

Loidl, J. 1991. Coming to grips with a complex matter. A multidisciplinary approach to the synaptonemal complex. *Chromosoma (Berl.).* 100:289–292.

Loidl, J. 1993. The questionable role of the synaptonemal complex in meiotic chromosome pairing and recombination. *Chromosomes Today.* 11:287–300.

Loidl, J., K. Nairz, and F. Klein. 1991. Meiotic chromosome synapsis in a haploid yeast. *Chromosoma (Berl.).* 100:221–228.

Loidl, J., and H. Länger. 1993. Evaluation of models of homologue search with respect to their efficiency on meiotic pairing. *Heredity.* 71:342–351.

Mosgöller, W., A. R. Leitch, J. K. M. Brown, and J. S. Heslop-Harrison. 1991. Chromosome arrangements in human fibroblasts at mitosis. *Hum. Genet.* 88:27–33.

Newlon, C. S., L. R. Lipchitz, I. Collins. A. Deshpande, R. J. Devenish, R. P. Green, H. L. Klein, T. G. Palzkill, R. Ren, S. Synn, and S. T. Woody. 1991. Analysis of a circular derivative of *Saccharomyces cerevisiae* chromosome *III*: a physical map and identification and location of *ARS* elements. *Genetics.* 129:343–357.

Padmore, R., L. Cao, and N. Kleckner. 1991. Temporal comparison of recombination and synaptonemal complex formation during meiosis in *S. cerevisiae. Cell.* 66:1239–1256.

Pinkel, D., J. Landegent, C. Collins, J. Fuscoe, R. Seagraves, J. Lucas, and J. W. Gray. 1988. Fluorescence in situ hybridization with human chromosome-specific libraries: detection of trisomy 21 and translocations of chromosome 4. *Proc. Natl. Acad. Sci. USA.* 85:9138–9142.

Rockmill, B., and G. S. Roeder. 1990. Meiosis in asynaptic yeast. *Genetics.* 126:563–574.

Roeder, G. S. 1990. Chromosome synapsis and genetic recombination: their roles in meiotic chromosome segregation. *Trends Genet.* 6:385–389.

Rothstein, R. 1983. One-step gene disruption in yeast. *Methods Enzymol.* 101:202–209.

Santos, J. L., A. L. del Cerro, and M. Diez. 1993. Spreading synaptonemal complexes from the grasshopper *Chorthippus jacobsi*: pachytene and zygotene observations. *Hereditas.* 118:235–241.

Scherthan, H., J. Loidl, T. Schuster, and D. Schweizer. 1992. Meiotic chromosome condensation and pairing in *Saccharomyces cerevisiae* studied by chromosome painting. *Chromosoma (Berl.).* 101:590–595.

Scherthan, H., D. Schweizer, and J. Loidl. 1993. Delineation of individual chromosomes of *Saccharomyces cerevisiae* by two-colour in situ hybridization. *Trends Genet.* 9:41.

Shinohara, A., H. Ogawa, and T. Ogawa. 1992. Rad51 protein involved in repair and recombination in *S. cerevisiae* is a recA-like protein. *Cell.* 69:457–470.

Smithies, O., and P. A. Powers. 1986. Gene conversions and their relation to homologous chromosome pairing. *Phil. Trans. R. Soc. Lond. B.* 312: 291–302.

Steensma, H. Y., J. C. Crowley, and D. B. Kaback. 1987. Molecular cloning of chromosome I DNA from *Saccharomyces cerevisiae*: isolation and analysis of the *CEN1-ADE1-CDC15* region. *Mol. Cell. Biol.* 7:410–419.

Stern, H., and Y. Hotta. 1987. The biochemistry of meiosis. *In* Meiosis. P. B. Moens, editor. Academic Press, Inc., Orlando. pp. 303–331.

Sun, H., D. Treco, and J. W. Szostak. 1991. Extensive 3′-overhanging, single-stranded DNA associated with the meiosis-specific double-strand breaks at the *arg4* recombination initiation site. *Cell.* 64:1155–1161.

von Wettstein, D., S. W. Rasmussen, and P. B. Holm. 1984. The synaptonemal complex in genetic segregation. *Annu. Rev. Genet.* 18:331–413.