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Cytological aspects of meiotic recombination

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Abstract. This article reviews current views on the mechanisms of meiotic homology searching and recombination. It discusses the relationship between molecular events at meiotic prophase and concomitant cytological processes. The role of the synaptonemal complex and other meiosis-specific structures is discussed. Whereas the relationship of crossovers, late recombination nodules, and chiasmata is well established, there is still some controversy about the temporal and causal relationships between double strand breaks, homologue recognition, heteroduplexes, early nodules and presynaptic alignment.

Key words. Synaptonemal complex; presynaptic alignment; homologue search; chromosome pairing; recombination nodule; chiasma.

Introduction

Meiosis is a special cell division which has evolved to reduce the chromosome number of diploid cells and thus to compensate the doubling that takes place during the fusion of gametes in sexually reproducing eukaryotes. A characteristic of extant meiosis is genetic recombination which produces progeny with new combinations of heritable traits that may be tested by natural selection. Recombination at meiosis is therefore an important factor in evolution.

The primary source of meiotic recombination in organisms with a haploid chromosome number greater than two is interchromosomal recombination, i.e., the independent assortment of unlinked genes[16]. The second source is crossing over which is the reciprocal 'intrachromosomal recombination of the intergenic type'[72]. There is an inconsistency in that it is sometimes referred to as interchromosomal recombination in order to differentiate it from recombination events within one and the same chromosome (see Heyer and Kohli, introduction to this issue). From the cytological point of view, the primary function of crossing over is to provide chiasmata as stable connections between homologous chromosomes in order to ensure their regular segregation. Another source of meiotic recombination is gene conversion, which might play a role in the meiotic homologue search process and which often is accompanying crossovers.

Mechanisms for recombinational repair in vegetative cells have to include processes which ensure the availability of an intact homologous DNA stretch for use as a template. Often lesions may hit DNA during G_1 when chromosomes exist in an unreplicated state where no sister DNA molecule can be employed as template (see ref. 39). In this case diploids can search for and make use of the corresponding sequence from the second set of chromosomes. Thus vegetative/somatic homology searching mechanisms may exist although they may only be activated sporadically. In contrast, extensive homology search as a provision for pairing, mutual exchange and disjunction of homologous chromosomes is characteristic of meiosis. (For the role of homology searching in the inactivation of repeated genes see Rossignol and Faugeron, this issue.) It may be assumed that vegetative repair mechanisms have been recruited in the evolution of meiosis (see, e.g., Maguire[54]). It has even been suggested that meiotic homology search might be triggered by deliberate DNA lesions (e.g., Carpenter[13]) and that recombination might merely be an occasional by-product of DNA repair[7].

The molecular process of crossing over is rougly concomitant with chromosome pairing at meiotic prophase. The common sequence of the cytological events related to recombination is about the following (fig. 1): First, axial elements are formed along the longitudinal axis of each chromosome. Homologous chromosomes align in parallel, whereby the axial elements can be seen to converge at individual sites along the chromosomes. In the electron microscope, nodules can often be seen between axial elements at these sites. Starting from one or several sites per bivalent, the axial elements zipper up to form a continuous synaptonemal complex (SC) which mediates a close contact. Along the SC, so-called recombination nodules indicate the positions where molecular processes of recombination take place. Later, the SC is decomposed and the intimate pairing of homologues is resolved. Their contact is maintained only at chiasmata where chromatids switch between homologues. Finally, chromosomes of chiasma-mediated bivalents separate during anaphase I whereby chromosomes of the parental sets are distributed randomly and newly combined chromatids are alloted to daughter nuclei.

This is only a rough outline of events. The individual processes may overlap or some of the participating

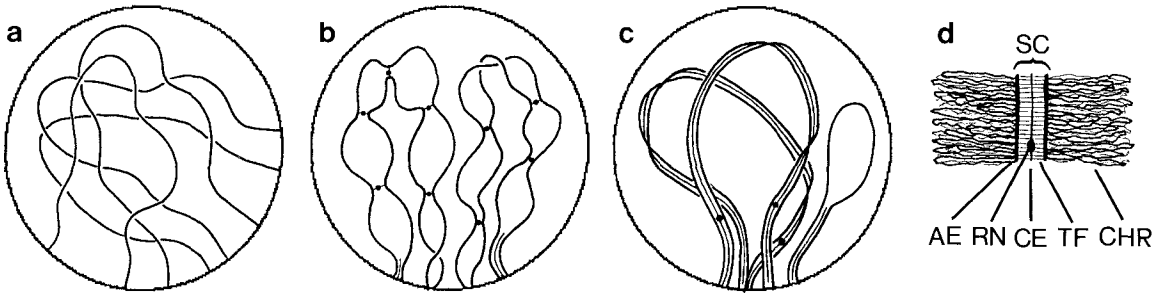

Figure 1. Hypothetical sequence of homologous alignment and synapsis.
a Chromosomes are attached with their ends to the nuclear envelope. Axial elements (AE) which form the backbones of chromosomes become visible at leptotene. There is an ongoing dispute whether or to which degree there is a predisposition of homologous chromosomes at this stage or even premeiotically[46].
b Presynaptic alignment at late leptotene/early zygotene. Homologues are aligned roughly in parallel (compare fig. 2). At sites where they converge 'early nodules' may be present. Presynaptic alignment overlaps in time with the development of the synaptonemal complex (SC) which usually commences near the chromosome ends by the appearance of central elements (CE) and transversal filaments (TF) between the axial elements.
c At pachytene homologous chromosomes are synapsed along their entire length. Recombination nodules (RN) occur at the sites of crossovers where chiasmata will be formed subsequently. Chromosomes which lack their partner (e.g., in haploids) or have failed to locate it may become involved in non-homologous synapsis (see hairpin-loop to the right).
d Segment of pachytene SC. Axial elements (AE) of pachytene SCs have previously been termed lateral elements which is still commonly used. However, the occasional presence of unsynapsed axial elements at pachytene and diplotene make this distinction questionable[47]. Chromatin (CHR) loops out from the axial elements; only a small fraction of the DNA projects into the space between the axial elements. Further explanations in the text.

structures may not always be distinct. In addition there are a number of striking variations to this scheme, like non-synaptic, achiasmatic, and inverted meiosis, not to mention rudimentary forms of meiosis in parthenogenetically reproducing organisms[34].

In the following I shall describe the structures presumed to be related to homology searching and recombination in more detail and discuss the relationship between events that can be monitored in the light and electron microscope and the underlying molecular processes.

The molecular basis of cytological events

Recognition of homology could be brought about by indirect DNA base comparison (e.g., via allosteric proteins[14]) or by comparison of arrays of histone and non-histone proteins or tertiary DNA configurations along DNA segments[73]. Most current models, however, employ direct DNA-DNA matching either between fully paired duplexes via additional hydrogen bonds or between locally opened DNA strands via Watson-Crick hydrogen bonds (for reviews see Stasiak[79] and the contributions by Stasiak and Egelman, and Heyer in this issue). Homology could be checked by sensing the similarity of parallel strands or the complementarity of antiparallel ones in paranemic joints in which the matching partners are not topologically interwound[27]. This mechanism may be imagined as analogous to RecA mediated homologue search. It would allow DNA segments to align and separate rapidly and with low energy-cost as has to be postulated for trial-and-error homology searching[8,51]. Another possibility of homology check is the formation of true heteroduplexes by exchange and plectonemic coiling of strands. The nicking or breakage involved would render this process recombinogenic. This would imply that homology search and early steps in crossover-generation share some molecular processes. This hypothesis is in keeping with the notion that meiotic pairing has evolved from vegetative recombinational repair systems.

Based on the concept of heteroduplex formation, several authors have proposed that single-strand DNA 'feelers' could invade double-helix DNA in search of complementary base sequences[13,18,78,81] and the increased rate of meiotic gene conversions has been interpreted as a sign of this event.

Artificially induced DNA breaks were found to promote recombination (reviewed by Petes et al.[69]) and double-strand breaks (DSBs) are widely induced during meiosis in *Saccharomyces cerevisiae*[86]. Thus it was suggested that meiotically induced DSBs might lead to heteroduplex formation as a recombination intermediate on the way to crossovers[82]. Comparative time courses showed that DSBs occur immediately before successful pairing is indicated by the appearance of synapsed stretches[41,64]. Furthermore, *rad50* mutants which are defective in the generation of meiotic DSBs lack tripartite SC structure[1]. Therefore, heteroduplex formation in the course of repair of gaps resulting from DSBs were proposed as an instrument of homology testing and SC formation, in addition to its putative function in crossing over[1]. This model calls for measures against the primary involvement of the sister chromatid as the template for repair, otherwise search for the homologue would be suppressed. (For recombinational repair during G_2, on the other hand, sister chromatids have been shown to be more readily utilized

than homologous chromosomes because of their spatial proximity[39].) Only in cases where no homologue is found (as might be the case in haploid meiosis), the sister DNA molecules could be recruited as templates for DSB repair. Indeed, this seems to be the case since sister chromatid recombination is enhanced by the lack of a homologue (see McKee and Handel[57] for literature).

An alternative to the DSB repair hypothesis of homology search is that DSBs occur only after an appropriate partner has been found at the site of a weak initial association (see refs 41, 86). In fact, both in *rad50* and *spo11* disrupted strains of *S. cerevisiae*, which seem to lack DSBs, and in the *rad50S* mutant, where DSBs are not processed to produce single-strand overhangs, homologous pairing is maintained to some degree as is evidenced by the fusion of homologous in situ hybridization signals (Loidl and Scherthan, unpublished). This contravenes the concept of heteroduplex formation at DSBs as the (only) search mechanism. Moreover, in a time course experiment, stable heteroduplexes (probably representing recombination intermediates in the course of crossover generation) have been observed relatively late during yeast meiotic prophase, well after the stage when homologous recognition takes place[25]. This led the authors to conclude that homology recognition either may not occur through the formation of recombination intermediates, or the recognition is effected by recombination intermediates that either are unstable or do not involve standard Watson-Crick base pairing (see above).

Based on the evidence presented above it was proposed that DSBs are not the primary events in homology search, but occur rather as part of a mechanism which stabilizes the weak initial homologous bonds and which may lead to crossovers[27]. It should be mentioned, however, that this model does not take into account the occurrence of ectopic recombination events which could be readily explained by a genome-wide homology search employing DSBs[26,80].

Homologous alignment and synapsis

Whatever molecular mechanisms may be involved, chromosomes appear to initiate homologous contacts during early meiotic prophase. There is an ongoing debate about strategies that may facilitate the encounter of chromosomes for mutual homology testing. Merely chance homologous contacts between randomly moving chromosomes may not suffice in view of the huge size of the genome that has to be screened for homology and the relatively moderate chromosome movements that have been observed[51]. Therefore several supportive structures and mechanisms have been invoked to reduce the role of randomness. These are non-random premeiotic chromosome disposition, attachment of telomeres to the inner nuclear membrane and hence the restriction of search movements to essentially two dimensions, or the clustering of chromosomes in small areas where mutual recognition can act within relatively close distances (for review see Loidl[46]). None of these possibilities, however, seem to be realized universally, and it is unclear whether a general search strategy common to a majority of organisms exists at all.

The first contact between homologues is made at individual sites along the chromosomes well before the SC is formed (see Loidl[46] and lit. cit. therein). If there is a sufficient number of these sites, homologous chromosomes appear arranged roughly in parallel at a distance which is considerably greater than the maximal distance of 300 nm that can be bridged by the transversal filaments of the SC[59]. This so-called presynaptic alignment is particularly obvious in ascomycetes like *Neurospora crassa*[56,77], *Sordaria macrospora*[87] and *S. cerevisiae*[74] but it seems to be a common though less distinct feature among other organisms, too [46,85] (fig. 2). In organisms where it cannot normally be observed, presynaptic alignment was visualized at pachytene of triploids as the non-synaptic association of chromosomes with their synapsed homologous partners[49,70]. At one or several regions where chromosomes have approached each other to within the critical distance of 300 nm, they initiate the typical tri-partite SC which then extends by zipper-like growth (fig. 1).

The SC was first independently described in 1956 by Fawcett[19] in pigeon, cat and man and by Moses[60] in crayfish primary spermatocytes. Since then it has proved almost universal in the meiosis of sexually reproducing eukaryotes (for review see von Wettstein et al.[85]). The elucidation of the molecular composition of the SC has made considerable progress over the last few years. It is mainly a proteinaceous structure consisting of parallel axial elements, 100 nm apart, in which the chromatin loops are anchored. The axial elements are connected by transversal filaments and halfway between them runs the central element (fig. 1). Monoclonal antibodies elicited to isolated rat SCs have recognized several major polypeptides. These proteins have been localized immunocytologically to SC substructures and several of them seem to have DNA binding properties (ref. 28, Heyting pers. commun.). Similarly, the Hop1 protein, which is an essential factor in SC formation in yeast, has a putative DNA-binding domain[29] and colocalizes with the axial elements (F. Klein and B. Byers pers. commun.). Recently, in the rat and in yeast presumptive coiled coil proteins were characterized that were proposed to constitute the major component of the transversal filaments of the SC[58,83]. In isolated rat pachytene chromosomes which were DNase II-treated, a fraction of the DNA was protected from digestion and it was suggested that this might be the portion which is in close association with the SC proteins.

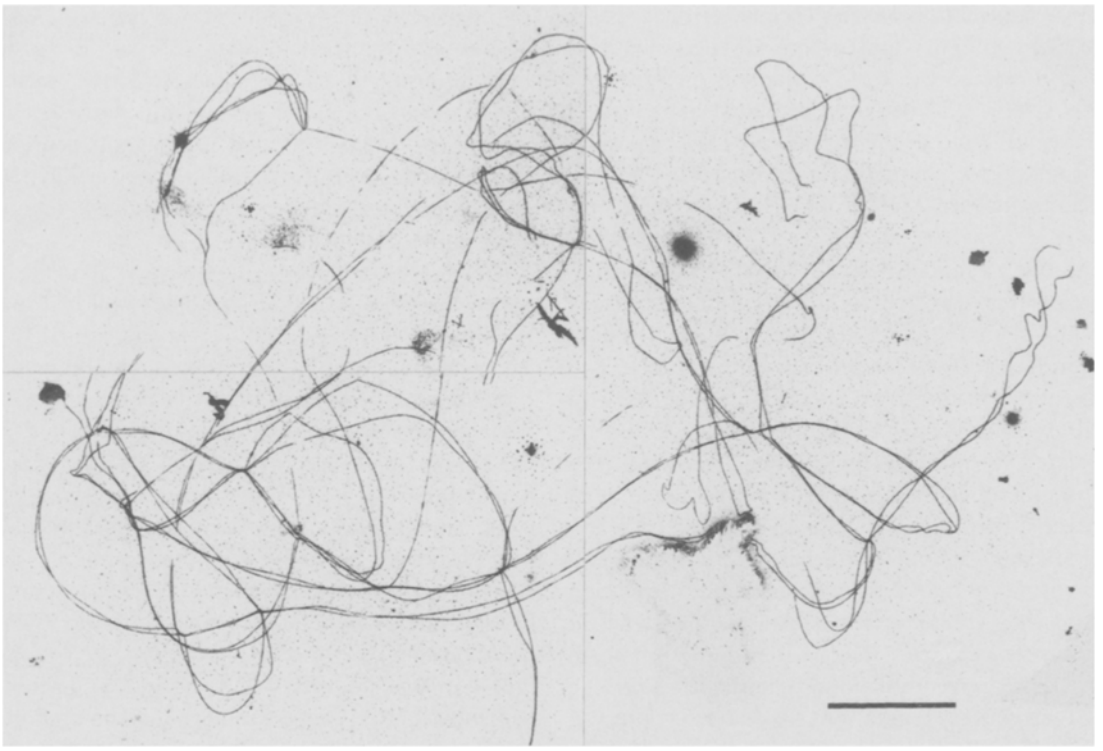

Figure 2. Presynaptic alignment in the plant *Paeonia tenuifolia*. Prepared by a SC spreading technique; silver staining, compound electron micrograph. Axial elements (SC precursors) can be seen associated in pairs along most of their length. This suggests that homologue recognition acts across some distance prior to the formation of a tripartite SC structure. Bar represents 10 µm.

Cloning and analysis of this residual DNA revealed that it is enriched in $(GT)_n$ tandem repeats and contains long and short interspersed repeated elements (LINE/SINEs)[68]. This suggests that a special fraction of DNA might constitute the bases of chromatin loops projecting from the axial elements.

Whereas presynaptic alignment is a good cytological indication of homology, this does not hold for the SC since there is ample evidence for non-homologous SC formation. Usually the SC forms between chromosomes and chromosomal regions that are preselected by presynaptic alignment. If chromosomes do not come in two homologous sets (e.g., in haploids[50]) or if the mechanisms for recognizing or moving together homologous chromosomes fail (see, e.g., Jenkins and Okumus[33]), extensive SCs may nevertheless be formed[46]. In these cases it is not clear whether the initiation of the SC is based on at least some short homologous stretch or homology of a low degree, or if it is totally independent of homology. The formation of so-called SC polycomplexes, i.e. stacks of SC material without the participation of chromosomes[23], suggests the capability of the SC to organize autonomously and hence of course in the absence of any homologous DNA segments. The neglect of homology by the SC is also illustrated by the elimination of duplication/deletion- and inversion-loops during the so-called synaptic adjustment where a straight but non-homologous SC is formed at the cost of a homologous one[61].

On the other hand, notably in higher eukaryotes, there are considerable amounts of dispersed repetitive DNA sequences which could serve as secondary sources of homology[76] for pairing between non-homologous chromosomes. Such minor local homologies may even allow crossing over as is evidenced by chiasma-formation in haploid rye[62]. Also in the genome of *S. cerevisiae*, more and more candidate sequences for potential secondary sources of homology are detected[63] and synapsis between non-homologous chromosomes does not seem to be totally arbitrary. In haploids, the SCs often have blunt ends[50], suggesting that non-homologous synapsis starts preferentially near chromosome ends. Also the meiotic association of chromosomes I and III in a double monosomic strain[52] may be based on low-level homologies of telomeres. It was found that the ratio of heterologous bivalents over auto-synapsed univalents of chromosomes I and III is 2:1. This may be neatly explained by the involvement of telomeres in non-homologous pairing because a telomeric pairing initiation site on one chromosome would encounter a corresponding site twice as frequently on the other chromosome than on the same. On the other hand, distal initiation of synapsis could be merely mechanical and independent of homology. Hence, the homology requirements for seemingly non-homologous synapsis are not yet clear.

The role of the SC in homology searching and crossing over

Cytological studies in a large number of species have shown that chiasmata do occur only in chromosomal regions where a SC is formed. This suggests that the SC is a prerequisite for meiotic levels of reciprocal recombination (see Rasmussen and Holm[71], von Wettstein et al.[85]) and one interpretation is that crossing over can be initiated only in the context of a completed tripartite SC (see Loidl[46]). Some cytological observations support this view. First, in many polyploids synapsis occurs initially between all members of a homologous chromosome set, but during zygotene multivalent synapsis is reorganized into exclusive bivalent synapsis[32]. This synaptic correction is possible only if multivalents are not fixed by crossovers until pachytene. Second, it was found that the proximal regions of homologous chromosomes in *Allium fistulosum* come into contact late, only by the zipper-like growth of the SC which is initiated distally. Since in *A. fistulosum* crossovers are localized proximally[3], it is likely that they form only when the SC has conferred the initial homologous contacts to proximal regions. These cases support the hypothesis that pairing occurs in two steps, whereby first homologous chromosomes are preselected (chromosome pairing) and then, within the stabilizing framework of the SC, sequences match precisely at the sites where recombination is to occur (exchange pairing)[81].

In opposition to this view it was held that early steps in meiotic recombination are initiated prior to synapsis[17,27,64]. This is because meiotic DSBs, commonly believed to represent an early stage in recombination, precede the formation of tripartite SC. Also, analysis of mutants in yeast has suggested that substantial levels of meiotic recombination can occur in the absence of full-length SCs (Sym et al.[83] and lit. cit. therein). The contradiction could be resolved by assuming that commitment to crossing over varies between organisms and may occur before, during, or just after initiation of synapsis[53].

Several proposals were made for possible functions of the SC in addition or alternatively to its questionable role as a structural support of the recombination machinery (see Loidl[48]). One is that it has to ensure chiasma maintenance. In order to maintain the chiasmatic association of chromosomes in bivalents up to metaphase I, sister chromatids must remain connected distal to a chiasma. In the desynaptic (*dy*) mutant of maize a high incidence of univalent chromosomes was found at diakinesis but these had undergone reciprocal recombination as was indicated by the equational separation of a heterozygous cytological marker[55]. This was taken as evidence that normal crossing-over had been followed by failure of chiasma maintenance owing to the defective SC of the mutant. Hence the conclusion was that prior involvement in completed synapsis somehow provides for reinforced sister chromatid-cohesiveness into later meiotic stages. Similarly, based on the segregation behaviour of crossover chromosomes in a SC-reduced mutant of yeast it was suggested that there might exist two kinds of crossovers, without and within the context of SC, where only the latter are effective on segregation[18]. Therefore it is now widely accepted that the SC helps to convert crossovers into functional chiasmata which assist orderly disjunction at the first meiotic division[64].

To ensure chiasma maintenance the SC need not extend continuously along the entire length of a bivalent. The presence of uninterrupted stretches of SC in most organisms suggests the additional or alternative possibility that it serves in signal transmission along bivalents. It could confer the homologous contact made at a few homology recognition sites all along bivalents, making remote sites also capable of crossing over (see the example above of *Allium fistulosum*). A continuous SC could also help to confine crossovers to allelic sequences. Whereas gene conversions between ectopic sequences frequently occur without detrimental consequences, ectopic crossovers would result in translocations. Formation of a continuous SC along a chromosome pair could join several individual segments, at which homology has been tested, in longer tracts and thereby verify the existence of extended and thus allelic homology[48].

In a few organisms including the fission yeast, *Schizosaccharomyces pombe*, no SC is formed and yet crossing over is frequent and meiosis performs regularly. However, no crossover inference exists (see below), which raises the possibility that the SC is also involved in the control of crossover distribution (Bähler et al.[5], see also Kohli and Bähler in this issue). In conclusion it can be said that the SC may exert a variety of different functions[46,48].

As expounded above, homology search precedes the formation of a mature tripartite SC in time. There is some reason to assume, however, that at least SC precursors, the axial elements, may play a supportive role in the homology search process. The axial elements could organize the chromatin threads in such a way that the main bulk projects as large loops on one side (fig. 1d) and only a fraction of the chromosomal DNA is available for mutual homology testing at the pairing face. Moreover, by the regular arrangement of chromatin loops along axial elements corresponding sequences on homologous chromosomes would be brought into register. Both preselection and disposition of pairing sites would facilitate the search for homologues[48]. In accordance with this hypothesis there seems to be a relationship between the density at which DNA is packed along axial elements and its susceptibility to recombination events. Comparison of a number of

1C DNA lengths (in μm), SC lengths (in μm), DNA/SC ratios and recombination rates (cM/kb) in different organisms. Notice that with increasing DNA content the DNA/SC ratio increases i.e., the chromatin appears to be more densely packed along the axial elements. This correlates roughly with a decrease in recombination rates.

	1C DNA (μm)	SC (μm)	DNA/SC	cM/kb
Saccharomyces cerevisiae	5×10^3	28 (ref. 50)	180	0.26
Neurospora crassa	1.4×10^4	46 (ref. 20)	300	0.05
Caenorhabditis elegans	3×10^4	41 (ref. 22)	730	0.004
Drosophila melanogaster	7×10^4	110	640	0.0017
Arabidopsis thaliana	2×10^5	117*	1710	0.005**
Homo sapiens	1×10^6	231	4330	0.0009
Mus musculus	1×10^6	156 (ref. 21)	6410	0.0006
Zea mays	2.5×10^6	353	7082	0.001

In parts compiled from tables in John[34].
*Loidl, unpublished
**A. Bachmair, pers. commun.

different organisms shows that with lower DNA/SC ratio, the recombination rate is higher (table). A possible explanation for this relationship is that a looser conformation of chromatin (i.e., more but smaller loops) along the axes of pairing chromosomes brings a higher portion of DNA into direct contact with the axial elements, allowing its interaction with corresponding sequences on the homologue.

Recombination nodules

Recombination nodules were first described as thickenings of the central element of the SC of ascomycetes, by Schrantz[75] in *Pustularia* and *Galactinia* and by Gillies[20] in *Neurospora*. Carpenter[9] found in *Drosophila* oocytes that they are discrete spherical structures that sit on top of the central elements rather than being a component of them. She also established a good agreement between the number and intrachromosomal distribution (notably their lack in heterochromatin) of these structures with the distribution of crossovers as determined genetically and hence designated them as recombination nodules (RNs).

It was subsequently found that these crossover-related nodules represent only a subset of SC-associated nodules. Carpenter[10] described ellipsoidal nodules which were more frequent and appeared during earlier stages in *Drosophila* oocytes. Later, in a variety of organisms, nodules were found from zygotene stage on at sites where the axial elements of homologues converge. In all cases the number of nodules decreases during pachytene whereby it cannot be determined whether the early nodules disappear and late nodules are formed anew, or if some of the early nodules persist and then become late nodules (see Anderson and Stack[4] and lit. cit. therein).

The relationship between late RNs and exchange events was confirmed by the parallel reduction of both in several recombination deficient mutants of *Drosophila* (see Carpenter[12]) and *Sordaria*[88]. *Mei-9* mutants of *Drosophila*, on the other hand, display wild-type numbers and locations of RNs, whereas crossing over is reduced to 8% of wild-type levels. This indicates that completion of recombination is not a precondition for formation of late RNs (see Carpenter[12]). Evidence for the correlation between late RNs and chiasmata came from their coincidental localization next to the centromere in the onion *Allium fistulosum*[2] and their coincidental near-terminal localization in the grasshopper *Chloealtis conspersa*[6]. However, there exists a deficit of late nodules with respect to chiasma number, which cannot solely be explained by technical losses of the nodules in the preparation procedure. Thus it was proposed that late RNs are ephemeral, meaning that they may disappear during pachytene well before the SC is decomposed (see Jones and Albini[37]).

Both early and late RNs showed enhanced ^{3}H-thymidine incorporation which is consistent with the hypothesis that recombination-related DNA synthesis takes place at the sites of RNs[11]. Whereas this is quite obvious for late RNs which correspond to crossovers, several hypotheses were put forward for the function of the early nodules (review in ref. 4): 1) they are involved in gene conversion events but not in crossing over; 2) they are capable both of gene conversion and of crossing over, depending on the resolution of the early recombination intermediate; and 3) together with fibers that connect axial elements during zygotene they have a role in the initiation of the SC. Specifically, Carpenter[13] proposed that the early nodules could be the sites where gene conversion events (which might result from transient heteroduplex formation) occur during homology search.

Chiasmata and the regulation of recombination at the level of chromosomes

The nature of the chiasma as the consequence of a crosswise reunion of nonsister chromatids and hence as the cytological manifestation of crossing over was con-

jectured as early as 1909 by Janssens[31]. The occurrence of 'cytological crossing over', i.e., the physical exchange of chromosome parts and its correspondence with genetical crossing over, was established by the observation of concerted intrachromosomal recombination behaviour of genetic and cytological markers in maize by Creighton and McClintock[15]. Many pieces of evidence for the correlation between crossing over and chiasmata have been collected since then, the clearest being provided by Tease[84]. He observed the exchange between differentially BrdU-labelled non-sister chromatids (visible as a change of colours) right in the center of the chiasma of metaphase I bivalents.

The distribution of crossovers/chiasmata is position-dependent rather than DNA-sequence-dependent, as was demonstrated by genetical and cytological experiments. In yeast, recombination in the *leu2* site varied when it was inserted at several different locations in the genome[42]. In the mouse, no change in the chromosomal positions of chiasmata was found when DNA sequences in these positions changed due to a homozygous inversion[24]. It was suggested that the level of recombination at a certain position is governed by local chromatin conformation (see Nicolas and Petes, and Smith in this issue). Deviations in crossover/chiasma distribution and frequency within and between chromosomes from random were found in most organisms[36]. These deviations include local suppression, localization, interference and over-representation in small chromosomes, of recombination events.

Heterochromatin is usually largely devoid of chiasmata. The exclusion of recombination events from heterochromatin or other specific chromosome regions could be attributed to a particular chromatin conformation which prevents the access of recombinatory enzymes. It was interpreted as a mechanism to avoid detrimental ectopic recombination[57]. In addition to being inhospitable to chiasmata, heterochromatic regions can exert effects beyond their borders and even on other chromosomes. Usually heterochromatin repels chiasmata but it can also have the opposite, attractive, effect[43]. Also, the increase in chiasma number by the presence of supernumerary heterochromatic segments was reported (for review see Loidl[44]). Similarly, the presence of B-chromosomes can raise chiasma frequency in the standard chromosome complement[67]. The interchromosomal effects of both heterochromatin and B chromosomes could be attributed to their influence on slowing down the cell cycle, thereby prolonging prophase and hence the time available for recombination to take place. An alternative explanation is that supernumerary chromatin has an effect on the packaging ratio of chromatin to the SC. It was shown that in the presence of B-chromosomes the SC is longer and consequently the ratio of DNA to SC length is decreased[67]. This agrees with the interpretation above that the looser the DNA is packed along axial elements, the more frequent is crossing over because there are more sites at which homologous DNAs can interact (see table).

Interference, i.e., the mutual exclusion of crossovers/chiasmata (and late recombination nodules) within a certain distance, causes the deviation of chromosomal distribution of chiasmata from random (see Kohli and Bähler, in this issue). Interference might serve to avoid closely spaced crossovers, which could cancel each other with respect to their recombinogenic effect. Several models for the mechanism of interference have been suggested: The limited supply of a substance necessary for crossing over could cause its reduction to below threshold concentration in the vicinity of a crossover. Alternatively, some structural or chemical information spreading along the chromosomes from the site where the first crossover is established (whereby the SC might act as the agent for the transmission) could impair the formation of additional crossovers (see Jones[35] for lit., ref. 40).

In general, there is a linear correlation between the size of a chromosome and the number of chiasmata/crossovers it harbours. However, even chromosomes which would be too small to get a chiasma allocated if crossover/chiasma distribution were random, obtain at least the single one which they need for orderly segregation. The bias in crossover/chiasma distribution toward small chromosomes was particularly clearly demonstrated by chromosome fissions in the plant *Hypochoeris radicata*[66] and in *Saccharomyces cerevisiae*[38]. In both cases, the total of chiasmata/crossovers in the two resulting small chromosomes was higher than in the original chromosome.

The over-representation of crossovers/chiasmata in small chromosomes could be explained by interference. If crossovers were initiated in abundance, even very small chromosomes could initiate several of them, but only one recombination event would become manifested as a chiasma because of interference. In larger chromosomes, on the other hand, a second and more crossovers/chiasmata can form if the interference distance is less than the residual length of the chromosome beyond the first crossover/chiasma. (For a discussion of this proposal by K. Mather see John[34].)

An alternative explanation for the formation of an obligatory chiasma by small chromosomes is based on the assumption that there are not many more crossovers initiated than are actually converted into chiasmata. As a consequence of interference, some recombination nodules containing the recombination machinery would become detached from larger bivalents and settle down on others, as proposed by the interference model of King and Mortimer[40]. Thus, as long as there are at least as many recombination nodules as bivalents, even the smaller bivalents would obtain one before supernumer-

ary recombination nodules start to fill up the larger bivalents with additional crossovers/chiasmata. This model would be preferable if it turned out that inter-bivalent competition for crossovers/chiasmata existed. There is, however, no evidence of inter-bivalent competition of chiasmata as a general phenomenon[34,35], although there have been a few examples provided[30,65].

There is also evidence of crossover/chiasma control at the level of individuals[35] where sex and age influence the rate of recombination through unknown mechanisms. Finally, recombination rate is influenced by environmental factors such as temperature and nutrition and is also subject to seasonal variation in plants and animals (for lit. see Loidl[45]).

For some species, which obviously are well adapted to their environment, meiotic recombination appears to be non-vital or even undesireable, so they have evolved strategies to reduce the recombinational effects of crossing over. Some achieve this by localizing crossovers/chiasmata near chromosome ends. Others restrict chiasmata to certain chromosome regions, which may be a measure to leave certain gene arrays unaffected by crossing over. Some organisms have obviated chiasmata altogether by developing non-recombinant modes of segregation ('distributive disjunction' – for lit. see Loidl et al.[52]). Members of the plant genera *Oenothera* and *Rhoeo* have even managed to eliminate interchromosomal recombination by non-random segregation of their parental chromosome complements[34].

Conclusions

At meiotic prophase homologous chromosomes recognize each other by base pair matching at individual sites and associate in parallel prior to SC formation. Some suprachromosomal nuclear organization may help homologous chromosomes to locate each other. It is unclear whether homology search is performed by intact DNA molecules or by single stranded DNA "tails" that arise at nicks or breaks. It is also unknown whether the sites of homology recognition and initiation of recombination are identical. DSBs seem to be part of a recombination pathway; it is doubtful whether they also serve in early homology search. Their chromosomal position depends on chromatin conformation rather than DNA sequence. This may be the reason why distribution of recombination events is susceptible to various extraneous factors.

Roughly simultaneously with the occurrence of DSBs, axial elements form along chromosomes, and homologues start to associate more intimately by forming a tripartite SC. The classical cytological view is that crossovers arise within the framework of the SC and that one function of the SC is to confer homologous contact from where it is originally made (usually near the telomeres) to the sites where crossovers are to be formed. This view is challenged by genetical and molecular evidence, mainly from yeast, which suggests that crossovers are initiated prior to synapsis and that they can occur in the absence of SCs. In this model, the SC has the function to stabilize early recombination intermediates and to transform them into functional chiasmata.

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