Journal of Cell Science 110, 1033-1040 (1997)
Printed in Great Britain © The Company of Biologists Limited 1997
JCS4395

COMMENTARY

The transcriptional basis of chromosome pairing

P. R. Cook

CRC Nuclear Structure and Function Research Group, Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford, OX1 3RE, UK

(e-mail: peter.cook@path.ox.ac.uk)

SUMMARY

Pairing between homologous chromosomes is essential for successful meiosis; generally only paired homologs recombine and segregate correctly into haploid germ cells. Homologs also pair in some somatic cells (e.g. in diploid and polytene cells of *Drosophila*). How homologs find their partners is a mystery. First, I review some explanations of how they might do so; most involve base-pairing (i.e. DNA-DNA) interactions. Then I discuss the remarkable fact that chromosomes only pair when they are transcriptionally active. Finally, I present a general model for pairing based upon the DNA-protein interactions involved in transcription. Each chromosome in the haploid set has a unique array of transcription units strung along its length. Therefore, each chromatin fibre will be folded into a unique array of loops associated with clusters of polymerases and transcription factors; only homologs share similar arrays. As these loops and clusters, or transcription factories, move continually, they make and break contact with others. Correct pairing would be nucleated when a promoter in a loop tethered to one factory binds to a homologous polymerizing site in another factory, before transcription stabilizes the association. This increases the chances that adjacent promoters will bind to their homologs, so that chromosomes eventually become zipped together with their partners. Pairing is then the inevitable consequence of transcription of partially-condensed chromosomes.

Key words: Leptotene, Meiosis, Recombination, Synaptonemal complex, Transvection, Silencing

INTRODUCTION

Homologous chromosomes associate to various degrees during the life of a diploid organism. Consider for example, the diverse types of pairing seen in *Drosophila*. As the fertilized egg develops, homologs are initially positioned at random relative to their partners; then, the probability that they will be found together increases at the mid-blastula transition but, even so, some remain apart (Hiraoka et al., 1993). Such an intermediate level of pairing probably persists subsequently in most diploid cells (Metz, 1916). However, in giant larval nuclei, each polytene chromosome usually becomes exactly aligned with its homolog along most, and usually all, of its length (Ashburner, 1989). And in the germ line, homologs search out their partners during meiotic leptotene to become intimately associated during pachytene (von Wettstein et al., 1984). Such pairing is essential for successful meiosis; generally only paired homologs can recombine, cross over, and form the bivalents required for correct segregation into haploid sets (Carpenter, 1994).

Many different mechanisms have been invoked to explain these various types of pairing (e.g. Comings and Riggs, 1971; Riley and Flavell, 1977; Kleckner and Weiner, 1993). Most involve hydrogen-bonding between bases in the DNA of the two homologs. Such DNA-DNA interactions undoubtedly play a role in the tight pairing that occurs at the point of genetic exchange during meiosis. (For reviews, see Camerini-Otero and Hsieh (1993) and Kowalczykowski and Eggleston (1994).) However, they are unlikely to be involved earlier during meiosis when homologs associate less intimately. Here, I review some explanations for this distant type of meiotic pairing. Then I discuss the remarkable fact that chromosomes only associate when they are transcriptionally active. Finally, I present a general model for pairing based upon the DNA-protein interactions involved in transcription.

DIFFERENT KINDS OF PAIRING DURING MEIOSIS

Meiosis involves at least four different kinds of pairing (Fig. 1; Kleckner, 1996). (1) During meiotic S phase, an initial duplication of the double helix generates two copies that remain together through the first meiotic division. Such pairing is simply explained if cohesion proteins hold the two together during the relevant period (Miyazaki and Orr-Weaver, 1994; Sekelsky and Hawley, 1995). (2) During leptotene, each duplicated pair searches for its (duplicated) homolog, so that by the beginning of zygotene most lie roughly parallel to their partners, usually between 300 and 1,000 nm away from them. The term '*alignment*' will be used to describe the remarkable

mechanism that creates this distant association. This mechanism is the central concern here. (3) During zygotene, the building of the synaptonemal complex draws the two even closer together, so that by pachytene the (duplicated) homologs lie ~100 nm apart in exact register (e.g. Loidl, 1994; Hasenkampf, 1996). The term '*synapsis*' will be used for this closer association. (4) Recombination takes place between two DNA duplexes that must lie within nanometers of each other; precise pairing depends on the complementarity that exists between bases in individual DNA strands (Kowalczykowski and Eggleston, 1994). Only when these four different types of association have taken place are the homologous pairs of duplicated chromosomes lined up on the spindle, and segregated by the two meiotic metaphases into the haploid germ cells. (Additional backup mechanisms are involved in pairing non-exchange chromosomes (Hawley et al., 1993; Wolf, 1994), but these are not discussed here.)

MEIOTIC 'ALIGNMENT' IS DISTINCT FROM 'SYNAPSIS' AND CAN OCCUR IN THE ABSENCE OF RECOMBINATION

It is now generally accepted that 'alignment' and 'synapsis' are different processes (e.g. Moses, 1968; Loidl, 1990; Hawley and Arbel, 1993; Scherthan et al., 1996; Kleckner, 1996). How can we be sure that they are not both mediated by DNA molecules stretching different distances away from the chromosomal axis? The main evidence is:

(1) The two types of association take place at different times, over different distances. Moreover, a distinct structure, the synaptonemal complex, separates the chromosomal axes by ~100 nm during 'synapsis', but no such complex is visible between 'aligned' chromosomes that may lie up to 4,000 nm apart (e.g. Moens, 1969; von Wettstein et al., 1984).

(2) In polyploids, all homologs 'align', but then 'synapse' in twos. For example, sets of three chromosomes 'co-align' in a triploid *Allium* before they 'synapse' in pairs. The excluded chromosomes can remain 'co-aligned' far into pachytene (Loidl and Jones, 1986). (This observation also shows that individual chromosomes cannot be molecularly marked as 'plus' or 'minus', because any two of the three homologs can 'synapse'.)

(3) Colchicine inhibits 'alignment', but not 'synapsis' (e.g. Loidl, 1990).

(4) Homology is required for 'alignment', but synaptonemal complexes are indifferent to homology; they form between non-homologs if no partner is available (e.g. Gillies, 1973; von Wettstein et al., 1984).

(5) The two processes can be distinguished genetically; chromosomes in many organisms 'align' even though synaptonemal complexes are not formed subsequently (e.g. the X and Y chromosomes in male *Drosophila*). They can also 'align' to some extent in yeast mutants (e.g. *hop1*, *mer1*, and *zip1* mutants) that cannot assemble synaptonemal complexes (Sym et al., 1993; Weiner and Kleckner, 1994; Loidl et al., 1994; Sym and Roeder, 1995).

Chromosomes also 'align' without recombining. Again, X and Y chromosomes in male *Drosophila* 'align', but never recombine. And yeast homologs 'align' in *hop1* mutants at 90% of the wild-type level, despite the generation of <10% of meiosis-specific double-strand breaks (Weiner and Kleckner, 1994).

MODELS FOR 'ALIGNMENT' IN MEIOSIS

Most models for meiotic 'alignment' contain some, or all, of the following features (Loidl, 1990; Kleckner, 1996):

(1) A 'stirring' mechanism to generate accidental contacts between homologs. Many authors have commented on how rapidly chromosomes move during leptotene, compared with pachytene; for example, both rat and yeast chromosomes rotate and reverse direction as they move among their neighbours at speeds of several microns per minute (Parvinen and Soderstrom, 1976; Chikashige et al., 1994).

(2) An initial non-random positioning of chromosomes to ease the search for homology (Weiner and Kleckner, 1994). Non-random positioning could arise from the partial pairing of homologs that occurs in somatic cells (e.g. Metz, 1916; Hiraoka et al., 1993), perhaps by aligning centromeres to centromeres and telomeres to telomeres through the 'Rabl' and 'bouquet' arrangements (e.g. Scherthan et al., 1996).

(3) Weak interactions (e.g. Kleckner and Weiner, 1993). Then pairing would result from a trial-and-error homology search as interactions between the 'wrong' partners are made and broken; only when the 'right' partner is found, would the two be zipped stably together. Such a zippering would also extrude any chromosomes accidentally caught between the two.

Despite a consensus on these issues, there is little agreement on which molecular interactions mediate pairing. In general terms, recognition must involve long-range interactions, where homologs 'feel' other chromosomes and sense specific sites in their partners before they move together. Many early models involved filamentous pairing proteins (Comings and Riggs, 1971; Holliday, 1977; Chandley, 1986). Most current models involve the same DNA-DNA interactions that we know occur later at the recombination site. Thus, a single-stranded break could enable a single-strand from one chromosome to extend through the nucleus to feel for its homolog and, once found, base-pairing would stabilize the connection (e.g. Sun et al., 1991; Stasiak, 1992). Then, as other single-stranded extensions succeeded in their searches for homology, the two partners would be zipped up ever more tightly together (e.g. Smithies and Powers, 1986). A variant of this model involves a double-strand break and resection of the 5′ terminus to leave a 3′ single strand that could feel for, and invade, a homologous duplex (Szostak et al., 1983; Kleckner, 1996).

Models involving DNA-DNA interactions are attractive for two reasons. First, there is a clear molecular precedent, base pairing, for the kind of interaction proposed. Second, they are minimalist models; the same event, a single, or a double-strand break, initiates both pairing and recombination. However, recent results in yeast genetics have compromised such models, and led to what has been described as the 'fall of the classical view of meiosis' (Hawley and Arbel, 1993); homologs 'align' before the relevant breaks can be detected (Fig. 1), and they do so to some extent in mutants (e.g. *rad50S*, *rad50* and *spo11*) that cannot form or process the double-strand breaks which probably initiate recombination (e.g. Loidl et al., 1994; Roeder, 1995). 'Alignment' clearly takes place without breakage.

Since models involving broken DNA can be eliminated, those involving interactions between intact DNA molecules

have come to the fore. For example, an intact duplex loop could 'kiss' chromatin loops in other chromosomes, and, once a partner had been found, a RecA-like protein could line them up through base pairing, stabilizing them (Kleckner and Weiner, 1993). Two candidate RecA-like proteins, RAD51 and DMC1, are found in the right place at the right time (Bishop, 1994; Heyer, 1994; Ashley et al., 1995; Terasawa et al., 1995). Unfortunately, chromosomes are now known to 'align' in mutants encoding defective RecA-like proteins (e.g. Weiner and Kleckner, 1994; Rockmill et al., 1995); therefore, although such proteins are required for recombination, no known ones are essential for 'alignment' (Kleckner, 1996). Alternatively, two intact duplexes could pair through the special hydrogen bonds that may form between certain base pairs (e.g. through Hoogsteen base pairs between four, parallel, DNA strands; Sen and Gilbert, 1988; Sundquist and Klug, 1989; see also McGavin, 1977; Hopkins, 1986). Although molecular precedents exist for such interactions, the appeal of such models is lessened because they invoke an additional mechanism that has no proven role during 'alignment'. Moreover, 'correct' pairing becomes more difficult the more repeated DNA sequences there are in the genome, since those repeats will pair with others on non-homologous chromosomes.

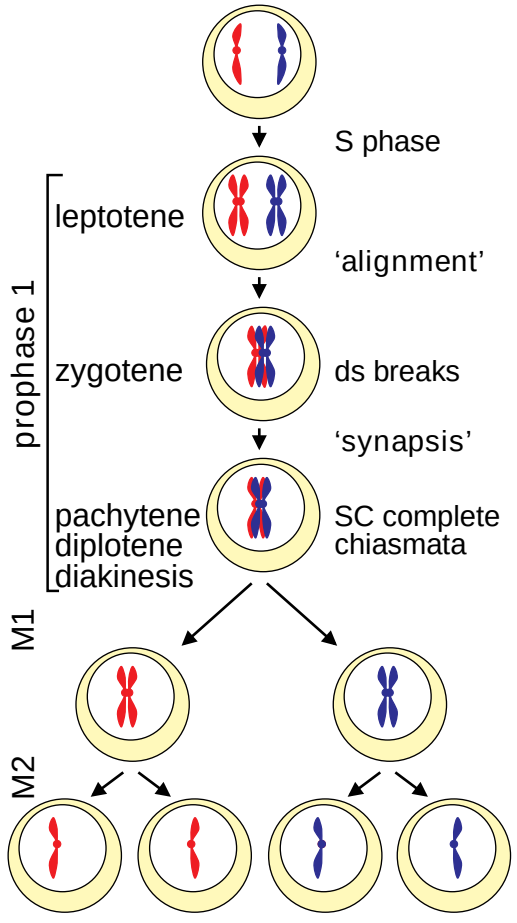


Fig. 1. Some events occurring as a diploid cell passes through meiosis; only a pair of homologous chromosomes is shown. M1 and 2, metaphases 1 and 2. ds breaks, double-strand breaks. SC, synaptonemal complex.

PAIRING IN SOMATIC CELLS

As long ago as 1916, Metz (1916) had seen paired chromosomes in diploid somatic cells of eighty Dipteran species. In general, such pairing is never as extensive as it is in the germ cells, the frequency varies from cell to cell, and it falls as the genome complexity increases (i.e. it is high in yeast, but low in man; Comings, 1980; Tartof and Henikoff, 1991). A recent study using fluorescence in situ hybridization to detect histone genes illustrates the range of pairing seen in *Drosophila* (Hiraoka et al., 1993). Before embryonic nuclear cycle 12, only 10-29% of nuclei give one, strong, fluorescent signal showing that the two loci are paired. Then, pairing increases during the mid-blastula transition, so that 63-86% are paired by cycle 14. Translocation alters the pairing frequency, so it also depends on global homology.

Pairing occurs more efficiently in some larval nuclei of *Drosophila*. Replication of individual DNA duplexes in each chromosome generates up to 1,000 copies; these all remain paired in exact register to give the enormous, banded, chromosomes that characterize such polytene nuclei (Ashburner, 1989). The two (polytenized) homologs are usually paired. Any general model should explain why chromosomes in both diploid and polytene cells also pair.

TRANSCRIPTION AND 'ALIGNMENT'

The condensation of chromatin into discernible chromosomes usually inhibits transcription; chromosomes always become *inactive* when they enter mitotic prophase (e.g. Shermoen and O'Farrell, 1991). In contrast, the 'aligned' chromosomes of meiotic prophase I, and polytene cells, are transcriptionally *active* (e.g. Monesi et al., 1978; Mitchell, 1994). In meiotic cells, this activity of the condensed chromosomes always persists for a considerable fraction of the cell cycle, lasting in newt oocytes for more than a year. Moreover, there have been odd reports that this transcription is promiscuous: satellite transcripts have been found in newt oocytes, and heat-shock proteins and brain-specific proenkephalin transcripts in unstressed mouse testis (Diaz et al., 1981; Gruppi and Wolgemuth, 1993; Davies and Willison, 1993). This correlation between 'alignment' and transcriptional activity is carried through to the somatic cells of *Drosophila* embryos; pairing and transcription increase together at the midblastula transition, as described above.

As far as I am aware, only one 'alignment' site has been mapped precisely; it turns out to be the key transcriptional element, a promoter (McKee et al., 1992). The site is responsible for the meiotic association of the X and Y chromosomes in male *Drosophila*; deleting rDNA sequences from heterochromatin on the X disrupts 'alignment', while reinserting them restores it. The critical pairing site contains multiple copies of a 240 bp repeat, and increasing the copy number increases pairing. Each copy contains a functional polymerase I promoter that enhances transcription in a way that depends on copy-number; 'alignment' and promoter activity are directly related.

Given these striking correlations between transcription and 'alignment', I now discuss how *transcription might inevitably cause pairing*. (Transcription also plays a role in recombination (Gangloff et al., 1994), but this will not be discussed here.)

TRANSCRIPTION FACTORIES AND CHROMOSOME STRUCTURE

The traditional model for transcription involves a polymerizing complex that tracks along the template; the enzyme moves while the DNA remains stationary. However, the size of the active polymerizing complex has grown steadily over the years, so that it now contains more than 80 different polypeptides (Pugh, 1996); therefore it dwarfs the template, and so is unlikely to track. Moreover, many active transcription units are associated with discrete structures, or 'factories' in the nucleus (for reviews, see Cook, 1994, 1995). The nucleolus contains the prototypic factory attached to the underlying nucleoskeleton (Hozák et al., 1994). When demand for rRNA is low, only a few transcription units can engage polymerases on the surface of a large fibrillar centre. As demand increases, this centre splits, increasing the surface area and so the number of accessible polymerases. When transcription is maximal, several hundred transcription units are each associated with only one centre and the ~80 polymerases on its surface (Haaf et al., 1991). Analogous factories containing polymerases II and III are found in extra-nucleolar regions; for example, ~35,000 active polymerases and their associated transcripts are found in ~2,000 factories in a HeLa nucleus (Iborra et al., 1996). Each such factory, which typically has a diameter of ~70 nm, would be associated with a surrounding 'cloud' of ~50 chromatin loops ranging in length from 5-200 kbp (Cook, 1995).

Transcription would then involve three basic steps (Fig. 2). First, a promoter/enhancer in a loop competes with others for polymerizing sites on the surface of a factory, and, if successful, it attaches. Concurrently, an initiation complex containing the appropriate transcription factors assembles around the attaching promoter. Second, the template is reeled in by a polymerase, as a transcript is extruded into the factory. Finally, the template dissociates so that the cycle can repeat. The chances that the promoter can attach again are now high; it lies near a factory containing the appropriate complex of transcription factors, and its chromatin has been 'opened' by the previous cycle. Other weaker promoters in longer transcriptionally-inactive loops, which have aggregated into denser heterochromatic clumps, will have little chance in the competition. This model implies that loops are transiently attached to factories through transcription units to polymerases and transcription factors. Various evidence shows that this is so, with two-thirds of the attachments being mediated through promoters/enhancers and one third through the body of a transcription unit (e.g. Jackson and Cook, 1993; Jackson et al., 1996).

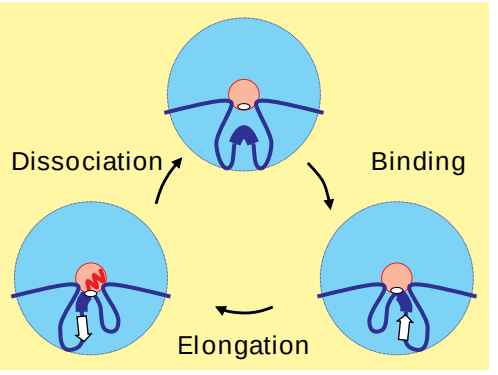


Fig. 2. A model for transcription. A chromatin fibre is tied in tens of loops (only one is shown) to a factory (pink circle) to give a surrounding cloud of chromatin (blue). Transcription initiates after a promoter in a transcription unit (thick regions) binds to the factory, and the appropriate transcription factors are assembled; then a transcript (wavy red line) is extruded as the template slides (arrows) through the polymerization site (white oval). The template dissociates on termination, and the cycle repeats.

Just as loops are in dynamic equilibrium, so are factories; they split as more promoters attach, and fuse as the transcription rate declines. Therefore, more long inactive loops surround large factories, and more short active loops surround small factories. The latter also contain high concentrations of transcription factors. Both kinds are attached to a nucleoskeleton, and new elements of this skeleton polymerize and depolymerize between factories as they split and fuse. When cells enter mitosis, the skeleton depolymerizes, transcription ceases, and factories disassemble. The remnants of the factories, still associated with loops, then form the chromomeres of the mitotic chromosome. An increased adhesiveness between nucleosomes and between factories drives condensation. Still surrounded by their cloud of loops, the chromomeres condense into the most compact and stable structure, an axial core surrounded by a cylinder of nucleosomes (Cook, 1995).

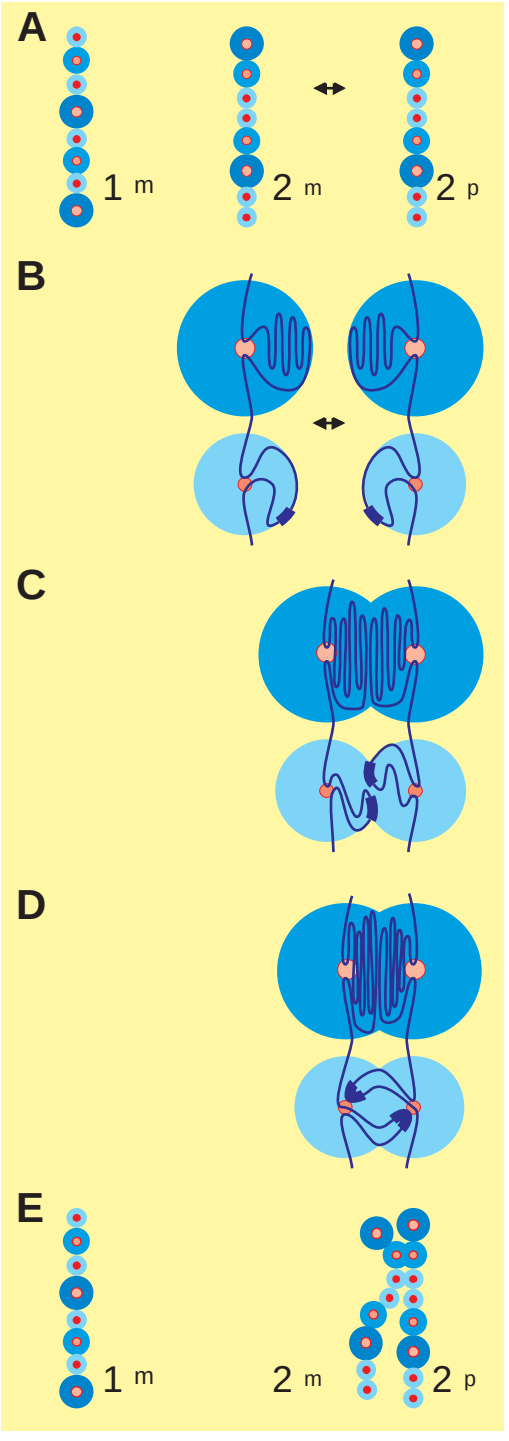
A MODEL FOR PAIRING IN SOMATIC CELLS

This model for chromosome structure is readily extended to explain how transcription might inevitably lead to pairing. I begin with a simple case, a diploid (non-meiotic) yeast cell during G_1 phase (Fig. 3). Each chromatin fibre in the haploid set has a unique array of transcription units strung along it, and each will be attached to factories to give a unique array of chromatin clouds (e.g. 1^m and 2^m in Fig. 3A). Some clouds will be rich in long heterochromatic loops, others in short active loops. Only homologs share similar arrays (e.g. 2^m and 2^p). (Their arrays may differ slightly, as individual loops can be at different stages in the attachment cycle, and factories split and fuse.)

Each cloud in an array moves continually, making and breaking contact with others. Interactions between nucleosomes, and between promoters/enhancers and factories, stabilize such contacts. A nucleosome in an inactive loop tends to aggregate with others into heterochromatin; inactive clouds also aggregate as individual nucleosomes cannot sense whether others belong to 'foreign' clouds. Similarly, a promoter or enhancer may bind to a 'foreign' factory, if it happens to contain the appropriate transcription factors. Both interactions are usually so weak that they are broken by Brownian motion. Occasionally, however, adjacent clouds in an array will contact their exact homologs, and now interactions between clouds reinforce each other. For example, in Fig. 3B-D, nucleosomes in the two long loops (at the top of each panel) aggregate; simultaneously, promoters in the two short, more dynamic, loops (at the bottom of each panel) attach to preassembled complexes of the appropriate transcription factors in the 'foreign' factory. Once transcription initiates, the two factories are tied together through some of the stablest interactions found in nuclei, the associations between engaged polymerases and their templates. This reciprocal aggregation/attachment gives time for other loops to aggregate/attach, so that the two clouds become difficult to separate by Brownian motion (Fig.

3E). Eventually the two homologous arrays are zipped together.

We can now see how the polytene chromosomes of *Drosophila* might form. Even before polytenization begins, heterochromatic clouds aggregate into the chromocentre (Fig. 4A). Then, transcription drives the zipping together of homologs (Fig. 4B). As polytenization generates more transcriptionally-active duplexes, clouds remain tied together laterally as heterochromatic clouds aggregate and transcription units bind to 'foreign' factories. Nevertheless, clouds split longitudinally into smaller clouds associated with fewer loops, lengthening the structure; eventually, long heterochromatic loops form bands, and short active loops form the interbands (Fig. 4C).



Various factors promote pairing in somatic cells:

(1) Increasing rates of contact and 'stirring'. Both are probably driven by Brownian motion, and so will be reduced by polymerizing skeletal elements between factories.

(2) Reducing cloud number. As most clouds in complex genomes are surrounded by others in the same array (Cremer et al., 1993), only those on the surface can encounter homologs; then, 'alignment' would rarely occur in complex genomes. Complex genomes also contain 'junk' DNA; fortunately this extra DNA is often transcriptionally inert and packaged into large clouds without a proportional increase in cloud number.

(3) Reducing cloud volume. As individual clouds condense, the nucleosomal density on the surface increases, promoting cloud fusion and so a reduction in cloud number. Condensation also reduces the amount of nuclear space occupied by chromatin, increasing the contact rate.

(4) Increasing the transcription rate. During the mid-blastula transition in fly embryos, the net effect of increased transcription is to boost pairing (as loops attach more often to factories), even though factories split (to increase cloud number).

A MODEL FOR MEIOTIC 'ALIGNMENT'

How can the moderate levels of pairing seen in somatic cells be increased during meiosis to ensure that all homologs 'align'? This is a significant problem in a human nucleus that may contain ~50 clouds per chromosome (Cook, 1995). Evolution seems to have solved this problem by condensing the chromosomes, while retaining the necessary transcription. And, of course, it will press into use any mechanisms that increase 'stirring', or pre-align chromosomes (e.g. through 'Rabl' orientations or 'bouquets').

Meiotic chromosomes would 'align' as follows. After pre-meiotic S phase, duplicated sister chromatids would be tied

Fig. 3. The basis of chromosomal alignment. (A) Segments of maternal chromosome 1 (1^m), and of maternal and paternal chromosomes 2 (2^m and 2^p) are represented as arrays of clouds and factories (blue rings surrounding pink circles). Each chromatin fibre in the haploid set has a unique array of transcription units strung along it, so each is folded into a unique array of clouds/factories of different size and content. Therefore 1^m and 2^m have different arrays, but 2^m and 2^p have similar arrays. (B) Detail of two homologous clouds in 2^m and 2^p. In both, a chromatin fibre runs from the top into the first factory where it is tied in many long, heterochromatic, loops (only one is shown) to give a large, dense, chromatin cloud; then, it runs down into a smaller, more active, factory, to which it is tied in many shorter loops (only one is shown) and so into a smaller cloud. (C) Two factories in 2^m have contacted their homologs in 2^p. Densely-packed nucleosomes associated with the top factories adhere to each other. Promoters (thick regions) on each of the lower loops now lie equidistant from their 'own' factory and the homologous factory in the 'wrong' array; the latter contains all factors necessary for promoter binding. (D) The two dense clouds at the top collapsed on to each other, as promoters in the bottom loops attached to the 'wrong' factories. (E) A low-power view of the resulting arrangement. Two clouds in 2^m and 2^p have paired, so that adjacent clouds have a high probability of being zipped together. Few clouds need pair to ensure identification of the correct homolog; for example, if 6 different types of cloud are randomly distributed among 1,000 in a haploid set, a run of 4 is enough to specify position uniquely within the genome (i.e. $6^4 > 1{,}000$).

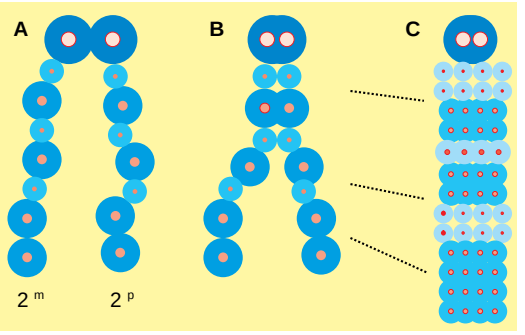


Fig. 4. The structure of a polytene chromosome. (A) Two homologs (2^m and 2^p) in a diploid cell are attached through terminal heterochromatic clouds. (B) Transcription leads to pairing, as in Fig. 3. (C) Polytenization generates more clouds; these remain tied together laterally through the aggregation of heterochromatin, and through the association of transcription units with 'foreign' factories. Factories also split as they become associated with fewer loops, lengthening the structure. Eventually, as polytenization continues and factories continue to split, long heterochromatic loops will form bands, and short active loops the interbands.

together in places where they remained attached to the same factory. On entry into meiotic prophase, chromosome condensation would promote 'alignment' by reducing the number of clouds (so increasing the chances of a productive contact), by increasing adhesion between heterochromatic clouds (so increasing the density of nucleosomes on their surface), and by decreasing chromatin volume (so increasing 'stirring'). Concurrently, the nucleoskeleton is probably depolymerized, which also raises the 'stirring' rate. Now the trick is to maintain, and even increase, transcription in the face of the chromatin condensation to promote attachment of loops to factories and so the trial-and-error homology search. Repeated transcription units (e.g. histone loci), and those that function normally in heterochromatin (e.g. rDNA) or when others are switched off (e.g. heat-shock loci), would be the most likely to nucleate 'alignment'. The general overstimulation of transcription would inevitably lead to the synthesis of some bizarre transcripts. Once a transcription unit had found its homologous factory (as in Fig. 3D, bottom), initiation would stabilize the association, to give time for other loops to become tightly bound together. In time, homologs are zipped together and synaptonemal complexes promote intimate 'synapsis' between DNA molecules that are then broken and recombined.

TRANSCRIPTION AND THE INITIATION OF RECOMBINATION

It is not my purpose here to discuss the roles that transcription might play in recombination, but the model immediately suggests two. First, factories may broadly specify where recombination occurs in the genome; they seem to nucleate the formation of sites of replication and repair (Hassan et al., 1994; Jackson et al., 1994) and so it is a small step to suggest that they also nucleate sites of recombination, the recombination nodules (Carpenter, 1987). Indeed, one key player in recombination, the recA-homolog Rad51, is concentrated during leptotene in sites that could well turn out to be factories (Ashley et al., 1995). Second, the model explains why sequences like promoters are local hot spots for recombination, and why there is a hot spot in every gene. (See Gangloff et al. (1994) and Lichten and Goldman (1995) for reviews.) It is natural to assume that recombination takes place at, or close to, the point where many loops are tethered to the underlying structure (i.e. where promoters and transcription units are attached to factories); then, some of the major pairing sites, places where maternal and paternal sequences are tied to the same factory, will become prime targets for the recombination machinery.

THE EVOLUTION OF MEIOTIC 'ALIGNMENT'

Cross-overs and two successive rounds of chromosome segregation differentiate meiotic from mitotic cell cycles. A recombination nodule that could process cross-overs might well have evolved from a recombination-based repair system housed in a factory, especially as so many transcription factors turn out to be involved in repair (Drapkin et al., 1994). A plausible route to a two-division cycle entails slowing the cycle by modifying the activity of a master regulator like maturation promoting factor. Slowing prophase would inevitably cause homologs to 'align' (as described above), and subsequent fluctuations in regulator levels could lead to a two-division segregation system that had to operate on those 'aligned' substrates. So 'alignment' is an inevitable consequence of slowing prophase, and not a mysterious mechanism that had to evolve separately.

TRANSVECTION AND SILENCING, EXAMPLES WHERE PAIRING AFFECTS TRANSCRIPTION

I have discussed how transcription affects pairing, but pairing also occasionally affects transcription (Tartof and Henikoff, 1991). In 1954, Lewis applied the term 'transvection' to the complementation seen when two alleles of the bithorax complex of *Drosophila* were paired, but which was lost when they were separated (for reviews, see Judd, 1988; Wu, 1993).

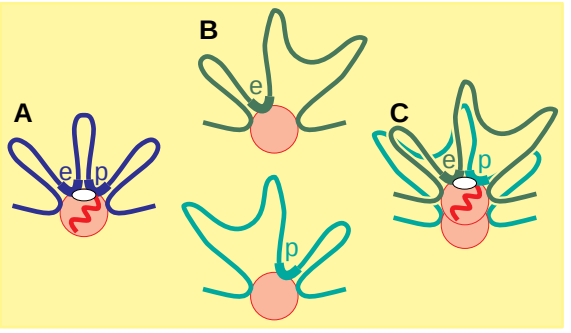


Fig. 5. Models for enhancer action and transvection at the *yellow* locus. (A) A wild-type chromosome with enhancer (e) and promoter (p) is attached to a factory; for simplicity, only one of the two enhancers in the locus is shown. A productive initiation complex (white oval) has assembled so that a transcript (wavy red line) can be made. (B) Mutation of either the promoter (top) or enhancer (bottom) prevents attachment to the factory and formation of a productive complex; no transcripts are made from unpaired chromosomes. (C) Pairing of the two factories and their loops allows the enhancer on one mutant chromosome and the promoter on the other to form a productive complex in one factory, so that a transcript is made.

Similar effects are seen at other loci, including *yellow*; transcription of a defective y^2 chromosome (which lacks functional enhancers but has an intact promoter) is rescued by pairing with a defective y^{59b} chromosome (which has the enhancers but lacks the promoter; Geyer et al., 1990). This phenomenon can be explained if transcription depends on the association of functional enhancers with a promoter in the same factory (Fig. 5).

Pairing can also suppress transcription so that genes that are active when unpaired become 'silenced' or 'co-suppressed' when paired (Pirotta and Rastelli, 1994; Vaucheret et al., 1995). In this case, binding to one factory could nucleate the formation of a stable inactivating complex around the now-neighbouring loops.

CONCLUSIONS

The successful segregation of meiotic chromosomes requires the 'alignment' of homologs during leptotene (Fig. 1). Which molecular interactions are involved in this homology search? Traditional models involve breaking DNA strands to allow single-stranded extensions to 'feel' for, and then base-pair with, their partners. However, recent evidence shows that homologs 'align' in the absence of the required breaks, so it no longer seems sensible to suggest that something as intrinsically dangerous as genome breakage should underpin the search. Traditional models must also explain how homologs find their partners in genomes containing many DNA repeats, which would dominate the kinetics of the search.

The alternative explored here is based on the remarkable fact that chromosomes only 'align' when they are transcriptionally active. As chromosomes enter mitotic prophase, they condense, lose transcriptional activity, but do not 'align'. (Nevertheless, vestiges of preexisting pairing may be retained into metaphase.) Chromosomes entering prophase I of meiosis also condense, *but they retain transcriptional activity, and they 'align'*. This correlation between activity and pairing is carried over into interphase in some somatic cells: in *Drosophila* embryos, homologs only pair when transcription begins at the midblastula transition, and, later, giant polytene chromosomes are both active and paired. Significantly, where a meiotic 'alignment' sequence has been mapped precisely, it turns out to be the key transcriptional element, a promoter, with the copy number of that promoter determining the degree of 'alignment' (McKee et al., 1992). These correlations suggest that pairing might be based upon the promoter-polymerase interactions involved in transcription.

How might such DNA-protein interactions determine 'alignment'. The traditional model for transcription involves a polymerizing complex that binds to a template and then tracks along it. The argument put forward here is based on the 'cart-before-the-horse' alternative in which promoters and enhancers bind to polymerizing sites fixed in transcription 'factories'; then transcripts are extruded into factories as templates are reeled in by polymerases (Cook, 1995). This implies that the chromatin fibre is tied in a 'cloud' of loops to a factory, and that individual loops in the cloud are continually attaching and detaching. It then follows that each chromatin fibre in the haploid set will be folded into a unique array of clouds, since each fibre has a unique array of transcription units strung along it. Only homologs will share similar arrays. If one cloud contacts its homolog, promoters and enhancers in that cloud can bind productively to the factory in the other cloud, as it contains all the appropriate transcription factors. Subsequent transcription will then stabilize the association between that loop and the factory, increasing the probability that adjacent clouds in the two arrays will be zipped together.

This model incorporates aspects of many others (e.g. Comings and Riggs, 1971; Riley and Flavell, 1977; Loidl, 1990; Kleckner and Weiner, 1993). It has several advantages. First, the number of sites to be scanned for homology is reduced. For example, an initial scan of the human genome with a base-pairing probe requires $>10^9$ interactions, but here thousands of promoters must scan a few thousand factories. Moreover, the homology scan is not defeated by repeats, which will usually be packaged into inert heterochromatin. Second, the proposed interactions, between transcription units and the polymerizing machinery, are well-characterized. Third, it is a general but economic model, explaining how homologs pair in meiotic, mitotic and polytene cells, and why transcription units are hot spots for recombination. Fourth, it is testable; for example, transcription must be concentrated in factories when chromosomes pair, and point mutations in the relevant promoters should disrupt pairing.

I thank my many colleagues for helpful discussions, and the Cancer Research Campaign and the Wellcome Trust for support.

REFERENCES

Ashburner, M. (1989). Drosophila: *a Laboratory Handbook*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Ashley, T., Plug, A. W., Xu, J., Solari, A. J., Reddy, G., Golub, E. I. and Ward, D. C. (1995). Dynamic changes in Rad51 distribution on chromatin during meiosis in male and female vertebrates. *Chromosoma* **104**, 19-28.

Bishop, D. K. (1994). RecA homologs Dmc1 and Rad51 interact to form multiple nuclear complexes prior to meiotic chromosome synapsis. *Cell* **79**, 1081-1092.

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

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

Carpenter, A. T. C. (1994). Chiasma function. *Cell* **77**, 959-962.

Chandley, A. C. (1986). A model for effective pairing and recombination at meiosis based on early replicating sites (R-bands) along chromosomes. *Hum. Genet*. **72**, 50-57.

Chikashige, Y., Ding, D. Q., Funabiki, H., Haraguchi, T., Mashiko, S., Yanagida, M. and Hiraoka, Y. (1994). Telomere-led premeiotic chromosome movement in fission yeast. *Science* **264**, 270-273.

Comings, D. E. and Riggs, A. D. (1971). Mechanisms of chromosome pairing during meiosis. *Nature* **227**, 451-456.

Comings, D. E. (1980). Arrangement of chromatin in the nucleus. *Hum. Genet*. **53**, 131-143.

Cook, P. R. (1994). RNA polymerase: structural determinant of the chromatin loop and the chromosome. *BioEssays* **16**, 425-430.

Cook, P. R. (1995). A chromomeric model for nuclear and chromosome structure. *J. Cell Sci*. **108**, 2927-2935.

Cremer, T., Kurz, A., Zirbel, R., Dietzel, S., Rinke, B., Schrock, E., Speicher, M. R., Mathieu, U., Jauch, A., Emmerich, P., Scherthan, H., Reid, T., Cremer, C. and Lichter, P. (1993). Role of chromosome territories in the functional compartmentalization of the cell nucleus. *Cold Spring Harb. Symp. Quant. Biol*. **58**, 777-792.

Davies, P. O. and Willison, K. R. (1993). Molecular mechanisms of differentiation in mammalian spermatogenesis. *Semin. Dev. Biol*. **4**, 179-188.

Diaz, M. O., Barsacchi-Pilone, G., Mahon, K. A. and Gall, J. G. (1981). Transcripts from both strands of a satellite DNA occur on lampbrush chromosome loops of the newt *Notophthalmus*. *Cell* **24**, 649-659.

Drapkin, R., Sancar, A. and Reinberg, D. (1994). Where transcription meets repair. *Cell* **77**, 9-12.
Gangloff, S., Lieber, M. R. and Rothstein, R. (1994). Transcription, topoisomerases and recombination. *Experentia* **50**, 261-269.
Geyer, P. M., Green, M. M. and Corces, V. G. (1990). Tissue-specific transcriptional enhancers may act in *trans* on the gene located in the homologous chromosome: the molecular basis of transvection in *Drosophila*. *EMBO J*. **9**, 2247-2256.
Gillies, C. B. (1973). Ultrastructural analysis of maize pachytene karyotypes by three dimensional reconstruction of the synaptonemal complexes. *Chromosoma* **43**, 145-176.
Gruppi, C. M. and Wolgemuth, D. J. (1993). HSP86 and HSP84 exhibit cellular specificity of expression and co-precipitate with an HSP70 family member in the murine testis. *Dev. Genet*. **14**, 119-126.
Haaf, T., Hayman, D. L. and Schmid, M. (1991). Quantitative determination of rDNA transcription units in vertebrate cells. *Exp. Cell Res*. **193**, 78-86.
Hasenkampf, C. A. (1996). The synaptonemal complex – a chaperone of crossing over. *Chromosome Res*. **4**, 133-140.
Hassan, A. B., Errington, R. J., White, N. S., Jackson, D. A. and Cook. P. R. (1994). Replication and transcription sites are colocalized in human cells. *J. Cell Sci*. **107**, 425-434.
Hawley, R. S. and Arbel, T. (1993). Yeast genetics and the fall of the classical view of meiosis. *Cell* **72**, 301-303.
Hawley, R. S., McKim, K. S. and Arbel, T. (1993). Meiotic segregation in *Drosophila melanogaster* females: molecules, mechanisms and myths. *Annu. Rev. Genet*. **27**, 281-317.
Heyer, W.-D. (1994). The search for the right partner: homologous pairing and DNA strand exchange proteins in eukaryotes. *Experentia* **50**, 223-233.
Hiraoka, Y., Durnburg, A. F., Parmalee, A. F., Rykowski, M. C., Agard, D. A. and Sedat, J. W. (1993). The onset of homologous chromosome pairing during *Drosophila melanogaster* embryogenesis. *J. Cell Biol*. **120**, 591-600.
Holliday, R. (1977). Recombination and meiosis. *Phil. Trans. R. Soc. Lond. B* **277**, 359-370.
Hopkins, R. C. (1986). A unique four-stranded model of a homologous recombination intermediate. *J. Theor. Biol*. **120**, 215-222.
Hozák, P., Cook, P. R., Schöfer, C., Mosgöller, W. and Wachtler, F. (1994). Site of transcription of ribosomal RNA and intra-nucleolar structure in HeLa cells. *J. Cell Sci*. **107**, 639-648.
Iborra, F. J., Pombo, A., Jackson, D. A. and Cook, P. R. (1996). Active RNA polymerases are localized within discrete transcription 'factories' in human nuclei. *J. Cell Sci*. **109**, 1427-1436.
Jackson, D. A. and Cook, P. R. (1993). Transcriptionally-active minichromosomes are attached transiently in nuclei through transcription units. *J. Cell Sci*. **105**, 1143-1150.
Jackson, D. A., Hassan, A. B., Errington, R. J. and Cook, P. R. (1994). Sites in human nuclei where damage induced by ultra-violet light is repaired: localization relative to transcription sites and concentrations of proliferating cell nuclear antigen and the tumour suppressor protein, p53. *J. Cell Sci*. **107**, 1753-1760.
Jackson, D. A., Bartlett, J. and Cook, P. R. (1996). Sequences attaching loops of nuclear and mitochondrial DNA to underlying structures in human cells: the role of transcription units. *Nucl. Acids Res*. **24**, 1212-1219.
Judd, B. H. (1988). Transvection: allelic cross talk. *Cell* **53**, 841-843.
Kleckner, N. and Weiner, B. M. (1993). Potential advantages of unstable interactions for pairing of chromosomes in meiotic, somatic, and premeiotic cells. *Cold Spring Harb. Symp. Quant. Biol*. **58**, 553-565.
Kleckner, N. (1996). Meiosis: how could it work? *Proc. Nat. Acad. Sci. USA* **93**, 8167-8174.
Kowalczykowski, S. C. and Eggleston, A. E. (1994). Homologous pairing and DNA strand-exchange proteins. *Annu. Rev. Biochem*. **63**, 991-1043.
Lichten, M. and Goldman, A. S. H. (1995). Meiotic recombination hotspots. *Annu. Rev. Genet*. **29**, 423-444.
Loidl, J. (1990). The initiation of meiotic chromosome pairing: the cytological view. *Genome* **33**, 759-778.
Loidl, J. (1994). Cytological aspects of meiotic recombination. *Experientia* **50**, 285-294.
Loidl, J. and Jones, E. H. (1986). Synaptonemal complex spreading in *Allium*, I: triploid *A. sphaerocephalon*. *Chromosoma* **93**, 420-428.
Loidl, J., Klein, F. and Scherthan, H. (1994). Homologous pairing is reduced but not abolished in asynaptic mutants of yeast. *J. Cell Biol*. **125**, 1191-1200.
McGavin, S. (1977). A model for the specific pairing of homologous double-stranded nucleic acid molecules during genetic recombination. *Heredity* **39**, 15-25.
McKee, B. D., Habera, L. and Vrana, J. A. (1992). Evidence that intergenic spacer repeats of Drosophila melanogaster rRNA genes function as X-Y pairing sites in male meiosis, and a general model for achiasmatic pairing. *Genetics* **132**, 529-544.
Metz, C. W. (1916). Chromosome studies on the Diptera, II: the paired association of chromosomes in the Diptera, and its significance. *J. Exp. Zool*. **21**, 213-279.
Mitchell, A. P. (1994). Control of meiotic gene expression in *Saccharomyces cerevisiae*. *Microbiol. Rev*. **58**, 56-70.
Miyazaki, W. Y. and Orr-Weaver, T. L. (1994). Sister-chromatid cohesion in mitosis and meiosis. *Annu. Rev. Genet*. **28**, 167-187.
Moens, P. B. (1969). The fine structure of meiotic chromosome polarization and pairing in *Locusta migratoria* spermatocytes. *Chromosoma* **28**, 1-25.
Monesi, V., Geremia, R., D'Agostino, A. and Boitani, C. (1978). Biochemistry of male germ cell differentiation in mammals: RNA synthesis in meiotic and post meiotic cells. *Curr. Top. Dev. Biol*. **12**, 11-36.
Moses, M. J. (1968). Synaptinemal complex. *Annu. Rev. Genet*. **2**, 363-412.
Parvinen, M. and Soderstrom, K.-O. (1976). Chromosome rotation and formation of synapsis. *Nature* **260**, 534-535.
Pirotta, V. and Rastelli, L. (1994). *white* gene expression, repressive chromatin domains and homeeotic gene regulation in *Drosophila*. *BioEssays* **16**, 549-556.
Pugh, B. F. (1996). Mechanism of transcription complex assembly. *Curr. Opin. Cell Biol*. **8**, 303-311.
Riley, R. and Flavell, R. B. (1977). A first view of the meiotic process. *Phil. Trans. R. Soc. London, Ser. B*, **277**, 191-199.
Rockmill, B., Sym, M., Scherthan, H. and Roeder, G. S. (1995). Roles of two RecA homologs in promoting meiotic chromosome synapsis. *Genes Dev*. **9**, 2684-2695.
Roeder, G. S. (1995). Sex and the single cell: meiosis in yeast. *Proc. Nat. Acad. Sci. USA* **92**, 10450-10456.
Scherthan, H., Weich, S., Schwegler, H., Heyting, C., Harle, M. and Cremer, T. (1996). Centromere and telomere movements during early meiotic prophase of mouse and man are associated with the onset of chromosome pairing. *J. Cell Biol*. **134**, 1109-1125.
Sekelsky, J. J. and Hawley, R. S. (1995). The bond between sisters. *Cell* **83**, 157-160.
Sen, D. and Gilbert, W. (1988). Formation of parallel four-stranded complexes by guanine rich motifs in DNA and its implications for meiosis. *Nature* **334**, 364-366.
Shermoen, A. W. and O'Farrell, P. H. (1991). Progression of the cell cycle through mitosis leads to abortion of nascent transcripts. *Cell* **67**, 303-310.
Sun, H., Treco, D. and Szostak, J. W. (1991). Extensive 3′-overhanging, single-stranded DNA associated with the meiosis-specific double-strand breaks at the *arg-4* recombination initiation site. *Cell* **64**, 1155-1161.
Sundquist, W. I. and Klug, A. (1989). Telomeric DNA dimerizes by formation of guanine tetrads between hairpin loops. *Nature* **342**, 825-829.
Smithies, O. and Powers, P. A. (1986). Gene conversions and their relation to homologous chromosome pairing. *Phil. Trans. R. Soc. Lond. B.* **312**, 291-302.
Stasiak, A. (1992). Three-stranded DNA structure; is this the secret of DNA homologous recognition? *Mol. Microbiol*. **6**, 3267-3276.
Sym, M., Engebrecht, J. and Roeder, G. S. (1993). *ZIP1* is a synaptonemal complex protein required for meiotic chromosome synapsis. *Cell* **72**, 365-378.
Sym, M. and Roeder, G. S. (1995). Zip1-induced changes in synaptonemal complex structure and polycomplex assembly. *J. Cell Biol*. **128**, 455-466.
Szostak, J. W., Orr-Weaver, T. L., Rothstein, R. J. and Stahl, F. W. (1993). The double-strand-break repair model for recombination. *Cell* **33**, 25-35.
Tartof, K. D. and Henikoff, S. (1991). Trans-sensing effects from Drosophila to humans. *Cell* **65**, 201-203.
Terasawa, M., Shinohara, A., Hotta, Y., Ogawa, H. and Ogawa, T. (1995). Localization of RecA-like recombination proteins on chromosomes of the lily at various meiotic stages. *Genes Dev*. **9**, 925-934.
von Wettstein, D., Rasmussen, S. W. and Holm, P. B. (1984). The synaptonemal complex. *Annu. Rev. Genet*. **18**, 331-413.
Vaucheret, H., Palauqui, J. C., Elmayan, T. and Moffatt, B. (1995). Molecular and genetic analysis of nitrite reductase co-suppression in transgenic tobacco plants. *Mol. Gen. Genet*. **248**, 311-317.
Weiner, B. M. and Kleckner, N. (1994). Chromosome pairing via multiple interstitial interactions before and during meiosis in yeast. *Cell* **77**, 977-991.
Wolf, K. W. (1994). How meiotic cells deal with non-exhange chromosomes. *BioEssays* **16**, 107-114.
Wu, C. (1993). Transvection, nuclear structure, and chromatin proteins. *J. Cell Biol*. **120**, 587-590.