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Review

Homologous pairing and chromosome dynamics in meiosis and mitosis

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Abstract

Pairing of homologous chromosomes is an essential feature of meiosis, acting to promote high levels of recombination and to ensure segregation of homologs. However, homologous pairing also occurs in somatic cells, most regularly in Dipterans such as *Drosophila*, but also to a lesser extent in other organisms, and it is not known how mitotic and meiotic pairing relate to each other. In this article, I summarize results of recent molecular studies of pairing in both mitosis and meiosis, focusing especially on studies using fluorescent in situ hybridization (FISH) and GFP-tagging of single loci, which have allowed investigators to assay the pairing status of chromosomes directly. These approaches have permitted the demonstration that pairing occurs throughout the cell cycle in mitotic cells in *Drosophila*, and that the transition from mitotic to meiotic pairing in spermatogenesis is accompanied by a dramatic increase in pairing frequency. Similar approaches in mammals, plants and fungi have established that with few exceptions, chromosomes enter meiosis unpaired and that chromosome movements involving the telomeric, and sometimes centromeric, regions often precede the onset of meiotic pairing. The possible roles of proteins involved in homologous recombination, synapsis and sister chromatid cohesion in homolog pairing are discussed with an emphasis on those for which mutant phenotypes have permitted an assessment of effects on homolog pairing. Finally, I consider the question of the distribution and identity of chromosomal pairing sites, using recent data to evaluate possible relationships between pairing sites and other chromosomal sites, such as centromeres, telomeres, promoters and heterochromatin. I cite evidence that may point to a relationship between matrix attachment sites and homologous pairing sites.
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1. Introduction

Pairing of homologous chromosomes is a fundamental event in meiosis, where it is normally accompanied by high levels of genetic recombination and results in the segregation of homologs into separate cells. However, homolog pairing can also occur in a variety of other contexts. Meiosis in some organisms proceeds without recombination but the homologs nevertheless pair and segregate with great regularity. Moreover, pairing also occurs in vegetative, somatic and germ-line mitotic cells of Dipteran insects. In these cases, the function of pairing is less clear as homologs do not segregate; various investigators have suggested a role in preparing for meiosis, promoting DSB repair, or facilitating interactions between homologous transcriptional regulatory sequences. Sister chromatids also associate with one another, usually quite closely, and these cohesive associations are essential for chromosome segregation in both mitosis and meiosis, as well as for DNA repair.

An important set of unanswered questions concerns whether these various meiotic and non-meiotic pairing processes are mechanistically similar, despite their often diverse morphological appearances. Is there a single underlying mechanism for pairing of homologous loci? Does the presence or absence of recombination in meiosis imply fundamentally different pairing processes, or is recombination a separate process that can be associated with pairing in certain pathways? Are there common mechanisms for linking sister chromatids and chromosomes in various segregation pathways? These and similar questions have been posed numerous times, and although there is a voluminous descriptive literature on pairing and related processes, the underlying mechanisms of homolog pairing have remained elusive. Progress has been hampered until recently by the inability to monitor pairing directly, so that most analyses have of necessity relied on indirect assays of downstream

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events such as homolog segregation, recombination, synaptonemal complex (SC) formation, or transvection. While in principle, abnormalities in these downstream processes could reflect failure of homologs to pair, in practice it has not been possible in most situations to determine whether this is the case. However, the development of molecular assays for pairing based on hybridization of fluorescently tagged DNA probes (FISH) and, more recently, GFP tagging of single loci have begun to circumvent this limitation. Studies based on these approaches, combined with a rapidly growing arsenal of mutations and other genetic and molecular reagents, have yielded a great deal of new information about pairing in both mitotic and meiotic contexts and how it relates to other processes such as recombination, synapsis and gene expression.

In this article I will review recent molecular studies of pairing and attempt to integrate the results with data from classical cytological and genetic approaches. My primary focus will be on homolog pairing in *Drosophila*, taking advantage of the presence in this organism of an unusual wealth of pairing pathways, including both recombinational and non-recombinational meiotic pathways in females and males, respectively, and "somatic" pairing in virtually all cell types. However, I will compare the *Drosophila* results with relevant data from other organisms, in an attempt to achieve as broad an overview as possible. The major purpose will be to explore similarities and differences in the dynamics of pairing and the distribution of pairing ability in different cellular contexts, in the hope of identifying key conserved features of chromosome pairing and gain clues to the underlying mechanism.

2. Homolog pairing in meiosis

The early stages of meiotic prophase in most organisms involve a gradual alignment of homologous axes and their subsequent incorporation into an elaborate meiosis-specific structure known as the synaptonemal complex (SC), which holds the homologs in close register as the events of recombination are being completed [1–3]. With the development of FISH methods for determining the locations of homologous loci, it has become possible to track the progress of pairing in early stages of prophase. In most organisms, homologs are unaligned at leptotene, when chromosomes first begin condensing, but achieve high levels of pairing in a relatively short period of time, coincident with the achievement of full synapsis at pachytene. However, as described below, there are interesting exceptions in which premeiotic pairing is thought to be an important prelude to meiotic pairing [4,5]. The SC is removed at mid-prophase and homologs from that point until anaphase I are connected only at discrete sites known as chiasmata, which are thought to be products of crossing over. There are numerous excellent reviews on synapsis (the process of forming SC), meiotic recombination, and chiasmata, to which the reader is referred for details [2,3,6–10].

Detailed and elegant studies in yeast have elucidated many of the details of meiotic recombination and synapsis and have shown those processes to be closely intertwined both temporally and mechanistically (reviewed in Ref. [7]). The early events of recombination, especially the formation and processing of meiotic double-strand breaks, which are induced by the Spo11 protein, coincide temporally with the initiation of synapsis, and mutations that disrupt those events, such as mutations in Spo11, also prevent synapsis [11–13]. Recent results have indicated that the basic processes of meiosis are conserved but have also pointed to substantial variation among organisms in the relationship between recombination and synapsis. Mammals and plants seem to follow the pattern in yeast [14,15], but in *Drosophila* and *C. elegans* synapsis can occur in the absence of meiotic double-strand breaks, which seems to indicate that homolog pairing is independent of recombination [16,17].

Indeed, it has long been known that homolog pairing and segregation can occur independently of recombination and chiasmata [18]. *Drosophila* males provide the best-studied example of "achiasmatic meiosis", and the only case in which crossing over has been demonstrated to be absent throughout the genome, but it is thought to be universal in Dipteran males and Lepidopteran females and has been described in numerous other taxa. Synaptonemal complex can be present, as in Lepidopteran females where it persists in modified form until anaphase, providing a chiasma substitute, but is absent in *Drosophila* males [1]. Until recently, little has been known about pairing patterns in achiasmatic meiosis, but the recent application of molecular methods has pointed to some unexpected and striking parallels between recombinational and non-recombinational meiosis.

3. Mitotic and meiotic homolog pairing in *Drosophila*

3.1. Mitotic pairing

Mitotic chromosomes in somatic and germ-line cells in *Drosophila* and other Dipterans have been known for almost a century to exhibit high levels of homolog pairing [19]. Homolog pairing is also the rule in polytene chromosomes which are arrested in an interphase-like state. However, it was not known until the development of FISH methodology whether homologs are also paired during interphase in mitotically cycling and other non-polytene cells. Fluorescent hybridization probes for the repetitive euchromatic *histones* locus, which contains 100–150 copies of a 5-kb repeat, were first used to address whether and when mitotic pairing occurs in cleavage-stage nuclei of *Drosophila* embryos, using a relatively non-disruptive whole-mount protocol [20]. Two separate signals, indicative of an unpaired condition, were seen in virtually all nuclei in the first 11

embryonic divisions. These divisions last only 10–17 min each and consist entirely of alternating S and M phases. However, single signals, indicative of pairing, were seen at low levels in cycle 12, which is slightly longer than the previous cycles, and then in the majority of cells in cycle 13, which is the first division in which there are significant gap phases. Subsequent FISH studies involving probes from different chromosomal regions have established that high levels of pairing occur at interphase throughout the chromosomes in all stages of development after the early embryonic cleavage stages, and in germ line as well as somatic cells [21–24].

A GFP tagging assay developed in yeast has recently been applied in *Drosophila*, and the first results provide strong confirmation for the FISH studies. In this method, a multimeric array of bacterial LacO repeats is inserted, via P-element transformation, at various chromosomal sites, which are then tagged by expression of a GFP-LacI chimeric protein [25,26]. In the initial study, 14 different euchromatic lacO insertions exhibited comparable frequencies of pairing, around 50%, in spermatogonia [27].

The cell cycle dynamics of mitotic pairing have not been very thoroughly investigated, but the general picture is that pairing is seen throughout most of the cell cycle. One FISH study reported a partial loss of pairing (from 80% to 60%) at S phase in mitotic larval brain nuclei at two loci. In this case, maximal pairing levels were not restored until G1 of the following cycle [22]. In another report, pairing frequencies at the histones locus were observed to be much lower in early G1 of embryonic cycle 14 than in late G2 of the preceding cell cycle, suggesting that pairing is disrupted during mitosis and then reestablished quickly during G1 of the next cell cycle. Interestingly, no loss of pairing was observed in prophase or metaphase, indicating that the disruption of pairing must occur at anaphase [21]. However, no disruption of pairing was detected in the GFP-tagging study of pairing in *Drosophila* spermatogonia [27]. Classical studies have documented the occurrence of anaphase pairing in somatic nuclei (e.g., Ref. [28]), but no reliable estimates of frequency have been available. Clearly, additional studies of mitotic pairing in the context of the cell cycle need to be carried out.

Another important unresolved question is whether pairing within a cell cycle (prior to anaphase) is reversible. In the Fung et al. [21] study, pairing frequencies were tracked from 2 h after egg deposition to 5 days, and found to increase throughout this period to frequencies in the 90–100% range. This was interpreted to indicate that pairing is irreversible. In support of this interpretation, it was found that the mean distance between unpaired loci increased over time, rather than decreasing as would be expected if many unpaired loci at later time points had recently been paired. However, other studies have detected maximum pairing frequencies below 100% at multiple loci, even in mitotically arrested cells, which would appear to more consistent with a scenario in which pairing and unpairing are in dynamic equilibrium [22–24,27]. However, a plausible alternative might be that pairing can occur only at the beginning of the cell cycle, and is permanent until anaphase. Resolution of this question will require tracking of single loci over time in living cells, which can now be done using the GFP-tagging system.

Somatic/mitotic pairing in *Drosophila* has been considered of significance because of the evidence for a variety of genetic phenomena that are thought to depend upon homolog pairing [29–31]. One of these is ‘‘transvection’’ which refers to situations in which mutant alleles of a gene complement one another, but this complementation is disrupted by heterozygosity for chromosome rearrangements with breakpoints on the same chromosome arm as the locus. Transvecting alleles are typically mutations in sequences that would normally be expected to be cis-acting, such as enhancers and promoters, suggesting that these sequences can interact in trans in certain circumstances, but only when the loci are well-paired [32]. An apparently related phenomenon is pairing-dependent silencing of transgenic reporter genes. In the most common examples, the reporter is silenced only when the transgene is homozygous. Two nonhomologous insertions can usually interact only weakly if at all, and in some cases, heterozygosity for a rearrangement on the same chromosome arm has been shown to disrupt the silencing interaction [29,31]. However, these phenomena are seen only in special mutant or rearranged genotypes and thus do not provide much insight into how pairing benefits wild-type organisms.

3.2. Premeiotic and meiotic pairing in Drosophila

It has been proposed by several investigators that mitotic pairing in the germ line of *Drosophila* and other Dipterans is a prelude to and important for the establishment of homolog pairing in meiosis (e.g., Ref. [33]). Both premeiotic oogonial and spermatogonial nuclei of *Drosophila* were shown by classical staining methods to exhibit homolog pairing in mitotic prophase, metaphase and anaphase, leading to the idea that the chromosomes enter meiosis fully aligned [33,34]. Mosquitoes also exhibit strong homolog pairing at mitotic metaphase and anaphase in spermatogonia [35,36], and this pairing has been reported to continue uninterrupted from premeiotic cells through pachytene [37]. However, until the development of molecular tagging methods, it was impossible to determine the arrangement of homologous loci in the interphase period immediately preceding meiotic prophase, or to track the changes that occur in chromosome organization and pairing during prophase.

Compelling evidence for premeiotic pairing in *Drosophila* was recently obtained from observations of the pairing behavior of GFP-tagged loci in cysts of spermatogonia and young spermatocytes [27]. Meiosis in *Drosophila* males takes place in cysts of 16 spermatocytes that are interconnected by cytoplasmic ring canals and that arise

from a single primary spermatogonium by a succession of four rapid mitotic divisions. Each primary spermatogonium in turn arises from an asymmetric division of a stem cell attached to the inner wall of the testis. Primary spermatocytes undergo S phase almost immediately after the completion of the last gonial mitotic division, then enter a prolonged G2 phase that lasts more than 4 days during which time they increase in volume more than 25-fold. At the end of the growth period, the chromosomes condense rapidly and enter the meiotic divisions, which last approximately 4 h [38]. Chromosomes in growing spermatocytes prior to the late condensation stage cannot be visualized by staining with non-fluorescent dyes because they are too decondensed. However, chromosomes in growing spermatocytes can be visualized by staining with fluorescent dyes such as DAPI. In the youngest spermatocytes, the chromatin appears as a brightly staining but indistinct mass that fills the interior of the nucleus. As the nucleus grows, the chromatin remains loosely associated with the expanding membrane, and a distinct nuclear lumen free of staining develops. At about the midpoint of G2, the chromatin separates into three distinct territories, each of which remains associated with the nuclear membrane as the nucleus continues to grow. After another 2 days or so of growth, the chromatin condenses into four compact masses at the onset of the division phases that are easily visualized by classical microscopy. Autosomes appear in both EM and light microscope analyses to be aligned in parallel, with the most stable connections being medial or distal and the centromeres clearly separated, and sex chromosomes appear to be connected at one or more discrete heterochromatic sites known as collochores [38–41]. The fact that there are three major territories from mid-G2 onward rather than six suggests that the homologs are paired in some way throughout G2, but the poor morphology of the chromosomes has precluded any detailed description of pairing, and it has not been possible to rule out the alternative that the two homologs are colocalized to a common territory by a mechanism independent of pairing, e.g., by binding to nearby sites on the nuclear membrane.

In the Vazquez et al. [27] study, 14 different insertions of LacO repeats at a variety of euchromatic loci on chromosomes 2 and 3 exhibited similar (about 50%) levels of pairing in spermatogonia. No loss of pairing in young spermatocytes was observed, suggesting that mitotic and meiotic pairing are continuous. However, the earliest distinguishable spermatocyte cysts have much higher pairing frequencies, >95%, indicative of a dramatic transition in pairing frequency. Unfortunately, it is difficult to distinguish the earliest spermatocyte cysts from 16-cell G1 spermatogonial cysts in living preparations. Consequently, it is not clear if this transition occurs before or after premeiotic S phase, although the authors of the study considered the former possibility more likely. The very high pairing frequencies of GFP-tagged loci that were observed in young spermatocytes persisted throughout the early G2 growth phase. However, a second dramatic change occurred at mid-G2: pairing of both homologous and sister loci at all 14 LacO insertions disappeared completely, with four spots becoming visible in most nuclei. This mid-G2 transition occurred shortly after the separation of the chromatin masses into distinct territories. Although a brief transition period was seen when nuclei in the same cyst can show one, two, three or four spots, the distribution of numbers and brightnesses indicate that there is no particular order to the loss of pairing and that homolog pairing and sister chromatid cohesion are lost essentially simultaneously. This unpaired state persisted through the end of G2. During this period, homologous and sister loci were confined to the chromosome territories but were on average no closer to one another than to nonhomologous loci on the same chromosome. At diakinesis/prometaphase, sister and homologous spots moved closer to one another as the chromosomes condensed, indicating that both the sister chromatids and homologs had remained connected somehow, but the spots never became fused again.

Three important conclusions emerge from this study. First, as has been proposed for years, *Drosophila* chromosomes apparently enter meiosis already paired; no interruption between premeiotic and meiotic pairing was observed. Second, nevertheless, meiotic pairing differs dramatically from mitotic pairing in frequency; nearly 100% pairing was observed at all 14 tagged loci in young spermatocytes, approximately double the frequency in spermatogonia. This is interesting because it shows that the very high levels of pairing associated with mid-prophase in meiosis do not depend upon synapsis or recombination. It also suggests that the pairing process in *Drosophila* males may be more similar to that in recombinational meiosis than had been suspected previously.

Finally, pairing is lost suddenly and completely at a point in mid-G2 after the homologs have separated into territories. Thus, intimate molecular pairing at most loci in *Drosophila* male meiosis is confined to, approximately, the first half of meiotic G2, despite the fact that chromosomes in late prophase and prometaphase appear in all classical light and electron microscope preparations to be very tightly linked. This result is surprising at first glance, but less so on reconsideration, as there is a similar transition in mid-prophase in recombinational meiosis. As described above, the transition from pachytene to diplotene involves removal of the SC and lateral elements, often during a "diffuse stage" in which axes lose resolution and chromatin becomes highly decondensed. When the chromosomes recondense at diplotene, homologous axes are clearly separate except for the chiasmata, which may be as few as one per chromosome; indeed, in several organisms, homologous axes appear to occupy positions as far from one another as possible, and are often said to be in "repulsion" (reviewed in Ref. [7]. FISH analyses show clearly that homologous spots are separate at this stage, although of course they are constrained by the chiasmata to remain within the confines of

the bivalent (e.g., Ref. [42]). As in the *Drosophila* case, homologous loci never become closely associated again. An important difference, however, is that sister loci remain paired in recombinational meiosis for the remainder of prophase I (two spots are seen, not four) and the sister axes remain closely associated. This persistent cohesion in the arms is now thought to be critical for maintenance of the chiasmata [43]. Presumably since male *Drosophila* lack chiasmata, they have no need for close sister chromatid cohesion after the end of the pairing phase of meiosis. Nevertheless, the parallel behavior of the homologs in the two types of meiosis is quite striking, and again suggests the possibility that the underlying homolog pairing pathways in recombinational and non-recombinational meiosis may be quite similar, despite the more obvious differences in associated structures.

4. Somatic and premeiotic pairing in other organisms

There has been considerable controversy about whether somatic and/or premeiotic pairing occurs in other organisms besides Dipterans. Although there have been reports of pairing of certain chromosomal regions in mammalian cells [44,45] most studies have been negative. There is strong evidence that chromosomes occupy discrete territories in interphase human cells, but homologous territories are typically separated from one another [46,47]. Moreover, there is clear evidence that homologs are unpaired in spermatogonia of humans and rodents and do not exhibit significant levels of association until late leptotene/zygotene of meiosis [48–52]. Pairing is preceded by a reorganization of chromosomes that features clustering of centromeres and of telomeres on the nuclear membrane, the so-called bouquet configuration [50–52].

The situation appears to be similar in *C. elegans* where FISH analysis has shown that homologs are unpaired in the premeiotic germ cells [16]. Significant levels of pairing are first seen in the transition stage nuclei which correspond to the leptotene and zygotene stages of meiosis, and are preceded by a reorganization of the nucleus in which the chromosomes cluster at one end. This arrangement differs from the bouquet arrangement in most organisms in that only one end of each chromosome is in contact with the nuclear membrane. Pairing frequencies average around 50–60% at most loci in the transition stage nuclei, then increase to nearly 100% in pachytene nuclei, which exhibit full synapsis [16,53,54].

There is evidence for somatic and premeiotic pairing in cereal species but it has been disputed (see Refs. [4,55] for reviews). In wheat, homologous chromosomes have been reported to be closer to each other than to homologous or heterologous chromosomes both in root tip metaphases and in early meiotic prophase [56–58]. More recently, FISH and GISH (genomic in situ hybridization) analyses have provided additional evidence for homologous pairing in premeiotic interphase (prior to leptotene), especially of centromeric regions [59–61]. This is followed by chromatin condensation and pairing at other loci, with telomeric pairing usually occurring prior to pairing at interstitial sites. However, several studies have failed to detect any homologous associations in premeiotic mitoses in cereal plants [62–64], suggesting that the preleptotene centromeric pairing may represent the initial onset of meiotic pairing, rather than a result of mitotic homolog alignment.

The situation appears to be somewhat different in *Arabidopsis*. Recent chromosome painting studies have documented preferential association of homologous chromosomes, especially in the proximal regions, in immature parenchymal (mitotic) cells [65–67]. In these cells, chromosomes occupy distinct territories centered about condensed chromocenters consisting mostly of pericentric heterochromatic, with the euchromatin forming one or several loops emanating from the chromocenters. Nonhomologous chromocenters were not significantly clustered, but homologous chromocenters were associated at frequencies ranging from 10% to almost 80%. Painting of the euchromatic region of one chromosome arm also detected close homolog pairing over a length of 500 kb to 2 Mb, but only at a frequency of 5–6%. Interestingly, euchromatic pairing was always accompanied by pairing of chromocenters, suggesting that pairing may initiate in centric regions and sometimes spread to the euchromatin. GFP-labelling of single loci has recently been achieved in *Arabidopsis*, and an initial report indicates significant levels of homologous pairing in guard cells, which are diploid [68]. However, the centric associations seen in somatic cells do not seem to contribute to meiotic pairing. At leptotene, centromeres are dispersed and telomeres take the lead in first clustering on the surface of the nucleolus, then pairing. Telomeric clustering on the nucleolus was suggested to be a functional substitute for the bouquet configuration in other organisms in which telomeres cluster on the nuclear membrane [69]. Thus, although there does seem to be at least some mitotic pairing in *Arabidopsis*, especially in centric regions, there is no evidence at this point that it contributes to meiotic pairing.

Maize meiosis appears to follow a similar pattern to that in yeast and mammals with initiation of pairing during meiotic prophase following telomere clustering on the nuclear membrane [70]. Maguire [71] claimed that homologs are associated in premeiotic mitosis in maize, but no such associations were detected by Palmer [72]. Recent FISH studies have also provided evidence against premeiotic homolog pairing in maize [70,73].

Somatic and premeiotic pairing have been reported in budding yeast, but the evidence is contradictory. Painting of chromosome V in spread meiotic nuclei demonstrated that many if not most homologs are unpaired in early prophase, prior to zygotene, and that close pairing at pachytene is preceded by parallel alignment at a distance [74]. However,

several other FISH studies have reported quite high levels of pairing of a substantial number of loci, at levels from 25–50%, in premeiotic interphase, i.e., at time 0 after transfer to sporulation medium [75–78], which was substantially disrupted by passage through premeiotic S phase [75,78]. Comparable levels of pairing were also reported in vegetative cells [77]. However, other FISH studies have failed to detect significant pairing, at least of centromere-distal probes, in vegetative cells [5,79]. Centromere-proximal sequences were found to be paired or nonrandomly close to each other at a significant frequency [79], but this was attributed to general centromere clustering in yeast nuclei throughout the cell cycle, an interpretation that has been confirmed by later studies [80,81]. Moreover, GFP tagging failed to detect any significant chromosome pairing in premeiotic cells [82]. Indirect assays for mitotic pairing in yeast have also been equivocal; site-specific recombination frequencies are somewhat higher between allelic pairs of insertions of Cre-Lox target sites than between nonhomologous insertions in mitotic cells, but the difference is relatively slight when the effects of the prominent clustering of yeast centromeres and the positions of the insertions relative to their centromeres are taken into account [83,84]. Homologous mitotic recombination frequencies are also very similar when the recombining loci are on homologous versus nonhomologous chromosomes as long as position relative to centromere is controlled [85,86]. A recently developed physical assay for proximity of DNA sequences shows some advantage for homologous loci, but again, the effect is not terribly dramatic [87]. These data contrast sharply with the data on site-specific recombination in *Drosophila* mitotic nuclei where FLP induced recombination frequencies can exceed 20% between allelic targets in the germ line [88], but are less than 1% between targets on nonhomologous chromosomes [89]. One interpretation of these data is that pairing in yeast is an artifact of the small size of the nuclei, the strongly polarized organization of its chromosomes and structural constraints that might act to sort chromosomes by size [81]. However, the issue of somatic/premeiotic pairing in yeast remains unresolved.

The strongest evidence for premeiotic pairing outside the Diptera is from the fungus, *S. pombe*, in which meiosis is normally azygotic, occurring in transient diploids in which the maternal and paternal chromosome sets do not encounter each other until after premeiotic S phase. However, diploids can be induced to undergo mitotic proliferation, and then to undergo an azygotic meiosis by manipulation of the media. FISH analysis in such diploids showed that the homologs occupy joint territories in more than 95% of mitotic nuclei, an arrangement that was proposed to occur also in azygotic meiosis as a preliminary to meiotic pairing [90]. It was also shown that both homologous and nonhomologous centromeres but not telomeres are typically clustered, and that sequences throughout the chromosomes are paired at frequencies ranging from less than 20% for interstitial probes to 60% for centromeric probes in vegetative nuclei. Induction of meiosis led to clustering of telomeres (bouquet formation) and to increases in pairing frequency for all but the centromeric probes. Interestingly, pairing at most sites never exceeded 50%, presumably reflecting the lack of synapsis in this organism. Pairing increased more rapidly and reached higher levels at telomeric than at interstitial sites, presumably reflecting the importance of telomeric clustering in early prophase, but the differences were fairly modest. Pairing occurred independently at all linked sites except the telomeres in both vegetative and meiotic cells, thus indicating that homology throughout the chromosome is used. Similar pairing frequencies were measured in an independent FISH study and in two applications of the GFP tagging method [91,92]. Interestingly, this latter method demonstrated that the intermediate pairing frequencies in *S. pombe* at meiotic prophase reflect dynamically unstable interactions, with GFP signals coming together and separating throughout meiotic prophase [91].

Overall, then, most eukaryotes do not exhibit high levels of homolog pairing in somatic and premeiotic cells, and apparently pair their chromosomes de novo after premeiotic S phase, but *S. pombe* and Dipterans generally provide exceptions in which chromosomes are paired premeiotically.

5. To what extent does pairing in recombinational meiosis depend upon the synapsis and/or recombination pathways?

It has been suggested that chromosome pairing is a consequence of the homology search process that underlies recombination, but the genetic data thus far are largely unsupportive of this idea. Mutations that disrupt processing of double-strand breaks, such as *rad50S*, or meiotic strand transfer, such as null mutations in *RAD51* and *DMC1*, have only mild effects on homolog pairing frequency despite severe effects on both recombination and synapsis [75,76,93,94], although Peoples et al. [84] report a stronger impact of *dmc1* mutations on meiotic site-specific recombination. Mutations in genes required for double-strand break formation such as *SPO11*, *RAD50*, *MEI4* and *REC102* tend to have stronger effects on prophase pairing frequencies, but typically do not eliminate pairing. The strongest effects have been seen in null mutations in *SPO11* which reduce pairing frequencies to 10–20% of wild-type levels [75,76]. However, this effect is not due to the role of Spo11 in induction of meiotic double-strand breaks; a missense mutation in the active site of Spo11 causes no reduction in pairing at prophase [95]. These results argue strongly that meiotic pairing is independent of the formation and repair of DSBs, although a significant deficit in pairing maintenance is manifested in several of the mutants.

Similar results have been reported in other organisms. Null mutations in homologs of *SPO11* completely block meiotic recombination but have no effect on synapsis in either *C.*

elegans or *Drosophila* [16,17]. The *spo11* mutant phenotype manifests only after pachytene, after removal of the SC. Homolog pairing occurs at normal frequencies in Spo11-deficient worms in both transition zone and pachytene nuclei [16]. Loss of Spo11 function in *S. pombe* reduces pairing frequencies in prophase but only slightly [91].

It is also clear that pairing precedes and is under separate genetic controls from synapsis. Homologs remain fully aligned but unsynapsed, and homolog pairing occurs at wild type frequencies in a null mutation in the *ZIP1* gene, which encodes a component of the transverse filaments of yeast SCs [93]; meiotic site-specific recombination frequencies are also unaffected by this mutation [84]. Mutations in other yeast genes required for synapsis and/or axial element formation, such as *HOP1* and *RED1*, also do not eliminate pairing, although they may reduce it [74,75,84,93]. Mutations in the *C. elegans syp1* gene, which encodes a coiled-coil protein that localizes to the central elements of the SCs, completely block synapsis but do not prevent pairing. Pairing initiates with normal timing and at normal levels in the transition stage nuclei, but subsequently decays, and is present only at low levels in nuclei that should be in pachytene [54]. However, the conserved Hop2/Mnd1 complex, which localizes to chromosomes in early meiosis, seems to play an important role in homolog pairing in both budding and fission yeast [91,96,97]. Mutations in the yeast homologs strongly reduce homolog pairing frequencies and meiotic recombination without affecting formation or processing of double-strand breaks and lead to extensive nonhomologous synapsis. Disruption of the fission yeast Hop2 homolog, Meu13 causes a significant reduction in pairing and recombination without effecting telomere clustering or movements [91]. It will be very interesting to learn what the functions of these proteins are in meiotic homolog pairing.

6. Do cohesins play a role in homolog pairing?

Cohesin is a four-member protein complex required for sister chromatid cohesion both in mitosis and meiosis [43,98]. It consists of two long coiled-coil proteins, SMC1 and SMC3, and two regulatory subunits, SCC1/RAD21 and SCC3. Cohesin is loaded onto chromosomes prior to or during S phase all along chromosomes and plays a crucial role in the vicinity of centromeres in mitosis and meiosis II, where it is needed to prevent premature separation of sister kinetochores. The removal of cohesin by proteolysis of its SCC1/RAD21 subunit at anaphase is thought to trigger chromatid separation.

During meiosis, cohesin plays other roles as well, and cohesin mutants therefore have multiple meiotic phenotypes. This has been most clearly established for the meiosis-specific REC8 subunit, a paralog of SCC1, found in most or all eukaryotes [43]. Yeast Rec8 protein largely replaces Scc1 and is present both on the chromosome arms throughout the first division, where it is essential to maintain chiasmata, and at the centromeres, where it is essential to prevent premature separation of sister centromeres [43,99]. Interestingly, *rec8* mutations also block axial element formation and synapsis, and severely reduce recombination, but do not affect double-strand break formation, suggesting that cohesin plays a fundamental role in establishing meiotic chromosome structure. Parallel results were obtained for *smc3* mutations [99]. *rec8* mutants also interfere with axial element formation and strongly reduce recombination in *S. pombe* [92,100,101] as do mutations in *rec11* which encodes a meiosis-specific homolog of Scc3 [102,103]. In other organisms, recombination has not been assayed directly, but depletion of *C. elegans REC8* by RNAi prevented synapsis and crossing over but not the initiation of recombination, based on the accumulation of small chromosome fragments at diakinesis [104].

The role of cohesins in meiotic chromosome pairing is not yet clear. In *rec8*$^-$ yeast, both sister chromatids and homologs are unpaired in approximately 70% of cells in prophase [99], but it is not clear if this represents a defect in initiation or maintenance of pairing. In *S. pombe*, mutations in *rec8* reduce homolog pairing about 2-fold during early to mid-prophase, but the reduction is limited to the interstitial regions of the chromosomes, consistent with the localized defect in recombination [100]. As with budding yeast, this effect is difficult to separate from the defects in cohesion and axial element formation [92]. In *Arabidopsis*, loss of Rec8 function has been reported to completely suppress sister chromatid cohesion, homolog pairing and synapsis [105,106], but another group has reported a milder phenotype in which synapsis appears normal, but homologs exhibit abnormal condensation in leptotene and extensive fragmentation and mis-segregation at anaphase I [107,108].

In *C. elegans*, the situation is clearer. The initiation of homolog pairing in transition zone nuclei was unaffected by depletion of REC8 by RNAi despite a strong defect in synapsis, but pairing was not maintained in pachytene [42,104]; similar results have recently been reported for a mutation in the single *C. elegans* SCC3 homolog [109]. An especially interesting observation is that in *C. elegans*, SMC1 and SMC3 localize to meiotic prophase chromosomes even in the absence of REC8, and depletion of SMC1 in a genetic background in which REC8 fails to localize to chromosomes caused a substantial reduction in homolog pairing in the transition zone [42], suggesting that SMC1 and perhaps SMC3 may have a function in homolog pairing independent of that of REC8. Recent reports indicate that REC8 and the SMC subunits are loaded and removed from chromosomes independently of one another in mammalian meiosis as well [110–112]. Another indication for a role of cohesins in chromosome pairing is that mutations in the *Coprinus* Rad9 protein, which is required for chromosomal loading of cohesin, causes an approximately twofold reduction in homolog pairing [113].

Taken together, these data indicate that cohesins may play crucial roles in meiotic chromosome pairing. However, a critical and as yet unanswered question is whether this role in homolog pairing is direct or is derivative of effects on sister chromatid cohesion. In all cases where homolog pairing has been disrupted in early meiosis by absence of a cohesin, that disruption has been accompanied by loss of sister chromatid cohesion as well. These data could indicate either that sister chromatid cohesion and homolog pairing share a common mechanism involving cohesins, or that sister chromatid cohesion is a necessary prerequisite for homolog pairing, perhaps due to formation of axial elements. If the latter explanation proves to be the case, it will point to divergent mitotic and meiotic pairing mechanisms, since mitotic pairing has been shown to occur at G1, when sister chromatids are absent. An argument against the latter explanation is that meiotic pairing and synapsis takes place at normal frequencies in Coprinus in *msh5-22* mutants which fail to undergo premeiotic DNA replication and therefore lack sister chromatids [114,115]. Interestingly, the *rad9* mutant still shows a partial defect in homolog pairing in a *msh5-22* background, indicating that the role of Rad9, and presumably cohesin, in homolog pairing is not entirely derivative of its role in sister chromatid cohesion [113].

7. Distribution of pairing sites

7.1. Pairing via general homology

In general, despite the important roles of telomere sequences and of a few specialized pairing sites in meiosis discussed below, homolog pairing in most organisms appears to utilize sequence homology throughout the chromosomes. Evidence for this includes the fact that ring chromosomes can pair and recombine despite lacking termini; that synaptic initiation can occur interstitially in most organisms, although subtelomeric sites are often the earliest synaptic sites; that homologous synapsis requiring interstitial initiation can and often does occur in individuals heterozygous for chromosome rearrangements such as inversions and translocations; and that homologous synaptic switches occur at interstitial sites, often at high frequency, in triploids and higher ploids of many organisms [1,4,116–118].

Similar observations have been made in *Drosophila* male meiosis. In males heterozygous for transpositions of euchromatic segments of chromosome 2 into the Y chromosome, the Y chromosomes were found to pair with a normal 2 at frequencies proportional to the size of the transposition. Pairing was monitored in late prophase or prometaphase by the presence of quadrivalents involving the X-Y and 2-2 pairs, which were seen at frequencies above background for all transpositions involving euchromatin, including one that involved only a few salivary bands (probably less than 100 kb). These quadrivalents were effective in orienting the connected chromosomes to opposite poles, as exhibited in excess segregation of the Y chromosome from the normal chromosome 2 [119]. It is not known whether the intermediate frequencies of quadrivalents seen with most transpositions directly reflect the pairing frequencies in early prophase. A plausible alternative could be that all loci become fully paired in prophase, but that the probability of forming (or maintaining) a stable connection between the transposed segment and the normal chromosome 2 is dependent on the length of the transposition. It would clearly be informative to evaluate pairing directly using the GFP-tagging protocol in these transposition heterozygotes.

This democratic approach is also characteristic of mitotic pairing in *Drosophila*. In embryonic and somatic cells, every locus or chromosomal region that has been tested by FISH has shown substantial frequencies (50% or higher) of pairing [20–24]. The FISH probes have included a variety of cosmid and BAC clones containing unique sequences as well as both complex and simple sequence repeats derived from euchromatic and heterochromatic regions. Moreover, 14 different LacO insertions in the autosomal euchromatin showed comparable levels of pairing (approximately 50%) in spermatogonia, and these same loci also paired at comparable levels in young primary spermatocytes [27].

As in political democracies, some individuals (or loci) are more equal than others. In the Fung et al. [21] study, pairing was observed at all 11 tested sites, but at different frequencies. The histones locus, a repetitive locus in the proximal euchromatin of 2L [120], was found to be the strongest mitotic pairing site, achieving high levels of pairing (61%) as early as embryonic cycle 13 when most other loci are still paired at less than 10% [21]. Intriguingly, the histones locus also appears to be especially potent in meiotic pairing, based on the fact that 2-Y transpositions that carry this region pair with a normal chromosome 2 at frequencies disproportionate to their size [119]. Unique-sequence euchromatic probes from proximal, medial and distal regions of the chromosome 2 arms were found to pair at more moderate rates and frequencies in both mitosis and meiosis [21,119]. Simple sequence satellite repeats also exhibited more moderate pairing frequencies.

Mitotic as well as meiotic pairing in *Drosophila* initiate at multiple sites rather than spreading from a single major site. One line of evidence for multiple initiations is the lack of significant inter-locus correlations when probes from the same chromosome are hybridized simultaneously [21,23]. Especially compelling are examples in which unpaired loci are flanked by paired loci. A second line of evidence for multiple independent initiation sites is that pairing of euchromatic regions in *Drosophila* mitotic cells is unaffected or only partially disrupted by heterozygosity for translocations or pericentric inversions with breakpoints on the same chromosome arm as the monitored locus. Pairing in the vicinity of the *brown* locus, which is near the tip of chromosome arm 2R,

seems to be almost completely independent of chromosome location, as relatively small transpositions of distal 2R sequences into the proximal heterochromatin pair with the native locus at the same frequency (70–80%) as do unrearranged copies in larval brain cells [24]. Pairing at the *BX-C* complex, which is located medially in chromosome arm 3R, is partially disrupted in embryonic cells by heterozygosity for two different translocations that move the *BX-C* complex either to the opposite arm of chromosome 3 or to the X chromosome. However, pairing still occurs at frequencies of 25–30% despite the fact that both translocations completely abrogate transvection at the *Ubx* locus [23,121,122]. Similar conclusions were reached using an indirect assay for pairing in which frequencies of FLP-induced recombination between homozygous target sites on chromosome 3 were measured in the male germ line. These events occur at high frequency (15–20%) between allelic insertions on unrearranged chromosomes, but at much lower frequencies between non-allelic insertions, indicating a dependence on chromosome pairing. Several inversions and translocations with breakpoints proximal to the target sites were tested and most were found to reduce recombination frequencies, but never by more than half, and some had only minor effects on recombination frequency. The severity of the effects correlated with the size of rearrangement and the proximity of the breakpoint to the FLP insertion [88]. These data indicate that euchromatic loci are capable of finding their homologs even when linked to different centromeres and/or at different distances from the centromere, but that these types of rearrangements do interfere with pairing to some degree.

7.2. Roles of telomeres and centromeres in pairing

There is little evidence for a special role of telomeres in mitotic pairing, but numerous recent observations indicate a prominent role for telomeres in the early stages of meiotic pairing. A common scenario is for telomeres to form a cluster on the nuclear membrane near the centrosome/spindle pole body in early prophase shortly before or commensurate with the onset of homologous pairing. The resulting bouquet configuration and associated chromosome movements have been postulated to promote the homology search process in interstitial as well as distal regions [123]. An interesting variation on this pattern is seen in *Arabidopsis* in which telomeres associate with the nucleolus prior to meiotic prophase, then pair homologously at leptotene, leading to initiation of synapsis in terminal or subterminal regions [69]. However, as noted above, there is a large body of evidence that pairing can initiate at interstitial sites independent of telomeric pairing, so the role of telomeres is likely to be more kinetic than essential. This is consistent with the observation that mutations in yeast that disrupt telomere clustering lead to delays in pairing and reductions in recombination but do not prevent either [124,125].

Roles for centromeres in pairing have been extensively discussed and investigated. As described above, in some organisms centromere clustering is a prominent feature of chromosome organization in mitotic cells, but it is often difficult to ascertain the role of homology in centromeric clusters. A common situation is for clusters to include variable numbers of homologous as well as heterologous centromeres [5,81]. However, in mitotic *Arabidopsis* cells, centromeric associations appear to be almost exclusively homologous; moreover, homologous centromeres were found to be paired whenever euchromatic regions of the same chromosome were paired, suggesting that pairing might spread from centric heterochromatin into the euchromatin, although other explanations have not been ruled out [66]. In *S. pombe*, both mitotic and premeiotic pairing frequencies are highest in centromere regions [90,92]. In *Drosophila* mitotic pairing, however, there is no obvious bias for or against heterochromatic sequences and no evidence for spreading, as discussed above. Moreover, pairing in the X heterochromatin was found to be almost completely disrupted by heterozygosity for a large inversion that moves the X heterochromatin to a distal location [126], suggesting that heterochromatic loci may not have the same independent ability to find their homologs that euchromatic loci evidently do.

Overall, there is relatively little evidence for a prominent role of centromeric heterochromatin in meiotic pairing, despite numerous suggestions for such a role; indeed, the bulk of the data suggests that centric heterochromatin is partially or completely excluded from an active role in pairing in most organisms, as well as being recombinationally inert. There is some evidence in wheat for the initiation of chromosome pairing in premeiotic interphase, with pairing of centric regions preceding that of more distal regions [59–61] but this seems to be rather untypical. In *Arabidopsis*, although some pairing of centric regions has been documented in mitotic cells, centromeres are clearly unpaired in early meiotic prophase [69,127] and pair later than distal regions. In yeast, centromere clustering is common in vegetative cells, but is rapidly lost upon induction of meiosis [78,81]. In humans and rodents, centromere movements to the nuclear membrane precede telomere movements in early prophase, but no evidence for homolog pairing is detected prior to telomere clustering and bouquet formation [50], and centric sequences were found to be the last to pair. Even in *S. pombe* in which centric heterochromatic regions appear to enter meiosis paired at high frequencies, early prophase is dominated by telomere-led chromosome movements [128], and both telomeric and interstitial regions pair independently of the centromeres [90,129]. These observations are consistent with numerous classical observations that SCs in heterochromatin are shorter than in euchromatin (or even absent in some cases), that heterochromatic SC is often structurally abnormal, and that synaptic initiation sites are absent from heterochromatin [1,4,130].

There is considerable evidence that centric heterochromatin is largely excluded from pairing in *Drosophila* male meiosis. Centromeres tagged with GFP-Cid (the *Drosophila*

homolog of CENP-A, a histone H3 variant specific for centromeres [131]) in young primary spermatocytes, the stage at which euchromatic loci exhibit nearly 100% pairing, were observed to form variable numbers of clusters but to be unpaired in a substantial fraction of cells. This is consistent with several classical studies analyzing pairing at prometaphase or metaphase and/or segregation patterns in heterozygotes for various types of chromosome rearrangements, all of which have failed to detect pairing ability in the centric heterochromatin of the autosomes (see Ref. [132]). Of particular relevance to the comparison with mitotic pairing is the finding that a large transposition of 2R heterochromatin into the Y chromosome proved unable to induce formation of quadrivalents despite being much larger than several euchromatic transpositions that did induce quadrivalents and despite carrying the Responder locus which was found to be a strong mitotic pairing site [21,119]. X-Y pairing clearly does take place in the heterochromatin in *Drosophila* [40,132], but as described in more detail below, the pairing site is a specific repeat, and most of the centromeric X heterochromatin is clearly devoid of pairing ability [132].

Distributive pairing in *Drosophila* females provides a major exception to the exclusion of centric heterochromatin from pairing. Distributive pairing is a back-up system that ensures the segregation of non-exchange homologs, especially the fourth chromosome pair which almost never undergoes exchange. Genetic evidence indicates that distributive pairing is based on heterochromatic homology [133,134]. Moreover, heterochromatic pairing has been directly documented by FISH in late prophase (after SC removal) in *Drosophila* females [135]. However, there is no evidence either for robust homologous distributive pairing systems or for post-synaptic pairing in the centric heterochromatin in other organisms; indeed, centric regions of homologs are usually widely separated in late prophase [7]. Although heterochromatic pairing clearly can exhibit at least some specificity, the limited ability of the distributive system to sort chromosomes homologously is shown by the chaos that ensues when exchange is drastically reduced in *Drosophila* females and most or all chromosomes are thrown into the distributive pool. In general, heterochromatic pairing is probably counterproductive in meiosis because of the high density of repeated sequences shared with other chromosomes, and the consequent danger of rearrangements and/or nonhomologous pairing and segregation.

7.3. Specialized pairing sites

Other than the rather limited evidence for centromere dominated pairing in *Arabidopsis* and *S. pombe* somatic cells, there is little evidence that mitotic pairing, as opposed to nonspecific heterochromatic stickiness, involves specialized pairing sites. Indeed as discussed above, there is substantial evidence that pairing initiates at multiple interstitial sites in *Drosophila* mitotic cells.

However, there is some genetic evidence for specialized pairing sites in meiosis. In both *Drosophila* and *C. elegans*, specific chromosomal sites are required in cis for normal levels of meiotic recombination. In *Drosophila* the X chromosome appears to be subdivided into at least three recombinationally independent intervals by four sites, based on the fact that heterozygosity for X-4 translocations suppresses recombination but only within the interrupted interval [136]. Reciprocal translocations in *C. elegans* typically suppress recombination to one side of each breakpoint, and the breakpoints of these translocations as well as of other rearrangements have been used to map a single Homologously Recombining Region (HRR) to one end of each chromosome (reviewed in Ref. [137]. These ends have been shown to contact the nuclear membrane at the onset of meiotic prophase in the transition zone nuclei [16,53,54]. Although both the X chromosomal sites in *Drosophila* and the HRRs in *C. elegans* have been suggested to be pairing sites, there is no direct evidence for such a role. In fact, deletions and mutations of HRRs have only minor effects on recombination when heterozygous, contrary to what would be expected if they functioned as pairing sites. An alternative might be that they function as loading sites for synaptic complexes or other proteins involved in pairing, synapsis or recombination [138]. Neither of these interesting types of sites has been molecularly identified as yet, although the HRRs are known to map in relatively gene-poor regions of the chromosomes [137].

The most thoroughly characterized specific meiotic pairing site is that of the X-Y pair in male *Drosophila*, which has been mapped to a 240-bp repeated sequence in the intergenic spacers of the rDNA genes. These genes are localized in two blocks in the heterochromatin of the X and the short arm of the Y chromosome. Each sex chromosome contains some 1000–2000 copies of the 240-bp IGS repeat, but transgenic studies showed that inserted arrays containing as few as six or seven repeats are capable of partially restoring pairing ability to an X chromosome deficient for the native pairing region [132]. This is clearly a special case in that the X and Y chromosomes in *Drosophila*, as in many other higher organisms, are highly diverged, and therefore unable to utilize the widespread homology available to other chromosomes. However, it is noteworthy that the pairing site nevertheless corresponds to a region of homology, as does the pseudoautosomal region of the mammalian X and Y chromosomes.

8. Where does pairing occur?

As we have seen, pairing ability is democratically distributed in the euchromatin in both mitosis and meiosis, and is present in the heterochromatin as well, although heterochromatic pairing is usually restricted in meiosis. Although specialized chromosomal sites have been shown to contribute to meiotic pairing in some organisms, the data in most

cases are rather strongly against the idea that pairing initiates at one or a few sites and spreads to the remainder of the chromosome. These data indicate that if pairing is restricted to certain types of sequences, those sequences must be widely distributed, more on the order of one or a few per gene than one or a few per chromosome.

The only meiotic pairing site that has been molecularly identified is the X-Y pairing site in *Drosophila* male meiosis (reviewed in Ref. [132]), which as noted above corresponds to arrays of 240-bp repeats located in the intergenic spacers between rDNA repeats. These repeats are present in arrays of 5–12 copies immediately upstream of each pre-rRNA transcription unit, and have been shown to function as enhancers of pre-rRNA transcription. Intriguingly, each 240-bp repeat contains a perfect copy of an approximately 80-bp region flanking the rRNA promoter, and these "spacer promoters" have been shown to be active, albeit weak, promoters. The most abundant spacer transcripts span the array from the upstream-most promoter to a termination site about 300 bp upstream of the pre-rRNA promoter. It has been shown that arrays of six or more such repeats, when inserted on an X chromosome deficient for its native pairing site, can stimulate X-Y pairing in the absence of any other rDNA-derived sequences. These data are consistent with the notion that enhancers and/or promoters may function as pairing sites.

The best-characterized mitotic pairing site is that encompassing the brown locus located near the tip of chromosome arm 2R. As described above, even small transpositions carrying this region pair with unrearranged homologs as avidly as do normally located copies, suggesting unusual pairing strength [24]. Mapping of the pairing site by deletion analysis indicated that it mapped broadly to the upstream regulatory region of the brown locus, coincident with the sequences that regulate transcription of brown, but could not be mapped more precisely [139,140]. Thus, the mapping of the mitotic pairing site in distal 2R agrees broadly with the mapping of the X-Y pairing site, and is consistent with the idea that pairing sites may correspond to enhancers and/or promoters.

Recent studies of pairing-dependent silencing of transgenes in *Drosophila* have also implicated upstream regulatory regions of genes in pairing. Transgenic insertions of constructs that carry a Polycomb Response Element (PRE) as well as a reporter gene often exhibit partial or full silencing of the reporter in homozygotes even though expression may be normal in hemizygotes [31]. Although trans-silencing interactions are usually limited to allelic insertions, two particular constructs containing regulatory regions from the *BX-C* complex proved to be able to undergo silencing interactions with copies located at distant sites in the genome. In each case, the transgene carries both an enhancer and the PRE, as well as one or more insulator sequences [141,142]. In one case, long-distance silencing was seen only when the insulator, derived from the gypsy element, was added to the construct. It is not clear whether these types of events have anything to do with mitotic chromosome pairing, but the fact that the sites map to complex upstream regulatory regions is intriguing.

It has been noted previously that the restriction of meiotic pairing ability to euchromatin in male meiosis is consistent with the notion of pairing at transcriptional regulatory sequences or promoters [132]. It is also consistent with the especially strong pairing ability of a region in proximal 2L encompassing the *histones* locus, which contains an unusually high density of strong promoters. Intriguingly, the single most avid pairing site in the mitotic pairing studies has also been the histones locus. It initiates pairing as early as cycle 12, before any other tested locus or region shows significant pairing, and achieves higher average pairing levels than any other locus [21]. This observation is consistent with the idea that pairing sites generally may correspond to promoters.

However, other data from the mitotic pairing studies are less easily incorporated into a promoter/enhancer pairing model. Particularly difficult to accommodate is the fact that heterochromatic repeats can pair mitotically. Although simple sequence repeats such as AACAC and AAGAC were not among the first sites to pair in *Drosophila* embryos, they did achieve high levels of pairing in cells with long cycling times, and apparently were able to do so independently of flanking sequences. Moreover, the second most potent pairing site in the Fung et al. [21] study of chromosome 2 pairing sites was the *Responder (Rsp)* locus which is adjacent to the chromosome 2 centromere and is a complex heterochromatic repeat not known to be transcribed or to possess a promoter. As discussed above, this same site was completely inactive in the assay for meiotic pairing ability. Another observation that is difficult to reconcile with transcription-based pairing models is that mitotic pairing frequencies seem to be entirely independent of the transcriptional status of the locus. This has been most clearly demonstrated for the *BX-C* complex, which pairs at equal frequencies in cells in which it is silenced as in cells in which it is actively transcribed [23]. Another pertinent observation is that mitotic pairing persists essentially throughout the cell cycle, although there is some evidence for partial disruption at anaphase and at S phase [21,22]. This suggests that the structure and function of mitotic pairing sites are not affected by chromatin condensation.

8.1. Could pairing sites be MARs?

From the above discussion, it seems likely that mitotic homolog pairing occurs at sites that are involved in overall organization of the chromatin fiber in both interphase and mitosis, and that are common to euchromatic and heterochromatic regions. This suggests that pairing sites might be MAR/SARs, short, AT-rich sequences that co-purify with

preparations of the nuclear matrix in interphase or the chromosome scaffolds in mitosis [143,144]. They are associated with DNAase hypersensitive sites, are commonly found in upstream regulatory regions where they can contribute to gene activation, but are present elsewhere as well, and have been suggested to be especially abundant in heterochromatin [145–151]. At least some MARs clearly have boundary element/insulator activity [152–159], and, conversely, some insulators have been shown to bind nuclear matrices [160]. Moreover, a recent ChIP study of some of the complex regulatory regions of the *BX-C* revealed that condensin and topoisomerase II, the canonical scaffold proteins, strongly colocalize with Polycomb-group proteins at PREs [161]. Several of these same regulatory regions have been shown to contain insulator activity, and, as noted above, at least two of these regions can promote long-distance interactions that are suggestive of pairing [141,142,162,163].

The idea that mitotic pairing sites might coincide with MAR/SAR sequences could account for the exceptional pairing strength of the histones locus in both mitosis and meiosis, as there is a strong MAR/SAR site located in the largest intergenic spacer of each repeat, which are thus spaced only 5 kb apart in the histones locus [145]. It is not clear whether other known pairing sites also contain MARs. The 240-bp IGS repeat that acts as the major X-Y pairing site in *Drosophila* contains regions that are highly AT rich and similar to consensus MAR sequences.

Unfortunately little is known about the actual functions of MARs. MARs have been postulated to bind to dispersed sites on the nuclear matrix during interphase, and to coalesce into linear arrays during mitosis to form the chromosome axes, forming the bases of chromatin loops that may comprise functional domains in interphase nuclei and structural domains in mitotic and/or meiotic chromosomes on the order of tens to hundreds of thousands of base pairs. Some insulators have been shown to form the bases of chromatin loops as well, and one idea about insulator function is that they act to separate enhancers and promoters into separate looped chromatin domains [164,165]. Recent data from yeast and *Drosophila* point to preferential localization of insulators at particular peripheral sites in the nucleus being essential to their function as insulators [165,166]).

How might MAR elements act as meiotic pairing sites? The ability to attach to nuclear matrices might contribute to homology searching but alone would not provide specificity. However, as noted above, MARs are associated with sites of open chromatin and tend to promote acetylation of surrounding DNA [150]. Moreover, at least some MARs have been shown to unwind readily in response to supercoiling [167]. These properties could be important in the context of a homology search as they could define sites at which homology testing could be carried out easily without a requirement for DNA breaks. They would also provide regulatory flexibility so that some elements could be active in pairing in some cells but not others.

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