Initiation of homologous chromosome pairing during meiosis

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Abstract

Following pre-meiotic DNA replication, homologous chromosomes must be paired and become tightly linked to ensure reductional segregation during meiosis I. Therefore initiation of homologous chromosome pairing is vital for meiosis to proceed correctly. A number of factors contribute to the initiation of homologous chromosome pairing including telomere and centromere dynamics, pairing centres, checkpoint proteins and components of the axial element. The present review briefly summarizes recent progress in our understanding of initiation of homologous chromosome pairing during meiosis and discusses the differences that are observed between research organisms.

Introduction

During mitosis, chromosomes are replicated and the resulting sister chromatids are segregated, generating two genetically identical daughter cells. Meiosis on the other hand is a specialized cell division that involves chromosome replication and two rounds of chromosome segregation (meiosis I and II), resulting in the formation of up to four haploid gametes. Meiosis I differs from mitosis because homologous chromosomes segregate, whereas sister chromatids remain associated until meiosis II. For successful chromosome segregation during meiosis I, homologous chromosomes need to become linked. Linkage of homologous chromosomes occurs during G_2; in cytological terms this stage is known as prophase I (Figure 1). In most organisms including humans, three co-ordinated events occur during prophase I that ensure this linkage, namely homologous chromosome pairing, recombination and synapsis.

During leptotene, homologous chromosomes pair and chromatin begins to condense. Proteins, including the meiosis-specific cohesin complex, form a structure called an axial element between sister chromatids [1]. In most organisms, chromosomes are also subjected to the action of the meiosis-specific topoisomerase-like enzyme Spo11 that introduces DSBs (double-strand breaks) during leptotene [2]. During zygotene, the pairing of homologous chromosomes is stabilized by the initiation of DSB repair via IH (inter-homologue) recombination [3] and the formation of proteinaceous bridges, which in most organisms is the SC (synaptonemal complex) [4]. The stabilized homologous chromosome structure is known as a bivalent. IH recombination can result in the formation of non-crossover or crossover events that are repaired by different homologous repair pathways [5]. All non-crossover events and a fraction of crossover events are repaired during zygotene; these events correspond in cytology to early recombination nodules [6]. Late recombination nodules that persist through zygotene are thought to be sites where SC formation initiates [7,8], although exceptions to this do exist. In *Drosophila melanogaster* [9,10] and *Caenorhabditis elegans* [11], homologues align and synapse independently of DSBs. By pachytene, the SC has formed along the entire length of homologous chromosomes (complete synapsis) and DSB repair of late recombination nodules has resulted in the formation of crossover events [12]. Further chromosome condensation occurs during diplotene/diakinesis, the SC is degraded, and homologous chromosomes remain associated via chiasmata that have formed as a result of crossover events [13]. At metaphase I, kinetochores of homologous chromosomes attach to microtubules emanating from opposite SPBs (spindle pole bodies)/centrosomes (bi-orientation). Chiasmata provide opposing tension to the pulling forces from the spindle poles to ensure correct homologous chromosome segregation during the metaphase to anaphase I transition.

Before stable bivalents can form, homologous chromosomes must pair. In the present review, I will discuss factors that promote initial pairing of homologous chromosomes that is maintained by IH recombination (reviewed in [5,14]) and SC formation (reviewed in [12,15]) during prophase I. Together, these events ensure correct homologous chromosome segregation during meiosis I.

Key words: homologous chromosome pairing, meiosis, pairing centre, recombination, synapsis, telomere bouquet.

Abbreviations used: CHK-2, checkpoint kinase 2; DSB, double-strand break; IH, inter-homologue; MEK1, mitogen-activated protein kinase/extracellular-signal-regulated kinase kinase; MNM, modifier of mdg4 in meiosis; SC, synaptonemal complex; SNM, stromalin in meiosis; SPB, spindle pole body.

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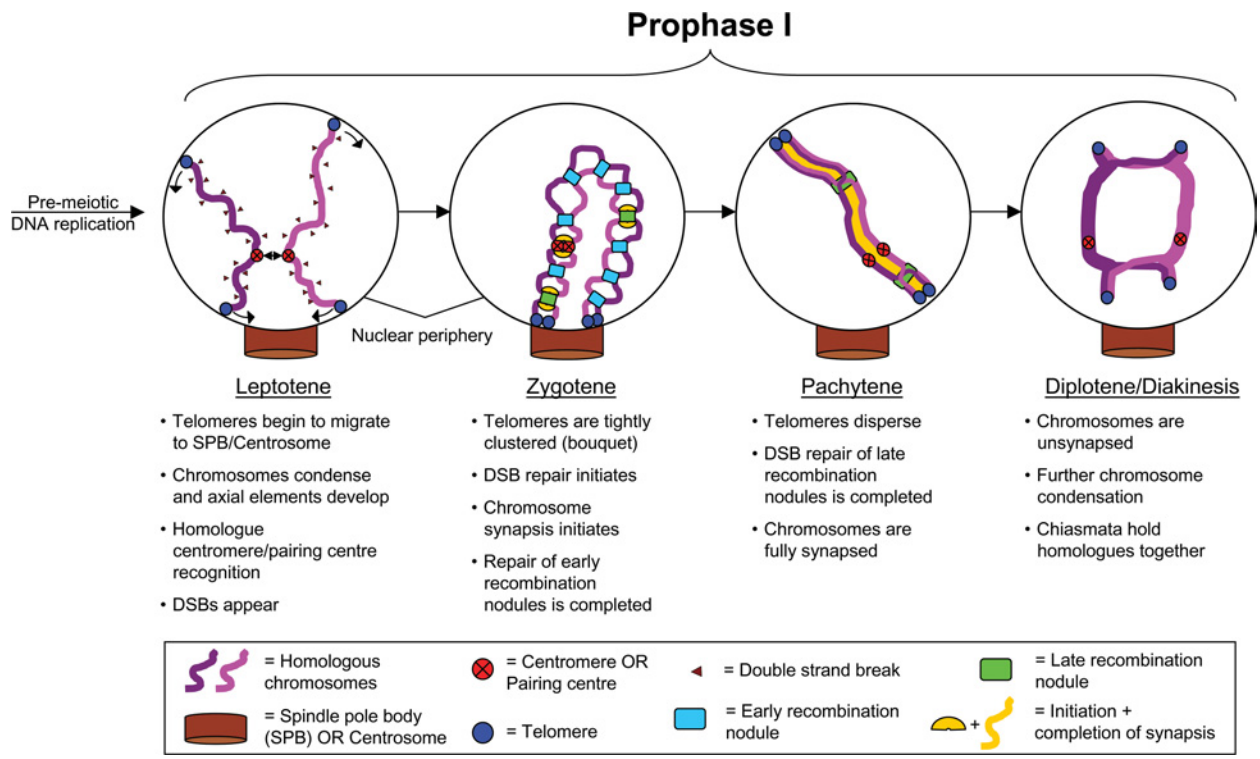
Initiation of homologous chromosome pairing

Pre-meiotic homologous chromosome pairing

Results obtained using *D. melanogaster* males [10], wheat [16], *Arabidopsis thaliana* [17] and *Schizosaccharomyces pombe* [18] show that pairing of homologous chromosomes

Figure 1 | **Diagrammatic representation of the main events that occur at each stage of prophase I**

Note that only a single pair of homologues has been depicted for simplicity. Leptotene: following pre-meiotic DNA replication, chromosomes begin to condense and axial elements form. Telomeres begin to migrate along the nuclear periphery towards the SPB/centrosome and homologous chromosomes become associated via centromeres (e.g. *S. cerevisiae*) or pairing centres (e.g. *C. elegans*). Following pairing, chromosomes are subjected to the formation of DNA DSBs catalysed by the meiosis-specific endonuclease Spo11. Zygotene: the telomere bouquet has formed at the SPB/centrosome, and homologous chromosome pairing is stabilized by the initiation of IH recombination and synapsis. Early IH recombination nodules are repaired during zygotene. Pachytene: telomeres disperse, homologous chromosomes become fully synapsed and repair of late recombination nodules is completed. Diplotene/Diakinesis: chromosomes are unsynapsed, chromosomes condense further and homologues are held together by chiasmata that have formed as a result of crossover recombination events from late recombination nodules.



occurs prior to pre-meiotic S-phase. Interestingly, with the exception of work based on *D. melanogaster*, pre-meiotic pairing of homologues is observed at or near the centromere. This association is thought to contribute to the efficiency of homologous chromosome pairing after pre-meiotic DNA replication by a mechanism that is currently unknown [10,19]. However, pre-meiotic pairing is not a completely conserved phenomenon; it is not observed in mammals [20] or *C. elegans* [11], and contradictory results have been presented for *Saccharomyces cerevisiae* [19,21] and maize [22,23].

Telomere dynamics

In most organisms, telomeres disperse over the nuclear periphery at the start of prophase I (leptotene) and migrate to the SPB/centrosome. By the leptotene–zygotene transition, telomeres have congregated at the SPB/centrosome; this structure is known as the telomere bouquet (Figure 1). The bouquet structure remains present during zygotene, and then telomeres disperse over the nuclear periphery at the beginning of pachytene. It is foreseeable that chromosome pairing could occur transiently while telomeres migrate towards the SPB (leptotene), and the bouquet stage represents a time when correct homologue pairing has been established and repair of DSBs is initiated (i.e. leptotene–zygotene transition). The bouquet structure is released when the pairing of homologous chromosomes is stabilized (early pachytene).

Genetic studies in *S. cerevisiae* (*ndj1*Δ), *Schizosaccharomyces pombe* (*taz1*Δ and *rap1*Δ) and maize (*pam1*Δ) have shown that failing to form a telomere bouquet causes inefficient homologous chromosome pairing, resulting in the formation of mainly infertile gametes [24–27]. However, these studies also show that, in the absence of bouquet formation, homologue pairing, recombination, synapsis and formation of fertile gametes still occur (albeit with lowered efficiency); therefore there must be other processes facilitating homologous chromosome pairing.

Pairing at the centromere

During vegetative growth, centromeres are congregated at the SPB/centrosome. Before pre-meiotic S-phase, the centromeres then disperse throughout the nucleus where they remain during meiosis. With the exception of wheat, it has been suggested that centromeres of homologous chromosomes are not associated with one another during the early stages of meiosis [28]. Nevertheless, studies in *D. melanogaster* females and *S. cerevisiae* have shown that pairing at the centromeric region is required for correct homologous chromosome segregation when chiasmata are absent [29,30]. With some exceptions such as *D. melanogaster* males, achiasmate chromosomes are rare; therefore it has been suggested that pairing of homologous chromosomes at centromeres is a 'back-up' pairing process [30]. However, a recent study using *S. cerevisiae* has shown that centromeric pairing plays an important role in homologous pairing [31]. This study [31] showed that initially centromeric interactions occur mainly between non-homologous chromosomes, and they then undergo switching until all homologous centromeres are paired prior to zygotene. Interestingly, centromeric interactions are dependent on a component of the SC, Zip1. Furthermore, transition from non-homologous to homologous centromere pairing is dependent on Spo11, the endonuclease required for DSB formation during meiosis. From this, it has been suggested that centromeres serve as sites of synapsis initiation [31], which is contradictory to previous observations [32,33]. Furthermore, it suggests that Spo11 is required for the recognition of homologues (see below). Future assessment of Spo11 and Zip1 with respect to centromere pairing during meiosis will result in a better understanding of the linkage between initiation and stabilization of homologous pairing.

As mentioned above, centromeric pairing of homologous chromosomes in wheat occurs early in meiosis; this phenomenon is essential for correct chromosome recombination and segregation [16]. Wheat is polyploid, therefore it contains homeologues as well as homologues. The *Ph1* locus on chromosome 5B of wheat ensures that pairing and recombination are restricted to true homologues rather than homeologues [34]. Much is still to be learnt about the *Ph1* locus, but recently it was found to contain subtelomeric heterochromatin that is inserted into a cluster of *cdc2*-type (cyclin-dependent kinase) genes; this *cdc2*/heterochromatin structure is required for the function of Ph1 [35].

Homologue pairing centres

Two organisms, namely *D. melanogaster* [36] and *C. elegans* [37], have specific chromosomal regions called 'pairing centres' that are required for pairing of homologous chromosomes during meiosis. Recent results suggest that they act as binding sites for proteins that are required for initiating and stabilizing pairing of homologous chromosomes [38,39].

Pairing centres are present on one end of each chromosome of *C. elegans*. Chromosomes of *C. elegans* are holocentric, meaning they do not possess a defined centromere. It has been proposed that transition from non-homologous pairing to homologous pairing occurs at the pairing centres in a similar way proposed for centromeric pairing in *S. cerevisiae* (described above) [37]. Recently, a *C. elegans* protein, HIM-8, was reported to localize specifically to the pairing centre of the X chromosome and was required for initiation of pairing between the two X chromosomes [38]. Finding proteins that bind to autosomal pairing centres will provide further understanding of the function of pairing centres of *C. elegans*.

D. melanogaster has a pairing centre that is shared between the X and Y chromosomes; it is required to initiate X–Y pairing during meiosis. This pairing centre is a repeated 240 bp sequence within the intragenic spacers of the rDNA genes [36]. Recently, two proteins, SNM (stromalin in meiosis) and MNM (modifier of mdg4 in meiosis), were found to be required for accurate chromosome segregation of X–Y and autosomal pairs [39]. SNM and MNM are required for stabilizing initial pairing of homologous chromosomes. They localize to the X–Y pairing centre, and MNM is also localized to autosomes. Currently, pairing sites on *D. melanogaster* autosomes are not well defined. They do not contain rDNA, and it is thought that pairing is most likely to occur at multiple discrete pairing sites [29,40]. Further assessment of SNM and MNM will increase our knowledge with respect to pairing of both sex chromosomes and autosomes.

Specific pairing centres have not been found in other research organisms. As stated, *C. elegans* and *D. melanogaster* do not require recombination to stabilize initial pairing of homologous chromosomes during meiosis. In fact, during meiosis in *D. melanogaster* males, homologous chromosomes efficiently segregate without recombination or an SC. Therefore it is conceivable that pairing centres are present in place of recombination and SC formation.

Checkpoint regulation

Mutation of *C. elegans* gene, *CHK-2* (checkpoint kinase 2), results in the formation of aneuploid gametes due to abnormal chromosome dynamics (e.g. telomere migration) and absence of homologue pairing during leptotene/zygotene [41]. CHK-2 is a member of a conserved family of checkpoint protein kinases that are generally required to arrest cell cycling in response to DNA damage or replication blockage. However, no other member has been shown to be required for pairing of homologues. Therefore CHK-2 has a unique checkpoint function that may couple the completion of pre-meiotic DNA replication with changes in chromosome dynamics and homologue pairing at the onset of prophase I. Localization of CHK-2 and identification of phosphorylation targets of CHK-2 would be very useful in uncovering mechanisms that influence chromosome dynamics and homologue pairing during the early stages of meiosis.

Axial element components required for homologous pairing

As described above, axial elements begin to form at the onset of leptotene. To date, most components of the axial element have been shown to be required for stabilizing initial pairing

of homologous chromosomes by regulating IH recombination and synapsis. For example, a meiosis-specific homologue of CHK-2 (described above) in *S. cerevisiae*, MEK1 (mitogen-activated protein kinase/extracellular-signal-regulated kinase kinase 1), is required for stabilizing paired homologues rather than initiating pairing. MEK1 localizes to the axis and, together with Hop1 and Red1, is required to stimulate IH recombination by inhibiting recombination between sister chromatids [42]. In contrast, HIM-3, a homologue of Hop1 in *C. elegans*, has been shown to be required for initial pairing of homologues [43]. It is not known whether CHK-2 and HIM-3 interact, and there are no obvious homologues of Red1 in higher eukaryotes. The difference in function between MEK1/Hop1 and CHK-2/HIM-3 could be linked to the fact that initial homologue pairing in *C. elegans* can be stabilized independently of IH recombination, which is not the case for *S. cerevisiae*.

DSB components

It has been suggested that proteins required for DSB formation also have an independent role in initiating homologous chromosome pairing. For example, in *S. cerevisiae*, it was observed that homologous chromosome pairing is absent in an *SPO11* null mutant, whereas a point mutation that makes Spo11 endonuclease catalytically inactive can support normal levels of pairing during meiosis [44]. Other meiotic DSB components have been shown to be required for normal levels of homologous pairing in *S. cerevisiae*, and results obtained from *SPO11* mutants in mouse and the fungus *Coprinus cinereus* support the findings in *S. cerevisiae* (reviewed in [2]). Additionally as described above, Spo11 is required for pairing centromeres of homologous chromosomes during leptotene [31].

Results from *C. elegans* [11] and *D. melanogaster* females [9,10] have shown that homologue pairing occurs in the absence of Spo11; however, this is not surprising considering SC formation is independent of the formation of DSBs.

Conclusions

It is apparent that successful initiation of homologous pairing during meiosis in all research organisms requires a number of contributing factors. It is also clear that these factors differ between research organisms. In particular, in organisms that require IH recombination to initiate synapsis (e.g. *S. cerevisiae* and mammals), it appears that proteins required for the formation of DSBs are also required to initiate pairing of homologous chromosomes (prior to their DSB function). However, this is not the case for organisms that do not require IH recombination to initiate synapsis (e.g. *C. elegans* and *D. melanogaster*). Instead these organisms have pairing centres that are required for initiating pairing of homologous chromosomes during meiosis.

In the present review, I have stated the main contributing factors that initiate homologous chromosome pairing during meiosis and discussed recent findings that have increased our knowledge of pairing of homologues. Although, in comparison with later steps in prophase I (IH recombination and synapsis) our understanding of the initiation of homologous chromosome pairing is small, a substantial amount of work is currently being done to dissect each contributing factor. For example, recent work based on *Schizosaccharomyces pombe* has provided significant insight into how the telomere bouquet is formed [45]. A SPB component (Sad1) was shown to transiently move from the SPB to the nuclear periphery. Sad1 then attaches to telomeres via two connector proteins (Bqt1 and Bqt2) and a telomere protein (Rap1), and then migrates back to the SPB, forming the telomere bouquet [45]. Research into the factors involved in the initiation of homologous chromosome pairing during meiosis is moving at a fast rate, as will our understanding.

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