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Repression of harmful meiotic recombination in centromeric regions

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Abstract

During the first division of meiosis, segregation of homologous chromosomes reduces the chromosome number by half. In most species, sister chromatid cohesion and reciprocal recombination (crossing-over) between homologous chromosomes are essential to provide tension to signal proper chromosome segregation during the first meiotic division. Crossovers are not distributed uniformly throughout the genome and are repressed at and near the centromeres. Rare crossovers that occur too near or in the centromere interfere with proper segregation and can give rise to aneuploid progeny, which can be severely defective or inviable. We review here how crossing-over occurs and how it is prevented in and around the centromeres. Molecular mechanisms of centromeric repression are only now being elucidated. However, rapid advances in understanding crossing-over, chromosome structure, and centromere functions promise to explain how potentially deleterious crossovers are avoided in certain chromosomal regions while allowing beneficial crossovers in others.

Keywords

meiosis; homologous recombination; crossing-over; centromeres; chromosome segregation; aneuploidy

Meiosis is a specialized form of cell division that consists of one round of chromosomal replication followed by two rounds of nuclear division to form gametes – eggs and sperm in animals; ovules and pollen in plants; and spores in fungi, such as yeast. It achieves the essential task of reducing the number of chromosomes to half by segregating the centromeres (and the connected chromosome arms) of homologous chromosomes (homologs) at the first meiotic division. The centromeres of sister chromatids, which are generated by chromosomal replication before the first meiotic division, separate subsequently in the second meiotic division. In most species separation of homologs requires the presence of a physical connection between the two chromosomes, which is genetically identified as a crossover. Crossovers are the sites of genetic exchange

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(recombination), by which shuffling of maternal and paternal alleles generates diversity in the progeny. They aid in breaking haplotypes and therefore can generate diversity within populations. Thus, crossovers have a dual role in meiosis – aiding immediate chromosome segregation and generating novel variants for long-term evolution.

Centromeres are specialized regions on the chromosomes that facilitate binding of a large protein complex (the kinetochore), which in turn attaches the chromosomes to microtubules arranged in an elongated polar structure (the spindle) [1]. Proper attachment of microtubules to centromeres is essential for faithful chromosome segregation. Centromeres can be defined as the regions that bind the centromere-specific histone H3 variant, CENP-A, which promotes kinetochore assembly. The regions in the immediate vicinity of the centromeres, the pericentric regions, in almost all species exist as heterochromatin containing H3 K9 methylated histones (H3 K9me). The pericentric region does not directly bind the kinetochore, but it nevertheless plays a crucial role in proper chromosomal segregation. Due to their indispensable function in segregation, the centromere and pericentric region are repressed for recombination because a crossover in either of these regions can interfere with proper segregation. Missegregation produces a chromosomal imbalance (aneuploidy) which can give rise to genetic disorders such as Down syndrome in humans [2]. In this review we describe how crossovers are formed and how their distribution is properly regulated to aid successful meiosis. In particular, we discuss how crossovers are avoided in and near the centromeres (the pericentric region).

1. Crossover formation and chromosome segregation in meiosis

Meiotic recombination is initiated by the induction of programmed DNA double-strand breaks (DSBs) by the meiosis-specific, highly conserved protein Spo11 and several partner proteins that, like Spo11, are essential for DSB formation and recombination [3] (Fig. 1). DSBs as initiators of meiotic recombination have been directly observed by DNA analysis, such as Southern blot hybridization, in both budding and fission yeasts. DSBs have been inferred in other organisms due to the requirement of Spo11 orthologs for recombination or for the formation of foci of the Rad51 and Dmc1 DNA strand-exchange proteins or the γH2AX modified histone; these foci represent sites of DSBs. After introduction of DSBs in a topoisomerase-like manner, Spo11 remains covalently attached to the DSB ends and is later cleaved off by an endonuclease, generating a short oligonucleotide attached to Spo11 [4]. The DSB ends are processed to generate an extended 3’ single-stranded DNA overhang bound by Rad51 strand-exchange protein and (in many species) its meiosis-specific paralog Dmc1. This single-stranded DNA-protein complex can invade an intact duplex (either the homolog or the sister chromatid) to produce a DNA joint molecule. The hybrid DNA intermediate formed with the homolog can be further processed and resolved into either a crossover, where the flanking DNA around the DSB site reciprocally recombines, or a non-crossover, where there is no exchange of the flanking DNA (Fig. 1) [5,6]. In addition, repair can occur such that one but not the other DNA is locally changed to the genotype of the other, resulting in a non-reciprocal exchange called gene conversion; a gene conversion can be part of either a crossover or a non-crossover.

DSBs are not induced uniformly across the genome in the species tested but tend to be concentrated at specific regions known as hotspots. Similarly, recombination events may also cluster to give rise to recombination hotspots, while some parts of the genome are "cold." In the species tested, the number of DSBs in meiotic cells exceeds the number of crossovers generated, implying that many DSBs get resolved into non-crossovers or are repaired with the sister chromatid, which cannot generate a genetic recombinant.

Proper chromosomal segregation involves equal distribution of chromosomes into daughter cells during nuclear division. In mitosis, the kinetochores of sister chromatids, generated by replication, attach to microtubules originating from *opposite* spindle poles (bipolar orientation) (Fig. 2) [7]. The sister chromatids are joined together by cohesion imparted by a complex of proteins (cohesins). The chromatids are then pulled toward the opposites poles; the proper direction of segregation is facilitated by the tension due to cohesion. Cohesin is cleaved as the sisters are separated into two diploid nuclei. In meiosis, by contrast, during the first meiotic division (MI), the kinetochores of sister chromatids attach to microtubules originating from the *same* pole (monopolar orientation) (Fig. 2), and homologous centromeres attach to microtubules from opposite poles [7]. Crossovers generated during recombination stabilize the oppositely-oriented pair of homologs (bivalents) and along with sister chromatid cohesion generate tension to allow proper pulling of the homologous centromeres and their attached chromosomal arms to opposite poles. At the onset of segregation in MI, cohesin in the arms is removed while that at the centromere is maintained. The centromeric cohesin facilitates bipolar orientation of sister centromeres during the second meiotic division (MII) (Fig.2) [7]. The end of meiosis generates four haploid nuclei. Hence, crossovers have a beneficial role in both generation of diversity and chromosome segregation, and therefore most species need to ensure at least one crossover per bivalent during meiosis.

2. Harmful roles of crossovers in meiotic segregation

Despite the important function of crossovers in meiosis, crossovers in certain genomic regions, such as near the centromere, can be as harmful as the lack of crossovers and result in missegregation. There are examples of proper segregation of chromosomes lacking crossovers (achiasmate segregation). However, in the absence of such backup systems, failure to form crossovers results in random segregation such that the homologous centromeres can migrate towards either the same or opposite poles. In MI, migration of homologs to the same pole (non-disjunction) followed by equational segregation at MII results in two MI disomes (nuclei with two copies of a chromosome, in this case having different sister centromeres) and two nullisomes (nuclei lacking a chromosome) (Fig. 3). These are rare in wild type but are more frequent in mutants with few or no crossovers. For example, trisomy for chromosome 21 in humans, associated with Down syndrome, often results from reduced levels of recombination during meiosis as analyzed in several conceptuses [8,9]. This is also true for human trisomies of chromosomes 15, 16 and 18, where the genetic maps (determined by recombination frequencies between genes) are significantly shorter, reflecting fewer crossovers, than controls [10].

Aberrant crossover positioning can also lead to missegregation. Crossovers too close to the centromere affect proper segregation and result in aneuploid inviable gametes. Centromere-proximal crossovers disrupt cohesion in the pericentric region or interfere with proper kinetochore orientation to the spindle poles during segregation. A significant number of maternally derived sex chromosome trisomies in humans possess meiotic exchanges at or close to the centromere [11,12]. Recombination proximal to a centromere is associated with as much as 60% of MI segregation errors in the budding yeast *Saccharomyces cerevisiae* [13]. In addition, crossovers near a centromere in *S. cerevisiae* are strongly correlated with precocious separation of sister chromatids (PSSC), whose effects can be seen in both MI and MII (Fig. 3) [14]. It has been proposed that PSSC is involved in the majority of human age-related trisomies and is a major source of missegregation events in humans [8,15,16]. Non-disjunction at MII, which can lead to formation of MII disomes (in this case having identical sister centromeres), are also associated with crossovers near the centromere (Fig. 3). This type represents ~22% of all missegregation events in humans and ~6% in Drosophila [8,15,17]. Because of the deleterious effects of pericentric crossovers, organisms have evolved mechanisms to stringently avoid them, as discussed below.

3. Diverse properties of centromeres

3.1 Repetitive DNA structure

As noted above, centromeres are the sites of kinetochore attachment and play a key role in establishing orientation during chromosomal segregation. Although the functions of centromeres in kinetochore binding, microtubule attachment, and segregation are conserved across species, centromeres and the flanking pericentric regions are structurally diverse. *S. cerevisiae* has a simple (“point”) centromere, which is approximately 125 bp in length and is subdivided into three conserved DNA elements – CDEI, II and III (Fig. 4) [18,19]. It binds a single nucleosome containing CENP-A (Cse4 in budding yeast) [20]. A single base-pair mutation in CDEIII can abolish centromere function, underscoring the simplicity of budding yeast centromere structure. *S. cerevisiae* and other budding yeasts do not contain heterochromatin around their centromeres, unlike other organisms.

In contrast, centromeres and the pericentric region in the distantly related fission yeast *Schizosaccharomyces pombe* are much larger and more complex (Fig. 4). They span 35 – 120 kb and consist of a unique 4 – 5 kb central core region (*cnt*) flanked by repetitive DNA elements, the ~5 kb innermost repeat (*imr*), and multiple 4 – 7 kb outermost repeats (*otr*) [21]. The *cnt* binds CENP-A (Cnp1 in *S. pombe*) and facilitates kinetochore assembly. A portion of the *imr* and the multiple *otr* repeats together constitute the pericentric region (Fig. 4). These repeat elements are organized into heterochromatin containing H3 K9me, which is essential for proper centromere function as discussed below.

Centromeres in plants and animals are even more complex and extend up to megabases in length. In humans they consist almost entirely of repetitive sequences, the most common of which is the alpha-satellite DNA [22]. It has a 171 bp consensus sequence that is arranged in complex higher order repeat patterns to form large arrays (Fig. 4). Some of the alpha-satellites are bound by CENP-A along with canonical H3 histones, whereas the pericentric monomeric repeats harbor heterochromatin with H3 K9me and the heterochromatin protein,

HP1 [23]. Collectively, these proteins establish the centromere-specific chromatin structure on which other centromere-specific proteins and the microtubule-binding complex assemble to form the functional kinetochore. (Some plants and animals have holocentric chromosomes, to which the spindle microtubules can attach at many points along the entire chromosome rather than to a discrete region, but these holocentrics are not discussed here.)

3.2. Pericentric heterochromatin

The pericentric region contains heterochromatin (in all species examined except budding yeasts, such as *S. cerevisiae*) and is essential for proper centromeric functions and sister chromatid cohesion. Heterochromatin formation is well-studied in *S. pombe* and largely depends on the RNA interference (RNAi) machinery [24], although an RNAi-independent pathway involving the histone deacetylase Sir2 has also been described [25]. Native transcripts from the *otr* repeats are processed by the RNA-dependent RNA polymerase and the Dicer ribonuclease complex into small interfering RNA (siRNA) duplexes that are guided back to the pericentric region via the Argonaute-containing RNA-induced silencing complex. This complex helps in recruiting Clr4, the only histone H3 K9 methyl transferase in *S. pombe* [26]. H3 K9me is bound by proteins such as Chp1 and the heterochromatin protein (HP1) homologs Swi6 and Chp2 via a protein domain (the chromodomain) that has a high affinity for H3 K9me [27]. Once bound, Swi6 helps to bring in more Clr4, which also contains a chromodomain, and by a positive feed-back loop aids in further establishment and spread of heterochromatin to neighboring regions. Apart from contributing to heterochromatin maintenance, Swi6 also helps recruit to the pericentric region cohesin subunits, such as Psc3 and Rec8, and other meiosis-specific proteins, such as Shugoshin, that preserve pericentric sister chromatid cohesin at meiosis I [28]. Swi6 also recruits Epe1, a protein that antagonizes heterochromatin formation and facilitates transcription of repeats within the pericentric region [29]. Chp2 brings in chromatin modulators, such as the histone deacetylase Clr3, that help maintain the transcriptionally repressed state in the pericentric region. A delicate balance between Epe1 and Clr3 activities ensures proper heterochromatinization at the pericentric regions [30].

In multicellular eukaryotes, heterochromatin is organized in a similar manner, although many steps in the pathway are poorly understood. SUV39H is the major H3 K9 methyl transferase at centromeres that helps in recruiting HP1, whose function is similar to that of its counterpart Swi6 in *S. pombe*. Histone deactelyation and DNA methylation also play important functions in heterochromatin maintenance in mammals and plants [31-33]. Many of these features bear on the repression of pericentric crossing-over, as discussed below.

4. Pericentric repression of meiotic crossing-over

Around 80 years ago, the centromeres or spindle attachment regions on the chromosomes in *Drosophila melanogaster* were observed to negatively affect the rates of crossing-over immediately around them [34-36]. This was termed the centromere effect. Apart from reduction of crossovers in the pericentric heterochromatin, the repression extended into the neighboring euchromatin as well. In *Drosophila*, placement of a segment from chromosome III near the centromere of chromosome IV led to a reduced crossover frequency in the

translocated segment. The repressing effect steadily weakened as the distance increased between the region assayed and the centromere [36].

Since these initial discoveries in *Drosophila*, the phenomenon of pericentric repression of meiotic recombination has been shown in several other species. Early genetic analysis in *S. cerevisiae* showed a reduced density (frequency per kb of DNA), relative to that in chromosomal arms, of both crossovers (up to 5-fold lower) and non-crossovers (4 – 7-fold lower) near *CEN3* [37,38]. More recent genome-wide microarray analyses with high resolution confirm this repression. The nearest crossover event on many chromosomes is at least 2 – 3 kb away from the centromere; on others it ranges from ~5 to 18 kb [39]. The nearest non-crossover event occurs ~2.5 kb from the centromeres. Another analysis exhibited an average 6-fold reduction of both crossover and non-crossover densities within 10 kb on each side of the centromeres [40].

The first evidence for pericentric repression in *S. pombe* came from studies that mapped the centromere on chromosome 2. A 50 kb centromeric region on chromosome 2 has a lower density of meiotic recombination than the genome average [41]. A more recent study showed that the crossover density across the largest centromere (on chromosome 3), using closely flanking markers, is ~200 fold lower than the genome average of 0.16 cM/kb (<0.1% across the ~120 kb *cen3*) [42]. There is evidence for pericentric repression in other fungi as well. In *Neurospora crassa*, crossover density decreases dramatically approaching the centromere [43]. Similarly, in *Aspergillus nidulans*, genetic mapping showed ~6-fold reduced recombination density in the centromere-proximal regions of chromosome IV [44].

Pericentric repression is also present in plants and animals. Calculation of recombination densities across large, highly repetitive centromeres, such as those in multicellular eukaryotes, can be problematic due to the difficulty of scoring many meiotic progeny, the absence of markers closely flanking the centromere, and the inability to accurately determine physical distances. Nevertheless, genome-wide and cytological data for the distribution of recombination events in plants demonstrate reduced meiotic exchange near centromeres in a number of species such as *Arabidopsis thaliana* [45,46], tomato [47], rice (*Oryza sativa*) [48,49], wheat [50] and maize [51]. This repressive effect is not restricted to the immediate vicinity of the centromere but may extend for megabases in plants. In *A. thaliana,* recombination in the ~2.5 Mb centromeric core regions is ~50-fold less dense (cM/Mb) than in the chromosomal arms [32,33,45]. Among animals, pericentric repression of recombination has been shown in *Drosophila* (see above), humans [52] and chickens [53]. Early studies on human chromosomes indicated reduced crossover-density near centromeres [54,55]. Integrated physical and genetic maps generated for human centromere 10 showed 9 – 11-fold reduced recombination [56] and ~8-fold reduced meiotic exchange near the human X centromere compared to the rest of the chromosome [52].

More recent studies have focused on understanding the factors causing pericentric repression of meiotic recombination. Possible explanations are centromeric sequence repetitiveness, function in kinetochore assembly, or surrounding highly condensed and inaccessible heterochromatin. *CEN8* of rice spans a 750 kb CENP-A rich region. Unlike other rice centromeres, it does not contain DNA repeats but appears otherwise similar to the other

DNA repeat-rich centromeres [57]. Despite this difference, it contains a ~2.3 Mb region that lacks detectable crossovers [48], indicating that, at least in this case, repetitive DNA is not necessary for pericentric repression. *S. cerevisiae* does not contain pericentric heterochromatin, yet centromere-proximal recombination densities are low, as noted above. Thus, at least in this case, heterochromatin is not necessary for pericentric repression. Furthermore, a single base-pair mutation in *S. cerevisiae* CDEIII that abolishes centromeric function stimulates proximal crossing-over and gene conversion by approximately 2.5-fold relative to wild type [38]. This suggests that centromeric function such as kinetochore assembly may be a factor in repression of recombination, at least in *S. cerevisiae*. Indeed, kinetochore components, such as the Ctf19 complex, reduce DSB formation within a ~6 kb region surrounding the centromeres [58]. The Ctf19 complex, by promoting enrichment of cohesin at the pericentric regions, may also influence partner choice (sister *vs.* homolog) for DSB repair. Current understanding of the mechanism of pericentric repression will be discussed later.

5. Gene conversion at centromeres

Although centromeres are often inert for crossovers, their evolution and higher order structure suggest that gene conversion events do occur in centromeres. In order to maintain the human centromeric structure with nearly identical tandem repeat arrays, it has been proposed that centromeres undergo recombination via random unequal exchange and accompanying gene conversion. This repeated recombination can remove variation arising from mutation in individual monomers, resulting in homogenization of the repeats, as well as promote their expansion or contraction. Evidence of such repeated exchanges leading to rearrangements and expansions within core centromeric structures of human alpha-satellites, rice and mouse centromeres has been observed in somatic tissues [59-63]. However, there is no direct evidence that such exchanges occur in the germ line and are stably passed on to the next generation.

Conversion events in the centromere might be too rare to be observed in the usual laboratory experiments but nevertheless be frequent enough to affect populations. Indeed, a population-based genetic analysis of 93 recombinant inbred lines of maize, using a retrotransposon polymorphism at centromeres, revealed two independent cases of gain of markers from one centromeric haplotype to the other [64]. Linkage disequilibrium and analysis of flanking genotypic markers indicated gene conversion rather than crossing-over. The conversion rates were estimated to be ~1×10^{-5} per marker per generation, similar to other regions on the maize chromosome; such low-frequency conversions would be undetectable in standard meiotic crosses. Nevertheless, these results suggest that pericentric recombination occurs at a low evolutionary rate to homogenize repeats but not frequently enough to cause significant missegregation during meiosis.

6. A role for heterochromatin in meiotic recombination repression near centromeres

Heterochromatic regions are transcriptionally inactive for artificially inserted protein coding genes, and therefore heterochromatin may repress recombination as well. As discussed

above, the RNAi pathway plays a major role in establishing heterochromatin formation in *S. pombe*. Recombination across *cen3,* the largest centromere in *S. pombe*, is <0.1% in wild type [42]. RNAi and heterochromatin mutants, such as *dcr1, ago1, clr4,* and *chp1,* manifest increased crossover density across *cen3* ~30 – 90-fold, to nearly that of the genome mean. As expected, the mutants have increased pericentric DSBs, as analyzed by Southern blot hybridization [42]. Surprisingly, recombination is not significantly increased in *swi6, chp2* and *clr3* mutants, despite the strong effects of these mutations on alleviating repression of gene expression. These results suggest that heterochromatin represses gene expression and recombination by distinct but overlapping mechanisms.

Like *S. pombe clr4,* the *Drosophila Su(var)3-9* gene encodes a histone H3 K9 methyl transferase. Mutations in *Su(var)3-9* increase the frequency of DSBs, measured as RAD51 foci, up to 2-fold in heterochromatin of germ cells (the oocytes and the surrounding nurse cells) relative to wild type [65]. The number of foci returns to the wild-type level when the Spo11-homolog MEI-W68 is removed, suggesting that at least half of the DSBs in the *Su(var)3-9* mutant are generated during meiotic recombination. A less severe effect is observed in a *dcr-2* mutant, disabled for the RNAi pathway that regulates heterochromatin formation in *Drosophila*. Mutations in *Su(var)3-9* and other S*u(var)* genes (9 out of the 16 studied) that are involved in heterochromatin establishment and propagation also enhance up to 6-fold crossing-over between markers flanking the pericentric-centromeric region on chromosome 3 [66]. Conversely, additional genome copies of *Su(var)3-7* and *Su(var)2-5* significantly reduce crossing-over in this interval. Therefore, heterochromatin, in particular H3 K9me, seems to repress both meiotic DSB formation and recombination in both *Drosophila* and *S. pombe* pericentric regions.

In *Tetrahymena* as well, heterochromatin appears to regulate DSB formation during meiosis. *Tetrahymena* cells have two nuclei – a large polyploid macronucleus and a small diploid micronucleus. Meiosis takes place inside the micronucleus, which has highly condensed, transcriptionally silent chromatin for most of its vegetative cell cycle [67]. In the early stages of meiosis, the centromeres and highly-repetitive pericentric regions move to one pole of the micronucleus, while the telomeric and gene-containing regions extend to the opposite pole. *Tetrahymena* lacks H3 K9me and H3 K4me, but its Ezl3 protein forms H3 K23me, which is characteristic of heterochromatin in meiotic prophase in this ciliate [68]. γH2AX foci generally accumulate in the genic regions during early meiosis and distribute throughout the micronucleus by late prophase. In *EZL3*Δ cells, γH2AX foci initially accumulate in the centromeric pole, persist throughout the meiotic lag, and finally distribute throughout the micronucleus [68]. This apparent mislocalization of DSBs is not seen in *EZL2*Δ cells, which lack H3 K27me, indicating specificity of H3 K23me formed by Ezl3 in pericentric repression. H3 K23me-enriched heterochromatin may block DSB formation by Spo11 during meiosis. Hence, histone modifications characteristic of heterochromatin other than H3 K9me also appear to impede meiotic DSB formation.

Apart from histone modifications, DNA methylation can also influence recombination rates during meiosis. *A. thaliana met1* mutants show strong defects in DNA cytosine methylation and ~3-fold increase, relative to wild type, in crossover density in the ~2.5 Mb core of the

centromere (averaged over all centromeres) [32,33]. The effects of these alterations on chromosome segregation remain to be elucidated.

To summarize, in diverse species heterochromatin appears to play a key but not universal role in repressing meiotic DSBs and recombination in pericentric regions.

7. Non-uniform genome-wide DSB and crossover distributions in meiosis

The distributions across the genome of both DSBs and crossovers are tightly regulated during meiosis. The regulatory mechanisms acting in the chromosomal arms may, with variations, also act in the centromeres to prevent harmful crossovers. Understanding how DSBs and crossovers are distributed throughout the genome may suggest potential mechanisms of pericentric repression, which is largely unknown so far.

As noted in section 1, Spo11 or its ortholog along with multiple accessory proteins is needed in most, if not all, organisms to initiate meiotic recombination (i.e., the formation of DSBs) [3]. The distribution of DSBs throughout the genome varies among species. In *S. cerevisiae,* DSBs are generally found in nucleosome-depleted regions, and histone H3 K4 methylation influences both DSB distribution and frequency [69,70]. In *S. pombe*, DSBs occur preferentially in large intergenic regions not necessarily enriched for nucleosome-depleted regions, and transcription factor-binding influences a small subset of hotspots [71,72]. In mice and humans, the chromosomal distribution of bound PRDM9 methyltransferase, which can methylate histone H3 K4, is a strong predictor of DSB formation, but PRDM9 is not essential for formation of DSBs, which are abundant but distributed differently in PRDM9 mutants than in wild type [73-76].

There is ample evidence that crossover distribution across the genome is also non-uniform but not absolutely correlated with DSB distribution. In mice and humans, there is a partial correlation between DSB maps and those generated for recombination [77,78]. Moreover, there are more DSBs than actual crossovers per meiotic cell in several species – the DSB:crossover ratio ranges from ~2:1 in budding and fission yeast to ~10:1 in mice and ~15:1 in *A. thaliana*. [71,79]. As noted in section 1, DSBs can be repaired into non-crossovers and thereby increase the DSB:crossover ratio and potentially influence crossover distribution. The choice of repairing a DSB with the sister chromatid as opposed to the homolog adds another level of regulation. One might expect interhomolog (IH) repair to be preferred over intersister (IS) repair to increase genetic diversity and to aid chromosome segregation. This is true in *S. cerevisiae,* with an IS:IH ratio of ~1:5 at the few DSB hotspots tested [80-82], but is reversed in *S. pombe,* with an IS:IH ratio of ~3 or 4:1 at the two hotspots tested [83,84]. In *S. pombe,* DSBs in non-hotspot (“cold”) chromosomal arm regions are inferred from genetic evidence to be repaired primarily with the homolog, resulting in a nearly uniform crossover distribution in spite of DSB hotspots (crossover invariance) [83,85]. In addition, in *S. cerevisiae* there is spatiotemporal regulation of DSB formation that can influence the IS:IH ratio and crossover distribution based on chromosomal position, presence of factors such as the synaptonemal complex-modifying SUMO E3 ligase Zip3, or time of DSB formation [86,87]. Crossover interference, which reduces the occurrence of one crossover near another, also adds to the complexity. Hence,

although DSBs are essential for recombination, there are multiple levels of regulation that determine crossover position and frequency. All these factors can have an effect on pericentric crossover repression.

8. Molecular mechanism of pericentric repression in meiosis

Although the paucity of crossovers at and near centromeres was discovered over 80 years ago, the molecular basis for pericentric repression is not yet explained in any organism. Heterochromatin, present at pericentric regions in most species, is often considered "inaccessible," for example to the transcription machinery, since genes inserted into heterochromatin are often not expressed [27]. But heterochromatic regions are acted on by the replication machinery every cell cycle, just like euchromatic regions, and are evidently accessible to proteins that form heterochromatin, such as histone-modifying enzymes and the RNAi machinery. In *S. pombe,* the Rec8 cohesin subunit is protected by Shugoshin in MI and phosphorylated by casein kinase homologs in MII specifically in pericentric regions, which are heterochromatic [88,89]. Thus, "inaccessibility" of recombination proteins due to heterochromatin is not a satisfactory explanation at the molecular level for the lack of DSBs and recombination. The limiting molecular step, not yet identified, could be either the absence of a positive effector (activator) or the presence of a negative effector (repressor) of recombination. For example, in wild-type cells heterochromatin could exclude an activator from the pericentric region or recruit a repressor to it. Distinguishing these possibilities is not simple, since a repressor could inactivate an activator that has a more immediate role in regulating the process than does the repressor. These possibilities must be kept in mind as one tries to determine the molecular mechanism of crossover repression in and near centromeres.

More generally, one can ask, is crossing-over limited by DSB formation or by the choice of repair mechanism? DSB formation could be limited by a lack of either Spo11 binding or Spo11 activation. If repair plays a role, is crossing-over limited by the choice of template (sister *vs.* homolog) or by resolution of recombination intermediates into crossovers *vs.* non-crossovers? Evidence for each of these mechanisms of repression is discussed next.

8.1. Blocking of meiotic DSB formation at centromeres

Formation of DSBs is a major determinant of where recombination events can occur. When centromeres are devoid of crossovers, they may lack DSBs as well. In *S. cerevisiae* DSBs are 2 – 3-fold less dense (number per kb per meiosis) than the genome-wide average in 5 – 10 kb intervals surrounding the centromeres [90]. A more recent, nucleotide-resolution DSB map estimates a 7-fold reduction in 3 kb intervals surrounding the centromeres [91]. *S. pombe* centromeres and pericentric regions lack detectable meiotic DSBs, observed both by direct physical analysis (Southern blot hybridization) and genome-wide mapping of Rec12 (Spo11 ortholog)-oligonucleotides [42,71]. γH2AX foci are limited in meiotic heterochromatin regions in *Drosophila* as well as in the *Tetrahymena* micronucleus, as noted above [65,68]. These data imply that the mechanism of repression of recombination around centromeres can be controlled at an early stage, the formation of DSBs.

Absence of pericentric DSBs might result from failure of Spo11 (or its ortholog) either to bind or to be activated for DSB formation in the pericentric regions. There is evidence for both possibilities. ChIP analysis for Rec12-binding following protein-DNA crosslinking showed strong Rec12 binding to all three centromeres in *S. pombe* [92]. Catalytically inactive Rec12 (Y98F) mutant protein also binds to the centromeres [93]. In *S. cerevisiae*, Spo11 binds first to centromeres during premeiotic replication without immediate recruitment of other essential partner proteins such as Mre11, part of the MRN complex also containing Rad50 and Nbs1 (Xrs2 in *S. cerevisiae*) [94]. Artificial tethering of Spo11 to centromere-proximal regions (and other cold regions) via fusion to the GAL4 DNA-binding domain in *S. cerevisiae* does not result in DSBs although in other regions it does [95,96]. These data from two diverse yeasts suggest that activation of Spo11 or Rec12, not their binding, can be limiting for DSB formation. In *A. thaliana*, SPO11 foci appear earlier than the actual γH2AX foci, indicative of a significant delay between SPO11 loading and break formation, which is seen in *S. cerevisiae* as well [97,98]. Thus, the mere presence of Spo11, perhaps without its partner proteins, is not sufficient to induce DSBs. Repression of pericentric meiotic recombination may be due to lack of Spo11-activating proteins rather than by lack of the DSB-inducing protein itself.

8.2. Choice of DSB repair mechanism

Crossover formation could also be limited by the mechanism of DSB repair. For example, DSB repair with the sister chromatid does not reassort parental alleles or produce crossovers joining homologs. In addition, resolution of repair intermediates could be limited to non-crossover outcomes. A few studies show pericentric DSBs, suggesting repair as the limiting step for crossing-over. In *S. cerevisiae*, centromere-proximal DSB hotspots are observed within 5 kb of the centromere [91,99], although their strength and density are lower than the genome average [91]. DSB hotspots near *CEN2*, *CEN4,* and *CEN15* were confirmed by Southern blot hybridization of DNA from *dmc1* mutants, which accumulate broken DNA [99]. Therefore, in pericentric regions with infrequent recombination, these DSBs may undergo intersister repair or be resolved as non-crossovers.

There are conflicting reports on the distribution of non-crossovers specifically around the centromeres. In *S. cerevisiae*, absence of Zip1, a synaptonemal complex component, alleviates pericentric repression during meiosis. Genome-wide analysis showed a significant increase in crossover density within 10 kb of the centromeres, up to the genome-wide level in *zip1* mutants [40]. A comparable increase in non-crossover levels suggests no alteration in the crossover/non-crossover ratio. Tetrad analyses for gene conversion and crossover events, using standard genetic markers both proximal and distal to *CEN3*, confirmed the genome-wide analysis [40]. Since neither an increase in DSBs nor a change in crossover/non-crossover ratio is observed, Zip1 may increase intersister over interhomolog repair during meiosis. In contrast, genome-wide high resolution maps for crossovers and non-crossovers in wild-type *Drosophila* show, for centromere-proximal regions, non-crossovers at the genome mean but crossovers at a considerably lower level [100]. This outcome indicates non-crossover products as a legitimate fate for DSBs formed in such crossover-cold regions. Therefore, regulation at the level of either DSB formation or repair, or both, could explain pericentric repression of meiotic recombination in different organisms.

9. Diversity among species

Although the basic mechanism of meiotic recombination (DSB formation by Spo11 or its ortholog followed by DSB repair) appears conserved, its regulation is highly diverse. For example, the MRN complex, which is required for DSB repair, is essential for DSB formation in *S. cerevisiae,* butnot in *S. pombe* or *A. thaliana* [101-103]. In *S. cerevisiae* the MRN complex is recruited last to the Spo11 binding site, perhaps to ensure its presence for efficient repair [104]. The cohesin subunit Rec8 is needed for DSB formation at most hotspots and for most recombination in *S. pombe* [93,105]. In *S. cerevisiae rec8Δ* mutants, however, DSBs occur at the wild-type (or higher) level at the artificial *HIS4:LEU2* hotspot as assayed by Southern blot hybridization [106]. A more comprehensive genome-wide analysis in *rec8*Δ shows a severe reduction of Spo11 binding and DSB formation at several intervals on different chromosomes including those near the centromeres [94,107]. Such species- and region-specific requirements for DSB formation indicate multiple levels of regulation of DSB formation. Therefore, it is quite tempting to speculate that there are several mechanisms to explain pericentric repression of meiotic recombination in different species. This view is supported by the wide range in the levels of crossover repression seen among various species.

10. Conclusions

Crossovers are important for proper segregation of meiotic chromosomes but are harmful when they occur too close to the centromeres. Crossovers at the centromere or in the pericentric region have a high propensity to cause missegregation, which can give rise to aneuploidy and cause genetic disorders such as Down syndrome. The phenomenon of pericentric repression of meiotic recombination was discovered more than 80 years ago and is now well established in several species. Yet the mechanistic basis for this repression is still unknown – the rate-limiting step at the molecular level has not been identified in any species. The role of heterochromatin in causing repression has been a major focus in many studies. However, not all mutants affecting heterochromatin function with respect to transcriptional silencing are similarly derepressed for recombination. Hence, there could be a different threshold or mechanism for heterochromatic repression of transcription and recombination. DSBs are not seen or indirectly inferred in the pericentric regions in the majority of organisms tested. Regulation of DSB formation by controlling activation of Spo11 or its homolog may be the rate-limiting step to prevent DSB formation, given that Spo11 and its homologs can bind to the pericentric regions in some species. But control at the level of DNA repair, by choice of partner or resolution mechanism for DSB repair, has also been inferred in a few cases. In the future, it will be essential to take into account the interplay between proteins recruited specifically to the pericentric heterochromatin and those of the recombination machinery in mediating repression of crossovers that would otherwise be deleterious for the organism.

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Abbreviations

DSB	DNA double-strand break
MI	first meiotic division
MII	second meiotic division
PSSC	precocious separation of sister chromatids
H3 K9me	histone H3 methylated on lysine 9
siRNA	small interfering RNA
IH	interhomolog
IS	intersister
MRN	Mre11-Rad50-Nbs1
NDJ	non-disjunction

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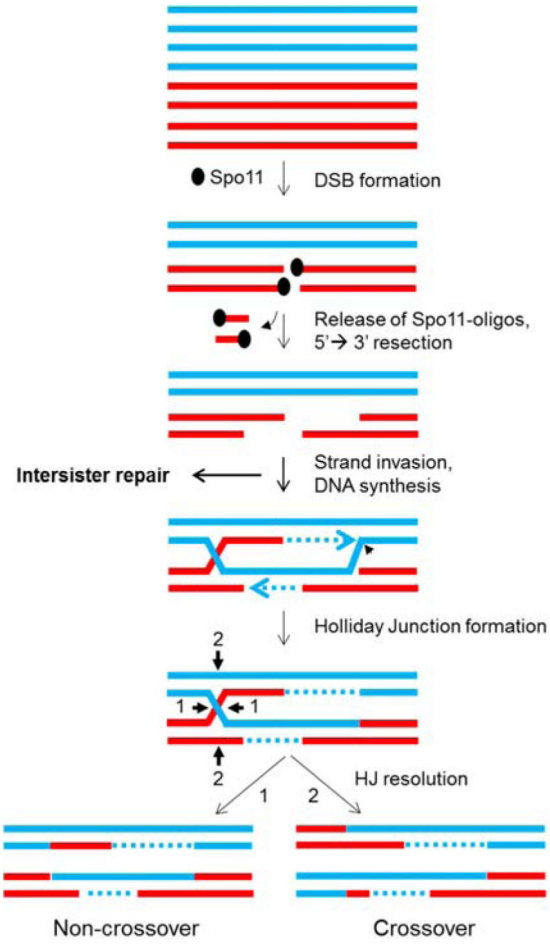

Figure 1. Formation of crossovers and non-crossovers at Spo11-induced DSBs

Red and blue lines are single DNA strands, and each pair depicts one sister chromatid of each homologous chromosome (the non-interacting sisters are shown only in the top panel); dotted lines are DNA synthesized during DSB repair. After meiotic replication, Spo11 or its ortholog induces DSBs and covalently attaches to the 5' ends. Spol11 is then cleaved from the ends by an endonuclease to release a short oligonucleotide attached to Spo11. The 5' ends are further resected to generate long 3' overhangs, which invade intact duplex DNA, either the homolog or the sister chromatid; this side reaction with the sister produces no genetic recombinants. Holliday junctions, either single (shown) or double (not shown), are formed and then resolved into either crossovers or non-crossovers (respectively with the non-parental or parental DNA configuration flanking the region of DNA exchange). These reactions have been demonstrated by direct DNA analysis in budding and fission yeasts and are inferred, from genetic and cytological data and limited DNA analysis, to occur in other species. In an alternative to Holliday junction formation, strand invasion at the third step can prime limited DNA synthesis; the unwound product can anneal with the other initially broken DNA end and produce a non-crossover. This proposed reaction is called synthesis-dependent strand-annealing.

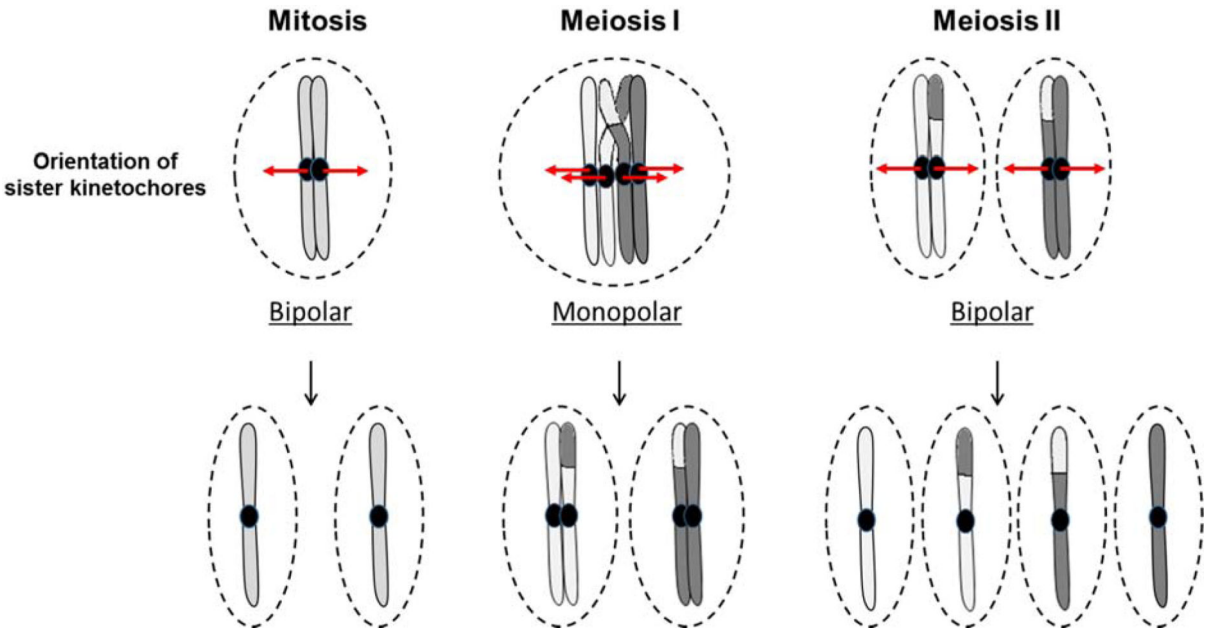

Figure 2. Orientation of kinetochores during mitosis and meiosis

In mitosis, kinetochores attach to the centromeres of the sister chromatids, orient towards opposite poles (bipolar orientation), and segregate into separate daughter nuclei. During the first meiotic division, however, both sister kinetochores orient towards the same pole (monopolar orientation) and segregate into the same daughter nucleus. Proper direction of segregation is ensured by sister chromatid cohesion and the tension generated due to crossovers (light grey-dark grey junction) between homologs. This reductional division results in separation of the homologous centromeres and the attached chromosomal arms and reduces the number of chromosomes by half. The second meiotic division is similar to mitosis, and bipolar orientation of the sister kinetochores results in separation of the chromatids into four haploid nuclei.

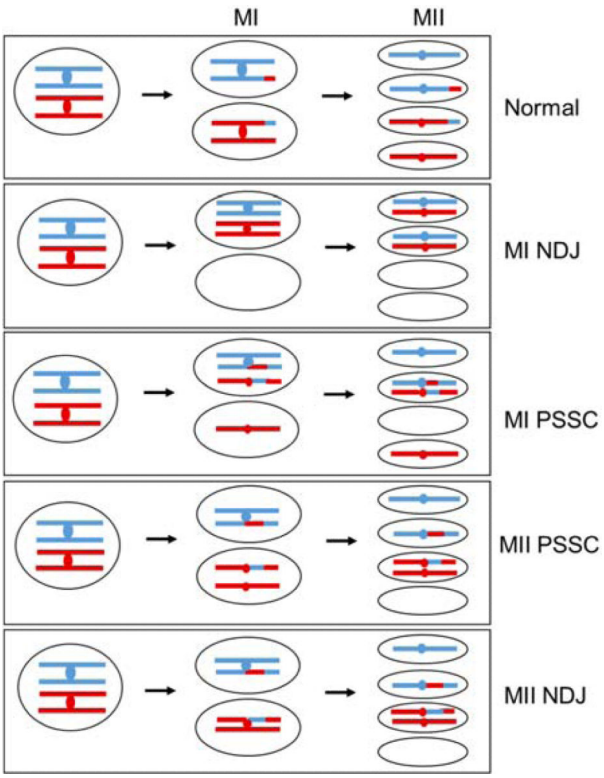

Figure 3. Meiotic chromosomal missegregation resulting from pericentric crossovers

Red and blue lines depict homologs (duplex DNA); red and blue dots depict centromeres. Crossovers, depicted by red-blue junctions, in chromosomal arms are essential for proper segregation of chromosomes during meiosis, but crossovers too near the centromere are harmful. Normally during MI, the centromeres of homologs with their attached chromosomal arms segregate to opposite poles, whereas in MII the sister centromeres segregate to opposite poles, giving rise to four haploid nuclei. During non-disjunction (NDJ) of homologs in MI, which primarily arises due to lack of crossovers in the arms, both homologs migrate to the same pole and then segregate properly at MII, giving rise to two nullisomes (nuclei lacking a chromosome) and two disomes (nuclei with two chromosome copies). Precocious separation of sister chromatids (PSSC) occurs when cohesion is lost between the sister chromatids and can occur in either MI or MII. Crossovers too close to the centromeres (pericentric crossovers) are associated with such PSSC events. In MI PSSC, sister centromeres of one homolog segregate to opposite poles at MI followed by proper MII, giving rise to a nullisome and a disome containing homologous centromeres. In MII PSSC, sisters stay in the same nucleus at MI but missegregate at MII, giving rise to a nullisome and a disome containing sister centromeres. MII NDJ has proper MI but aberrant segregation at MII, resulting in a fate similar to MII PSSC. These aberrant events are also linked to the presence of pericentric crossovers. Some of these missegregation events give rise to similar types of aneuploids, but careful tetrad analysis with multiple markers can distinguish them.

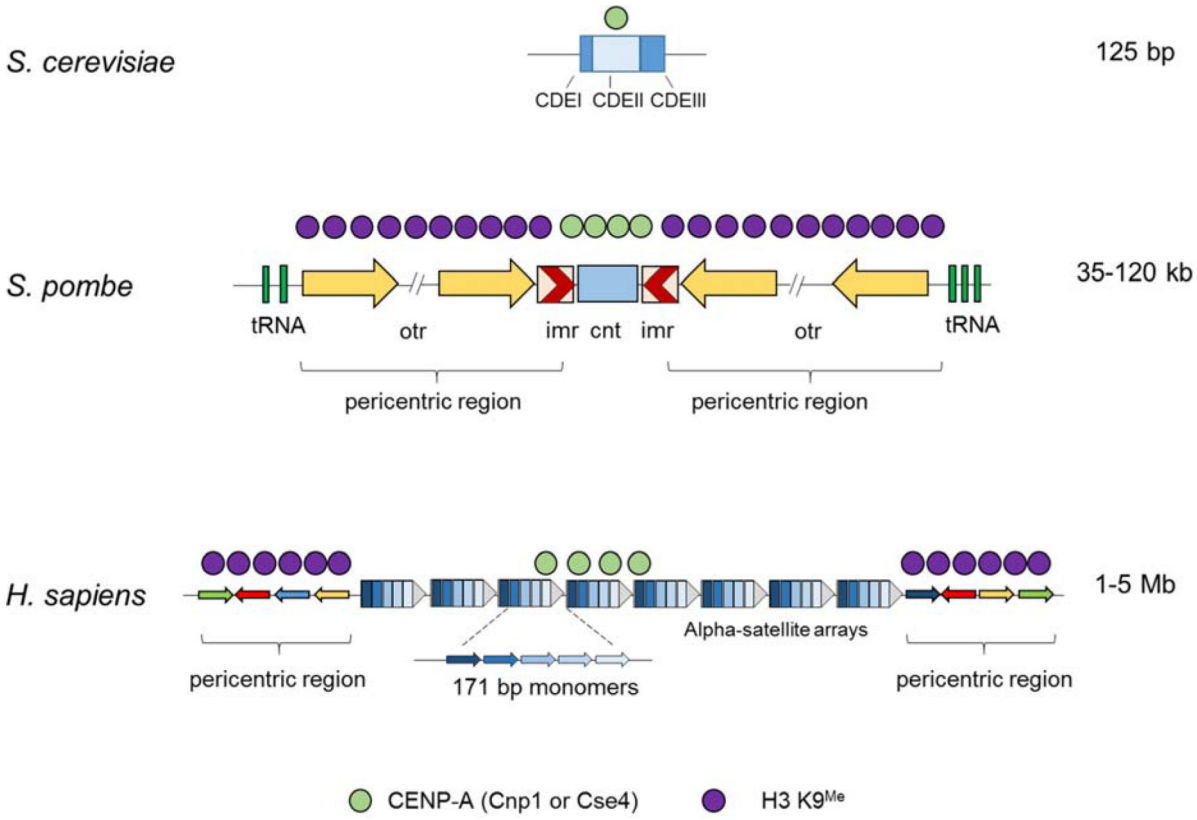

Figure 4. Structure of centromeres in yeasts and humans

The budding yeast *S. cerevisiae* has 125 bp ("point") centromeres with three conserved regions – CDEI, II and III. A single nucleosome containing the centromere-specific histone H3 variant Cse4 (CENP-A in most other organisms) occupies the whole centromere. The fission yeast *S. pombe* contains large 35 – 120 kb ("regional") centromeres consisting of a central core (*cnt*) surrounded by innermost repeats (*imr*) and outermost repeats (*otr*). The central core contains nucleosomes with CENP-A (Cnp1 in *S. pombe*), while the inverted repeats bear H3 K9me histones that form the pericentric heterochromatin. Human centromeres have large 1 – 5 Mb arrays of repetitive DNA consisting of 171 bp alpha-satellite repeats as the basic repeating unit. An array consists of multiple copies of higher order repeats which themselves are made up of multiple alpha-satellites that are diverged from each other. These arrays contain CENP-A interspersed with H3 K4me histones. The pericentric region contains stretches of monomeric alpha-satellite repeats that are in random orientation and are bound by H3 K9me histones that form the heterochromatin. The complete organization of human centromeres is still being worked out, and the schematic shown here is a model based on current understanding.