Factors underlying restricted crossover localization in barley meiosis

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ABSTRACT

Meiotic recombination results in the formation of cytological structures known as chiasmata at the sites of genetic crossovers (COs). The formation of at least one, chiasma/CO between homologous chromosome pairs is essential for accurate chromosome segregation at the first meiotic division as well as generating genetic variation. Although DNA double-strand breaks which initiate recombination are widely distributed along the chromosomes, this is not necessarily reflected in the chiasma distribution. While in many species there is a tendency for chiasmata to be distributed in favoured regions along the chromosomes, in others, such as barley and some other grasses, chiasma localization is extremely pronounced. Localization of chiasma to the distal regions of barley chromosomes restricts the genetic variation available to breeders. Studies reviewed herein are beginning to provide an explanation for chiasma localization in barley. Moreover, they suggest a potential route to manipulating chiasma distribution that could be of value to plant breeders.

INTRODUCTION

A brief overview of meiosis

The formation of genetic crossovers (COs) is essential for the accurate segregation of chromosomes during meiosis in most sexually reproducing eukaryotes. Moreover, it provides a source of genetic variation between the generations which has long been exploited by both plant and animal breeders. During prophase I of meiosis, physical links between homologous chromosomes (homologs), referred to cytologically as chiasmata, arise at the sites of COs through homologous recombination (45, 47). These enable the homologs to correctly orientate on the meiotic spindle equator at metaphase I. Homolog disjunction at the first meiotic division is directly followed by a second division which segregates the sister chromatids to form four haploid gametes. In mutants defective for chiasma formation, homologs segregate randomly at the first meiotic division leading to the formation of aneuploid gametes. Although meiosis has been investigated for over a century, significant progress towards understanding the underlying molecular processes has been comparatively recent. Studies in budding yeast have been instrumental in this respect and have provided the basis for functional analyses in a range of organisms, including plants. These studies have revealed that many aspects of meiotic recombination are highly conserved; nevertheless there are also intriguing differences.

Meiotic recombination is initiated by the programmed formation of numerous DNA double-strand breaks (DSBs) catalysed by the topoisomerase-like protein SPO11 (49). DSBs preferentially occur in short DNA regions termed recombination hotspots that are distributed along the chromosomes but with significantly reduced frequency in the centromeric and telomeric regions (78). In budding yeast, hotspots are associated with regions of low nucleosome density associated with gene promoters (79). In mammals, DSBs are directed away from gene promoters to intergenic sequence motifs through the activity of PRDM9, a rapidly evolving zinc-finger protein containing a histone 3 lysine 4 methyltransferase (8, 71, 80, 85). In *Arabidopsis thaliana*, hotspots overlap gene promoters and are linked with a number of chromatin features. These include the presence of histone H2A.Z nucleosomes at the +1 position, low nucleosome density, low DNA methylation and H3K4 trimethylation (21). Repair of the DSBs is controlled to ensure that each pair of homologs receives at least one, obligate CO (referred to as CO assurance), which is essential for accurate segregation. In budding yeast around 50% of the DSBs are repaired to form 80-90 COs per cell, the remainder being repaired as non-COs. However, in plants and animals only about 5% of DSBs are repaired as COs whilst the vast majority are repaired via a non-CO pathway (76). The important, yet poorly understood phenomenon termed CO interference ensures that multiple COs do not occur in adjacent regions along the

chromosomes (11, 48). As a result of these control mechanisms the numerical distribution of most COs is strikingly non-random. In addition, a small proportion of COs, estimated to be around 15% in *A. thaliana*, arise via a non-interference pathway (38, 69, 76).

Studies have established that recombination is closely coordinated with the extensive remodelling of the homologous chromosomes that characterizes prophase I of meiosis (51). At leptotene, the first sub-stage of prophase I, a linear protein axis is formed along each homolog. This organizes the sister-chromatids that comprise each homolog into linear looped arrays of chromatin conjoined at the loop bases. Some of the proteins that comprise the axis, for example Hop1/Red1 in budding yeast and the corresponding proteins ASY1/ASY3 in Arabidopsis and PAIR2/PAIR3 in rice, play a key role by influencing DSB repair through the creation of a repair template bias in favour of using one of the non-sister chromatids, thus promoting inter-homolog recombination (18, 31, 42, 74, 89, 91). As prophase I progresses from leptotene to zygotene the homolog pairs become increasingly aligned. During zygotene the paired homologs undergo synapsis through the formation of the synaptonemal complex (SC) (77). The SC has a tri-partite structure consisting of the aligned linear axes linked by overlapping transverse filaments that lie perpendicular to the axes bringing them in close apposition at a distance of 100nm and at pachytene stage, SC formation is complete. Recombination is on-going throughout prophase I. Importantly, in most species chromosome pairing, synapsis and recombination progression are interdependent (95). During diplotene/diakinesis when the homologs have recombined to form COs the SC breaks down. At this stage the homolog pairs appear cytologically as condensed bivalent structures linked by one or more chiasmata. Subsequently, at metaphase I the bivalents align on the equator before undergoing the first meiotic division.

It might be assumed that providing the constraints of the obligate CO and interference are fulfilled, the position of the COs along the chromosomes could be quite variable from cell to cell. While positional variation does occur, it is clear that distribution is somehow influenced such that COs tend to arise in favoured chromosomal regions. In some species localized distribution is highly pronounced (24, 45, 47). This is particularly true for members of the grass family including cereal crops and has important implications for plant breeding (29, 54, 63). Although the phenomenon of CO/chiasma localization has been known for many years from genetic and cytogenetic studies, an understanding of the basis for this has been lacking. However, the development of molecular cytogenetic tools for the analysis of plant meiosis is beginning to provide a route towards unravelling this question. Here we review the factors that may account for localized CO formation in the grasses, with particular reference to recent insights into how CO distribution in barley (*Hordeum vulgare*), a key member of the Poaceae is influenced.

The phenomenon of crossover localization

The early construction of genetic maps in species such as *Drosophila* and maize during the early part of the 20th century, was predicated on the assumption that genetic crossovers were more or less evenly distributed across chromosomes so that the crossover frequency between any two genetic markers could be regarded as an indication of the physical distance separating them (28, 86). Almost contemporaneously, cytogenetic observations were increasingly suggesting that crossovers, visualised as chiasmata, were in many instances, far from even in their distribution along chromosomes (24).

These cytogenetic observations led to the development of the concept of chiasma/crossover localization whereby chiasmata may be, depending on species, preferentially and non-randomly restricted to certain chromosome regions (24). Two forms of pronounced localization were recognized viz. distal localization where chiasmata are restricted to the terminal regions of chromosomes, usually remote from centromeric regions, and proximal localization where chiasmata are restricted to regions bordering centromeres (47). Despite some early reservations that distal chiasmata may have originated in more central chromosome regions and migrated, by a process called terminalization, to distal regions, it is now widely accepted that chiasmata do not terminalize and hence their locations reflect their actual sites of origin (87, 88).

Examples of such localization have been recorded across a diverse range of animal and plant species (47). In several cases of pronounced chiasma localization, restriction of chiasmata to certain regions is associated with restricted synapsis of homologous chromosomes. Probably the earliest demonstration of this association was in the plant genus *Fritillaria* (73). The snake's head fritillary (*F. meleagris*) exhibits localization of chiasmata in pollen mother cells (PMCs), to proximal chromosome regions, bordering the centromeres, which in this species occupy mid-chromosome locations. A study of prophase I chromosomes in this species revealed that chromosome pairing at the light microscopical level is also restricted to proximal regions. At this time the existence of the SC (see above) was not known. In later studies, involving electron microscopical analysis, an association between chiasma localization and restricted synapsis was confirmed. For example, in male meiosis of the Large Marsh Grasshopper *Stethophyma grossum* extreme proximal chiasma localization in eight of the eleven chromosome pairs is associated with restricted synapsis to proximal regions of the same chromosomes (16, 32, 90). A similar association of chiasma localization, this time distal, and restricted synapsis is found in male meiosis of the Rhabdocoel planarian worm *Mesostoma ehrenbergii* (75). Thus, an association between restricted synapsis and chiasma localization has been observed in both plant and animal kingdoms. Nevertheless, in most recorded cases of chiasma localization chromosome

synapsis is complete. An instructive example is the case of the closely related onion species *Allium cepa* and *A. fistulosum* (Figure 1). *A. fistulosum* exhibits proximal chiasma localization (Figure 1a) with over 90% occurring within the proximal 25% of the SC length, whereas *A. cepa* has distal to unrestricted chiasmata (Figure 1b) (2). In both species chromosome synapsis is complete and, furthermore, there is no discernible difference in the initiation or progression of synapsis between the two species.

It is worth noting that many species of plants and animals, while not exhibiting pronounced localization of chiasmata, nevertheless do exhibit some degree of localization so that when chiasma positions are carefully measured and mapped it is evident that some regions have elevated chiasma frequencies compared to others as, for example in the Orthopteran insects *Schistocerca gregaria* (34) and *Chorthippus brunneus* (57) which exhibit polarity in chiasma distribution from the telomeres to the centromeric regions. Sub-telomeric and distal chiasmata are also strongly predominant in the human male, with proximal chiasmata rare other than on acrocentric chromosomes (43). A final point of interest is that chiasma localization within a species can vary between the sexes. For example, chiasmata in males of the newt, *Triturus helveticus* show a marked distal localization whereas in females they are interstitial (93). Similarly, when female meiosis was finally analyzed in *F. meleagris* chiasmata were frequently found to occupy interstitial positions rather than the highly proximal position observed in male meiosis (33).

Chiasma localization in members of the grass family

The grass family (Poaceae) comprises over 10,000 species classified into sub-families each comprising a number of tribes amongst which are all the major cereal species, forage grasses, many minor grains (eg. millet) and other economically important crops (eg. sugar cane) (50). Given their major importance as sources of human and animal nutrition these species have been the subject of extensive genetical analysis particularly in relation to crop improvement. Aside from their practical importance, some of the key members of the Poaceae are particularly well suited to cytogenetic analysis since they possess very large chromosomes. Thus since the early part of the 20th century cytogenetic studies in the cereals have made important contributions to our understanding of chromosome behaviour during meiosis.

The grass tribe Triticeae includes the cereal crops, wheat (*T. aestivum*), rye (*Secale cereale* L.), oats (*Avena sativa*) and barley (*Hordeum vulgare* L.). A striking feature of these species is that CO formation along the chromosomes is non-uniform. Allohexaploid bread wheat (*Triticum aestivum*) ($2n = 6x = 42$) is the product of hybridizations between progenitor species carrying the AA, BB and DD genomes (50). A common feature of the Poaceae in

general, reflected in wheat, is the dominance of distally located chiasmata. Chiasma counts on several published figures by Lukaszewski et al (61) on wheat metaphase I chromosome spreads revealed that among 40–41 paired arms per PMC, 34–35 had terminal chiasmata, 1–2 had interstitial chiasmata and none had proximal chiasmata. COs are restricted to the distal half of each arm, where they increase exponentially within proximity to the telomeres (61). An analysis of inverted wheat chromosome arms 2BS and 4AL revealed that COs are biased to particular chromosome segments independent of their locations along the chromosomes (64). The positions of chiasmata moved with the chromosome segments from distal to interstitial regions.

Rye (*Secale cereal* L.) is also a member of the wheat family and has been extensively studied at the cytological level due to possessing a small number of very large chromosomes ($2n = 2x = 14$) (62). The distal bias of chiasmata is so skewed that homologous chromosome associations at metaphase I are often referred to as ‘end-to-end’ (45, 46). However, using Giemsa-banding, Jones (46) revealed that the distal chiasmata were actually adjacent and proximal in location to the terminal heterochromatic repeats that are present in rye chromosomes. Analysis of a line in which the long arm of chromosome 1 was nearly entirely inverted revealed a shift to proximal chiasma formation on the inverted arm rather than the normal distal localization (62). The author therefore reasoned that the recombination frequency along a chromosome is position-independent and segment-specific. In addition, instances of synapsis being limited to the chiasma proficient chromosomal regions was observed, but it is not clear why full synapsis was not achieved. Analysis of the behaviour of a rye deletion chromosome in a wheat addition line also highlights the link between chromosomal segment and chiasma formation. Deletion of all but the proximal 30% of the long arm of rye chromosome 5 resulted in loss of COs in the remaining region (72). However, chromosome pairing and synapsis in the deleted arm appeared normal. This finding is consistent with those in barley (see later), which show that recombination initiation is not deficient in the interstitial/proximal regions but rather that these initiation events are unlikely to progress to form COs.

In the diploid oat (*Avena strigosa*) and tetraploid oat (*A. barbata*) a skewed distal bias of chiasmata was also observed, although the frequency was not quantified (56). In twelve cultivated hexaploid oat (*A. sativa*) varieties an over-representation of terminal chiasmata was observed (6). Despite the low frequency of interstitial chiasmata, there was considerable variation among varieties suggesting that they could differ at the genetic recombination level.

Maize (*Zea mays* L.) possesses ten large ($2n=2x=20$) chromosomes (2300 Mb genome) characterised by large heterochromatic DNA knobs (15). The bulk of the maize genome is composed of highly repetitive transposable elements (TEs) (67). Analysis of CO

frequency and distribution has largely been carried out using recombination nodule (RN) based assays. RNs are electron dense protein complexes detected using electron microscopy that are closely correlated with COs and lie at sites where chiasmata will form later (3, 17). A detailed analysis of RNs in maize revealed a high number in the distal regions that dramatically declined with increased proximity to the centromeres. The gradient of RN distribution correlated with SC length. SCs that were longer (SC1) had a weak gradient, whereas shorter SCs (SC9 & SC10) had very steep gradients (3). These data were in accordance with chiasma counts performed concurrently. In addition, Falque et al. (30) using an antibody against the MutL homolog MLH1 which as a heterodimer with MLH3, marks the sites of interfering COs (19, 44, 65), revealed that ~85% of the RNs belonged to the interference sensitive pathway and the remaining ~15% to the interference independent pathway. The number of residual non-interfering COs also correlated with SC length.

Brachypodium distachyon is a temperate grass species closely related to the cereal crops (barley, wheat and rye) as well as forage grasses such as ryegrass (27). However, in contrast to the cereals, it has a relatively small genome for a grass species (~355 Mb) containing ten small diploid ($2n = 2x=10$) chromosomes (10). A cytological analysis of meiotic metaphase I chromosome spreads revealed that the majority of nuclei possess five ring bivalents, indicating at least one CO in each chromosome arm (16/20) and the remaining four nuclei contained four rings and one rod bivalent per cell (27). Moreover, chiasmata were not strictly localized to any particular region of the chromosomes and were observed in proximal, distal and interstitial positions.

Although chromosome size may be a major contributory factor in determining CO number and position, evidence from rice suggests that meiotic genes that control important steps in the process also have a substantial influence. Compared to the large chromosomes of the cereal crops, rice (*Oryza sativa*) has a larger number of relatively small chromosomes ($2n=2x=24$) and a small genome ~420 Mb (14). However, during meiosis, wild-type metaphase I chromosome spreads revealed that bivalents consisted of a mixture of rods and rings with chiasma location biased towards the ends of chromosomes (59, 92). The chiasma frequency and distribution was dramatically altered in a mutant of the rice synaptonemal complex transverse filament protein ZEP1 (92). Although the analysis lacked immunolocalization of MLH1/3 or marker based recombination assays, the metaphase I bivalents appeared to contain greater numbers of chiasmata that were observed in proximal, distal and interstitial chromosomal regions (92).

CROSSOVER LOCALIZATION IN BARLEY: IDENTIFYING THE CONTRIBUTARY FACTORS

Barley (*Hordeum vulgare* L.) is a diploid member of the Triticeae, and the fourth most abundant cereal after wheat, maize and rice. It has a haploid genome size of 5100 Mb comprising seven chromosomes encoding a putative 53,220 genes of which around 50% have been identified with high confidence (66). Genetical analysis of recombination frequencies in barley mapping populations has revealed that similar to its near relatives the distribution of CO events is not uniform, with a strong bias towards distal chromosomal regions (54). This has been confirmed by cytological analysis including chiasma counts and immunolocalization of MLH1 on pachytene chromosome spreads (Figure 2a,b). CO formation is strongly suppressed in the centromeric and pericentromeric chromosomal regions which data indicate corresponds to almost 50% of the physical map (55, 66). The heterogeneity of CO formation along the chromosomes is mirrored by the gene distribution which shows a strong enrichment in the distal regions. Nevertheless, studies indicate that in the order of 30% of the genes lie outside the recombinogenic distal DNA. It is suggested that this may be a significant barrier for plant breeders as it has the potential to limit available genetic variability, creating difficulties for both gene introgression through linkage drag and map-based cloning.

Despite the long awareness of the strong bias towards distal localization of COs in the cereals, identifying the factors responsible for this has until recently proved difficult. However, in recent years the application of molecular cytological approaches based around immunocytochemistry using antibodies that recognize meiotic proteins has led to substantial progress in the understanding of meiosis in the model plant *Arabidopsis thaliana* (5, 19, 68). By combining immunolocalization studies with 5'-bromo-2'-deoxyuridine (BrdU) labelling of the DNA during meiotic S-phase (4) it has been possible to establish an accurate chronology of progress through meiosis. Fortuitously, many of the antibodies raised against the *Arabidopsis* meiotic proteins also recognize the corresponding proteins in barley, thus permitting a more detailed analysis of barley than was hitherto possible. These studies are beginning to reveal the factors that contribute to the skewed distribution of COs in barley and likely other members of the Poaceae.

Spatio-temporal asymmetry of chromosome axis formation and synapsis during prophase I of meiosis

An earlier study based on sequential sampling of developing spikelets estimated that meiosis in barley occupied 39.4 h (9). A more recent analysis applying a modified version of the BrdU labelling method developed for *Arabidopsis* indicated a figure of 43 h, which is not too different from the earlier study (39). The majority of this time is accounted for by prophase I with the two division stages completed in around 3 h. In common with many species, early

prophase I in barley is characterized by the appearance of the telomere bouquet, one of the early landmarks in the meiotic pathway (39, 82, 84). The bouquet arises through the attachment and clustering of the telomeres in a restricted region on the nuclear envelope (84). As a result physical contacts between the sub-telomeric/distal regions of chromosomes are promoted. This is thought to lead to stable association and pairing of homologous chromosomes at these distal sites prior to interstitial and proximal regions of the chromosomes. This is supported by analyzes of chromosome spread preparations from PMCs using electron microscopy in a range of species including members of the Poaceae. Formation of the telomere bouquet is completed by late G2, some 8 h post-S-phase. Immunolocalization of the chromosome axis-associated protein ASY1 (39) reveals that appearance of the telomere bouquet coincides with the elaboration of a linear chromosome axis initiating in the sub-telomeric/distal regions of the chromosomes. The linearization of the axis continues into interstitial/proximal regions, such that by 13 h post S-phase axis formation is complete. Synapsis of the homologous chromosomes denoting the onset of zygotene can be monitored using electron microscopy or an antibody raised against the SC transverse filament protein, ZYP1 (40) (Figure 2c,d). ZYP1 is first observed around 25 h after S-phase again initiating in the sub-telomeric/distal regions. As these signals extend, additional interstitial synapsis initiation sites are observed, which also extend and coalesce to form a complete synaptonemal complex at pachytene. The SC persists until diplotene, 39 h post S-phase at which point the chromosomes desynapse. The immunolocalization studies are in accord with earlier analyzes regarding chromosome synapsis in the Poaceae based on electron microscopy. Thus, it is clear that barley chromosomes complete synapsis at pachytene. Hence, the skewed spatial distribution of chiasmata cannot be due to synapsis being restricted to a limited region of the homologous chromosomes as in male meiosis of the grasshopper, *Stethophyma grossum* (16, 32, 90).

An interesting observation from the analysis of synapsis in barley is that there is nearly a four-fold excess of synapsis initiation sites relative to COs/chiasmata (55 v ~17). In budding yeast, it appears that each synapsis initiation site corresponds to a future CO site (36). However, this is clearly not the case in barley. Indeed, it seems likely that barley may reflect the norm for multi-cellular eukaryotes, since observations in *Arabidopsis* suggest a similar excess of synapsis initiation sites over COs (38). One could suppose that limiting synapsis initiation to sites of recombination intermediates destined to form COs in barley and other species with large chromosomes would compromise the ability to undergo efficient synapsis. Conversely, high levels of CO formation could lead to insurmountable problems due to chromosome entanglements during meiotic prophase I. Thus, it may be that CO control in most species has been adjusted such that synapsis is initiated efficiently, but CO

formation is limited to a relatively low level. That said, recent studies in *Arabidopsis* have shown that mutation of the *FANCM* gene can result in a substantial elevation of CO formation without any deleterious impact on chromosome stability (23, 53). Studies to knockout the barley *FANCM* homolog (currently in progress, Isabelle Colas, James Hutton Institute personal communication) will determine whether this is also the case in plants with large chromosomes.

Recombination pathway progression

The chronology of the early recombination pathway in barley has been investigated by immunolocalization studies in conjunction with a meiotic time course (39). Immunolocalization of γ H2AX, the phosphorylated form of the histone 2 variant, H2AX which is widely used as a proxy for DSB formation and the recombinases RAD51 and DMC1 which are required for strand-exchange has revealed that recombination is initiated in the same spatially biased manner as chromosome axis formation and synapsis. At about 4 h post S phase around 200 γ H2AX foci are detected in the sub-telomeric/distal regions coincident with the appearance of the axis marker ASY1. The number of foci then progressively increases, concurrent with their appearance in the interstitial and proximal regions of the chromosomes. Maximum numbers are reached at about 13 h post-S phase when around 450 foci per nucleus are detectable and chromosome axis formation is complete. Immunolocalization of RAD51 and DMC1 foci follows a similar spatial and numerical pattern, although they are initially detected at around 10 h post-S phase (Figure 2e). Thus, overall recombination initiation and progression appears to occur in a spatio-temporal wave across the nucleus with events in distal regions preceding those in proximal regions by up to 3 h. Immunolocalization of the MutS homolog, MSH4 which is thought to stabilize progenitor Holliday junction intermediates, also follows this spatio-temporal distribution. Nevertheless, progression to form COs in interstitial/proximal DNA is clearly rare, since chiasmata are generally not found in these regions. Moreover, immunolocalization with an anti-MLH3 antibody, which localizes to CO sites in pachytene, confirms this distribution suggesting that the initiating DSBs in interstitial/proximal regions are repaired prior to dHj formation by an alternative repair pathway, possibly via synthesis-dependent strand annealing or using the sister-chromatid as the repair template (83).

Meiotic progression and crossover formation is correlated with chromatin organization

It is well established that the chromosomes in eukaryotes show linear differentiation into regions of euchromatin and heterochromatin. In barley and some other members of the

Poaceae with similarly large chromosomes, the euchromatin-rich DNA is distributed along the distal regions of the chromosomes, whereas the heterochromatic DNA is localized to the centromeric region. Additional heterochromatic DNA may also occur at interstitial sites or in the telomeric region, as is the case in rye. Immunolocalization using antibodies that recognize histone modifications K3K9me3, H3K27me3, H3K4me3 (Figure 2f) and H4K16ac that are associated with transcriptionally active, gene-rich DNA reveals a high degree of enrichment in the distal regions (35, 39). Whereas heterochromatic marks such as H3K9me2, H3K27me2 and H4K20me1 are abundant along the entire length of the chromosomes, save for the distal regions where they are depleted. These observations suggest a link between euchromatin and CO formation. This association is also supported by the studies in rye, outlined earlier, which indicate that CO formation is excluded from heterochromatic DNA.

Effect of temperature on chiasma frequency and distribution

Further insight into the underlying control of CO distribution in barley has come from temperature shift experiments. That meiosis is sensitive to elevated temperature has been known for many years. Unsurprisingly, extreme temperature during meiosis leads to a complete disruption of the process (60). However, studies in *Tradescantia bracteata* and *Uvularia perfoliata* revealed that while high temperature, above 35°C, resulted in a rapid decrease in chiasma formation, plants exposed to a range of temperatures below this exhibit a progressive shift of chiasma distribution with an increased frequency of interstitial crossovers (26). Similarly in barley exposure to 35°C also resulted in complete meiotic failure, while comparison of the chiasma distribution in plants exposed to 22°C and 30°C during meiosis revealed a significant decrease in distal crossovers and a coincident increase in interstitial events at the higher temperature (39). Interestingly, this effect was not uniform across all the chromosomes, hinting at a possible influence of chromosome structure. The shift in distribution at 30°C was also accompanied by a slight reduction in the mean chiasma frequency per PMC from 14.8 to 13.5. It would seem reasonable to suppose that the reduction in COs and change in chiasma distribution are in some way linked and further studies have been conducted to try to address this.

Effect of temperature on meiotic recombination

As PMCs progress through prophase I extensive chromosome remodelling occurs which evidence suggests, is closely coupled with meiotic recombination and vice-versa. Thus mutants affecting one of these processes can have profound effects on the other. This is illustrated in a range of Arabidopsis and rice mutants. For example, mutation of the

chromosome axis protein, ASY3/PAIR3, leads to defects in recombination resulting in a reduction of COs (31, 94). In some instances, mutations in components of the recombination machinery, such as the *MutS* genes *MSH4* and *MSH5*, lead to a delay in meiotic progression of several hours (38, 41). Also, a mutant allele of the cell cycle control gene *RETINOBLASTOMA* (*RBR*) affects meiotic progression resulting in a defect in synapsis and CO formation (20). An Arabidopsis *arp6* mutant that is defective in deposition of H2A.Z into nucleosomes at DSB sites during meiosis phenocopies H2A.Z localization at higher temperatures and exhibits a small but significant reduction in COs (21). Hence, the reduction in COs in barley at 30°C could be accounted for by one or more factors.

Immunolocalization studies conducted on chromosome spread preparations from PMCs isolated from plants held at 30°C compared to those from plants at 22°C suggest that there are no obvious defects in the chromosome axes and that overall levels of DSBs and early recombination intermediates are not significantly different. However, localization of the SC transverse filament protein, ZYP1, revealed a defect in installation of the SC. Formation was delayed and accumulations of ZYP1 protein, possibly corresponding to polycomplexes, were apparent (39). It is known from studies in budding yeast that the corresponding Zip1 protein acts at CO-designated recombination intermediates to impose the CO fate. Assuming ZYP1 fulfils a similar role in barley, it seems likely that the loading defect may result in an occasional failure in CO imposition. Presumably, the magnitude of the defect increases as the temperature rises beyond 30°C, since it seems that no COs are formed at 35°C and the chromosomes are completely asynaptic. Moreover, in barley ZYP1 RNAi knockdown lines in which SC formation is absent or reduced, there is a corresponding reduction in CO formation (7). Although the impact on SC formation at 30°C likely explains the slight reduction in COs at this temperature, additional defects in other components of the meiotic machinery or meiotic progression (see below) cannot be ruled out based on existing evidence.

Effect of temperature on chromatin and meiotic progression

While the reduction in CO frequency at 30°C is likely related to the SC defect, the change in chiasma distribution may be due other factors. Since, most COs in barley are associated with euchromatin-rich chromosomal regions one explanation for the change in CO distribution could be a shift in the pattern of histone modifications at the elevated temperature. There is strong evidence from a variety of species that meiotic recombination is influenced by the chromatin landscape. For example, studies in mammals and budding yeast have demonstrated that recombination hotspots are associated with trimethylation of the lysine 4 residue in histone 3 (8, 13, 71, 80, 85). Histone acetylation has also been shown to

influence recombination in both budding yeast and Arabidopsis (70, 81). In the former, deletion of the *SIR2* gene which encodes a histone deacetylase, changes the genomic distribution of recombination. In Arabidopsis, hyperacetylation arising through over expression of a histone acetylase altered the frequency and distribution of chiasmata.

However, in barley PMCs there are no obvious changes in the distribution of the euchromatic and heterochromatic marks at 30°C compared to 22°C (39). While, subtle short range changes cannot be excluded, there is as yet no evidence to indicate that the change in distribution is directly driven by altered chromatin marks. Nevertheless, the global chromatin organization in barley does appear to have a significant part to play in influencing chiasma distribution. Comparison of meiotic time courses at the two temperatures reveals that the duration is approximately 43 h in both instances. This suggests that meiotic progression in barley is buffered against fluctuations in temperature. Although the duration of meiosis does not appear to be affected by a moderate shift in temperature, dual labelling of the chromosomal DNA during meiotic S-phase with BrdU and 5-ethynyl-2'-deoxyuridine (EdU) reveals a significant effect on replication. It is well established that euchromatin-rich chromosomal segments are replicated earlier than heterochromatin-rich regions. BrdU/EdU dual labelling has revealed that in barley at 22°C the distal DNA is replicated within 4 h of the initiation of S-phase; interstitial DNA is replicated by 6 h. However, replication of the heterochromatin-rich, proximal DNA is not completed until 13 h. Shifting the temperature to 30°C reduces the length of S-phase. This does not seem to have an influence on replication of the distal euchromatic DNA but replication of the interstitial and proximal DNA is completed by 9 h, some 4 h earlier than at 22°C. It is proposed that the increase in temperature makes the heterochromatic DNA more accessible to the replication machinery, possibly through reducing the occupancy of the histone H2A.Z which acts a thermosensor. As mentioned earlier, aside from the effect on SC formation the temperature shift does not compromise the ability to form the chromosome axes or reduce the overall number of recombination initiation events. That said, there is a clear effect on the localization of the meiotic proteins during the early stages of meiosis. In particular, the strong bias towards initiation of both axis formation and recombination in the distal regions of the chromosomes in the vicinity of the telomere bouquet seen at 22°C is less pronounced at 30°C. As a result, elaboration of the chromosome axes and initiation of recombination takes place in distal and interstitial regions at a similar time, although a degree of bias still exists. As a consequence, DSBs that occur in interstitial/proximal DNA are repaired more frequently via the CO pathway, such that the 25:1 ratio of distal to interstitial chiasmata observed at 22°C is reduced to 11:1 at 30°C. Thus it seems that elevated temperature tends to synchronize early meiotic events along the chromosomes and that this increases the probability that a DSB

occurring in interstitial DNA may be repaired as a CO rather than a non-CO. Why this should be the case remains to be established, nevertheless there are a number of factors that may influence events.

Can CO frequency and distribution in barley be explained by CO interference?

The distribution of meiotic COs is highly controlled such that each pair of homologous chromosomes receives a minimum of one, obligate, CO with most additional COs subject to interference (see earlier). As a result, COs are well-spaced along the chromosomes, with often only a single CO per chromosome arm. Studies in budding yeast indicate that the fate of individual DSBs to be repaired as either a CO or a non-CO is taken early in prophase I implying that CO interference is established at this point (12). Since there are many commonalities between meiotic control in budding yeast and plants it seems likely that CO designation also occurs in early prophase I in plants. This could suggest that in barley under normal conditions there would be a strong bias for CO designation at distal sites since recombination initiates in this region 2-3 h before interstitial/proximal sites. Thus, interference would be established at distal sites thus disfavouring interstitial/proximal DSBs from progressing to form COs. Although this hypothesis may seem attractive, there are a number of observations that suggest the explanation may lie elsewhere.

Immunolocalization of MLH3 foci along barley chromosomes at pachytene has revealed that the mean inter-focus distances range from 29.2% to 44.35% of arm length for chromosomes 2H and 3H, with a minimum distance of 6.1% (83). Overall, nearly 40% of the MLH3 foci were separated by less than 20% of arm length. These data were analyzed using the CODA gamma distribution method to quantify the strength of interference (nu), whereby a value for $nu = 1$ indicates no interference; >1 positive interference; <1 negative interference (37). This analysis gave values for nu of 1.44 and 1.58 for 2H and 3H respectively. These figures are substantially lower than interference calculations in some other species, including tomato where nu values of 7.9 and 6.9 for chromosomes 1 and 2 respectively were recorded (58). Although this could be interpreted as indicating that interference in barley is relatively weak in these chromosomal regions, some caution in direct comparisons may be required due to the nature of meiotic progression in barley and the proposed role of the chromosome axis in mediating interference. The gamma distribution method is based on the relative separation of MLH3 foci along the chromosome axes when synapsis is complete at pachytene. However, the differential timing of events along barley chromosomes could allow CO designation to take place at recombination intermediates along a distal chromosome segment before axis elaboration has been completed in the interstitial/proximal region. If so, then it could be argued that interference should be

measured in the context of the degree of axis formation at the time of CO designation, rather than when axis formation has proceeded to completion. In this context it is arguable that CO interference in barley may actually be stronger than currently estimated. Counter to this argument, mutation of the axial element protein, SYCP3, in mouse does not affect interference between MLH1 foci (25). However, the cohesin complex which is a key component and organizer of the chromosome axes was present in these mice, hence it is unclear if axis function in relation to any role in mediating interference was compromised.

Although inter-focus separation between MLH3 foci can be relatively short, the majority of foci are nevertheless separated by >70% of the total chromosome length because most COs are restricted to the distal regions of the chromosomes. Since CO interference is known to operate across the centromeric region (22), this would imply that interference over the interstitial/proximal regions is stronger than that in distal regions. In fact in their study, Philips et al (83) calculated *nu* across the centromeric region for 2H and 3H to be 5.86 and 6.42 respectively. This raises the question of whether interference is imposed differentially along the chromosomes or alternatively, the repair of interstitial/proximal recombination intermediates is mediated via a non-CO route. While no categorical answer yet exists, studies indicate that dynamic changes in the chromatin environment may be a significant influence.

Based on data from a range of species, it appears that during mitosis and meiotic prophase I, chromosomes undergo a programmed set of cycles of chromatin expansion and contraction (52). These observations have led to the development of the “mechanical basis of chromosome function” model which in relation to meiosis, proposes a functional inter-relationship between the chromatin cycles and the four key transitions in the meiotic pathway, namely DSB formation, single-end invasion, second-end capture and dHj resolution that lead to CO formation. It is proposed that the four transitions are coordinated by three rounds of mechanical stress and relaxation generated during prophase I by the chromatin cycles, with each transition coincident with a phase of chromatin expansion. Studies confirm that the chromosome cycles are also conserved in barley (39). Analyzing these in conjunction with meiotic progression has led to an interesting observation concerning the spatial differentiation across the barley chromosomes. The 2-3 h time difference between meiotic transitions, such as axis formation and DSB formation, in distal versus interstitial/proximal regions monitored using immunocytochemistry to detect meiotic proteins, reveals that while the transitions in distal regions occur in synchrony with a chromatin expansion phase, interstitial events occur during periods of contraction. If it is a requirement that imposition of a CO fate on a designated recombination intermediate is coincident with a chromatin expansion phase, then it would explain why interstitial/proximal

intermediates are not repaired via a CO route. There is no direct experimental evidence to confirm this supposition. Nevertheless, one of the effects of exposing barley PMCs to 30°C is that the spatial differentiation in meiotic progression observed at 22°C is much less pronounced. As a result, the timing of the meiotic transitions in the interstitial regions tends to be more coincident with those in the distal DNA and in phase with periods of chromatin expansion. Importantly, this is accompanied by a significant increase in CO formation at interstitial sites.

CONCLUSION

The phenomenon of chiasma localization has been recognized for many years, in some instances since the early days of cytogenetics. It is clear that it occurs widely throughout the different eukaryotic kingdoms. Examples of both distal and proximal localization have been observed. In some cases extreme localization is directly associated with limited or incomplete chromosome synapsis. There is now compelling evidence to indicate that in many organisms SC formation is essential to ensure a CO fate is imposed on CO-designated recombination intermediates. Hence, by restricting the degree of synapsis recombination is concomitantly limited. However, in many species, including the cereals, chiasma localization is not linked to limited SC formation. An explanation has therefore proved less tractable. Elucidating the basis for chiasma localization, particularly in the cereals, is potentially important from a plant breeding viewpoint. The studies outlined above provide a tantalizing indication that the global organization of the chromatin in relation to timing of replication is a key influence. Moreover they suggest some scope for modifying the chiasma distribution which may provide a simple basis for manipulating recombination through temperature.

An additional, intriguing question, which has previously been raised, is whether the chromosome architecture in the cereals determines CO position or whether crossing over has been a major factor in the evolution of genome organization. Akhunov et al. (1) has shown that wheat loci derived by duplication were most frequently located in distal, high-recombination chromosome regions whereas ancestral loci were most frequently located proximal to them. These authors suggest that recombination has played a central role in the evolution of the wheat genome structure and that gradients of recombination rates along chromosome arms promote more rapid rates of genome evolution in distal, high-recombination regions than in proximal, low-recombination regions. Similarly to wheat, it has been argued that meiotic recombination has been one of the main factors driving maize genome evolution and the two may be intimately linked. Meiotic drive, the subversion of meiosis so that particular genes are preferentially transmitted to the progeny, appears to

affect heterochromatin knob chromosomal position and size. Hence, it is likely that meiotic recombination influences genome organisation and possibly vice versa but further study is required to resolve the issue.

SUMMARY POINTS

1. The formation of crossovers (COs), which are cytologically manifested as chiasmata at metaphase I of meiosis, is carefully regulated to ensure a minimum of at least one obligate CO between homologous chromosome pairs (bivalents). CO interference ensures that additional COs along a bivalent are widely spaced.
2. Although most species studied show a tendency for COs/chiasmata to be localized in favoured chromosomal regions, in some species this localization is highly pronounced.
3. A number of important members of the grass family including cereals, such as barley and forage grasses exhibit CO localization which effectively limits COs to the distal regions of the chromosomes. This presents a potential barrier for plant breeders.
4. In some species CO localization is associated with restricted chromosome synapsis. However, this is not the case in barley and other cereals.
5. Immunocytochemistry in conjunction with fluorescence microscopy and super-resolution microscopy using a panel of antibodies against meiotic proteins has revealed that spatio-temporal asymmetry of meiotic chromosome remodelling and recombination progression underlies CO/chiasma localization in barley. Studies show that chromosome axis formation, chromosome pairing and synapsis and recombination are initiated in the distal chromosome regions 2-3h in advance of the corresponding events in proximal DNA. As a consequence, a proportion of recombination events in the distal regions progress to form COs. Whereas virtually all those in the proximal regions are repaired without CO formation.
6. Studies indicate that late replication of the heterochromatic DNA (relative to euchromatic DNA) which is enriched in the proximal regions of the barley chromosomes is an important factor in establishing the asymmetry of meiotic progression.
7. The application of a moderate temperature pulse during meiosis has been found to alter chiasma distribution, leading to a greater proportion of interstitial/proximal COs. It seems that the differential timing of replication between the euchromatic and heterochromatic DNA is less marked, such that recombination is initiated more synchronously along the chromosomes. This provides a potential route for plant breeders to manipulate recombination.

FUTURE ISSUES

1. While an elevation of temperature to 30°C during meiosis in barley leads to an increase in interstitial/proximal COs, this does not appear to affect all the chromosomes to the same

degree. One hypothesis to test is that the effect of temperature is governed by the organization of the individual chromosomes. Hence it will be of interest to determine whether factors such as chromosome size, the proportion and distribution of heterochromatin underlie this variation. The effect of different temperatures could be explored, or similarly, the effect of modification of chromatin through chemical treatments. For example, application of trichostatin A to modify histone acetylation has been shown to affect chiasma distribution in *Arabidopsis* (81). Whether or not a heat-pulse strategy could be used to modify chiasma distribution in other cereals could also be investigated.

2. The fact that a modest increase in temperature also reduces CO frequency is also significant as it could contribute to yield reduction in areas affected by climate change. It will be interesting to determine whether accessions can be identified that are resilient to elevated temperatures. At present the mechanistic basis of the temperature susceptibility remains to be determined. Work on barley has identified a problem with SC formation but whether this is due to an impact on the SC proteins themselves, synapsis initiation or remodelling of the chromosome axis during zygotene has yet to be established.

3. Why DSBs that form in the interstitial/proximal regions do not progress to form COs remains to be resolved. The observation that there is a time delay in DSB formation relative to distal regions which alters the relationship between the repair processes and the conserved chromatin cycles appears significant. Clearly, this influences how the breaks are repaired. It is conceivable that CO interference may be involved. However, studies in other species, with large chromosomes, such as grasshopper, indicates that interference is dissipated over a region of 30% of a chromosome arm. Also, the MLH3 inter-focus distance observed in barley itself can be less than 20% of arm length. Hence, more interstitial COs may be anticipated. One possibility is that by the time interstitial and proximal DSBs are undergoing repair, the bias towards inter-homolog repair mediated by the chromosome axis proteins is lifted such that a switch to using the sister chromatid as the repair template occurs, precluding additional COs from forming. It is also possible that the local chromatin environment directs repair down a non-CO route.

Figure Legends

Figure 1

Squash preparations of *Allium* chromosomes at metaphase I of meiosis stained with orcein. (a) *A. fistulosum* note stellate shaped bivalents due to the extreme proximal position of the chiasmata (example arrowed). (b) *A. cepa* chiasmata are distal (arrowed) or interstitial. Bars = 10µm. Reproduced with permission reference 2 (NRC Research Press. License: 3318821336122)

Figure 2

Barley chromosomes reveal a spatio-temporal progression of meiotic events which initiate at the distal ends and consequently form crossovers in these regions. (a) During pachytene, immunolocalization of MLH1 foci (red) which mark the future sites of interfering crossovers are observed at the ends of the synaptonemal complexes marked by the transverse filament protein ZYP1 (green). (b) At metaphase I, chiasmata are detected at the distal ends of the chromosomes (white arrows), highlighted by the location of fluorescence *in situ* hybridization ribosomal DNA probes 5S (red) and 45S (green). (c) Structured illumination microscopy of

an early zygotene nucleus showing extensive polymerisation of ZYP1 (green) in a specific region of the nucleus with small stretches throughout the nucleus and the chromosome axes are marked by ASY1 (red). (d) An electron micrograph of silver-stained chromosomes at zygotene showing the initiation of synapsis in the distal regions (highlighted by the dotted circle). (e) The strand-exchange protein DMC1 initially localizes in the vicinity of the telomeres (green) (highlighted with the white dotted circle) in a leptotene nucleus. (f) The chromosome axis protein ASY1 (red) initially localizes and extends in the euchromatic hyper-abundant regions marked by histone 3 lysine 94 tri-methylation (green) (highlighted by white dotted line). Bars = 10µm and the chromosomes are counterstained with DAPI (blue).

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REFERENCES

1. Akhunov ED, Akhunova AR, Linkiewicz AM, Dubcovsky J, Hummel D, et al. 2003. Synteny perturbations between wheat homoeologous chromosomes caused by locus duplications and deletions correlate with recombination rates. *Proceedings of the National Academy of Sciences of the United States of America* 100: 10836-41
2. Albin SM, Jones GH. 1988. Synaptonemal Complex Spreading in *Allium-Cepa* and *Allium-Fistulosum* .2. Pachytene Observations - the SC Karyotype and the Correspondence of Late Recombination Nodules and Chiasmata. *Genome* 30: 399-410
3. Anderson LK, Doyle GG, Brigham B, Carter J, Hooker KD, et al. 2003. High-resolution crossover maps for each bivalent of *Zea mays* using recombination nodules. *Genetics* 165: 849-65
4. Armstrong SJ, Franklin FCH, Jones GH. 2003. A meiotic time-course for *Arabidopsis thaliana*. *Sexual Plant Reproduction* 16: 141-9
5. Armstrong SJ, Jones GH. 2003. Meiotic cytology and chromosome behaviour in wild-type *Arabidopsis thaliana*. *Journal of Experimental Botany* 54: 1-10
6. Baptista-Giacomelli FR, Pagliarini MS, de Almeida JL. 2000. Meiotic behavior in several Brazilian oat cultivars (*Avena sativa* L.). *Cytologia (Tokyo)* 65: 371-8
7. Barakate A, Higgins JD, Vivera S, Stephens J, Perry RM, et al. 2014. The synaptonemal complex protein ZYP1 is required for imposition of meiotic crossovers in barley. *The Plant Cell*
8. Baudat F, Buard J, Grey C, Fledel-Alon A, Ober C, et al. 2010. PRDM9 is a major determinant of meiotic recombination hotspots in humans and mice. *Science* 327: 836-40
9. Bennett MD, Finch RA. 1971. Duration of meiosis in barley. *Genetical Research* 17: 209-&

10. Bennett MD, Leitch IJ. 2005. Nuclear DNA amounts in angiosperms: Progress, problems and prospects. *Annals of Botany* 95: 45-90
11. Berchowitz LE, Copenhaver GP. Genetic Interference: Don't Stand So Close to Me. *Current Genomics* 11: 91-102
12. Bishop DK, Zickler D. 2004. Early decision: Meiotic crossover interference prior to stable strand exchange and synapsis. *Cell* 117: 9-15
13. Borde V, Robine N, Lin W, Bonfils S, Geli V, Nicolas A. 2009. Histone H3 lysine 4 trimethylation marks meiotic recombination initiation sites. *Embo Journal* 28: 99-111
14. Bowers JE, Arias MA, Asher R, Avise JA, Ball RT, et al. 2005. Comparative physical mapping links conservation of microsynteny to chromosome structure and recombination in grasses. *Proceedings of the National Academy of Sciences of the United States of America* 102: 13206-11
15. Buckler ES, Phelps-Durr TL, Buckler CSK, Dawe RK, Doebley JF, Holtsford TP. 1999. Meiotic drive of chromosomal knobs reshaped the maize genome. *Genetics* 153: 415-26
16. Calvente A, Viera A, Page J, Parra MT, Gomez R, et al. 2005. DNA double-strand breaks and homology search: inferences from a species with incomplete pairing and synapsis. *Journal of Cell Science* 118: 2957-63
17. Carpenter ATC. 1975. Electron-microscopy of meiosis in *Drosophila melanogaster* females: II: The recombination nodule - a recombination-associated structure at pachytene? *Proceedings of the National Academy of Sciences of the United States of America* 72: 3186-9
18. Caryl AP, Armstrong SJ, Jones GH, Franklin FCH. 2000. A homologue of the yeast HOP1 gene is inactivated in the Arabidopsis meiotic mutant *asy1*. *Chromosoma* 109: 62-71
19. Chelysheva L, Grandont L, Vrielynck N, le Guin S, Mercier R, Grelon M. 2010. An Easy Protocol for Studying Chromatin and Recombination Protein Dynamics during Arabidopsis thaliana Meiosis: Immunodetection of Cohesins, Histones and MLH1. *Cytogenetic and Genome Research* 129: 143-53
20. Chen Z, Higgins JD, Hui JTL, Li J, Franklin FCH, Berger F. 2011. Retinoblastoma protein is essential for early meiotic events in Arabidopsis. *Embo Journal* 30: 744-55
21. Choi K, Zhao X, Kelly KA, Venn O, Higgins JD, et al. Arabidopsis meiotic crossover hot spots overlap with H2A. Z nucleosomes at gene promoters. *Nature Genetics* 45: 1327-+
22. Colombo PC, Jones GH. 1997. Chiasma interference is blind to centromeres. *Heredity* 79: 214-27
23. Crismani W, Girard C, Froger N, Pradillo M, Luis Santos J, et al. 2012. FANCM Limits Meiotic Crossovers. *Science* 336: 1588-90
24. Darlington CD. 1931. Meiosis. *Biological Reviews and Biological Proceedings of the Cambridge Philosophical Society* 6: 221-64
25. de Boer E, Dietrich AJJ, Hoog C, Stam P, Heyting C. 2007. Meiotic interference among MLH1 foci requires neither an intact axial element structure nor full synapsis. *Journal of Cell Science* 120: 731-6
26. Dowrick G. 1957. The influence of temperature on meiosis. *Heredity* 11: 37-49
27. Draper J, Mur LAJ, Jenkins G, Ghosh-Biswas GC, Bablak P, et al. 2001. Brachypodium distachyon. A new model system for functional genomics in grasses. *Plant Physiology* 127: 1539-55
28. Emerson RA, Beadle GW, Fraser AC. 1935. A summary of linkage studies in maize. *Cornell Univ, Agric. Stn. Memoir* 180: 1-83
29. Erayman M, Sandhu D, Sidhu D, Dilbirli M, Baenziger PS, Gill KS. 2004. Demarcating the gene-rich regions of the wheat genome. *Nucleic Acids Research* 32: 3546-65
30. Falque M, Anderson LK, Stack SM, Gauthier F, Martin OC. 2009. Two Types of Meiotic Crossovers Coexist in Maize. *Plant Cell* 21: 3915-25

31. Ferdous M, Higgins JD, Osman K, Lambing C, Roitinger E, et al. 2012. Inter-Homolog Crossing-Over and Synapsis in Arabidopsis Meiosis Are Dependent on the Chromosome Axis Protein AtASY3. *Plos Genetics* 8
32. Fletcher HL. 1978. Localized chiasmata due to partial pairing - 3D reconstruction of synaptonemal complexes in male *Stethophyma grossum*. *Chromosoma* 65: 247-69
33. Fogwill M. 1958. Differences In Crossing-Over And Chromosome Size In The Sex Cells Of Lilium And Fritillaria. *Chromosoma* 9: 493-504
34. Fox DP. 1973. Control Of Chiasma Distribution In Locust, *Schistocerca gregaria* (Forsk.). *Chromosoma* 43: 289-328
35. Fuchs J, Demidov D, Houben A, Schubert I. 2006. Chromosomal histone modification patterns - from conservation to diversity. *Trends in Plant Science* 11: 199-208
36. Fung JC, Rockmill B, Odell M, Roeder GS. 2004. Imposition of crossover interference through the nonrandom distribution of synapsis initiation complexes. *Cell* 116: 795-802
37. Gauthier F, Martin OC, Falque M. CODA (crossover distribution analyzer): quantitative characterization of crossover position patterns along chromosomes. *Bmc Bioinformatics* 12
38. Higgins JD, Armstrong SJ, Franklin FCH, Jones GH. 2004. The Arabidopsis MutS homolog AtMSH4 functions at an early step in recombination: evidence for two classes of recombination in Arabidopsis. *Genes & Development* 18: 2557-70
39. Higgins JD, Perry RM, Barakat A, Ramsay L, Waugh R, et al. 2012. Spatiotemporal asymmetry of the meiotic program underlies the predominantly distal distribution of meiotic crossovers in barley. . *The Plant Cell* 24: 4096-109
40. Higgins JD, Sanchez-Moran E, Armstrong SJ, Jones GH, Franklin FCH. 2005. The Arabidopsis synaptonemal complex protein ZYP1 is required for chromosome synapsis and normal fidelity of crossing over. *Genes Dev.* 19: 2488-500
41. Higgins JD, Vignard J, Mercier R, Pugh AG, Franklin FCH, Jones GH. 2008. AtMSH5 partners AtMSH4 in the class I meiotic crossover pathway in Arabidopsis thaliana, but is not required for synapsis. *Plant Journal* 55: 28-39
42. Hollingsworth NM, Byers B. 1989. Hop1 - a yeast meiotic pairing gene. *Genetics* 121: 445-62
43. Hulten M. 1974. Chiasma Distribution At Diakinesis In Normal Human Male. *Hereditas* 76: 55-78
44. Jackson N, Sanchez-Moran E, Buckling E, Armstrong SJ, Jones GH, Franklin FCH. 2006. Reduced meiotic crossovers and delayed prophase I progression in AtMLH3-deficient Arabidopsis. *Embo Journal* 25: 1315-23
45. Jones G. 1987. Chiasmata. *Meiosis*: 213-44
46. Jones GH. 1978. Giemsa C-Banding Of Rye Meiotic Chromosomes And Nature Of Terminal Chiasmata. *Chromosoma* 66: 45-57
47. Jones GH. 1984. The control of chiasma distribution. *SEB Symposium* 38: 293-320
48. Jones GH, Franklin FC. 2006. Meiotic crossing-over: obligation and interference. *Cell* 126: 246-8
49. Keeney S, Giroux CN, Kleckner N. 1997. Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. *Cell* 88: 375-84
50. Kellogg EA. 1998. Relationships of cereal crops and other grasses. *Proceedings of the National Academy of Sciences of the United States of America* 95: 2005-10
51. Kleckner N. 2006. Chiasma formation: chromatin/axis interplay and the role(s) of the synaptonemal complex. *Chromosoma* 115: 175-94
52. Kleckner N, Zickler D, Jones GH, Dekker J, Padmore R, et al. 2004. A mechanical basis for chromosome function. *Proceedings of the National Academy of Sciences of the United States of America* 101: 12592-7

53. Knoll A, Higgins JD, Seeliger K, Reha SJ, Dangel NJ, et al. 2012. The Fanconi Anemia Ortholog FANCM Ensures Ordered Homologous Recombination in Both Somatic and Meiotic Cells in Arabidopsis. *Plant Cell* 24: 1448-64
54. Kunzel G, Korzun L, Meister A. 2000. Cytologically integrated physical restriction fragment length polymorphism maps for the barley genome based on translocation breakpoints. *Genetics* 154: 397-412
55. Kunzel G, Waugh R. 2002. Integration of microsatellite markers into the translocation-based physical RFLP map of barley chromosome 3H. *Theoretical and Applied Genetics* 105: 660-5
56. Ladizinsky G. 2012. Studies in oat evolution: A man's life with Avena. *Springer Briefs in Agriculture*: 1-87
57. Laurie DA, Jones GH. 1981. Interindividual Variation In Chiasma Distribution In Chorthippus-Brunneus (*Orthoptera, Acrididae*). *Heredity* 47: 409-&
58. Lhuissier FGP, Offenberger HH, Wittich PE, Vischer NOE, Heyting C. 2007. The mismatch repair protein MLH1 marks a subset of strongly interfering crossovers in tomato. *Plant Cell* 19: 862-76
59. Li X, Chang Y, Xin X, Zhu C, Li X, et al. Replication Protein A2c Coupled with Replication Protein A1c Regulates Crossover Formation during Meiosis in Rice. *Plant Cell* 25: 3885-99
60. Loidl J. 1989. Effects of elevated-temperature on meiotic chromosome synapsis in *Allium ursinum*. *Chromosoma* 97: 449-58
61. Lukaszewski AJ. 1992. A comparison of physical distribution of recombination in chromosome 1R in diploid rye and in hexaploid triticale. *Theoretical and Applied Genetics* 83: 1048-53
62. Lukaszewski AJ. 2008. Unexpected behavior of an inverted rye chromosome arm in wheat. *Chromosoma* 117: 569-78
63. Lukaszewski AJ, Curtis CA. 1993. Physical Distribution Of Recombination In B-Genome Chromosomes Of Tetraploid Wheat. *Theoretical and Applied Genetics* 86: 121-7
64. Lukaszewski AJ, Kopecky D, Linc G. Inversions of chromosome arms 4AL and 2BS in wheat invert the patterns of chiasma distribution. *Chromosoma* 121: 201-8
65. Marcon E, Moens P. 2003. MLH1p and MLH3p localize to precociously induced chiasmata of okadaic-acid-treated mouse spermatocytes. *Genetics* 165: 2283-7
66. Mayer KFX, Waugh R, Langridge P, Close TJ, Wise RP, et al. 2012. A physical, genetic and functional sequence assembly of the barley genome. *Nature* 491: 711-+
67. McClintock B. 1948. Mutable Loci in Maize. *Carnegie Institute of Washington Year Book* 47: 155-69
68. Mercier R, Grelon M. 2008. Meiosis in plants: ten years of gene discovery. *Cytogenetic and Genome Research* 120: 281-90
69. Mercier R, Jolivet S, Vezon D, Huppe E, Chelysheva L, et al. 2005. Two meiotic crossover classes cohabit in Arabidopsis: One is dependent on MER3, whereas the other one is not. *Current Biology* 15: 692-701
70. Mieczkowski PA, Dominska M, Buck MJ, Gerton JL, Lieb JD, Petes TD. 2006. Global analysis of the relationship between the binding of the Bas1p transcription factor and meiosis-specific double-strand DNA breaks in *Saccharomyces cerevisiae*. *Molecular and Cellular Biology* 26: 1014-27
71. Myers S, Bowden R, Tumian A, Bontrop RE, Freeman C, et al. 2010. Drive against hotspot motifs in primates implicates the PRDM9 gene in meiotic recombination. *Science* 327: 876-9
72. Naranjo T, Valenzuela NT, Perera E. 2010. Chiasma Frequency Is Region Specific and Chromosome Conformation Dependent in a Rye Chromosome Added to Wheat. *Cytogenetic and Genome Research* 129: 133-42
73. Newton WCF, Darlington CD. 1930. Firitillaria meleagris chiasmaformation and distribution. *Journal of Genetics* 22: 1-14

74. Nonomura KI, Nakano M, Eiguchi M, Suzuki T, Kurata N. 2006. PAIR2 is essential for homologous chromosome synapsis in rice meiosis I. *Journal of Cell Science* 119: 217-25
75. Oakley HA, Jones GH. 1982. Meiosis in *Mesostoma ehrenbergii ehrenbergii* (Turbellaria, Rhabdocoela) .1. Chromosoma pairing, synaptonemal complexes and chiasma localization in spermatogenesis. *Chromosoma* 85: 311-22
76. Osman K, Higgins JD, Sanchez-Moran E, Armstrong SJ, Franklin FCH. 2011. Pathways to meiotic recombination in *Arabidopsis thaliana*. *New Phytologist* 190: 523-44
77. Page SL, Hawley RS. 2004. The genetics and molecular biology of the synaptonemal complex. *Annual Review of Cell and Developmental Biology* 20: 525-58
78. Paigen K, Petkov P. 2010. Mammalian recombination hot spots: properties, control and evolution. *Nature Reviews Genetics* 11: 221-33
79. Pan J, Sasaki M, Kniewel R, Murakami H, Blitzblau HG, et al. A Hierarchical Combination of Factors Shapes the Genome-wide Topography of Yeast Meiotic Recombination Initiation. *Cell* 144: 719-31
80. Parvanov ED, Petkov PM, Paigen K. 2010. Prdm9 controls activation of mammalian recombination hotspots. *Science* 327: 835-
81. Perrella G, Consiglio MF, Aiese-Cigliano R, Cremona G, Sanchez-Moran E, et al. 2010. Histone hyperacetylation affects meiotic recombination and chromosome segregation in *Arabidopsis*. *Plant Journal* 62: 796-806
82. Phillips D, Nibau C, Wnetrzak J, Jenkins G. High Resolution Analysis of Meiotic Chromosome Structure and Behaviour in Barley (*Hordeum vulgare* L.). *Plos One* 7
83. Phillips D, Wnetrzak J, Nibau C, Barakate A, Ramsay L, et al. 2013. Quantitative high resolution mapping of HvMLH3 foci in barley pachytene nuclei reveals a strong distal bias and weak interference. *Journal of Experimental Botany* 64: 2139-54
84. Ronceret A, Pawlowski WP. 2010. Chromosome Dynamics in Meiotic Prophase I in Plants. *Cytogenetic and Genome Research* 129: 173-83
85. Smagulova F, Gregoret IV, Brick K, Khil P, Camerini-Otero RD, Petukhova GV. 2011. Genome-wide analysis reveals novel molecular features of mouse recombination hotspots. *Nature* 472: 375-8
86. Sturtevant AH. 1913. The linear arrangement of six sex-linked factors in *Drosophila*, as shown by their mode of association. *Journal of Experimental Zoology* 14: 43-59
87. Tease C. 1978. Cytological detection of crossing-over in BrdU substituted meiotic chromosomes using the fluorescent plus giemsa technique. *Nature* 272: 823-4
88. Tease C, Jones GH. 1978. Analysis of exchanges in differentially stained meiotic chromosomes of *Locusta migratoria* after BrdU-substitution and FPG staining .1. Crossover exchanges in monochiasmate bivalents. *Chromosoma* 69: 163-78
89. Thompson EA, Roeder GS. 1989. Expression and DNA sequence of RED1, a gene required for meiosis I chromosome segregation in yeast. *Molecular & General Genetics* 218: 293-301
90. Wallace BMN, Jones GH. 1978. Incomplete chromosome-pairing and its relation to chiasma localization in *Stethophyma grossum* spermocytes. *Heredity* 40: 385-&
91. Wang KJ, Wang M, Tang D, Shen Y, Qin BX, et al. PAIR3, an axis-associated protein, is essential for the recruitment of recombination elements onto meiotic chromosomes in rice. *Molecular Biology of the Cell* 22: 12-9
92. Wang M, Wang KJ, Tang D, Wei CX, Li M, et al. 2010. The Central Element Protein ZEP1 of the Synaptonemal Complex Regulates the Number of Crossovers during Meiosis in Rice. *Plant Cell* 22: 417-30
93. Watson JD, Callan HG. 1963. The form of bivalent chromosomes in newt oocytes at first metaphase of meiosis. *Quart. J. Micro. Sci.* 104: 281-95
94. Yuan WY, Li XW, Chang YX, Wen RY, Chen GX, et al. 2009. Mutation of the rice gene PAIR3 results in lack of bivalent formation in meiosis. *Plant Journal* 59: 303-15

95. Zickler D, Kleckner N. 1999. Meiotic chromosomes: Integrating structure and function. *Annual Review of Genetics* 33: 603-754