INTRODUCTION

Meiosis is the specialized cell division program that ensures that gametes carry the correct number of chromosomes. Wheat carries two copies (homologues) of each chromosome that are separated during meiosis resulting in the formation of haploid gametes. Before meiosis, each homologue is replicated, forming two sister chromatids that remain linked together. In order to achieve the correct segregation of chromosomes to each gamete, each chromosome (composed of two sister chromatids) must recognise its homologue from amongst all the chromosomes present in the nucleus. In the case of wheat, homologues need to be distinguished from homoeologues. Having recognised its partner, the two homologues must then intimately align along their entire lengths. As part of this alignment, a proteinaceous structure known as the synaptonemal complex (SC) is assembled between the homologues, in a process called synapsis. Within this structure, meiotic recombination is completed, resulting in the formation of crossovers between the DNA strands of the parental chromosomes, thereby reshuffling genetic information. Crossovers, together with sister chromatid cohesion, provide physical links (visualized as chiasma) that hold the homologues together after disassembly of the SC and allow the correct orientation of the centromeres of the homologues on the first meiotic spindle. The homologues are then correctly segregated via their centromeres during meiosis I and this is followed by the second meiotic division in which sister chromatids are segregated so that each gamete carries only a single copy of each chromosome. Therefore for correct segregation to occur, centromeres must pair homologously. Some species lack the ability to form chiasma between their chromosomes but can still pair their centromeres homologously enabling correct segregation to occur.

MODE OF ACTION

Over the last 50 years, as each new method of analysing meiosis in wheat has become available, it has been deployed to understand the mode of action of \( \text{Ph1} \). The first experiments as stated above exploited classical cytogenetics or scoring pairing configurations in squashed meiocytes at metaphase I. Riley showed that supernumerary chromosomes (B) could compensate for the absence of \( \text{Ph1} \) in interspecific hybrids by reducing chromosome pairing at metaphase I between the homoeologous chromosomes (Dover and Riley 1972). It was surprising that apparently “inert” lumps of heterochromatin which lacked gene activity could compensate for the \( \text{Ph1} \) locus. However a later study in maize revealed that synchronised replication of heterochromatin was disrupted by the presence of B chromosomes (Pryor et al., 1980). The implication was
that \textit{Ph1} affected in someway heterochromatin and replication.

The advent of the ability to visualise chromosome synapsis in hexaploid wheat enabled Holm to make three observations (Holm 1986; Holm and Wang 1988). First, he made a general observation about wheat meiosis, namely that the chromosomes started synapsing from the telomeres in wheat which were clustered in one location at the start of meiosis. As described above, we now know that chromosomes in many species are sorted into homologous pairs via their telomeres which group together to form a telomeric bouquet at the onset of meiosis. Holm also observed that in wild type wheat on average 5 of the 42 chromosomes engaged in multiple meiosis. Holm also observed that in wild type wheat on average 5 of the 42 chromosomes engaged in multiple associations but that this increased to more than 19 of the chromosomes engaging in multiple associations in the absence of \textit{Ph1}. At later meiotic stages in the presence of \textit{Ph1}, these associations were resolved or corrected while in the absence of \textit{Ph1}, such associations were maintained. Thus \textit{Ph1} reduced the initial level of homoeologous associations as chromosomes interacted at the onset of meiosis and secondly \textit{Ph1} affected the stringency at which associations were resolved later in prophase I.

The advent of RFLP analysis and the ability to generate RFLP genetic maps revealed that \textit{Ph1} could affect the stringency of recombination (Dubcovsky et al., 1995; Luo et al., 1996). The \textit{Ph1} locus was able to block recombination from occurring between similar but distinct chromosome segments located within otherwise identical chromosomes. This implied that \textit{Ph1} may be affecting the mismatch repair process which is involved in controlling the stringency at which recombination occurs. Thus there were essentially three earlier observations, \textit{Ph1} affected heterochromatin behaviour possibly via replication, \textit{Ph1} affected the initial associations of chromosomes at the onset of meiosis and finally \textit{Ph1} affected the stringency of recombination (possibly the mismatch repair process).

As described above, squashed preparations of meiocytes were and are routinely used to score chromosome pairing at metaphase I in wheat, where they provide an accurate score of the level of pairing, as the pairing at this stage is unaffected by the procedures used to generate the preparations. However such preparations also have been used to study early stages in meiosis and even in premeiosis. For me, the problem with using such “squashed” preparations to study these early stages is that the process of squashing disrupts the chromosome structures and initial interactions between chromosomes. Moreover cell types such meiocytes, tapetal and cell wall cells are different to distinguish after squashing particularly in premeiotic stages. Put simply- if a block of stone is dropped on your head and you are flattened, your head will pair with your feet when of course it should not normally do this! Thus the exploitation of such preparations has often led to contradictory observations with one study initially reporting altered chromosome structure after squashing (Mikhailova, et al., 1998; Maestra et al., 2002) only for a later study to indicate that there is in fact no altered structure (Kopecky et al., 2007). In collaboration with Peter Shaw, we have exploited cell biological approaches over the last 10 years to solve such problems and this has enabled us to study the effects of \textit{Ph1} on heterochromatin in more detail. Such approaches exploiting \textit{in situ} hybridisation, anther sectioning and confocal microscopy enables the behaviour of chromosomes to be analysed in 3D in all the cells in an anther. These 3-D projections can then be rotated so that the process can be visualized more easily from any angle. Examples of whole sections can be seen for each stage described below on the following websites (http://www.jic.ac.uk/staff/graham-moore/ and then go to meiosis gallery or http://www.jic.ac.uk/staff/peter-shaw/meiosisGallery .html). The studies showed that wheat chromosomes can also associate in pairs prior to meiosis during floral development by their centromeres (Martinez-Perez et al., 1999, 2001 and 2003; Prieto et al., 2004). The 42 centromeres of hexaploid wheat are visualised as 21 sites prior to meiosis, thus implying pairing of the centromeres. Also the 28 centromeres of tetraploid wheat are visualised as 14 sites prior to meiosis which again indicated pairing. The reduction in the number of centromeric sites prior to meiosis happens in the presence or absence of the \textit{Ph1} locus.

The paired sites in hexaploid and tetraploid wheat then reduce in just 7 sites in the meiocytes as the telomeres cluster into a bouquet. These 7 centromere sites then undergo some chromatin changes (the centromeric heterochromatin elongates) and resolve back to 21 sites in hexaploid wheat or 14 sites in tetraploid wheat. This centromere pairing is largely unaltered whether \textit{Ph1} is present or absent. We wanted to know whether the wheat centromeres were pairing homologously or homoeologously or just randomly prior to meiosis and at the 7 site stage at the onset of meiosis, but unfortunately there are no probes available which would specifically mark homologous and homoeologous wheat centromeres. However we assessed the randomness of centromere pairing prior to meiosis in a different way using wheat-rye hybrids in the presence and absence of \textit{Ph1} which have 21 homoeologous wheat centromeres and 7 rye centromeres (but no homologous centromeres). The 21 wheat centromeres reduce to 7 sites in all anther cells prior to meiosis whether \textit{Ph1} is present or absent. Thus as the telomeres cluster at the onset of meiosis, the wheat centromeres are as 7 sites. If premeiotic centromere pairing was random, different numbers of sites would be formed in every cell which is not the case. This premeiotic centromere pairing is non-random with each wheat centromere only able to pair with two of the 20 possible wheat centromeres in the cell. We have therefore proposed that wheat centromeres pair either homologously or homoeologously prior to meiosis. This pairing of centromeres independently from the rest of the chromosome may allow chromosomes to be segregated later in meiosis even if the chromosomes fail to
crossover. The rye centromeres pair with the 7 wheat sites prior to meiosis only in the presence of Ph1. Thus prior to meiosis, Ph1 is specifically affecting the behaviour of rye centromeres with respect wheat centromeres. Various studies, for example Corredor et al., 2007, have used rye centromeres as a marker for wheat centromere behaviour prior to meiosis, however unfortunately rye centromeres don’t mirror wheat centromere behaviour prior to meiosis. Premeiotic centromere pairing occurs during replication (Jasencakova et al., 2001). Therefore by implication, Ph1 is affecting the timing of replication of rye centromere regions with respect to wheat centromeres.

At the onset of meiosis as the telomere bouquet forms, the wheat centromeres are as 7 sites and each site contains a rye centromere whether Ph1 is present or absent. These 7 sites then undergo the chromatin structural change (the centromeric heterochromatin elongates) as observed in hexaploid and tetraploid wheat. The 7 sites resolve as 28 unpaired centromeres in elongates) as observed in hexaploid and tetraploid wheat. The 7 sites resolve as 28 unpaired centromeres in the presence of Ph1 and as 14 sites initially in the absence of Ph1, 7 of which contain a rye centromere. Later but still at the telomere bouquet stage, the rye centromeres are resolved from the 14 wheat sites. Thus after the chromatin changes at the onset of meiosis, Ph1 reduces the ability of homoeologous associations to occur between the wheat centromeres. So initially there were three observations connected with Ph1, namely Ph1 affected heterochromatin behaviour possibly via replication, Ph1 affected the initial associations of chromosomes and finally Ph1 affected the mismatch repair process in some way. Our centromere behaviour studies exploiting cell biology approaches provided further support for two of these observations. Ph1 is affecting replication as indicated by rye and wheat centromere behaviour and that Ph1 is reducing the ability to engage in homoeologous associations after a chromatin remodelling of centromere heterochromatin at the onset of meiosis. Importantly, this data also indicated that the chromatin remodelling stage at the onset of meiosis may have an important role.

At the onset of meiosis, chromosomes start the process of condensing. However the subtelomeric constitutive heterochromatin in maize and rye which is already condensed, undergoes chromatin remodeling and elongates as the telomeres cluster to form a bouquet (Bass et al., 1997; Prieto et al., 2005). The constitutive heterochromatin at the centromere and subtelomeric regions undergoes similar conformation changes at a time when the rest of the chromosomes are initiating the condensation process. Recent data indicates that subtelomeric heterochromatin must undergo these conformational changes at the onset of meiosis in order for pairing and recombination to occur between chromosomes. In the presence of Ph1, the heterochromatin must be identical or near identical for the chromatin remodeling of the heterochromatin to occur on both homologues (Prieto et al., 2004; Colas, et a.l., 2008). If the heterochromatin shows too much divergence then they are unable to remodel and therefore the homologues are unable to pair and recombine via their telomere regions. The implication is that the homoeologous segments are unable to undergo remodeling in the presence of Ph1 and therefore do not pair and recombine in contrast to the homologous segments. However, in the absence of Ph1, all related heterochromatin can remodel without the requirement for identical or near identical heterochromatin on another chromosome. Thus Ph1 is diploidising the behaviour of heterochromatin. In the presence of Ph1, only near or identical heterochromatin can remodel. Since near or identical heterochromatin is found on homologues, only heterochromatin on homologues will be remodeled simultaneously. In the hybrids between wheat and related species, where there are no homologues, in the absence of Ph1, related heterochromatin is remodeled synchronously and the chromosomes pair, while in the presence of Ph1, heterochromatin does not undergo this synchronized remodeling and the chromosomes don’t pair. The presence of B chromosomes which can compensate for absence of Ph1 in wheat hybrids, disrupt the synchronized replication of related heterochromatin (Pryor et al., 1980). Thus Ph1’s effect on heterochromatin links the process of chromatin remodeling with replication. Does Ph1 affect the behaviour of heterochromatin along the rest of the chromosome arms? Studies reveal that the initiation of condensation is more coordinated in the presence than the absence of Ph1 (Prieto et al., 2004). This effect on coordination of initiation of condensation may simply reflect a change in the coordination of an earlier process, namely premeiotic replication. Thus true homologues can initially be in different conformational states in the absence of Ph1. This increases the chance of homoeologous pairing associations initially. At later stages, the condensation of the homologues is similar. The diploidisation of heterochromatin and the coordination of the initial phases of condensation explain why there are fewer associations between multiple chromosomes observed in Holm’s synopsis studies in the presence of Ph1 than its absence. In summary, the Ph1 locus affects a process during replication as revealed by centromere behaviour, heterochromatin remodeling, coordination of the initiation of condensation (which may be linked also to replication) and the mismatch repair process during meiosis.

**WHAT IS Ph1?**

Recent molecular analyses have defined the Ph1 locus to a region containing a cyclin-dependent kinase complex (Cdk2-like genes) related to Cdk2 from humans and mouse which has been disrupted by the insertion of a segment of subtelomeric heterochromatin (Griffiths, et al., 2006; Al-Kaff et al., 2007). Cdk2 has been extensively studied because it controls chromatin remodeling at replication (Cohen et al., 2006). Cdk2 has also been shown to control the remodeling of heterochromatin by altering the binding of the
heterochromatin protein (HP1) to such regions (Hale, et al., 2006). This change in binding means that the heterochromatin "elongates". However importantly for meiotic studies, it has been recently shown that disruption of Cdk2 has a major effect on meiosis resulting in sterility and non-homologous synapsis. More recently still, Cdk2 has been shown to recruit mismatch repair proteins to double stand breaks during early stages of meiosis (Ward, et al., 2007). These observations on Cdk2 function are similar to that described above for the Ph1 locus, namely in affecting replication, chromatin remodeling and mismatch repair system.

The 5B Cdk2-like gene complex is also suppressing expression of the corresponding Cdk2-like loci on 5A and 5D in bread wheat (Al-Kaff et al., 2007). The 5B Cdk2-like complex contains pseudogenes which are being transcribed. The presence of multiple copies of Cdk2-like genes including expressed pseudogenes may explain why it is difficult to generate mutant Ph1 phenotypes with EMS treatments which only yield point mutations. Transcriptional analysis from the homoeologous genes reveals that most of it comes from the B genome genes. If these genes are deleted, then the loss of transcription is compensated by an increase in transcription from either or both on the genes in the 5A or 5D genomes. Deletion of the 5B Cdk2-like locus therefore results in activation of the 5A and 5D Cdk2-like loci. Thus the 5B locus is dominant with respect to the 5A and 5D loci. Feldman reported that chromosome 5A and 5D may carry homoeologous loci related to the Ph1 locus which affect chromosome pairing (Feldman, 1966). Deletion of 5D or multiple copies of the 5A chromosome could affect the regular pairing observed in wheat. The progenitor of the B genome of hexaploid and tetraploid wheat is likely to be related to Ae. speltoides. However its genome does not carry anything which can substitute or compensate for Ph1 activity on 5B. As Feldman (1966) proposed, it is likely that Ae. speltoides and T. urartu produced a hybrid exhibiting homoeologous pairing. Therefore a polyploidisation event on 5B gave rise to the Ph1 locus which suppresses homoeologous pairing activity.

Further dissection of the region containing the Cdk2-like locus and heterochromatin is required. This would assess the involvement of all the Cdk2-like genes (seven of them) in the 5B locus in producing the Ph1 phenotype, which of the 5B copies suppresses the expression of those in the 5A and 5D loci, whether the Ph1 mutant phenotype involves a contribution from 5A and 5D loci or whether it simply reflects the loss of the 5B locus, and finally the role of the segment of heterochromatin. However at present, it is difficult to envisage how to further dissect the 5B region as well as the 5A and 5D regions. Identification of more deletion breakpoints which fall within the 5B region would require screening mutagenised populations of more than half a million individuals in size in order to generate deletion breakpoints distributed every 250 Kb. This also assumes that the distribution of breakpoints will be random. However the presence of the segment of heterochromatin may affect the occurrence of breakpoints both within the 5B locus and in the flanking regions. There are a total of 14 Cdk2-like genes on 5B, 5A and 5D. Therefore it is also difficult to envisage mutagenising each Cdk2-like gene individually through EMS treatment and then recombining different combinations of mutagenised genes and assessing the resulting effect on the Ph1 phenotype. Equally exploiting a RNAi based approach would knock out all expression of copies with the 5A, 5B and 5D loci and could well lead to a gross phenotype similar to that observed in mice, namely gross disruption of meiosis and sterility rather than the subtle Ph1 effect observed with deletion just the 5B locus. It also does not dissect the contribution of individual Cdk2-like genes in the locus. If it is possible to design methods that selectively silence the whole 5B Cdk2-like complex rather than the 5A or 5D loci, then transgenic plants expressing dsRNA targeting the 5B locus could be generated. Such constitutive silencing of the 5B locus must also be accompanied by the activation of the 5A and 5D loci whose overexpression may be contributing to the Ph1 mutant phenotype rather than a silencing of all Cdk2-like expression from the 5A, 5B and 5D loci. For breeding purposes, ideally one would like to be able to switch off Ph1 in hybrid plants between wheat and a wild relative and then switch it back on in the BC plants so that stable recombinant lines could be recovered. If Ph1 Cdk2-like genes do function similar to CDK2 then it should be possible to design drugs which could be delivered directly into the immature inflorescence of the hybrid plant, thereby inhibiting Ph1 function only during meiosis. There are many compounds which are known to activate CDK2 in mammals and yeast, such chemicals could be delivered into the inflorescence and then scored for the ability to reproduce the Ph1 mutant phenotype. This approach could have a major impact on breeding strategies as it may not just affect homoeologous recombination but also homologous recombination as well. The alternative approach is to exploit the suppressors of Ph1 activity which have been described in the genome of A. speltoides (Dvorak et. al., 2006). When the chromosomes carrying these loci are introduced into wheat, they induce homoeologous pairing even in the presence of the Ph1 locus. The identification of genes responsible may allow them to be exploited to regulate Ph1 expression and hence pairing and recombination in wheat.

REFERENCES


