Homologous chromosome pairing in wheat

Enrique Martínez-Pérez, Peter Shaw, Steve Reader, Luis Aragón-Alcaide*, Terry Miller and Graham Moore†

John Innes Centre, Colney, Norwich NR4 7UH, UK
*Present address: NIH, Bethesda, Maryland 20892, USA
†Author for correspondence (e-mail: foote@bbsrc.ac.uk)

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SUMMARY

Bread wheat is a hexaploid (AABBDD, 2n=6x=42) containing three related ancestral genomes, each having 7 chromosomes, giving 42 chromosomes in diploid cells. During meiosis true homologues are correctly associated in wild-type wheat, but a degree of association of related chromosomes (homoeologues) occurs in a mutant (ph1b). We show that the centromeres are associated in non-homologous pairs in all floral tissues studied, both in wild-type wheat and the ph1b mutant. The non-homologous centromere associations then become homologous premeiotically in wild-type wheat in both meiocytes and the tapetal cells, but not in the mutant. In wild-type wheat, the homologues are colocalised along their length at this stage, but the telomeres remain distinct. A single telomere cluster (bouquet) is formed in the meiocytes only by the onset of leptotene. The sub-telomeric regions of the homologues associate as the telomere cluster forms. The homologous associations at the telomeres and centromeres are maintained through meiotic prophase, although, during leptotene, the two homologues and also the sister chromatids within each homologue are separate along the rest of their length. As meiosis progresses, first the sister chromatids and then the homologues associate intimately. In wild-type wheat, first the centromere grouping, then the bouquet disperse by the end of zygotene.

Key words: Wheat, Chromosome pairing, Centromere, Telomere, Ph1

INTRODUCTION

Chromosome pairing has been studied in detail in budding yeast, fission yeast, maize and humans (Dawe et al., 1994; Bass et al., 1997; Scherthan et al., 1996, 1998; Chikashige et al., 1997; Weiner and Kleckner, 1994). The chromosomes of hexaploid wheat (Abranches et al., 1998) and other Triticeae, budding yeast (Jin et al., 1998) and fission yeast (Chikashige et al., 1997) are organised in the Rabl configuration in interphase cells, with their centromeres clustered at one pole and telomeres spread around the other pole. However, this is not the case for humans (Scherthan et al., 1996), maize (Dong and Jiang, 1998) and many other species. In all studied species, a telomere clustering or bouquet is observed during meiotic prophase (Scherthan et al., 1996; Bass et al., 1997). In maize, humans and budding yeast (Trelles-Sticken et al., 1999), the bouquet occurs during the transition between leptotene and zygotene. In maize and humans, where there is no pre-meiotic association of homologues, this is suggested to be the first stage of homologue pairing.

Bread wheat, Triticum aestivum (AABBDD, 2n = 6x = 42) has seven pairs of chromosomes derived from three ancestral diploid progenitors (i.e. it is an allopolyploid). In contrast, maize is proposed to be either a degenerate autotetraploid or a segmental allopolyploid, having arisen through a whole genome duplication event (Skrabanek and Wolfe, 1998). Although each chromosome of hexaploid wheat has the potential to pair with either its homologue or one of the four equivalent chromosomes from the other two genomes (homoeologues), chromosome pairing is in fact largely restricted to homologues. Thus hexaploid wheat behaves as a diploid with 21 bivalents observed at metaphase 1 (Riley and Chapman, 1958; Sears, 1976). The major locus (Ph1) controlling this pairing behaviour is located on chromosome 5B. A mutant (ph1b) carrying a deletion of the Ph1 locus exhibits a degree of pairing of homoeologous chromosomes and hence multivalent formation at metaphase 1 (Sears, 1976). However, most chromosomes are still observed as bivalents at metaphase 1 in the ph1b mutant and it is stable for several generations. In wheat hybrids where the opportunity for homologue pairing does not exist, homoeologues pair and synapse in either the presence or absence of the Ph1 locus (Gillies, 1987). Thus the Ph1 locus must function by actively promoting homologous pairing, and cannot simply be preventing homoeologous pairing.

Studies on chromosome pairing in yeast, humans or Drosophila have been able to use single copy probes (Weiner and Kleckner, 1994; Fung et al., 1998; Scherthan et al., 1996) to follow the pairing of individual sites on pairs of homologous chromosomes as against non-homologous chromosomes. This approach is not possible for hexaploid wheat. In the simplest case, for each pair of genes on the homologues, there are four genes on the two homoeologous pairs. Therefore, up to six sites will be visualized by in situ methods, with no means of
assessing whether any observed association involves the genes on homoeologues or homologues. In practice, the situation is more complicated; detailed comparative mapping reveals that many regions in wheat, including the region covering the Ph1 locus, are not only found on the four homoeologues, but are duplicated on other ‘non-homologous’ chromosomes (chromosome groups 1, 2 and 7 in the case of the Ph1 region; Foote et al., 1997). Thus any associations of related genes could potentially involve 24 different relatives located on homologous, homoeologous and non-homologous chromosomes.

Because of the polyploid genome in wheat, it is possible to substitute or add single chromosome pairs from other related species (listed in Shepherd and Islam, 1988). For example, many modern feed wheats possess a pair of rye chromosome arms. We have taken advantage of wheat carrying either rye or barley chromosomes to study chromosome dynamics during meiosis by fluorescence in situ hybridization and confocal microscopy. We have determined the behaviour of centromeres, telomeres and three different pairs of labelled chromosomes, allowing us to formulate a detailed scheme for meiotic homologue pairing in wheat.

MATERIALS AND METHODS

Wheat genotypes

Anthers used in this study came from 6 different genotypes: Ph1/Ph1 wheat (Triticum aestivum, cv Chinese Spring), termed wild-type wheat below; ph1b/ph1b homozygous mutant, termed ph1b mutant below (Sears, 1976); addition lines 3RS and 1RL (hexaploid wheat carrying an extra pair of 3RS or 1RL rye (Secale cereale) telocentric chromosomes) (listed in Shepherd and Islam, 1988, 1RL: Sears P60-28.2-1; 3RS: Sears P67-29.2-4); a 3RS addition line carrying a single 3RS rye telocentric chromosome; substitution line 5H inaccessible (hexaploid wheat with the 5A pair of chromosomes substituted by a pair of 5H accessible wild barley (Hordeum chilense) chromosomes. All experiments described were carried out on summer (May-August) grown plants.

Anther sections

Anther sections were prepared as described by Aragon-Alcaide et al. (1996). Whenever barley or rye total genomic DNA was used as a probe, sonicated wheat DNA was used as blocking DNA. If only centromeric and telomeric probes were used, salmon sperm DNA was used as blocking DNA. The hybridization mix was denatured at 100°C for 7 minutes, cooled on ice for 5 minutes and then immediately added to the sections. The slides were placed in a modified thermocycler (Omnislide; Hybaid Ltd, Long Island, NY). Denaturation was carried out at 77°C for 10 minutes, and then hybridization overnight at 37°C. Post-hybridization washes were carried out at 42°C with 20% formamide in 0.1x SSC for ten minutes. Probes were labelled with digoxigenin-11-dUTP (Boehringer Mannheim Corp., Indianapolis, IN) or biotin-16-dUTP (Boehringer Mannheim). Probes labelled with digoxigenin were detected using fluorescein isothiocyanate (FITC)-conjugated sheep anti-digoxigenin antibody (Boehringer Mannheim), and biotin-labelled probes were detected with extravidin-Cy3 (Sigma Chemical Co.). Both antibodies were prepared in 4x SSC, 0.1% Tween-20, 5% BSA. Antibody incubations were carried out for 1 hour in a humid chamber at 37°C. After 3 washes with 4x SSC, 0.1% Tween-20, slides were counterstained with 4',6-diamidino-2-phenylindole (DAPI; Sigma), and then mounted in antifade solution (Vectorshield; Vector Laboratories Inc., Burlingame, CA). In the case of the root squashes, in situ hybridization was carried out as described by Aragon-Alcaide et al. (1996).

Confocal, fluorescence microscopy and imaging processing

All the slides were checked after in situ hybridization in a Nikon Microphot-SA fluorescence microscope (Nikon Inc., Melville, NY). Confocal optical sections stacks were collected using a Leica TCS SP confocal microscope (Leica Microsystems, Heidelberg GmbH, Germany) equipped with a Krypton and an Argon laser. All the DAPI confocal images were collected using a Bio-Rad MRC-1000 confocal microscope (Bio-Rad Laboratories, Hercules, CA) equipped with an UV laser. Images were then transferred to a Macintosh computer, and composite images were produced using Photoshop (Adobe Systems Inc., Mountain view, CA) or NIH image (public domain program for the Macintosh available via anonymous ftp from zippy.nimh.nih.gov).

RESULTS

Staging of meiocyte development

Meiocytes are visually indistinguishable from the neighbouring somatic cells until a tapetum forms around the developing meiocytes. Early in premeiotic interphase, the meiocytes contain two or more nucleoli and have a similar
volume to the surrounding somatic cells. Determination of the timing of pre-meiotic S phase is problematic (Bennett et al., 1973, 1979), but the identification of sister chromatids can provide an unequivocal marker for completion of S phase. Leptotene is classically defined as the stage at which lateral element formation occurs between the sister chromatids. By leptotene there is only a single nucleolus, which is located adjacent to the nuclear membrane and the nucleus is significantly larger than somatic cell nuclei. Squashing of the meiocytes at this stage reveals that the chromatin is starting to become thread-like. By zygotene, the nucleolus is flattened against the nuclear membrane and the tapetal cells are binucleate (Bennett et al., 1973, 1979). By the end of zygotene, the central element of the synaptonemal complex has formed and synopsis is complete.

### Premeiotic chromosome alignment

Fig. 1 shows different premeiotic stages of meiocyte development from a wheat genotype which carries an additional pair of rye telocentric (1RL) chromosomes. The presence of a sub-telomeric heterochromatin knob on this rye chromosome allows easy identification of the distal telomere. In the earliest stages, the two homologous chromosomes are separated, as they are in all other somatic cells we have examined (Fig. 1a,b). Next, the centromeres associate, giving a V-shaped configuration (arrow in Fig. 1a). Finally, the two chromosomes colocalise along their entire length (Fig. 1b). However, the two telomeric heterochromatin knobs remain distinct, implying that the chromosomes are not intimately associated. Throughout these stages, the chromosomes remain in a typical interphase configuration (Abranches et al., 1998). These results confirm and extend our previous observations on a wheat genotype containing a pair of homologous wild barley 5H<sup>th</sup> chromosomes (Aragon-Alcaide et al., 1997b). Premeiotic pairing in wheat has also been confirmed by the studies by Schwarzer (1997) and Michailova et al. (1998). In the Ph1 mutant, this pre-meiotic association is at a much lower level (Aragon-Alcaide et al., 1997b).

### Centromere and telomere dynamics

In all somatic wheat cells so far examined, the centromeres are grouped at the nuclear periphery at one pole of the nucleus and the telomeres are spread around the opposite side of the nucleus, in a typical Rabl configuration (Abranches et al., 1998). In the early stages of meiocyte development, as shown in Fig. 1, this centromere/telomere organization is maintained in both wild-type and ph1b mutant wheat (Fig. 2a,b). Later, the telomeres begin to cluster to form a single telomere bouquet, at the opposite pole to the centromere cluster. In wild-type wheat, the centromeres remain grouped at the nuclear membrane during telomere clustering (Fig. 2c), whereas in the ph1b mutant, the centromeres disperse from the membrane during telomere clustering (Fig. 2d). At the stage when the bouquet is fully formed, the centromeres remain grouped on the membrane in the wild-type wheat, but have completely dispersed in the ph1b mutant (Fig. 2e,f). From the staging defined by Bennett et al. (1973, 1979) telomere clustering would occur in wild-type wheat during late pre-meiotic interphase and in the ph1b mutant during early meiotic prophase. Later during meiotic prophase, both telomeres and centromeres disperse (Fig. 2g-j).

Using 3-D data stacks we counted the numbers of telomeres and centromeres, both in meiocytes at different stages and in surrounding tapetal and other somatic cells in the flowers (Table 1). Although hexaploid wheat has 42 centromeres, all the cells examined from floral tissue, whether somatic cells or meiocytes, or from wild-type or ph1b mutant, had close to 21 centromere sites. The number of centromere sites remained unchanged through to meiotic prophase (Table 1). Seedling roots displayed the diploid number of 42 centromere sites (data not shown). At present we do not know at which precise stage of development the centromeres reduce to 21. However, the presence of only 21 centromeric sites in floral tissue implies the centromeric regions are associated in pairs.

In contrast to the centromeres, during premeiotic interphase, telomeres remain spread around the opposite pole of the

### Table 1. Average number of centromeres and telomeres in different cell types from wheat flowers

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<th>Somatic cells</th>
<th>Meiocytes</th>
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<tr>
<td></td>
<td>Non tapetal</td>
<td>Tapetal</td>
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<tr>
<td></td>
<td>Cent. Tel.</td>
<td>Cent. Tel.</td>
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<tr>
<td>Wild-type wheat</td>
<td>20.7 (2.3)</td>
<td>19.9 (1.5)</td>
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<tr>
<td>ph1b mutant</td>
<td>22.5 (2.1)</td>
<td>21.9 (2.1)</td>
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3-D focal section stacks from 15 individual cells were analysed for each cell type or stage. Standard deviation is given in brackets.

*In all cases indicated, the number of telomere sites observed in 100% of the cells was greater than 60.
nucleus to the centromeres, but unassociated with each other (of the 84 telomeres present, more than 60 could be counted in every nucleus examined, Table 1). Formation of the bouquet in wild-type wheat is completed in most meiocytes at the two

**Fig. 2.** Fluorescence in situ hybridization to centromeres (green) and telomeres (red) of wheat floral tissue at different stages. Chromatin was counterstained with DAPI. Confocal image stacks were recorded with a section spacing of 1 μm. In the panels shown here 5-15 sections have been projected to give a clearer view of the centromere and telomere distributions, but it is not possible to show the entire depth of the nuclei of interest without obscuration from overlying and underlying cells. The counting of telomeres and centromeres was carried out on the original 3-D focal section stacks. In each panel a region of the anther section including the developing meiocytes and some of the surrounding tapetum is shown. A single meiocyte from the section is inset, and one or more single DAPI sections from the same meiocyte are shown in order to demonstrate the nuclear chromatin organization. The nucleoli, visible as dark holes in the DAPI image are indicated by arrowheads. Bar, 5 μm, for all panels. (a) Wild-type wheat at an early pre-meiotic stage. Meiocytes are barely distinguishable. The nuclei display a clear Rabl configuration with centromeres clustered at one nuclear pole, and telomeres at the opposite pole. Three nucleoli are visible in the inset cell, the number of centromeres is haploid and the number of telomeres is diploid (not all visible on this projection). (b) Equivalent stage in the ph1b mutant. Two nucleoli present, at different depths, Rabl configuration with a haploid number of centromeres and a diploid number of telomeres. (c) Wild-type wheat at a slightly later stage than a. Rabl configuration maintained, telomeres beginning to cluster. Two nucleoli, haploid number of centromeres. (d) ph1b mutant. Telomeres are beginning to cluster, centromeres (haploid number) have declustered. One central nucleolus is present. (e) Wild-type wheat at a later stage than c, showing a tight telomere bouquet at the opposite pole to the centromeres, which remain clustered at the nuclear periphery (haploid number). Two nucleoli, indicating pre-meiotic interphase. (f) ph1b mutant. Fully formed telomere bouquet. Centromeres (haploid number) dispersed. Nucleolus against nuclear periphery, indicating late pre-meiotic interphase/early leptotene. (g) Meiocyte from wild-type wheat at later stage than e showing completely dispersed telomeres and centromeres (both in a haploid number). (h) DAPI image of the meiocyte shown in g. A single nucleolus is flattened against the nuclear periphery (arrowhead). (i) ph1b mutant meiocyte at the same stage as g. Centromeres and telomeres are dispersed and in haploid number. (j) DAPI image of i.
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nucleoli stage (31 out of 50 nuclei examined) when the centromeres are still located in 21 pairs at the other pole. In the ph1b mutant it is completed in meiocytes at the single nucleolus stage (41 nuclei out of 50 examined) when their centromeres are dispersed.

**Behaviour of homologous chromosomes during meiotic prophase**

To relate the association of homologues with telomere clustering, we carried out double labelling on the wheat genotype carrying a pair of 3RS chromosomes (Fig. 3). Of 20 nuclei examined containing a tight telomere cluster, all possessed a single rye heterochromatin knob, indicating association of the homologues in the distal regions at the onset of meiotic prophase. Fig. 3a shows two meiocytes in different stages of telomere clustering. In the left hand meiocyte, clustering has just begun. The two telomeric heterochromatin knobs are apart, although within the developing cluster. In the right hand meiocyte, the cluster is fully formed and the two knobs have fused together. In the right hand meiocyte in Fig. 3a, the two homologues are colocalised for most of their length, but in some regions there is clearly more than one strand visible.

**Fig. 3.** Confocal sections from 3RS anthers labelled with rye genomic DNA (green), and telomeres (red). The sections are shown in consecutive order and the last panel of each series is the projection of the whole stack. Because of the overlapping of the red and the green channel when the signals are very strong, the heterochromatin knobs present in the telomere cluster appear as yellow signals. (a) Two meiocytes at different stages of the telomere cluster. On panel 7, the arrow indicates two DNA threads visible. In panel 8 (projection) the two heterochromatin knobs on the left hand meiocyte are clearly separated (arrowheads), the telomere cluster is still forming, and the two homologous chromosomes are separated along most of their length. In the right hand side meiocyte the telomere cluster is very tight, the two knobs are only seen as one structure, and the two homologous chromosomes are more condensed than the left meiocyte, and colocalize along their length. In b and c the consecutive sections only shows the green channel (rye chromosome), and the projection shows the green and the red (telomeres) channels. (b) Single meiocyte displaying a telomere cluster. The two homologues appear as a circular structure, linked at their centromeres and telomeres. At some points (indicated by arrows in panels 3 and 5) the two sister chromatids can be observed. On panel 6 the two heterochromatin knobs can still be identified individually (arrowheads). On the top right hand corner the telomere cluster, with a single heterochromatin knob, from another meiocyte can be seen. (c) Single meiocyte at a later stage of the telomere cluster than in b. The two knobs are visible as a single site, and in the distal regions the two homologues appear as a single thick thread. The two sister chromatids of both homologues can be seen in the proximal region (arrows in panels 5 and 9). Section spacing 1 μm. Bars, 5 μm.
In Fig. 3b, the two telomeric knobs are very close together (arrowheads in panel 6). The two homologues then run apart along different sides of the nucleus, and then must join at their centromeres since there are no breaks in the labelled structure, and we have already shown that the centromeres at this stage are located at the opposite pole of the nucleus in pairs (Fig. 2). In some places along the chromosomes, two strands are visible, indicating the presence of sister chromatids after DNA replication (arrows in panels 3 and 5). Fig. 3c shows another example, but with the homologues more closely associated. In the region near the telomere cluster, only one thick strand is visible, but further along four separate strands can be clearly seen (arrows in panels 5 and 9).

To demonstrate that the individual strands must correspond to sister chromatids, we also studied a wheat genotype carrying a single telocentric rye chromosome (3RS), rather than a homologous pair. Fig. 4a shows somatic root tip cells from this line, confirming the presence of only a single rye chromosome. Fig. 4 b-d shows different examples of the single rye chromosome at the telomere cluster stage. In all cases, two strands are visible for at least part of the length of the chromosome, and in Fig. 4d the two chromatids are separated along their whole length, and only joined at the centromere and telomere.

Finally, Fig. 5 shows a wheat genotype containing a pair of wild barley 5H<sup>ch</sup> metacentric chromosomes at the stage when the telomeric bouquet is beginning to decluster, and the centromeres have already dispersed from the membrane (arrows in panel 10). The homologues are intimately associated along virtually their entire length. The telomeres from the two arms are located in different parts of the telomere cluster.

**DISCUSSION**

We have shown that centromeres associate in non-homologous pairs in wheat in all floral tissues, and that in wild-type wheat, but not in ph1b, these associations become homologous pre-meiotically in tapetum and meiocyte precursors. Telomeres are not associated prior to meiosis, but are brought together into a single bouquet at the onset of leptotene, resulting in the association of homologous telomeric and sub-telomeric...
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Thus both centromeres and telomeres are clustered at opposite nuclear poles, in an extension of the interphase Rabl configuration. This configuration and the centromere and telomere associations are maintained through to the completion of homologue association at the end of zygotene, but at the onset of leptotene, both sister chromatids and homologues are visibly separated along the rest of their length. Our results are based on the analysis of all wheat centromeres and telomeres and on lines containing either rye telocentric or barley metacentric chromosomes. All these probes show a consistent behaviour, so we are confident that the rye and barley chromosomes behave in a similar way to the endogenous wheat chromosomes.

Some of the data presented contradict results reported by previous studies using spread and squashed preparations. For instance, Michailova et al. (1998) did not observe pre-meiotic association of homologues in tapetal cells. However, the results here confirm our previous observations that homologues in both pre-meioocytes and tapetal cells associate via initial centromere contacts. We can attribute the contradictions to a difference in the techniques used. It has been shown both by Bennett et al. (1979) and by Aragon-Alcaide et al. (1998) that it is essential to examine structurally well-preserved material, both to be confident of the 3-D preservation of delicate nuclear structure, and to be confident of the cell types which are being analysed. For example, tapetal cells and meiocytes can be misclassified, and polarised centromere or telomere clusters can easily be displaced or brought together artefactually by the process of squashing. We overcame these problems by using anther sections, which preserves 3-D structure and allow easy classification of cell types based on their position in the anther.

We show a detailed scheme for premeiotic and meiotic regions. Thus both centromeres and telomeres are clustered at opposite nuclear poles, in an extension of the interphase Rabl configuration. This configuration and the centromere and telomere associations are maintained through to the completion of homologue association at the end of zygotene, but at the onset of leptotene, both sister chromatids and homologues are visibly separated along the rest of their length. Our results are based on the analysis of all wheat centromeres and telomeres and on lines containing either rye telocentric or barley metacentric chromosomes. All these probes show a consistent behaviour, so we are confident that the rye and barley chromosomes behave in a similar way to the endogenous wheat chromosomes.

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We show a detailed scheme for premeiotic and meiotic
centsomeres can pair in the presence of the non-homologous telocentric chromosomes can fuse at their stage is represented in Fig. 6a. Sears (1972) demonstrated that development, the initial pairing must be non-homologous. This since this occurs before homologues are associated in floral development, occasionally one can count fewer than 21 sites, with some sites being brighter than the others. This implies that associations of more than two centromeres can occur. We do not as yet know at what stage this pairing occurs, or whether it is between unrelated or homoeologous centromeres. Later, these centromere associations become homologous in both the meiocytes and tapetal cells, implying a sorting mechanism, and an intermediate V-shaped chromosome arrangement is often observed (Fig. 6b). The homologous chromosomes become colocalised along their length, but, unlike the centromeres, the telomeres and heterochromatin knobs remain distinct (Fig. 6c), since the diploid number of telomere sites (more than 60) are seen. Pre-meiotic association of homologous chromosomes and pairing of centromeres has also been recently supported by the observation that pre-meiotic treatment with colchicine disrupts chromosome pairing in wild-type wheat (Vega and Feldman, 1998). A haploid number of discrete centromere sites have also been observed in the diploid Lilium during pre-meiotic interphase (Suzuki et al., 1997), suggesting a similar centromere pre-association mechanism.

Then, in the meiocytes only, the telomeres cluster to form a bouquet by the onset of meiotic prophase. Homologous telomeres are brought together as the bouquet forms. By the time the telomeres cluster, the chromosomes have replicated, and both sister chromatids and homologues are often visibly separated along their length, although they remain associated at centromeres and telomeres (Fig. 6d). Since we have used a telocentric chromosome to monitor the separation, it might be suggested that the two telomeres have both become located within the telomere bouquet, resulting in a ‘loop-back’ configuration. This is unlikely for several reasons. First, we have never observed the centromere of the telocentric chromosome at the telomere cluster, which would be implied by this explanation. Second, the chromosome remains linear with the heterochromatic knob end in the telomere cluster and the other end in the centromere cluster region, and there is no sign of a looped conformation. Third, the telocentric chromosome stretches across the nucleus, as do all the other chromosomes, and so, if looped back, would have to stretch to twice its normal length.

The sister chromatids then associate from the distal regions, followed by the intimate association of the homologues (Fig. 6e). The most obvious interpretation of these observations is that sister chromatids associate as the lateral elements are inserted, and homologues associate as central elements are formed. A conclusion from our work is that the central elements can be formed before lateral element formation is complete, and that these two processes, which are usually regarded as separate stages of meiotic prophase in other species, may be a continuum in wild-type wheat. Dawe et al. (1994) have shown previously that during the leptotene/zygotene transition stage, sister chromatids partially separate at a time of intimate homologue association in maize. This suggests this phenomenon may be widespread.

Gillies (1987) noted the difficulty of obtaining synaptonemal complex spreads from wild-type wheat compared to the phlb mutant and concluded that the spreading was causing breakage of the lateral elements. However an alternative explanation, which is suggested by our results, is that the lateral element formation is not complete before central element formation occurs from the telomere regions. This is also consistent with the observation that meiotic prophase is shortened in wild-type wheat compared to its diploid relatives (and the phlb mutant) (Bennett et al., 1974).

When homologous chromosome pairing is virtually complete, first the centromeres and then the telomeres disperse (Fig. 6f). By the time all the telomeres have dispersed, they are seen at a haploid number of sites, and homologues are completely paired (Fig. 6g). Thus, according to classical definitions, the telomere cluster begins prior to leptotene, and lasts until the end of zygotene in this species. By the staging criteria of Bennett et al. (1973, 1979), the single telomere bouquet would be initially formed during pre-meiotic interphase. The occurrence of sister chromatid separation with the formation of the telomere bouquet places its initial formation during the pre-meiotic S phase/meiotic prophase transition and before the stage which has been classically defined as leptotene. But the labelled chromosomes at this stage clearly differ from a typical interphase configuration, and therefore we would include it as part of meiotic prophase.

In budding yeast Weiner and Kleckner (1994) and Loidl et al. (1994) reported up to 50% premeiotic association of homologous chromosomes. However, the chromosomes dissociate during S phase and then reassociate intimately during meiotic prophase (reviewed by Kleckner, 1996; Roeder, 1997). In wild-type wheat, the premeiotic association of homologues is much higher (90%) than budding yeast while in the phlb mutant it is reduced to 25%. In vegetative cells of budding yeast, centromeres are clustered at the spindle pole body with the telomeres separated around the opposite pole of the nucleus (Jin et al., 1998). In both wild-type wheat and the phlb mutant, centromeres are grouped at one pole of the nucleus and telomeres are spread around the opposite pole. In wild-type wheat, the association of centromeres in pairs either in pre-meiotic or meiotic cells is maintained after squashing. However, diffuse centromere structures, in which individual centromeres are not well enough differentiated to count, are observable after squashing nuclei from the phlb mutant (Aragon-Alcaide et al., 1997a). This implies that the Phl locus affects the centromere structure.

In both budding and fission yeast, the centromeres decluster from the spindle pole body as the telomeres cluster to the spindle pole body (Chikashige et al., 1997; Trelles-Sticken et al., 1999). This implies a major reorganization of the chromosomes and loss of the Rabl configuration. In the phlb mutant, the telomeres cluster when the centromeres are no longer grouped. Thus the telomere cluster occurs at a later stage than in wild-type wheat, and is accompanied by the loss of the Rabl configuration in the meiocytes. This is similar to the situations described in maize (Bass et al., 1997) and humans (Scherthan et al., 1996, 1998).
To date meiosis has been described in detail in two plants, wheat and maize (Dawe et al., 1994; Bass et al., 1997), and significant differences in the mechanism of homologue pairing have been discovered. Both species are polyploid, but maize has recently been proposed to be either a degenerate autotetraploid or a segmental allopolyploid (Skrabanek and Wolfe, 1998), while wheat is clearly an allopolyploid. The requirement for stable maintenance of these two different types of polyploids could account for the differences in meiotic mechanism. Our studies have shown that the presence of the Ph1 locus results in a premeiotic association of homologues via centromeres during floral development and an earlier association of sub-telomeric regions via telomeric clustering at the onset of meiotic prophase. Other studies have shown that the presence of the locus eliminates recombination between homoeologous chromosomes or segments (Gillies et al., 1987; Luo et al., 1996). As yet it is unclear whether these two effects of the Ph1 locus are functionally related, or whether the Ph1 locus compromises two or more genes which have differing effects on premeiotic and meiotic events. The recent identification of new deletions of the Ph1 locus should resolve this issue (Miller et al., 1998; Snape et al., 1998).

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