Homologue recognition during meiosis is associated with a change in chromatin conformation.

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During meiosis, homologues are sorted into pairs and then intimately aligned, or synapsed, along their lengths as a proteinaceous structure, the synaptonemal complex (SC) is assembled between them. However little is known about how chromosomes first recognise each other[1]. By comparing the behaviour of *Ph1* mutant and wild-type wheat, we show that when chromosomes recognise a partner to pair with, a conformational change of the chromatin is triggered in both partners. This is followed by their intimate alignment. Thus, a conformational change in the chromosomes at the onset of meiosis can be correlated directly with recognition.

At the onset of meiosis, chromosomes undergo conformational changes [2,3], which, however, have yet to be correlated directly with homologue recognition. In hexaploid wheat (AABBDD, 2*n* = 6x = 42) the *Ph1* locus ensures that pairing and recombination are restricted to true homologues rather than homoeologues (equivalent chromosomes from the other genomes) [4]. A line has been generated in which a rye segment covering

15% of the distal chromosome arm has been substituted for the equivalent region in the 1D pair of wheat chromosomes [5]. This enables us to distinguish homologues from homoeologues, as well as following conformational changes (Fig 1a).

By visualising the rye segments using genomic *in situ* hybridization, the homologues bearing these segments are shown paired with each other at metaphase I in 100% of the (20) meiocytes examined from wild-type wheat (Fig. 1b). In contrast, these homologues are not paired with one another in 66% of the (20) meiocytes examined from the *Ph1* mutant. It has been reported that on average 40 of the 42 chromosomes are still paired at metaphase I in the *Ph1* mutant [6]. Thus if most homologues are not pairing with each other in the *Ph1* mutant and there is little change in the overall level of pairing, the homologue recognition process is likely to be affected.

The conformational state of the labelled segments is the same in the two homologues throughout premeiosis and early meiotic prophase in all the meiocytes examined from wild type wheat (Fig. 1). In particular, the segments elongate just prior to telomere bouquet formation and their intimate pairing. In contrast, in the mutant, the homologues show different conformations during early meiosis in 64% of the meiocytes (Fig. 1, Supplementary Information Table 1 online) - the same percentage as are incorrectly paired. The correlation between the proportion of meiocytes containing the labelled chromosome segments in a similar conformation (36%) and the level of pairing between these homologues at meiotic metaphase I (33%) suggests that the homologues can only pair when they are in the same conformational state.

If the elongation is linked to intimate pairing, then in the wild type, only interactions between homologues can trigger this chromatin conformational change; in the mutant, interactions between related chromosomes will suffice. We tested this

prediction using wheat-rye hybrids, which contain a haploid set of 21 wheat chromosomes and a haploid set of 7 rye chromosomes, making 28 homoeologues and no homologues. In the presence of *Ph1*, the heterochromatin knobs on each rye chromosome remain as tight foci, showing that the chromatin conformation in this chromosome region does not change either before or during the telomere bouquet in the (50) meiocytes examined (Fig. 2a). In contrast, in meiocytes from the hybrid lacking *Ph1*, the knobs are seen as groups of elongated structures in all the (50) meiocytes examined, either as the telomeres cluster to form the bouquet or decluster after the bouquet stage, indicating a conformational change (Fig. 2c). In 3 meiocytes out of 50 examined, all the heterochromatin knobs were found as a single elongated structure at the telomere bouquet stage implying an interaction with one another (Fig. 2b).

If telomeric heterochromatin regions of rye associate with themselves, what about other heterochromatin regions? Centromeres also associate in groups for meiosis in the wheat-rye hybrids [7,8]. The availability of specific probes for wheat and rye centromeres now enables us to characterise these interactions [9]. In the presence of *Ph1*, 7 of the 14 signals are labelled with both probes, showing a wheat-rye association (Fig. 2d,e,f). The wheat and rye centromeres then coalesce to 7 groups, each group containing a rye centromere, by the time the telomere bouquet is fully formed (Fig. 2g,h,i). The distribution of rye centromeres between the 7 groups supports our previous hypothesis that these groups comprise homoeologues from the 4 genomes [8]. In the hybrid lacking *Ph1*, 7 of the 14 signals seen as the telomeres cluster correspond to rye centromeres alone, and the remaining 7 signals to the 21 wheat centromeres (Fig. 2j,k,l). Thus, the 21 wheat centromeres cluster into 7 groups but the rye centromeres do not join these groups in most meiocytes. In only 3

meiocytes, out of more than 50 examined at the telomere bouquet stage, had the wheat and rye centromeres coalesced into 7 groups (Fig. 2m,n,o). Thus not only are the telomere regions of the rye chromosomes interacting with themselves, but their centromeres rarely interact with the wheat centromeres. This explains the low level of success for transferring chromosome segments from rye into wheat using interspecific hybrids, even when the conformational changes occur. A summary model of the pairing events in the wheat hybrids is provided (Supplementary information Fig 1 online).

The changes in chromatin conformation cannot involve a signal diffusing throughout the nucleus. If this were the case, homologues would always be visualised with similar conformations within any given meiocyte. The changes in conformation must occur chromosome by chromosome. Moreover the fact that the elongated segments so closely mirror each other in the presence of *Ph1* implies the signal to initiate the conformational change occurs at the same time in the two homologues, suggesting an interaction between them (Supplementary information Table 1 online). In a series of chromosomal deletions in wheat, only those of the sub-telomeric region of one homologue eliminated the subsequent pairing between the homologues [10]. Thus it seems likely that, as the telomeres cluster, it is the interaction between sub-telomeric regions of the homologues that triggers the conformational change enabling them to pair.

In hexaploid wheat, centromeres pair premeiotically and then sort into 7 groups at the beginning of meiosis [8,12] and *Ph1* affects the specificity of the interactions [7]. The present study clearly demonstrates the effect of *Ph1* on these interactions and that the 7 centromere groups are indeed formed from the related chromosomes. However more importantly, we also now show that *Ph1* affects the

specificity of the interactions of the telomeric regions at early meiosis. This has consequences in turn for whether interactions between pairs of chromosomes can trigger a conformational change in their telomeric regions, enabling them to intimately pair with each other. Thus as shown here, *Ph1* does not have a major effect on the overall level of pairing, just which chromosomes pair with each other. A summary model of these pairing events in wheat is presented (Fig. 3). Given the effects on different chromosomal regions in different species, it seems likely that *Ph1* is binding to these regions and modulating their chromatin structure.

Methods

Plant material

The anthers used in this study came from 60 plants of wheat (*Triticum aestivum* cv. 'Chinese Spring') either carrying or lacking the *Ph1* locus and carrying two rye segments substituted for the equivalent region of the 1D pair of wheat chromosomes (these chromosomes still have wheat telomeres and sub-telomeric regions and the rye segment possesses a similar gene content but different repetitive content compared to the equivalent wheat region [5]) and 30 plants of Chinese Spring/*Secale cereale* cv. Petkus F_1 hybrids with and without the *Ph1* locus. All *Ph1* mutant lines used here carried the *ph1b* deficiency.

Sectioning and Fluorescence *in situ* hybridization

The wheat centromere, rye centromere, the rye heterochromatin knob probes used, tissue sectioning and specimen preparation, *in situ* hybridisation and probe preparation and the labelling of the rye segments have all been described previously [8,9,11]. Some 1500 tissue sections have been used for this study. Preparation of the

meiotic and root metaphase chromosome spreads, their labelling by *in situ* hybridisation and subsequent scoring have all been described previously [6,13].

Fluorescence microscopy and image processing

We collected confocal optical stacks using a Leica TCS SP as described previously [8]. Confocal images were processed by the public domain program ImageJ written by Wayne Rasband (wayne@codon.nih.gov), at the Research Services Branch, National Institute of Mental Health, Bethesda, Maryland, USA. Final figures were prepared using Adobe Photoshop. All the images of single meiocytes are taken from whole anther sections which are two layers thick. The meiocytes were analysed from 3D confocal data stacks. Projections were made for the images shown in this paper.

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Table 1 Statistics for the homologue condensation

	***Ph1* present**			***Ph1* absent**		
	segment 1	**segment 2**	**ratio**	**segment 1**	**segment 2**	**ratio**
Length of the 2 rye	4.165	4.163	1.0	5.033	1.222	4.0
segments per cell (μm)	4.759	4.713	1.0	2.946	0.972	3.0
	4.672	4.577	1.0	3.475	1.361	2.5
	5.057	5.005	1.0	5.115	2.050	2.4
	5.600	5.934	1.0	3.091	0.856	3.6
	6.345	6.314	1.0	7.209	1.755	4.1
	4.978	4.880	1.0	6.900	2.255	3.0
	4.706	4.037	1.1	5.087	1.901	2.6
	5.759	5.709	1.0	5.300	1.892	2.8
	6.178	6.175	1.0	6.185	2.412	2.5
	5.302	5.625	1.0	4.976	4.676	1.0
	6.334	5.700	1.1	2.745	2.530	1.0
	8.287	8.576	1.0	5.147	5.007	1.0
	3.755	3.857	1.0	3.806	3.770	1.0
	7.434	7.329	1.0	2.979	2.576	1.0
t-test	P=0.5			P<0.001		

The lengths of the homologous segments were measured in 3-D confocal stacks of randomly selected meiocytes in which the telomere bouquet was present. T-tests were carried out for the null hypothesis that there is no difference between the length of the two rye segments, in the presence and in the absence of *Ph1*. The null hypothesis can be rejected with more than 99% confidence in the absence of *Ph1* and be accepted in its presence. Data in the box show that the two homologous segments are at the same level of elongation in 36% of the meiocytes (the proportion that would be expected to

be correctly paired at metaphase I) in the absence of *Ph1*. All the data including that within the box was included in the statistical analysis.

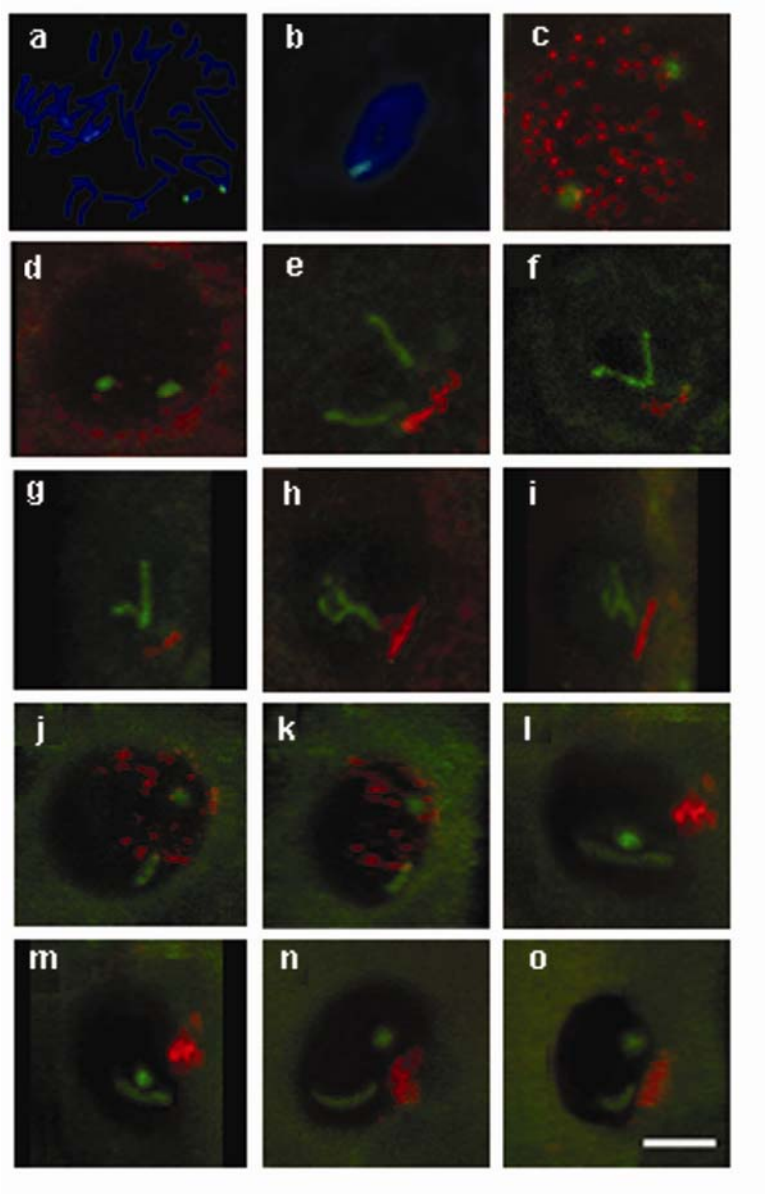

Figure 1 Homologous segment behaviour during premeiotic interphase and early meiosis in wheat. Pairs of rotations (**f-o**) separated by 45°. The two rye segments are green and telomeres red. **a**, Root metaphase spread. **b-i**, Pollen mother cells from wild-type wheat. **b**, Pairing of homologues carrying the rye segments at metaphase I. **c**, Premeiotic interphase nucleus. **d**, Early meiotic nucleus. **e-i**, Early meiotic nuclei at the telomere bouquet stage showing the rye segments elongating and then associating. **j-o**, Early meiotic nuclei from the *Ph1* mutant showing only one of the rye segments

elongating at the telomere bouquet stage. Scale bar, 5 μm for panel **b** and 10μm for the rest of the panels.

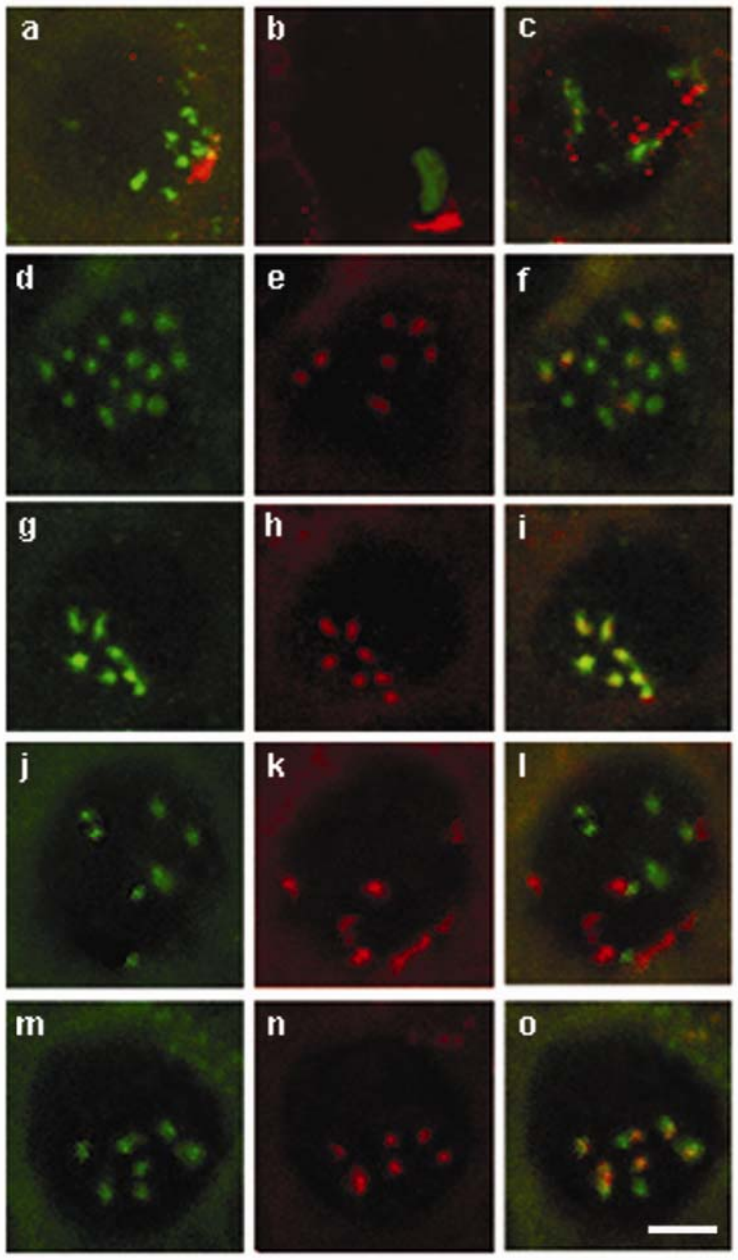

Figure 2 Centromere and heterochromatin behaviour in the wheat-rye hybrids. **a-c**, Rye heterochromatin knobs are green and telomeres red. **a**, Early meiotic nucleus, *Ph1* present. **b**, Early meiotic nucleus, *Ph1* absent. **c**, Later meiotic nucleus, *Ph1* absent. **d-i**, Pollen mother cells with *Ph1* and **j-o** lacking *Ph1* with 21 wheat (green) and 7 rye (red) centromeres . **d-f**, Early meiotic nucleus as the telomeres cluster. **d**, 14 wheat signals. **e**, 7 rye signals. **f**, Overlay of d and e. **g-i**, Early meiotic nucleus with

the telomere bouquet formed. **g**, 7 wheat signals. **h**, 7 rye signals. **i**, Overlay of g and h. **j**-**l**, Early meiotic nucleus as the telomeres cluster. **j,** 7 wheat signals. **k**, 7 rye signals. **l**, Overlay of j and k. **m**-**o**, Early meiotic nucleus with the telomere bouquet formed. **m**, 7 wheat signals. **n**, 7 rye signals. **o**, Overlay of m and n. Scale bar 10 µm.

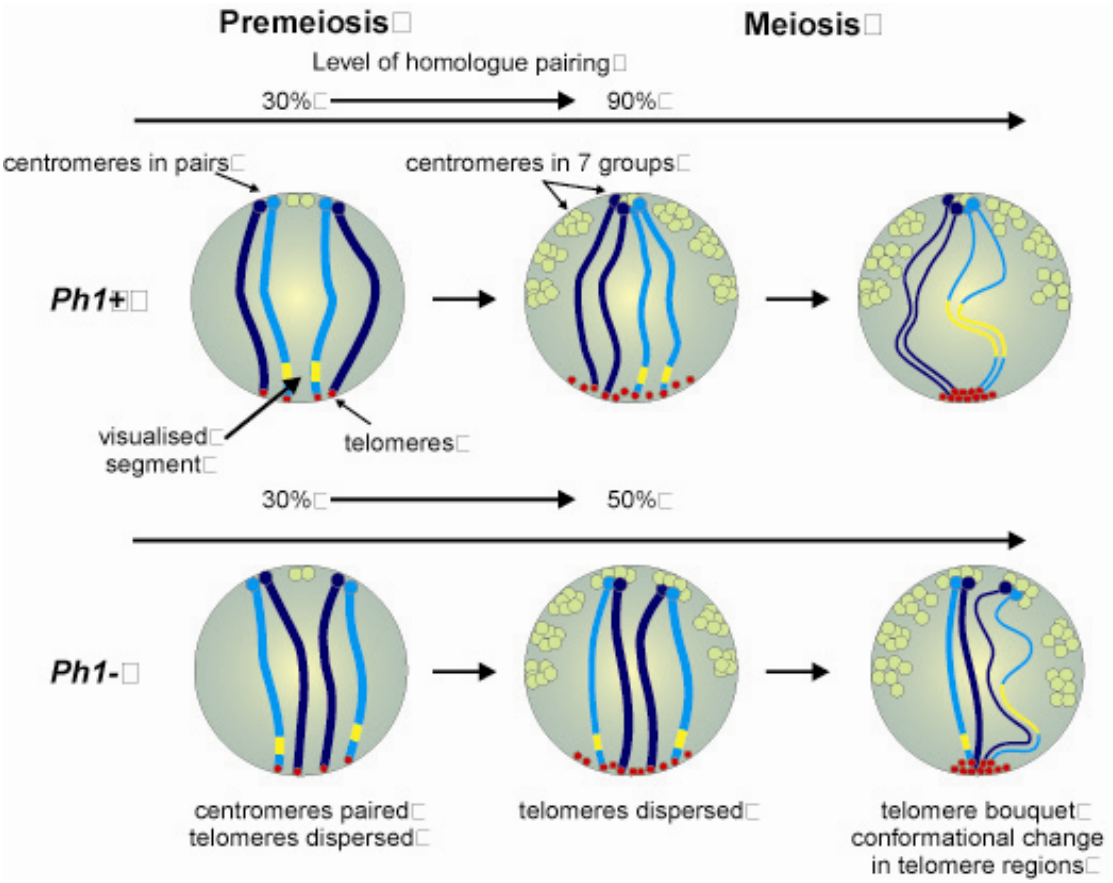

Figure 3 Diagram of the chromosome pairing events in hexaploid wheat in the presence and absence of *Ph1*. The pairing of single chromosome arms of two pairs of homologues (four homoeologues) are shown just prior to and during the early stages of meiosis.

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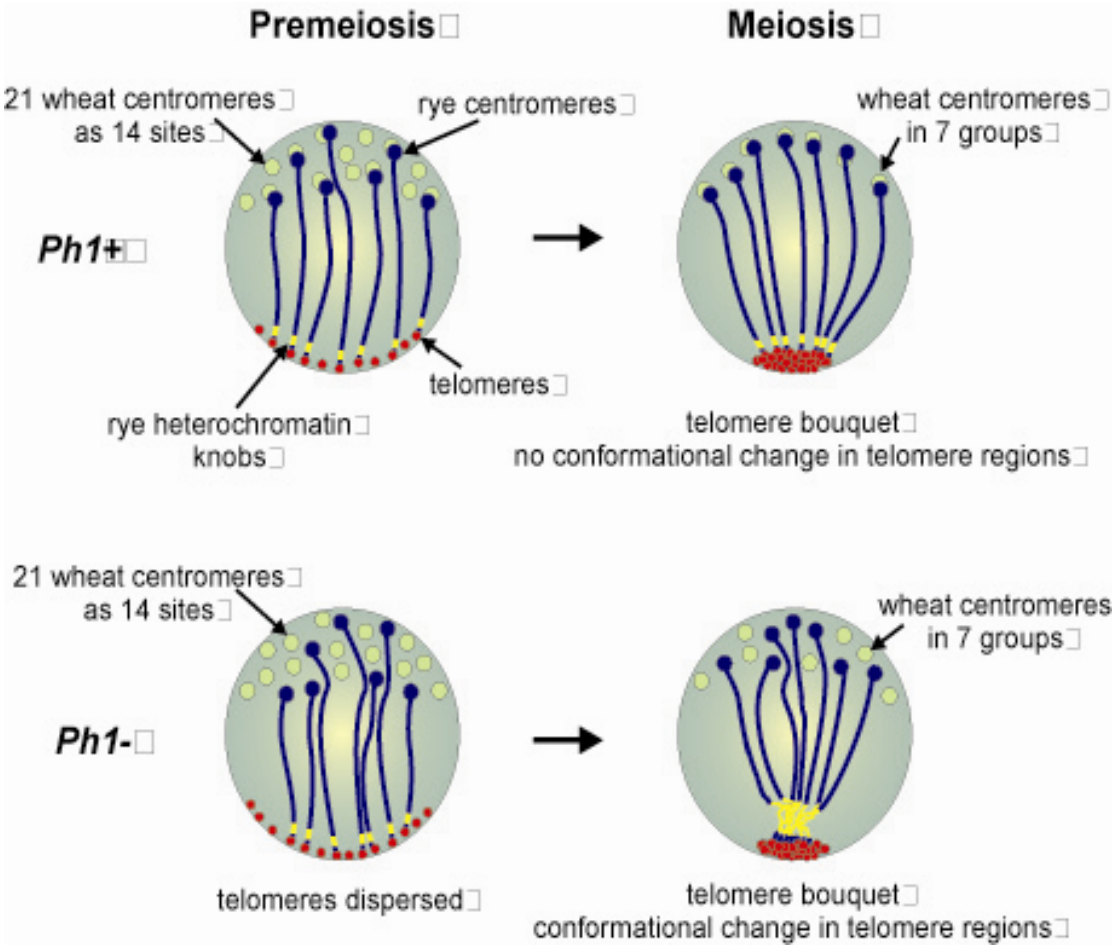

Supplementary Figure 1 Diagram of the chromosome pairing events in wheat-rye hybrids in the presence and absence of *Ph1*. The behaviour of the 21 wheat and 7 rye centromeres as well as the rye telomeric heterochromatin knobs is shown. The rye chromosomes are represented as single arms.