

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

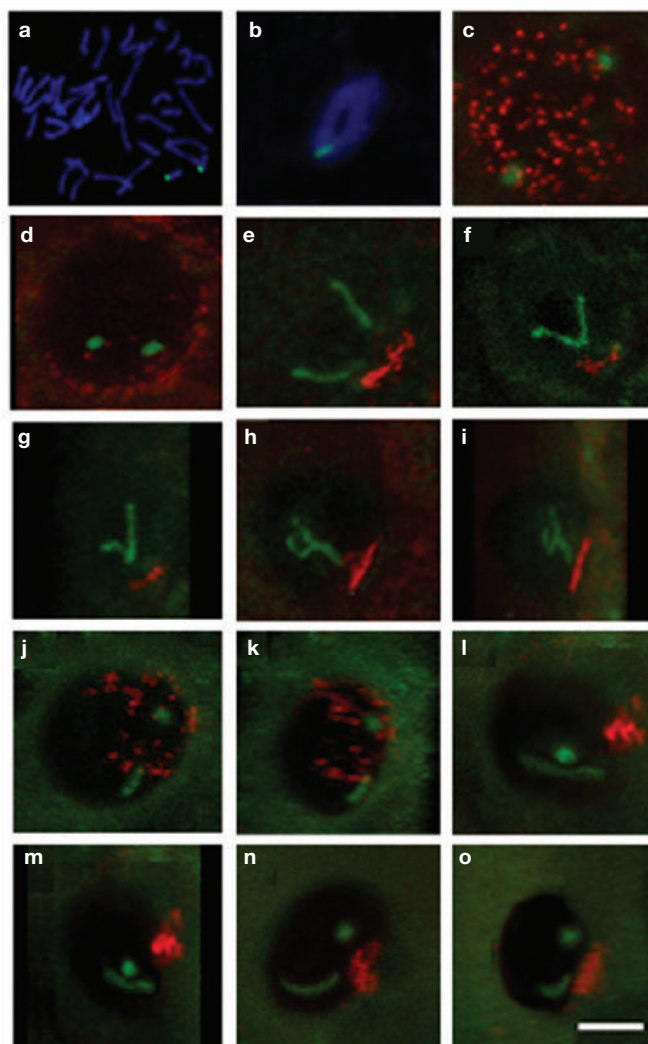
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During meiosis, homologous chromosomes are sorted into pairs and are then intimately aligned, or synapsed, along their lengths while a proteinaceous structure, the synaptonemal complex, is assembled between them. However, little is known about how chromosomes first recognise each other<sup>1</sup>. Here we show, by comparing the behaviour of wild-type wheat and wheat mutant for *Ph1* (a suppressor of homologous chromosome pairing), that when chromosomes recognise a partner to pair with, a conformational change to the chromatin is triggered in both partners that 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<sup>2,3</sup>, which, however, do not correlate directly with homologue recognition. In hexaploid wheat ( $2n = 42$ ) possessing three related genomes (A, B and D), the *Ph1* locus ensures that pairing and recombination are restricted to true homologues rather than homoeologues (equivalent chromosomes from other genomes)<sup>4</sup>. A wheat line has been generated in which a rye segment that covers 15% of the distal chromosome arm has been substituted for the equivalent region in the 1D pair of wheat chromosomes<sup>5</sup>. This enabled homologues to be distinguished from homoeologues, and conformational changes to be observed (Fig. 1a).

By visualising the rye segments using genomic *in situ* hybridization, the homologues bearing these segments paired with each other at metaphase I in all (20/20) of the meiocytes examined from wild-type wheat (Fig. 1b). In contrast, these homologues did not pair in 66% (13/20) of the meiocytes examined from the *Ph1* mutant. On average, 40 of the 42 chromosomes are still paired at metaphase I in the *Ph1* mutant<sup>6</sup>. Thus, if most homologues do not pair with each other in the *Ph1* mutant, and there is little change in the overall level of pairing, the homologue recognition process will most probably be affected.

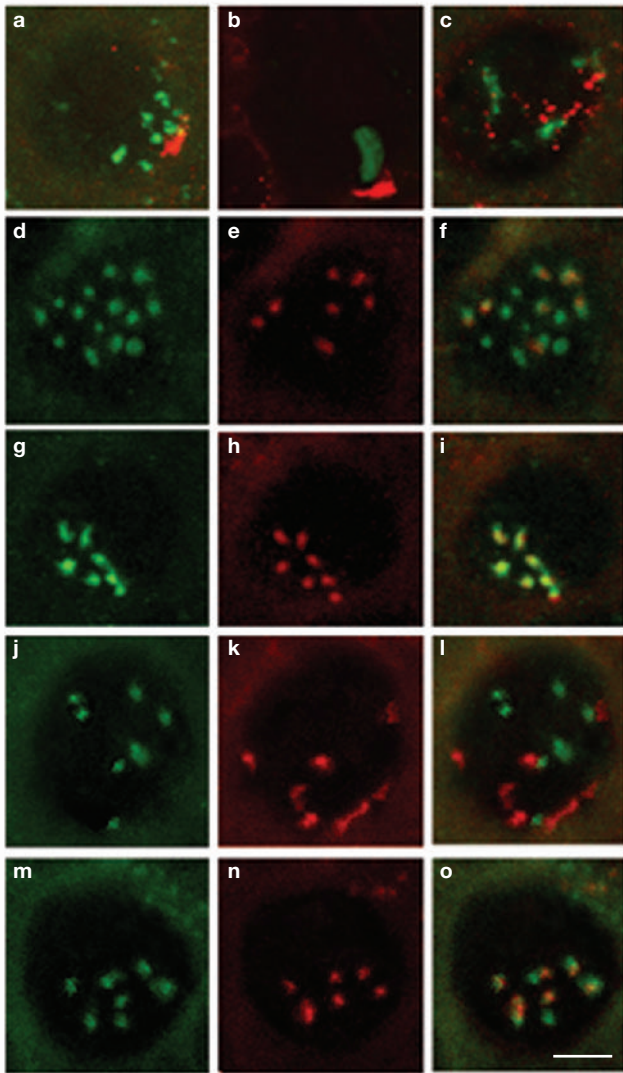
The conformational state of the labelled segments was the same in the two homologues throughout pre-meiosis and early meiotic prophase in all of the meiocytes examined from wild-type wheat (Fig. 1). In particular, the segments elongated immediately before telomere bouquet formation and their intimate pairing. In contrast, in the mutant, the homologues showed different conformations during early meiosis in 64% of the meiocytes (Fig. 1; also see Supplementary



**Figure 1** Homologous segment behaviour during pre-meiotic interphase and early meiosis in wheat. **(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)** Pre-meiotic 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. Pairs of rotations **(f–o)** are separated by 45°. Green, rye segments; red, telomeres. Scale bar represents 5  $\mu\text{m}$  for panel **b**, 10  $\mu\text{m}$  for all others.

Information, Table 1) — the same percentage as were incorrectly paired. The correlation between the proportion of meiocytes containing the labelled chromosome segments in a similar conformation

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**Figure 2** Centromere and heterochromatin behaviour in the wheat-rye hybrids. (a) Early meiotic nucleus, *Ph1* present. (b) Early meiotic nucleus, *Ph1* absent. (c) Later meiotic nucleus, *Ph1* absent. In a–c, green, rye heterochromatin knobs; red, telomeres. (d–f) 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 represents 10  $\mu\text{m}$ .

(36%), and the level of pairing between these homologues at meiotic metaphase I (33%), suggested 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 that contain a haploid set of 21 wheat chromosomes and a haploid set of 7 rye chromosomes, producing 28 homoeologues and no homologues. In the presence of *Ph1*, the heterochromatin knobs on each rye chromosome remained as tight foci, showing that the

chromatin conformation in this chromosome region did 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 were seen as groups of elongated structures in all (50) of the meiocytes examined, either as the telomeres clustered to form the bouquet or as they declustered after the bouquet stage, suggesting a conformational change (Fig. 2c). In 3 out of the 50 meiocytes examined, all of the heterochromatin knobs were found as a single elongated structure at the telomere bouquet stage, suggesting that they had interacted with each other (Fig. 2b).

If telomeric heterochromatin regions of rye associate with themselves, the question of what occurs with other heterochromatin regions arises. Centromeres also associate in groups for meiosis in the wheat-rye hybrids<sup>7,8</sup>. The recent availability of specific probes for wheat and rye centromeres has enabled the characterization of these interactions<sup>9</sup>. In the presence of *Ph1*, 7 out of the 14 signals labelled with both probes, demonstrating a wheat-rye association (Fig. 2d, e, f). The wheat and rye centromeres then coalesced into seven groups, with each group containing a rye centromere, by the time the telomere bouquet was fully formed (Fig. 2g, h, i). The distribution of rye centromeres between the seven groups supports our previous hypothesis that these groups comprise homoeologues from the four genomes<sup>8</sup>. In the hybrid lacking *Ph1*, 7 out of the 14 signals seen as the telomeres cluster corresponded to rye centromeres alone, and the remaining 7 signals corresponded to the 21 wheat centromeres (Fig. 2j, k, l). Thus, the 21 wheat centromeres clustered into 7 groups, but the rye centromeres did not join these groups in most meiocytes. The wheat and rye centromeres coalesced into 7 groups in only 3 meiocytes (out of more than 50 examined) at the telomere bouquet stage (Fig. 2m, n, o). Thus, not only were the telomeric regions of the rye chromosomes interacting with themselves, but their centromeres rarely interacted 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 (see Supplementary information, Fig. S1).

The changes in chromatin conformation cannot involve a signal diffusing throughout the nucleus. If this were true, homologues would always be visualised with similar conformations within any given meiocyte. The changes in conformation must occur chromosome by chromosome. Moreover, as the elongated segments so closely mirror each other in the presence of *Ph1*, this suggests that the signal to initiate the conformational change occurs at the same time in the two homologues, and thus also suggests an interaction between them (see Supplementary Information, Table 1). In a series of chromosomal deletions in wheat, only those of the sub-telomeric region of one homologue eliminate the subsequent pairing between the homologues<sup>10</sup>. Thus, it seems probable that, as the telomeres cluster, it is the interaction between sub-telomeric regions of the homologues that triggers the conformational change that enables them to pair.

In hexaploid wheat, centromeres pair pre-meiotically and then sort into seven groups at the beginning of meiosis<sup>8,12</sup>, and *Ph1* affects the specificity of the interactions<sup>7</sup>. The present study demonstrates the effect of *Ph1* on these interactions, and shows that the seven centromere groups are indeed formed from the related chromosomes. However, and more importantly, we have also shown that *Ph1* affects the specificity of interaction between the telomeric regions at early meiosis. This, in turn, has consequences 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, only on which chromosomes pair with each other (a summary model of these pairing events in wheat is shown in Supplementary Information, Fig. S2). Because of the effects on different chromosomal regions in different species, it seems probable that *Ph1* binds to these regions and modulates their chromatin structure. □

## METHODS

**Plant material.** The anthers used in this study came from 60 wheat plants (*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<sup>5</sup>. Also used were 30 plants of Chinese Spring/*Secale cereale* cv. Petkus F<sub>1</sub> 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; the rye centromere; the rye heterochromatin knob probes; tissue sectioning and specimen preparation; *in situ* hybridisation and probe preparation; and the labelling of the rye segments have all been described previously<sup>8,9,11</sup>. Preparation of the meiotic and root metaphase chromosome spreads, labelling of them by *in situ* hybridisation and subsequent scoring have all been described previously<sup>6,13</sup>.

**Fluorescence microscopy and image processing.** Confocal optical stacks were collected using a TCS SP (Leica, Heidelberg, Germany) as described<sup>8</sup>. Confocal images were processed by the public domain program ImageJ written by W. Rasband (wayne@codon.nih.gov), at the Research Services Branch, National Institute of Mental Health, Bethesda, Maryland, USA. All the images of single

meiocytes were taken from whole anther sections that were two layers thick. The meiocytes were analysed from three-dimensional confocal data stacks. Projections were made for the images shown in this paper.

*Note: Supplementary Information is available on the Nature Cell Biology website.*

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## COMPETING FINANCIAL INTERESTS

The authors declare that they have no competing financial interests.

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