Ph1 affects remodelling of the heterochromatin at the telomeres and the centromeres at the onset of meiosis

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ABSTRACT

Meiosis is a specialized type of cell division in which two rounds of chromosome segregation follow a single round of DNA replication. The *Ph1* locus is the major locus in wheat that ensures that pairing is restricted to true homologues; in lines carrying a deletion of *Ph1*, homoeologous pairing occurs. We have applied fluorescence *in situ* hybridization (FISH) on three-dimensionally preserved anther sections derived from intact florets of wheat-rye hybrids, in the presence and in the absence of the *Ph1* locus. Our observations suggest that *Ph1* affects chromatin remodeling at the onset of meiosis, and occurs when chromosomes are associated via centromeres and telomeres at the telomere bouquet stage.

INTRODUCTION

Wheat is an allopolyploid which possesses two or more sets of related chromosomes as a result of doubling of chromosomes following sexual hybridization between closely related species. At the onset of meiosis, homologous chromosomes (having the same gene order and repetitive DNA content) need to be distinguished from the related chromosomes (homoeologues), which have a similar gene content and order but have divergent repetitive DNA content. Hexaploid (bread) wheat contains A, B and D genomes, and tetraploid (pasta) wheat contains A and B genomes. Despite their genome complexity, both wheats behave as diploids during meiosis, so bread wheat chromosome 1A only pairs with 1A, and not with either 1B or 1D. This holds true for all seven chromosome groups. The accuracy and efficiency of the mechanisms used to achieve this diploidization have a profound influence on the fertility of the wheat plant, which is of major importance for success in breeding. The *Ph1* locus, on wheat chromosome 5B, ensures that pairing and recombination are restricted to true homologues rather than homoeologues[1].

The *Ph1* locus has been defined by deletion mapping and BAC sequencing to a cluster of *Cdk2-like (CDK2L)* genes, which show close homology to Cdk2 in humans and mice[2,3]. This kinase is required for meiosis and it co-localizes with mismatch repair proteins to recombination nodules and to the telomere regions during early meiosis[4-6]. Cdk2 has been proposed to be involved in licensing origins of replication through histone phosphorylation and chromatin remodelling[7].

The control of such biological processes during pre-meiotic replication and at the start of meiosis are consistent with the initiation and co-ordination of chromatin remodelling during the onset of meiosis[8-10]. In this paper we describe how *Ph1* is affects remodelling of the heterochromatin at the centromeres and telomeres and therefore we suggest that *Ph1* is affecting replication during pre-meiotic S-phase.

MATERIAL AND METHODS

Plant material

The anthers used in this study came from *Secale cereale* cv. Petkus (diploid rye) and *T. aestivum* cv. Chinese Spring (CS)/*S. cereale* cv. Petkus F_1 hybrids with and without the *Ph1* locus (carrying the *ph1b* deficiency).

Sectioning and Fluorescence *in situ* hybridization

The wheat centromere, rye centromere, the rye heterochromatin knob, the telomere probes used, tissue sectioning and specimen preparation, *in situ* hybridization, probe preparation and labelling have all been described previously[10-14]. More than 2000 tissue sections have been used.

Fluorescence microscopy and image processing

Confocal optical stacks have been collected using a Leica TCS SP as described previously[12]. Confocal images were processed by the public domain program ImageJ written by Wayne Rasband at the NIH (Bethesda, Maryland, USA). Final figures were prepared using Adobe Photoshop.

RESULTS AND DISCUSSION

Cell biological and synapsis studies reveal that *Ph1 CDK2L* affects processes during replication, controls chromatin remodelling and affects recombination and synapsis. To study chromatin remodelling we have analysed 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. Using *in situ* hybridisation in meiocytes from these hybrids in the presence and in the absence of *Ph1* either just prior to or during early stages of meiosis, we have visualized the behaviour of the heterochromatin at the centromeres and at the telomeres by analysing the rye heterochromatin knobs on the rye chromosomes which are adjacent to the rye telomeres. The anthers used in this study came from more than 40 F_1 hybrids either in the presence or in the absence of *Ph1* with 100s of them being analysed in 3D.

In hexaploid wheat, prior to meiosis, the centromeres pair and then form seven clusters corresponding to the seven sets of related chromosomes. At the onset of meiosis, the telomeres cluster to form a telomere bouquet and chromatin of chromosomes is remodelled to enable the homologues to pair[9,12]. In the wheat-rye hybrids, centromeres also associate in seven groups but only in the presence of *Ph1*[10]. We have observed that wheat centromeres start to associate at the onset of meiosis in the absence and in the presence of *Ph1*. As prophase progress, centromeres remodel in the absence of *Ph1* and remodelling reaches the maximum at the telomere cluster stage. At this stage, wheat centromeres are associated in seven groups and the seven rye centromeres are dispersed (Figure 1a). In contrast, at the telomere bouquet stage in the presence of *Ph1*, wheat and rye centromeres still remain unchanged as tight foci and they are associated in seven groups (Fig. 1b). These observations suggest that *Ph1* is affecting chromatin remodelling in wheat-rye hybrids and therefore affecting replication of the chromosomes.

To analyse chromosome remodelling at the other end of the chromosomes, we visualised the rye heterochromatin knobs present in all rye chromosomes at the distal regions, close to the telomeres by *in situ* hybridisation. In the diploid rye, we observed that the rye heterochromatin knobs were tight in appearance in premeiosis and during meiosis before the telomere bouquet[10] (Fig. 1c). When meiosis reaches the telomere bouquet stage, the rye heterochromatin knobs can be seen as diffuse elongated structures (Fig. 1d), but they do not associate as a single structure. Rye heterochromatin knobs are also seen as tight structures at early meiosis stages in wheat-rye hybrids in the presence of *Ph1* (Fig. 1e). In contrast with the results found in diploid rye, when meiosis reaches the telomere bouquet stage, rye heterochromatin knobs remained unchanged as tight foci in the hybrids in the presence of *Ph1* (Fig. 1f). Remodelling of the rye heterochromatin knobs at the telomere bouquet stage in wheat-rye hybrids was only observed in the absence of *Ph1*. Rye heterochromatin knobs are tight in appearance in the hybrids in the absence of *Ph1* before the telomere cluster stage (Fig. 1g). When the telomeres associated to form the telomere cluster, the knobs were observed as groups of elongated structures in the wheat-rye hybrids in the absence of *Ph1* (Fig. 1h). At this stage rye heterochromatin knobs do associate in a single structure in the wheat-rye hybrids (Fig. 1h) contrary to the behaviour of the rye heterochromatin knobs in the diploid rye.

The *Ph1* phenotype was observed for first time in hybrids and its effect is dramatically more evident in the absence of homologues[15]. We have shown that *Ph1* affects centromere pairing in the hybrid prior to meiosis.

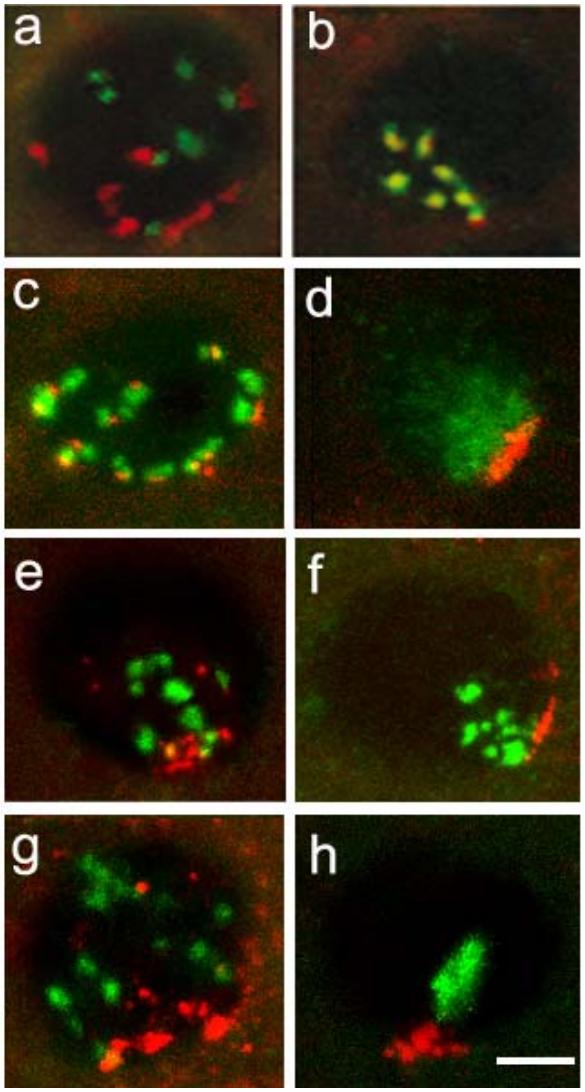

Figure 1. Chromatin remodelling in diploid rye and in wheat-rye hybrids in the presence and in the absence of *Ph1*. Wheat and rye centromeres are shown in green and red respectively in panels a and b. Telomeres are shown in red and rye heterochromatin knobs are shown in green in panels c-h. a) Centromeres are remodelled in the absence of *Ph1* at the telomere cluster stage. Wheat centromeres do not associate with rye centromeres. b) Meiocyte in the presence of *Ph1* at the telomere bouquet stage. Centromeres remain unchanged but they do associate into seven groups. c) Early meiotic nucleus in diploid rye. d) Diploid rye nucleus at the telomere bouquet stage. Heterochromatin knobs are remodelled and they are seen as elongated structures. e) Early wheat-rye meiotic nucleus in the presence of *Ph1*. f) Wheat-rye meiotic nucleus in the presence of *Ph1* at the telomere bouquet stage. Heterochromatin knobs remain unchanged as tight foci. g) Early wheat-rye meiotic nucleus in the absence of *Ph1*. h) Early wheat-rye meiotic nucleus at the telomere bouquet stage in the absence of *Ph1*. Rye heterochromatin knobs are remodelled and they associate in a single structure. Scale bar represents 10 μm.

Although the 21 wheat centromeres are able to reduce to 7 sites prior to meiosis in all anther cells, the rye centromeres are only able to associate with wheat

centromeres in the presence of *Ph1* and not in its absence. Moreover the timing of the remodelling of the centromeric heterochromatin is different in the presence and absence of *Ph1*. Previous studies have shown that *Ph1* affects centromere pairing in cells undergoing endoreduplication in roots[16]. At the other end of the chromosome, the subtelomeric heterochromatin is able to remodel in the absence of *Ph1* during the telomere bouquet stage but not in its presence. A number of studies have shown that heterochromatin is remodelled prior to the replication of these regions. Cdk2, the closest homologue to *Ph1*, is known to be involved in chromatin remodelling during replication, phosphorylating histone H1. Furthermore it has been shown that centromeres associate during replication[17]. All these observations point to the conclusion that *Ph1* affecting chromatin remodelling which is having an effect on replication.

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