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The subtelomeric region is important for chromosome recognition and pairing during meiosis

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The process of meiosis results in the formation of haploid daughter cells, each of which inherit a half of the diploid parental cells' genetic material. The ordered association of homologues (identical chromosomes) is a critical prerequisite for a successful outcome of meiosis. Homologue recognition and pairing are initiated at the chromosome ends, which comprise the telomere dominated by generic repetitive sequences, and the adjacent subtelomeric region, which harbours chromosome-specific sequences. In many organisms telomeres are responsible for bringing the ends of the chromosomes close together during early meiosis, but little is known regarding the role of the subtelomeric region sequence during meiosis. Here, the observation of homologue pairing between a pair of *Hordeum chilense* chromosomes lacking the subtelomeric region on one chromosome arm indicates that the subtelomeric region is important for the process of homologous chromosome recognition and pairing.

The outcome of meiosis is the generation of balanced gametes each carrying a full haploid complement. Proper homologue recognition is required in order to ensure ordered pairing and legitimate recombination. In a polyploid such as bread wheat, (hexaploid, 2n = 6x = 42), which has three related genomes (A, B and D), the presence of homoeologous (related) chromosomes complicates the picture, since homologues also need to be distinguished from homoeologues before the chromosomes can pair in an ordered way. The mechanism by which homologues identify one another is the most poorly understood aspect of meiosis[1]. It is accepted that the distal region of the chromosomes, which encompasses the telomere and the subtelomeric region, is critical to the process of homologue recognition and pairing in many organisms, but the specific role of these two structures is still unclear[2]. Telomeric sequence is highly conserved across the eukaryotes, underlining the importance of the telomeres in cell division. In many organisms, at an early stage of meiosis, the telomeres appear to cluster at the nuclear envelope to form a "bouquet"; the effect of this clustering is to bring the ends of the chromosomes close together, thereby facilitating the initiation of homologue recognition and pairing[3,4]. Once initiated, pairing triggers a conformational change in the chromatin which advances in a proximal direction along the length of the chromosome arm, inducing the necessary intimate contact between the two homologues along their entire chromosome length[5]. How chromosomes identify their homologous partners to pair, however, remains unknown, since the DNA sequence of the telomeres is largely generic and not at all chromosome-specific. The polymorphic nature of subtelomeres is an exciting area for study, but also presents a difficult challenge from the technical perspective. Subtelomeres are the transition between chromosome-specific sequences and the arrays of telomeric repeats, gene-rich, less evolutionary conserved than telomeres, and represents hot spots of recombination[6,7]. These features have contributed to the difficulty in assessing the potential conserved functions of these high-polymorphic regions, which are one of the most exciting frontiers left in genomics.

The addition of a pair of "alien" chromosomes to the full genome complement of a crop species is a commonly used first step for accessing genetic variation from the secondary gene pool[8]. Such addition lines have a long history of use for locating genes and markers, characterising the regulation of alien genes, isolating individual chromosomes and understanding meiotic pairing behaviour and chromosome structure[9-11]. Sets of both cultivated (*Hordeum vulgare)* and wild (*H. chilense*) barley addition lines in a hexaploid wheat background have been established for some time[12,13]. *Hordeum chilense* is highly polymorphic both morphologically and biochemically[14], and has been used as a donor of various traits of relevance to wheat improvement[15]. In this study, chromosome pairing in wheat was analysed at the onset of meiosis by following an extra pair of chromosomes from this wild

barley. One of the added *H. chilense* chromosomes appears to have suffered a terminal chromosome deletion on its short arm, which has removed the subtelomeric region but retained the telomere, while a sister line carries a deletion on the long arm, but has retained both the long arm telomere and subtelomeric region[16]. Since non-wheat chromosomes present in a wheat line can be readily tracked via *in situ* hybridisation[17], these materials provide an excellent opportunity to analyse the influence of the subtelomeric region on chromosome pairing and conformational changes during meiosis.

Results

Detection of *H. chilense* subtelomeric regions in the wheat background. The presence/absence of the *H. chilense* subtelomeric regions in the wheat background was visualised using fluorescence *in situ* hybridisation. Mitotic chromosome spreads obtained from root tips were made from both the addition line carrying the terminal *H. chilense* chromosome short arm deletion (lacking the subtelomeric region) and the one carrying the deleted version of the long arm (subtelomeric region retained)(Fig. 1). When probed with the *Arabidopsis thaliana* telomeric sequence pAt74, all of the wheat chromosomes and the *H. chilense* pair generated positive hybridisation signals. In contrast, only the barley subtelomeric regions were labeled when the barley-specific subtelomeric repetitive sequence HvT01 was used as the probe. Both the telomere and the subtelomeric region were present on both arms of the 3H^ch chromosome carrying the long arm deletion (Fig. 1a), while the telomere but not the subtelomeric region were present on 3H^ch chromosome carrying the short arm deletion (Fig. 1b). These wheat lines and the visualisation of the satellite regions in only one pair of chromosomes were used as a tool to help in understanding the role of the subtelomeric regions in chromosome recognition/pairing at early meiosis in wheat.

Homologous chromosomes failed to recognise and pair with one another in the absence of the subtelomeric region. Chromosome

pairing was analysed during early meiosis by fluorescence *in situ* hybridisation, visualising telomeres and subtelomeres on the wild barley chromosomes in the wheat background. The *in situ* hybridisation analysis of more than 500 pachytene meiocytes of each of the two addition lines showed that the *H. chilense* homologues were associated with one another along the whole of their length provided that the sequences recognised by HvT01 were present on both chromosome arms (Fig. 2a). In the absence of the *H. chilense* subtelomeric region on the 3H^ch short arm, homologous chromosomes were only associated by the distal region of long arm where the HvT01 sequence was present (Fig. 2b). No chromosome remodeling was observed where the homologous arms were not associated with one another. A result, the short arms without the subtelomeric region were unable to initiate pairing. However, as meiosis progressed, the pairing signal initiated at the other end of the chromosome was propagated along the chromosome, even crossing the centromere into the short arm (Fig. 2c), so that the homologues became fully associated by late pachytene (Fig. 2d). No other chromosome patterns were visualised during pachytene. The implication was that DNA sequence(s) within the subtelomeric region must be important for the process of homologue recognition and pairing.

Recombination does not occur in the absence of the subtelomeric region. Chromosome pairing was also analysed during meiotic metaphase I. Meanwhile homologous chromosomes remained associated correctly in ring bivalents by two chiasmata (the cytological equivalent of genetic crossing-over) at metaphase I when the subtelomeric region was present on both chromosome arms (Fig. 3a), rod bivalents were always observed in all the cells analysed when the short arm subtelomeric region was absent, keeping homologues associated only by the long arm having subtelomeres (Fig. 3b; Table 1). These observations implied that, even though in the absence of the subtelomeric regions the short arms were still able to associate with one another late in pachytene
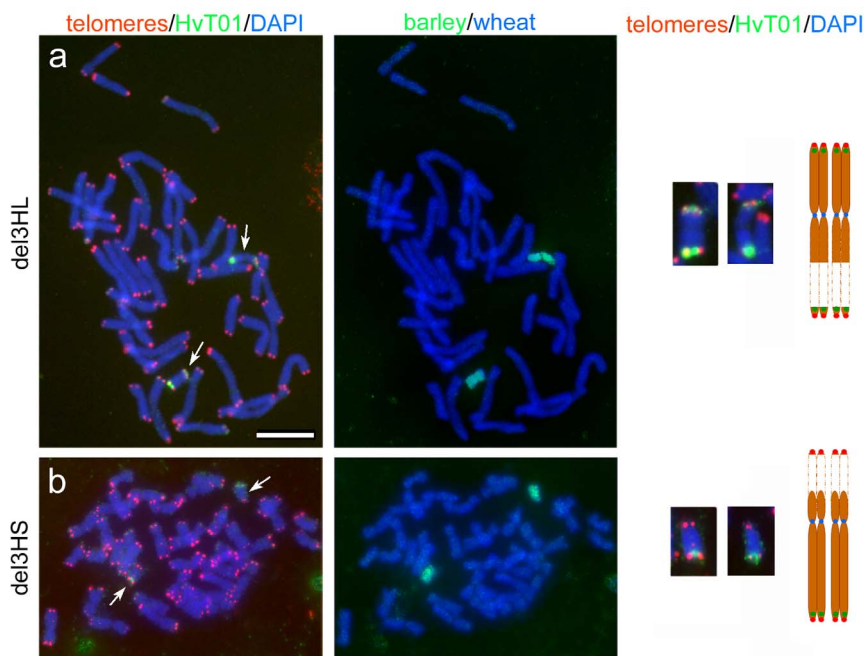

Figure 1 | *In situ* hybridisation-based detection of telomeres and the *H. chilense* chromosome 3H^ch subtelomeric regions in addition line root tip metaphase spreads. (a) The line carrying the version of chromosome 3H^ch in which a deletion on the long arm has removed neither the long arm telomere nor the subtelomeric region, (b) the line carrying the version of chromosome 3H^ch in which a deletion on the short arm has removed the subtelomeric region. The 3H^ch chromosomes (indicated by a white arrow) can be distinguished by the presence of signal following hybridisation with HvT01, as confirmed by probing with labelled *H. chilense* genomic DNA. Bar: 10 μm.
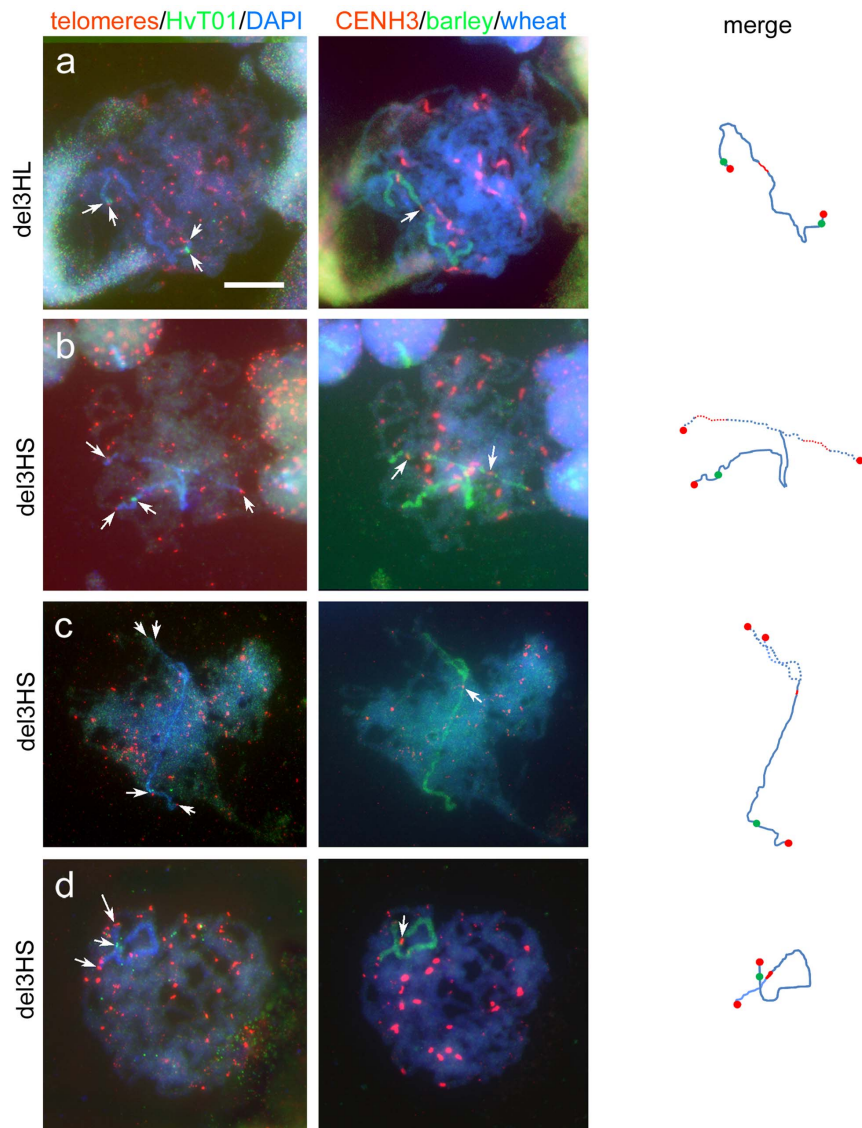
Figure 2 | The behaviour of the chromosome 3H[ch] homologues during pachytene. Telomeres, subtelomeric regions (first line) and centromeres (second line) of the two homologues are indicated by a white arrow. Solid and broken lines in the diagrams represent the paired and unpaired regions of the homologous chromosomes, respectively. (a) In the line carrying the version of chromosome 3H[ch] in which a deletion on the long arm has removed neither the long arm telomere nor the subtelomeric region, the two homologues are fully paired, while (b) in the line carrying the version of chromosome 3H[ch] in which a deletion on the short arm has removed the subtelomeric region, they are only associated along the distal region of the long arm, leaving the centromeric region and the short arm unpaired. (c) At a later stage during meiosis, pairing is extended in the line carrying the version of chromosome 3H[ch] in which a deletion on the short arm has removed the subtelomeric region. The homologues are fully associated along their long arm and the pairing signal has been transmitted through the centromeres, although the short arms remain largely unpaired. (d) At an even later stage, the homologues become fully associated. Bar: 10 μm.

(Fig. 2d), chiasma formation (and hence recombination) was not possible. The absence of the subtelomeric region therefore had an indirect effect on recombination.

The ZYP1 and MLH1 proteins are known to be deposited in, respectively, the synaptonemal complex and the chiasma. Immunolocalization experiments based on antibodies recognising each of these proteins were performed on the pachytene meiocytes of the addition line carrying the pair of 3H[ch] chromosomes with the short arm deletion (the subtelomeric region is absent). ZYP1 was associated with both arms, so appeared not to be affected by the absence of the subtelomeric region (Fig. 4a). However, MLH1 was only deposited on the long arm of the alien chromosome (Fig. 4b); consistent with the conclusion that recombination depended on the presence of the subtelomeric region at pachytene, the stage during which chromosome remodelling occurred and pairing is initiated.

Discussion

In silico modeling has suggested that telomere interactions alone may not be sufficient to assure the pairing of large chromosomes, so that interactions at additional chromosome sites are likely required[18]. In addition, even when the telomere bouquet fails to form homologous pairs, recombination, synapsis and the formation of balanced gametes can still occur[19,20], suggesting that other mechanisms involving subtelomeres, which also form a bouquet in rye[21], can determine homologue recognition and pairing. Because telomeres are highly conserved structures, it is logical that a less well conserved structure, such as the subtelomeric region, probably controls homologue recognition and pairing once the clustering of the telomeres has ensured that the chromosome ends lie close to one another. Homologue recognition may depend not only on the molecular DNA-DNA interactions but also on chromatin structure, which is determined
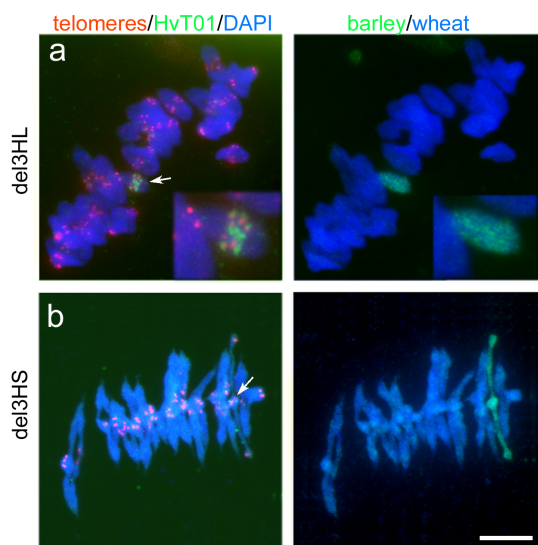
Figure 3 | **The behaviour of the chromosome 3H^{ch} homologues (labelled by genomic *in situ* hybridisation) during metaphase I.** (a) In the line carrying the version of chromosome 3H^{ch} in which a deletion on the long arm has removed neither the long arm telomere nor the subtelomeric region, the homologues form a ring bivalent (see inset), reflecting chiasma formation on both arms. (b) In the line carrying the version of chromosome 3H^{ch} in which a deletion on the short arm has removed the subtelomeric region, a rod bivalent forms, reflecting the lack of chiasma formation on the short arm (arrowed). Scale bar 10 μm.

by the DNA sequence within the subtelomeric region. The formation of a homologue-specific structure close to its telomere could serve as a chromosome identifier, which would facilitate homologue recognition and the initiation of pairing at its distal ends; thereafter, the trigger to pair can pass along the chromosome arm[5]. In addition, it cannot be discarded the ability that the subtelomeric region might have in physical interaction between homologous chromosomes. The subtelomeric region can be able to bind with a specific set of proteins, so that recognition and pairing could be driven by protein-protein or DNA-protein, rather than DNA-DNA interactions[2].

Although chromosomes associate via centromeres into seven groups of homoeologues at the onset of meiosis in wheat[22], association of homologous barley centromeres have not been visualised at this stage until the pairing signal initiated at the chromosome ends was transmitted along the chromosome arms up to the centromeric regions. Sister chromatid cohesion and full synapsis among the arm of a pair of homologues are known to be a prerequisite for homologous centromere pairing at early prophase I to occur in both wheat and maize[23,24]. The present data suggest that centromeres are not sufficient to trigger chromosome pairing along the chromosome arms. Centromere associations might act to stabilise the centromere pole, to maintain a reference point for the oriented telomere migration, providing a physical structure for chromosome movements that
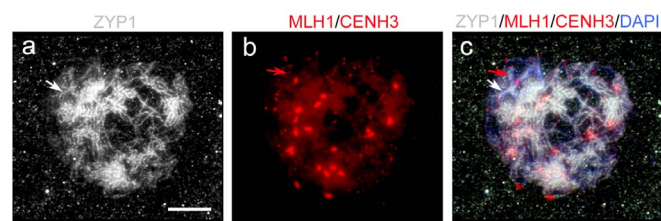

Figure 4 | **Immunolocalisation of ZYP1, MLH1 and CENH3 in the pachytene meiocyte of the line carrying the version of chromosome 3H^{ch} in which a deletion on the short arm has removed the subtelomeric region (the same preparations were assayed as described in Fig. 2d).** (a) ZYP1 is deposited along the synapsed homologues chromosomes, even in the short arm of the barley chromosome (green arrow) were the subtelomeric region is absent. (b) Foci of MLH1 deposition only show along the long arms having the subtelomeric region (red arrow). The large red signals relate to sites of CENH3 deposition and mark the centromere. (c) A merged image of (a) and (b). Bar: 10 μm.

facilitate pairing interactions along chromosome arms, as suggested for maize or wheat[24,11]. Our observations of the behaviour of the wheat addition line which carries the 3H^{ch} chromosome lacking its short arm subtelomeric region have illustrated that, although the centromeres do not trigger a signal inducing the homologues to pair, the signal which has been initiated at one end of the chromosome can be transmitted through the centromere, thereby allowing homologues to become fully associated by the end of prophase I.

It has recently become clear that rather than location on the chromosome *per se*, it is either the DNA sequence or chromatin organisation which governs the site of chiasma formation[25]. Regions which frequently feature crossing-over, such as the subtelomeric region, are more intimately involved in homologue recognition and synapsis than are cross-over poor regions[6,7,25]. Thus, the absence of a subtelomeric region, as in the short arm deletion version of chromosome 3H^{ch}, would be expected to suppress chiasma formation, and consequently this arm would be less likely to be associated during meiotic metaphase I. During the telomere bouquet stage, the chromosome distal ends undergo remodeling, following which the homologue pairs associate with one another[5]. Chromatin remodeling has been shown to be a prerequisite for chromosome pairing and recombination in wheat[5,26]. The requirement of chromatin remodelling for chromosome pairing and recombination has been also shown in other species such as *Caenorhabditis elengans*, which does not even display a telomere bouquet during early meiosis[27]. The present observations of the behaviour of the version of chromosome 3H^{ch} which lacks the short arm subtelomeric region suggest that it fails to undergo remodelling at the bouquet stage, and the homologous arms do not pair until the pairing signal has been transmitted along the chromosome from the long arm. The lack of chromatin remodeling in the short arm and the consequent delay in pairing together can explain the localised absence of recombination, a process which needs to be completed before the meiocyte progresses beyond pachytene. The implication is that it is not so much the lack of the subtelomeric sequences that is important, but rather the consequent

Table 1 | Chromosome 3H^{ch} pairing at metaphase I in the first ear sampled from four independent plants per addition line. Applying a χ^2 test to test whether chromosome pairing was similar in the two lines suggested that the hypothesis should be rejected ($p < 0.001$). del3H^{ch}L: the line carrying the version of chromosome 3H^{ch} in which a deletion on the long arm has removed neither the long arm telomere nor the subtelomeric region, del3H^{ch}S: the line carrying the version of chromosome 3H^{ch} in which a deletion on the short arm has removed the subtelomeric region. n: number of cells analysed

Line	Number of spikes	Total n	Ring bivalents n (%)	Open bivalents n (%)	Univalents n (%)	P
del3**H^{ch}**L	4	275	273 (99.27)	2 (0.73)	0	2.2×10^{-16}
del3**H^{ch}**S	4	511	0	503 (98.43)	8 (1.57)	

delay in chromatin remodeling and pairing which is responsible for the absence of recombination.

The present observations have shown that the subtelomeric region plays a key role in the processes of homologue recognition and subsequent pairing during early meiosis in wheat. It was also confirmed that it acts to trigger a conformational change to the chromatin, an event which allows the homologue pairs to associate intimately with one another, thereby permitting recombination later in meiosis. Unraveling the underlying molecular mechanisms involved will shed light to our understanding of how each chromosome associates with 'the right partner' during meiosis in wheat.

Methods

Plant material. Root tips and anthers were harvested from two addition lines constructed in the experimental bread wheat variety 'Chinese Spring' and involved two versions of *H. chilense* chromosome 3H[ch]. One of these carried a terminal deletion of the short arm (44% of the chromosome arm deleted, including the subtelomeric region) and the other a terminal deletion within the long arm (58% of the chromosome arm deleted, but retaining the subtelomeric region)[16].

Fluorescence *in situ* hybridisation. Three *in situ* hybridisation probes were fluorescently labelled following standard procedures: these comprised (1) the barley subtelomeric sequence HvT01[28], (2) the highly conserved telomeric sequence pAt74[29], originally isolated from *A. thaliana*, and (3) total genomic *H. chilense* DNA[17]. Methods for preparing meiotic and mitotic chromosomes spreads, *in situ* hybridisation and subsequent scoring have been described elsewhere[17,30].

Immunocytological detection of CENH3, ZYP1 and MLH1 during wheat meiosis. To visualise centromeres, samples were rinsed in PBS buffer following the *in situ* hybridisation procedure, and then incubated with the α-CENH3 antibody[31], kindly supply by Dr. J.M. Vega from the Complutense University of Madrid (Spain). The hybridisation signal was detected following incubation with a secondary incubation with the α-rabbit-ROD (Millipore, MA, USA). After rinsing once more in PBS buffer, a second immunolocalisation was carried out in the same meiocytes to detect the ZYP1 and MLH1 proteins following the protocols previously described[32,33]. The anti-ZYP1 and the anti-MLH1 were kindly supplied by Dr. J.A. Able from the University of Adelaide (Australia) and Dr. Chelysheva from the INRA (France), respectively.

Fluorescence microscopy and image processing. The fluorescence optical images were collected using the fluorescence microscope Eclipse 80i, Nikon UK. The images were processed by the Photoshop version 11.0.2 software.

Ethics statement. The authors declare that the experiments comply with the current laws of the country (Spain) in which they were performed.

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Author contributions

M.C.C., M.D.R., A.C. and P.P. conceived the project and planned the experiments. M.C.C., M.D.R. and P.P. conducted the experiments. M.C.C., M.D.R., A.C. and P.P. wrote the paper (all authors provided constructive feedback).

Additional information