Meiotic metaphase I pairing behavior of a 5BL recombinant isochromosome in wheat

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Abstract
A recombinant isochromosome i5BL rec of wheat was developed with one arm and the proximal 36% of the other arm of Chinese Spring (CS) origin and the distal 64% of the recombined arm of Triticum turgidum subsp. dicoccoides origin. The i5BL rec provides an unusual opportunity to analyze the role of the centromere or arm heterozygosity in chromosome prealignment and synapsis during meiosis. In monosomic condition, the i5BL rec formed a ring univalent in 86.8% of the pollen mother cells (PMCs) at meiotic metaphase I. In the disomic condition, the two i5BL rec preferentially paired as a normal bivalent in 74.8% of the PMCs, which differed significantly (p < 0.01) from the normal bivalent pairing of 51% observed in diisosomic 5BL chromosomes of the CS (Di5BL CS) control plants. In plants with one i5BL rec and a normal 5B CS, the long arm of 5B CS paired with the homologous arm of i5BL rec in 54.4% of the PMCs, and 40.4% of the PMCs had a 5B CS univalent and a i5BL rec ring univalent. The implications of the i5BL rec pairing data on the mechanism of Ph1 gene action are discussed.

Introduction
In allohexaploid bread wheat, Triticum aestivum L. (2n = 6X = 42, genomically AABBDD), the Ph1 gene is located on the long arm of chromosome 5B and ensures that only homologous but not homoeologous chromosomes pair and recombine at meiosis (Sears & Okamoto 1958, Riley & Chapman 1958, Riley 1968). Therefore, common wheat is a diploid-like allopolyploid in which only bivalents between homologous chromosomes are formed at metaphase I of meiosis (for review, see Sears 1976).

The mechanism of the Ph1 gene action has been studied intensively by analyzing pairing behavior of isochromosomes with or without colchicine treatment (Feldman 1966, Driscoll & Darvey 1970, Kato & Yamagata 1982, Vega & Feldman 1998a, 1998b). Three main hypotheses have been put forward in light of the information collected...

from these studies: (1) Ph1 affects the time available for synapsis; thus, only homologues have the opportunity to pair (Riley 1968); (2) Ph1 regulates strict diploid-like pairing at the prealignment phase by acting on the centromeres (for review, see Feldman 1993, Vega & Feldman 1998a); and (3) Ph1 processes homology along the entire length of the chromosome at the DNA heteroduplex level during synapsis (Holm & Wang 1988, Dubcovsky et al. 1995, Luo et al. 1996).

Recently, we isolated a recombinant (rec) isochromosome for the long arm of chromosome 5B (i5BLrec) of common wheat. The i5BLrec is heterogenetic for the distal 64% of the long arm and provides an unusual opportunity to analyze the role of centromere and arm heterozygosity in chromosome prealignment and synapsis during meiosis. The meiotic pairing in plants with monoisosomic, disisomic 5BLrec, and monoisosomic 5BLrec and a normal chromosome 5B, is reported here. The implications of the i5BLrec pairing data on the mechanism of Ph1 gene action are discussed.

Materials and methods

A plant monosomic for a recombinant isochromosome i5BLrec and trisomic for chromosome 5D (Mi5BLrecTri5D) was available from a previous study (Qi et al. 2000). One arm and the proximal 36% of the other arm of the i5BLrec is of Chinese Spring (CS) origin, and the distal 64% of the recombinated arm is of T. turgidum subsp. dicoccoides origin (Figure 1). Other genetic stocks used in the study were 5BL mono- and diisosomics of T. aestivum cv. Chinese Spring (CS), a nullisomic 5B–tetrasomic 5D (N5BT5D) line, and a ph1a ph2b line (Sears 1954). The 5BL chromosomes of CS and T. dicoccoides are designated as 5BLCS and 5BLTdic, and the 5BL isochromosomes of CS, and the CS–T. dicoccoides recombinant as i5BLCS and i5BLrec, respectively.

Cytogenetic analysis

Chromosome identification and N- and C-banding analyses were as described by Gill et al. (1991).
Figure 2. N-banded mitotic metaphases (a, c, e) and C-banded meiotic metaphase I PMCs (b, d, f) of plants with different chromosome constitutions: (a & b) M5iBL5c5D paired as a ring univalent; (c & d) Di5BL5c5D paired as two ring univalents; (e & f) M5iBL5c5D plus 5B5c5 paired as a ring (top i5BL5c5) and a rod (bottom 5B5c5) univalent. Arrows point to the isochromosomes and 5B5c5. Scale bar = 20 μm.
quency of i5BL<sub>rec</sub> bivalent pairing in Di5BL<sub>rec</sub> plants was significantly different (p < 0.01) from that of the control Di5BL<sub>CS</sub> (Table 2).

In plants with one normal 5B<sub>CS</sub> and one i5BL<sub>rec</sub> chromosome, the long arm of 5B<sub>CS</sub> paired with the homologous arm of i5BL<sub>rec</sub> in 54.4% (74/136) of the PMCs, and 40.4% of the PMCs had a 5B<sub>CS</sub> univalent and an i5BL<sub>rec</sub> ring univalent (Figure 2f). Chromosomes 5B<sub>CS</sub> and i5BL<sub>rec</sub> remained univalent in 5.2% of the PMCs.

Discussion

In meiotic prophase, homologous chromosomes prealign by an active process followed by synapsis and crossover, which results in a chiasmata association at metaphase I that facilitates coorientation and chromosome segregation at anaphase I. In polyploid wheat, the additional distinction between homologous vs. homoeologous chromosome prealignment, synapsis, and crossover must be made. The pairing homoeologous gene Ph1 suppresses homoeologous chromosome pairing but promotes strict homologous pairing leading to diploid-like inheritance. Ph1 is believed to regulate strict diploid-like pairing either at the prealignment phase by acting on the centromeres (Vega & Feldman 1998b) or during synapsis at the DNA heteroduplex level by processing homology along the entire length of the chromosome (Holm & Wang 1988, Dubcovsky et al. 1995).

The i5BL<sub>rec</sub> chromosome has two arms attached to the same centromere. In our i5BL<sub>rec</sub>, the proximal 36% of both arms are strictly homologous (homogenetic), whereas the distal 64% of one arm is heterogenetic (homologous/homoeologous). Analysis of meiotic metaphase I pairing in i5BL<sub>rec</sub> plants in the absence of Ph1 is not possible because the 5BL arm itself carries the gene. However, the role of centromeres in homologous vs. homoeologous pairing can be analyzed.

In disomic i5BL plants with four doses of the Ph1 gene, the metaphase I pairing data are consistent if Ph1 processes DNA homology rather than acts on the centromeres. The four 5BL arms are homogenetic in disomic i5BL<sub>CS</sub> plants and are expected to pair in equal frequencies as bivalents and univalents. Indeed, this was observed (51.1% vs. 48.9%, see Table 2) indicating that attachment to the same centromere and extra doses of Ph1 (reported to reduce pairing even among homologous arms by altering the spatial arrangements of homologous chromosomes, see Feldman 1966) did not reduce homologous pairing. However, in disomic i5BL<sub>rec</sub> plants, homologous arms attached to different centromeres paired preferentially and formed a regular bivalent in 75% of the PMCs (Table 2). This result demonstrates that the primary mechanism of Ph1 gene action is to process DNA homology and promote strictly homologous synapsis.

Recently, chromosome painting was used to analyze the behavior of alien chromosomes or chromosome arms in wheat. The data showed that homologous chromosomes associate during the last premeiotic interphase (Aragón-Alcaide et al. 1997b, Schwarzacher 1997, Mikhailova et al.

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Table 1. Chromosome pairing at meiotic metaphase I of an isochromosome in monosomic i5BL<sub>rec</sub> and i5BL<sub>CS</sub> plants.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of PMCs</th>
<th>Univalents (%)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ring</td>
<td>Rod</td>
<td></td>
</tr>
<tr>
<td>Mi5BL&lt;sub&gt;rec&lt;/sub&gt;Tri5D</td>
<td>174</td>
<td>86.8*</td>
<td>13.2</td>
<td></td>
</tr>
<tr>
<td>Mi5BL&lt;sub&gt;CS&lt;/sub&gt;</td>
<td>40</td>
<td>95.0*</td>
<td>5.0</td>
<td></td>
</tr>
</tbody>
</table>

* Differences in the frequencies of ring univalent formation are not significant (p<0.15).

Table 2. Chromosome pairing at meiotic metaphase I of isochromosomes in plants disomic for i5BL<sub>rec</sub> and i5BL<sub>CS</sub>.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of PMCs</th>
<th>Bivalent (%)</th>
<th>Univalents (%)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>2 rings</td>
<td>Rod + ring</td>
<td>2 rods</td>
</tr>
<tr>
<td>Di5BL&lt;sub&gt;rec&lt;/sub&gt;</td>
<td>103</td>
<td>74.8*</td>
<td>14.6</td>
<td>4.8</td>
<td>5.8</td>
</tr>
<tr>
<td>Di5BL&lt;sub&gt;CS&lt;/sub&gt;</td>
<td>45</td>
<td>51.1*</td>
<td>37.8</td>
<td>11.1</td>
<td>0.0</td>
</tr>
</tbody>
</table>

* The difference in the frequencies of bivalent formation are significant (p<0.01).
1998) and that this process is disrupted in genotypes lacking the Ph1 gene (Aragón-Alcaide et al. 1997b, Mikhailova et al. 1998). Fluorescence in-situ hybridization (FISH) analysis further shows that the centromeres also associate during premeiotic interphase and have a more diffuse appearance in genotypes lacking Ph1 (Aragón-Alcaide et al. 1997a), supporting earlier observations by Upadhyya & Swaminathan (1967).

However, there also is strong evidence suggesting that critical regions determining metaphase I pairing are telomere ends of the chromosomes (Lukaszewski 1997, Gill & Friebe 1998).

Because the ph1 mutant is a large deletion (Gill et al. 1993), we cannot preclude the possibility that abnormal chromosome condensation is caused by a gene or genes different from Ph1. Disruption in chromosome pairing also is caused by colchicine treatment prior to premeiotic S-phase (Vega & Feldman 1998b). These experiments also favor the primary role of centromeres in the process of chromosome prealignment. However, the data presented here, other cytogenetic evidence (Lukaszewski 1997, Gill et al. 1997, Gill & Friebe 1998), and data from mouse, humans, and maize (Scherthan et al. 1996, Bass et al. 1997) indicate that the process of alignment and synopsis begins at the telomeric ends. The telomeres appear to be anchored to the nuclear matrix and facilitate their movement in search of homology. Thus, it is tempting to speculate that colchicine may act by disrupting this vital process (rather than acting on the attachment of microtubules to centromeres) by preventing the prealignment of homologous chromosomes. The primary role of Ph1 would then be to promote homology and to promote strict homologous pairing. Thus, the nuclear mechanism used to search for DNA homology rather than the effects of centromeres plays a role in chromosome prealignment and synopsis.

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References


