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

LiLi Qi, Bernd Friebe* & Bikram S. Gill
Wheat Genetics Resource Center, Department of Plant Pathology, Throckmorton Plant Sciences Center, Kansas State University, Manhattan, KS 66506-5502, USA; Tel: (+1) 785 5322364; Fax: (+1) 785 5325692; E-mail: friebe@ksu.edu
** Correspondence*

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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 (i5BL^{rec}) of common wheat. The i5BL^{rec} 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, diisosomic 5BL^{rec}, and monoisosomic 5BL^{rec} and a normal chromosome 5B, is reported here. The implications of the i5BL^{rec} pairing data on the mechanism of *Ph1* gene action are discussed.

Materials and methods

A plant monosomic for a recombinant isochromosome i5BL^{rec} and trisomic for chromosome 5D (Mi5BL^{rec}Tri5D) was available from a previous study (Qi *et al.* 2000). One arm and the proximal 36% of the other arm of the i5BL^{rec} is of Chinese Spring (CS) origin, and the distal 64% of the recombined 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 5BL^{CS} and 5BL$^{T.dic}$, and the 5BL isochromosomes of CS, and the CS–*T. dicoccoides* recombinant as i5BL^{CS} and i5BL^{rec}, respectively.

Cytogenetic analysis

Chromosome identification and N- and C-banding analyses were as described by Gill *et al.* (1991).

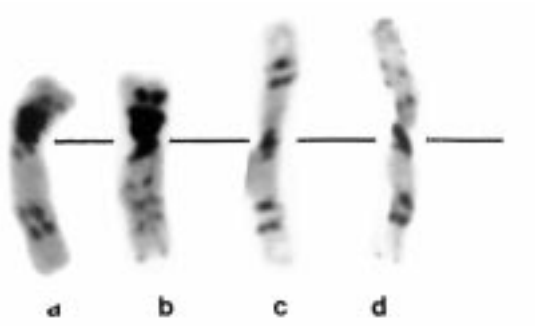

Figure 1. N-banding patterns of chromosomes: (**a**) 5B^{CS}, (**b**) 5B$^{T.dic}$, (**c**) i5BL^{CS}, (**d**) i5BL^{rec}.

Results

Meiotic pairing of the i5BL^{rec}

Three genotypes, one monosomic for i5BL^{rec}, one disomic for i5BL^{rec}, and one monosomic for both i5BL^{rec} and a normal chromosome 5B^{CS}, were selected in the self-progeny of a Mi5BL^{rec}Tri5D plant and from the progeny of the cross Mi5BL^{rec}Tri5D/CS*ph1aph2b*. The meiotic metaphase I pairing of the i5BL^{rec} was compared with control plants that were monosomic or disomic for i5BL^{CS}.

In monosomic i5BL^{rec} plants, a ring univalent resulting from intrachromosomal pairing between the two arms of i5BL^{rec} was observed in 86.8% of the 174 PMCs analyzed (Figure 2b). The i5BL^{rec} remained unpaired and formed a rod univalent in the remaining PMCs (13.2%). The i5BL^{CS} control formed a ring univalent in 95% of the PMCs (Table 1). The frequency of ring univalents formed by i5BL^{rec} was not significantly different from the control i5BL^{CS} ($p = 0.05$).

In Di5BL^{rec} plants, pairing occurred preferentially between homologous arms. The two i5BL^{rec} chromosomes paired as normal bivalents in 74.8%, as two ring univalents in 14.6% (Figure 2d), as one rod and one ring univalent in 4.8%, and as two rod univalents in 5.8% of the 103 PMCs analyzed (Table 2). In Di5BL^{CS} plants, the two i5BL^{CS} paired as normal bivalents and as two ring univalents in 51.1% and 37.8% of the PMCs, respectively. A rod and a ring univalent were formed in 11.1% PMCs (Table 2). The fre-

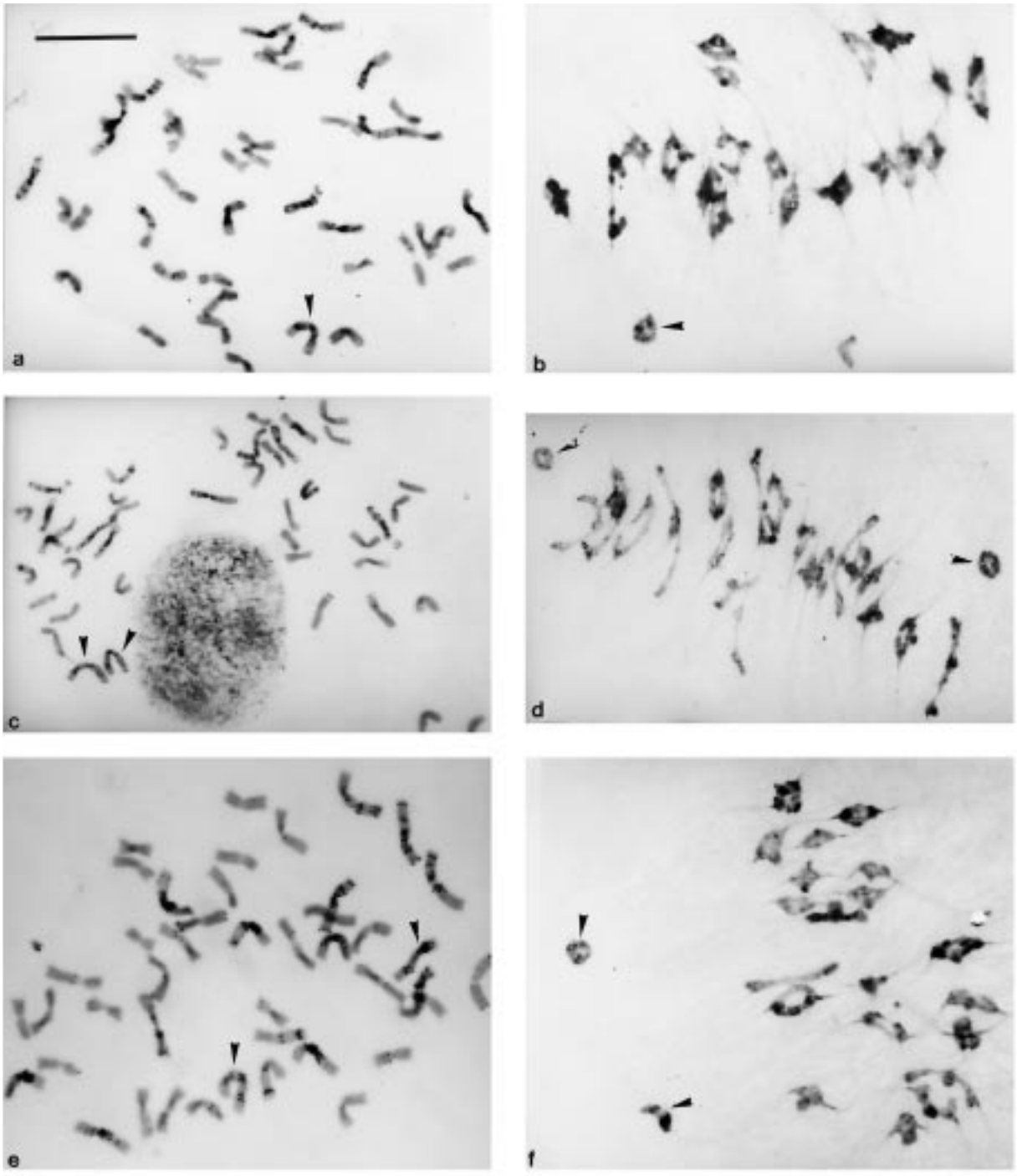

Figure 2. N-banded mitotic metaphases (**a**, **c**, **e**) and C-banded meiotic metaphase I PMCs (**b**, **d**, **f**) of plants with different chromosome constitutions: (**a & b**) Mi5BL^{rec}Tri5D paired as a ring univalent; (**c & d**) Di5BL^{rec} paired as two ring univalents; (**e & f**) Mi5BL^{rec} plus 5B^{CS} paired as a ring (top i5BL^{rec}) and a rod (bottom 5B^{CS}) univalent. Arrows point to the isochromosomes and 5B^{CS}. Scale bar = 20 μm.

Table 1. Chromosome pairing at meiotic metaphase I of an isochromosome in monosomic i5BL^{rec} and i5BL^{CS} plants.

Genotype	No. of PMCs	Univalents (%)	
		Ring	Rod
Mi5BL^{rec}Tri5D	174	86.8*	13.2
Mi5BL^{CS}	40	95.0*	5.0

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

quency of i5BL^{rec} bivalent pairing in Di5BL^{rec} plants was significantly different ($p<0.01$) from that of the control Di5BL^{CS} (Table 2).

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

Discusion

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^{rec} chromosome has two arms attached to the same centromere. In our i5BL^{rec}, 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^{rec} 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^{CS} 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^{rec} 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.*

Table 2. Chromosome pairing at meiotic metaphase I of isochromosomes in plants disomic for i5BL^{rec} and i5BL^{CS}.

Genotype	No. of PMCs	Bivalent (%)	Univalents (%)		
			2 rings	Rod + ring	2 rods
Di5BL^{rec}	103	74.8*	14.6	4.8	5.8
Di5BL^{CS}	45	51.1*	37.8	11.1	0.0

* 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 Upadhya & 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 synapsis 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 process 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 synapsis.

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