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ORIGINAL ARTICLE

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Recombination in an isochromosome preferentially occurs between *cis* isochromatids

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Abstract An isochromosome has identical arms attached to the same centromere. At the pachytene stage of meiosis, it has four isochromatids and recombination can occur either between *cis* isochromatids (attached to the same half-centromere) or *trans* isochromatids (attached to different half-centromeres). Normally such recombination cannot be detected because all four chromatids are homogenetic (arose from misdivision of a centromere to which genetically identical sister chromatids were attached). We isolated an isochromosome of wheat that is heterogenetic for the distal 64% of the long arm. The heterogenetic isochromosome was recovered from the progeny of a cross between *Triticum aestivum* cv. Chinese Spring containing an isochromosome for the long arm of chromosome 5B (i5BL) and a disomic substitution line of *Triticum turgidum ssp. dicoccoides* chromosome 5B in Chinese Spring wheat. New recombinants were produced when the two arms of i5BL^{rec} paired at metaphase I of meiosis. Only *trans* isochromatid exchanges led to some homozygous loci in i5BL^{rec}, whereas exchanges between *cis* isochromatids resulted in heterozygosity at all loci similar to the parental type. There was an average frequency of 0.87 chiasmata per pollen mother cell for the heterogenetic i5BL, which will result in 0.44 *cis* and 0.44 *trans* isochromatid exchanges, assuming that both are occurring at the same frequency. The average crossover frequency based on recombination between *trans* isochromatid exchange detected by restriction fragment length polymorphism analysis in 98 plants was 0.29. This observed value is significantly lower (P<0.01) than the value of 0.44 as expected from chiasmata counts. Our study provides the first experimental evidence that crossovers preferentially occur between *cis* isochromatids rather than *trans* isochromatids.

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Introduction

Bread wheat, *Triticum aestivum* L. (2*n*=6*x*=42, AABBDD), is an allohexaploid species in which the three chromosome pairs of a homoeologous group genetically compensate for one another. The genetic buffering capacity of wheat was exploited to isolate a series of whole-chromosome, chromosome-arm, and sub-arm (deficiencies) aneuploids in the cultivar Chinese Spring (CS) (Sears 1954; Endo and Gill 1996). The development of aneuploid stocks has greatly facilitated mapping of phenotypic and molecular markers (Werner et al. 1992; Kota et al. 1993; Gill KS et al. 1993a, b, 1996; Hohmann et al. 1994; Delaney et al. 1995a, b; Mickelson-Young et al. 1995). Furthermore, aneuploid stocks permit the study of meiotic metaphase I pairing behavior of individual chromosomes. Combining metaphase I pairing and genetic mapping data can provide unique insights into the meiotic process, as reported here for an isochromosome of wheat.

Isochromosomes (i) consist of two homologous arms connected by the same centromere and result from centromere misdivision or fusion of homologous telocentric chromosomes at meiosis (Fig. 1a). A pseudo-isochromosome induced by X-ray was described by Caldecott and Smith (1952). In this case, the distal regions of the two arms of the isochromosome were identical, whereas the proximal regions were different. Lukaszewski (1997) developed asymmetrical isochromosomes with identical proximal regions, but one arm was deficient for a terminal segment. Isochromosomes were used to study the mechanisms of chromosome pairing and synapsis in wheat (Feldman 1966; Driscoll and Darvey 1970; Kato and Yamagata 1982; Lukaszewski 1997), centromere structure (Kaszás and Birchler 1996), and molecular mapping of centromeres in genetic maps of rice (Singh et al. 1996), tomato (Frary et al. 1996), and maize (Schreerman et al. 1998).

Recently, we developed a recombinant (rec) isochromosome for the long arm of chromosome 5B (i5BL^{rec}) of common wheat. In this paper, we report on the identifi-

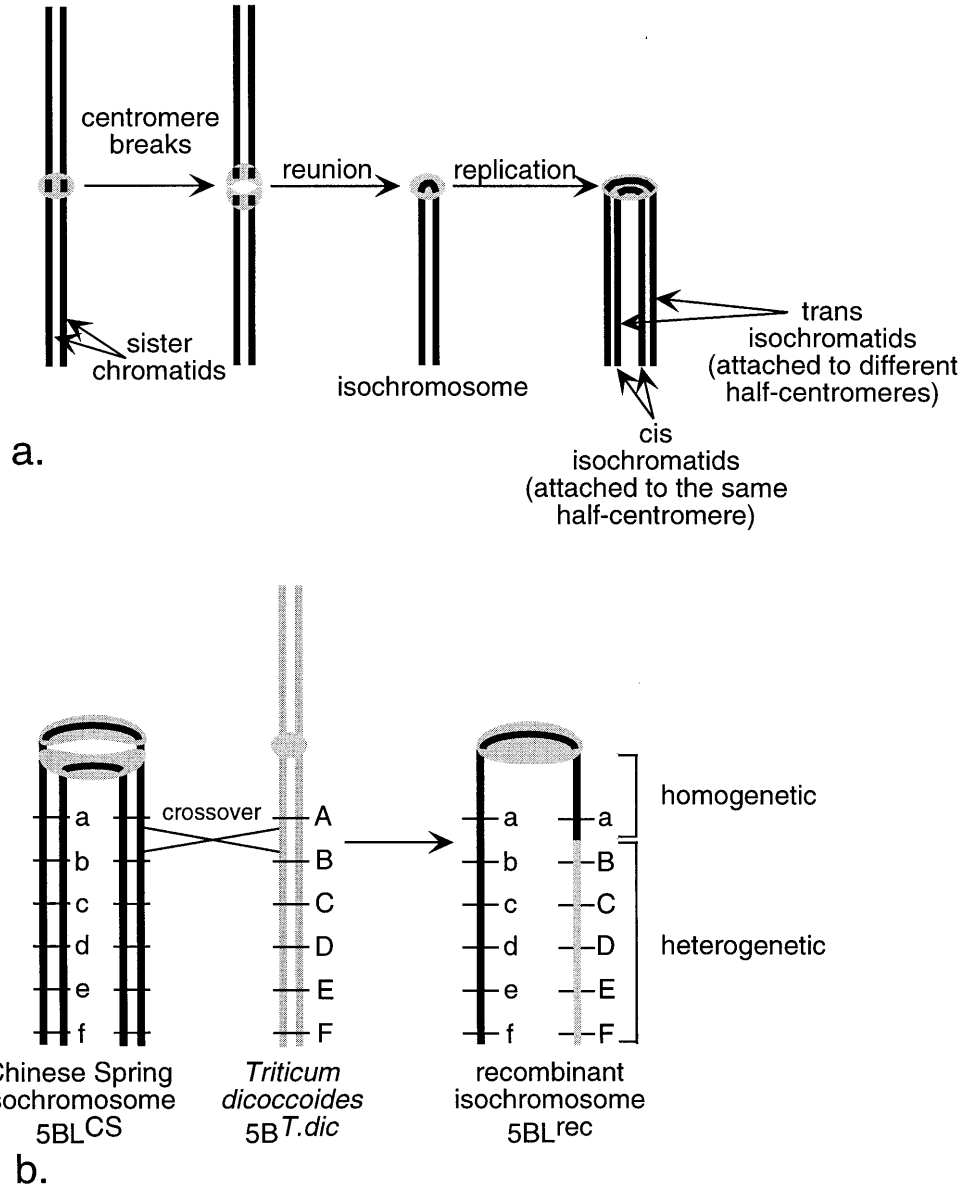

Fig. 1 Origin of an isochromosome (**a**), and the origin of the recombinant isochromosome 5BL^{rec} (**b**). **a** A break at the centromere of a bibranchial chromosome followed by the fusion of the sister chromatids at the breakpoint results in an isochromosome that, after DNA replication, consists of four genetically identical chromatids; chromatids connected by the same half-centromere are termed *cis* isochromatids, whereas those with different half-centromeres are termed *trans* isochromatids. **b** Crossover between a Chinese Spring isochromosome 5BL^{CS} and a *Triticum dicoccoides* chromosome 5B$^{T.\,dic}$ results in a recombinant isochromosome 5BL^{rec} in which one arm and the proximal region of the other arm are of CS origin, whereas the remaining part of the latter arm is derived from 5BL$^{T.\,dic}$. Loci present in the proximal region are homogenetic, whereas loci distal to the crossover event are heterogenetic

cation, meiotic metaphase I pairing behavior and crossover frequencies of i5BL^{rec}. The implications of these results for the recombination process are discussed.

Materials and methods

Cytogenetic stocks

Various cytogenetic stocks in a CS background were employed in this study, including a line diisosomic for 5BL (Di5BL^{CS}) (normal chromosome pair 5B is replaced by two isochromosomes consisting of the long arm of 5B of CS origin), a disomic substitution (DS) of *Triticum turgidum* ssp. *dicoccoides* (2*n*=4*x*=28, AABB, referred to as *Triticum dicoccoides* from here on) chromosome 5B for chromosome 5B of CS, DS5B$^{T.dic}$ (5B^{CS}) (disomic substitution line where the normal chromosome pair 5B of CS origin is replaced by a 5B pair of *T. dicoccoides* origin) and a nullisomic 5B-tetrasomic 5D (N5BT5D) line (line is lacking chromosome pair 5B and this loss is compensated by four copies of chromosome 5D). All the lines were developed by Dr. E.R. Sears (Sears 1954), and are maintained by the Wheat Genetics Resource Center at Kansas State University. 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 is based on the N- and C-banded standard karyotypes as described (Gill BS and Kimber 1974; Gill BS et al. 1991). Anthers at meiotic metaphase I were fixed in a 3:1 solution of absolute ethanol:glacial acetic acid for 5–7 days, squashed in 45% acetic acid, and C-banded.

DNA probes

Twenty-one wheat homoeologous chromosome group 5-specific probes were used for restriction fragment length polymorphism (RFLP) analysis. These clones were BCD (barley cDNA), CDO

(oat cDNA), and WG (wheat genomic DNA) obtained from Dr. M.E. Sorrells, Ithaca, NY, USA; PSR (wheat cDNA or genomic DNA) obtained from Dr. M.D. Gale, Norwich, UK; KSU, *Aegilops tauschii* genomic DNA as described in Gill KS et al. (1991); MWG (barley genomic DNA) obtained from Dr. A. Graner, Grünbach, Germany; RZ (rice cDNA) obtained from Dr. S.R. McCouch, Ithaca, NY, USA; and FBA (wheat genomic DNA) obtained from Dr. F. Quetier, Paris, France.

Restriction fragment length polymorphism analysis

Ninety-eight plants with an i5BL^{rec} and lacking a normal 5B^{CS} were selected by N-banding analysis from the cross N5BT5D/Mi5BL^{rec}Tri5D and the self-pollinated progeny of Mi5BL^{rec}Tri5D, and were used as a mapping population. The DNA of these plants was digested with four different restriction enzymes (EcoRI, EcoRV, HindIII, and DraI) and screened with RFLP clones that hybridize within the fraction length (FL) 0.37–1.00 interval of 5BL. Methods for gel-blot DNA hybridization were as previously described by Qi et al. (1997).

Statistical analysis

All data were subjected to a *z* statistic test to determine the probability of getting a sample proportion as divergent as another one (Ott 1993).

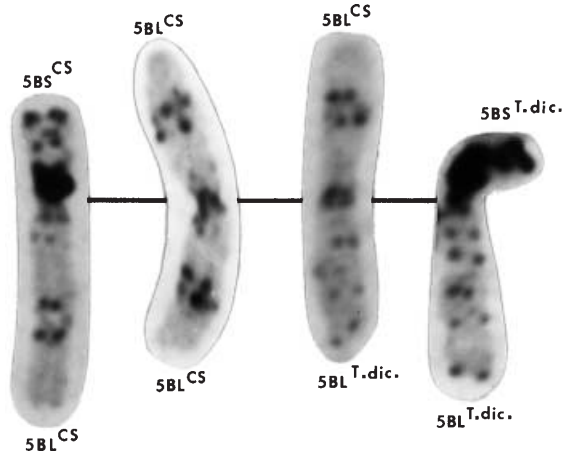

Fig. 2 C-banding patterns of chromosomes 5B^{CS} (*left*), 5B$^{T.\ dic}$ (*right*), i5BL^{CS} (*second from left*), and i5BL^{rec} (*second from right*). Note the polymorphic C-bands on 5BL of *T. dicoccoides* origin that are clearly observed in one arm of the recombinant isochromosome i5BL^{rec}

Results

Development and identification of the heterogenetic isochromosome 5BL

Line Di5BL^{CS} was crossed with DS5B$^{T.\ dic}$ (5B^{CS}). The long arm of chromosome 5BL$^{T.dic}$ differs from 5BL^{CS} by additional proximal and telomeric C-bands (Fig. 2). The F_1 was crossed as a female to CS N5BT5D. Thirty-seven progeny plants were analyzed by C-banding, and one 42-chromosome plant monosomic for i5BL^{rec} and trisomic for 5D was identified. One arm of the i5BL^{rec} is of CS origin and most of the other arm is derived from *T. dicoccoides* (Fig. 1b, Fig. 2).

The crossover site in the recombinant chromatid of i5BL^{rec} was identified by molecular marker analysis. Three DNA markers, *XksuA3*, *Xpsr360*, and *Xbcd204*, previously mapped within the proximal region of the long arm of chromosome 5B (FL 0.26–0.36) (Gill KS et al. 1996), detected homozygous loci of CS in i5BL^{rec}. Heterozygotes were detected by the probes *Xbcd926* and *Xpsr128* (Fig. 3), which were closely linked on the genetic map and mapped in the FL 0.37–0.43 interval (Gill KS et al. 1996). The results indicated that the i5BL^{rec} arose from genetic recombination between i5BL^{CS} and 5B$^{T.dic}$ (Fig. 1b). The crossover site is located between markers *Xbcd204* and *Xbcd926* at FL 0.36.

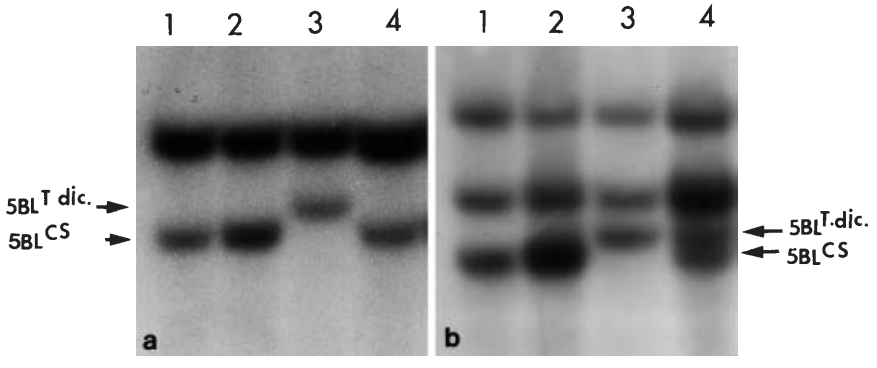

Fig. 3 **a** Southern hybridization of probe PSR360 to *Hind*III-digested genomic DNA of Chinese Spring (*CS*) and various cytogenetic stocks. *Lane 1* CS; *lane 2* Di5BL^{CS} (normal chromosome pair 5B is replaced by two isochromosomes consisting of the long arm of 5B of CS origin); *lane 3* DS5B$^{T.\ dic}$ (5B^{CS}) (disomic substitution line where the normal chromosome pair 5B of CS origin is replaced by 5B of *T. dicoccoides* origin); *lane 4* Mi5BL^{rec}Tri5D (line has three copies of chromosomes 5D and one recombined isochromosome i5BL^{rec} replacing normal chromosome pair 5B). Only the CS pattern was detected by PSR360 in i5BL^{rec} (*lane 4*), showing that both arms of i5BL^{rec} in this region are derived from CS. **b** Southern hybridization of probe PSR128 to *Dra*I-digested genomic DNA; *lanes 1–4* are the same as in **a**. Both CS and *T. dicoccoides* 5BL bands were detected by PSR128 in plants with i5BL^{rec} (*lane 4*), indicating that, in this region, one arm of i5BL^{rec} is of CS origin and other is derived from *T. dicoccoides*

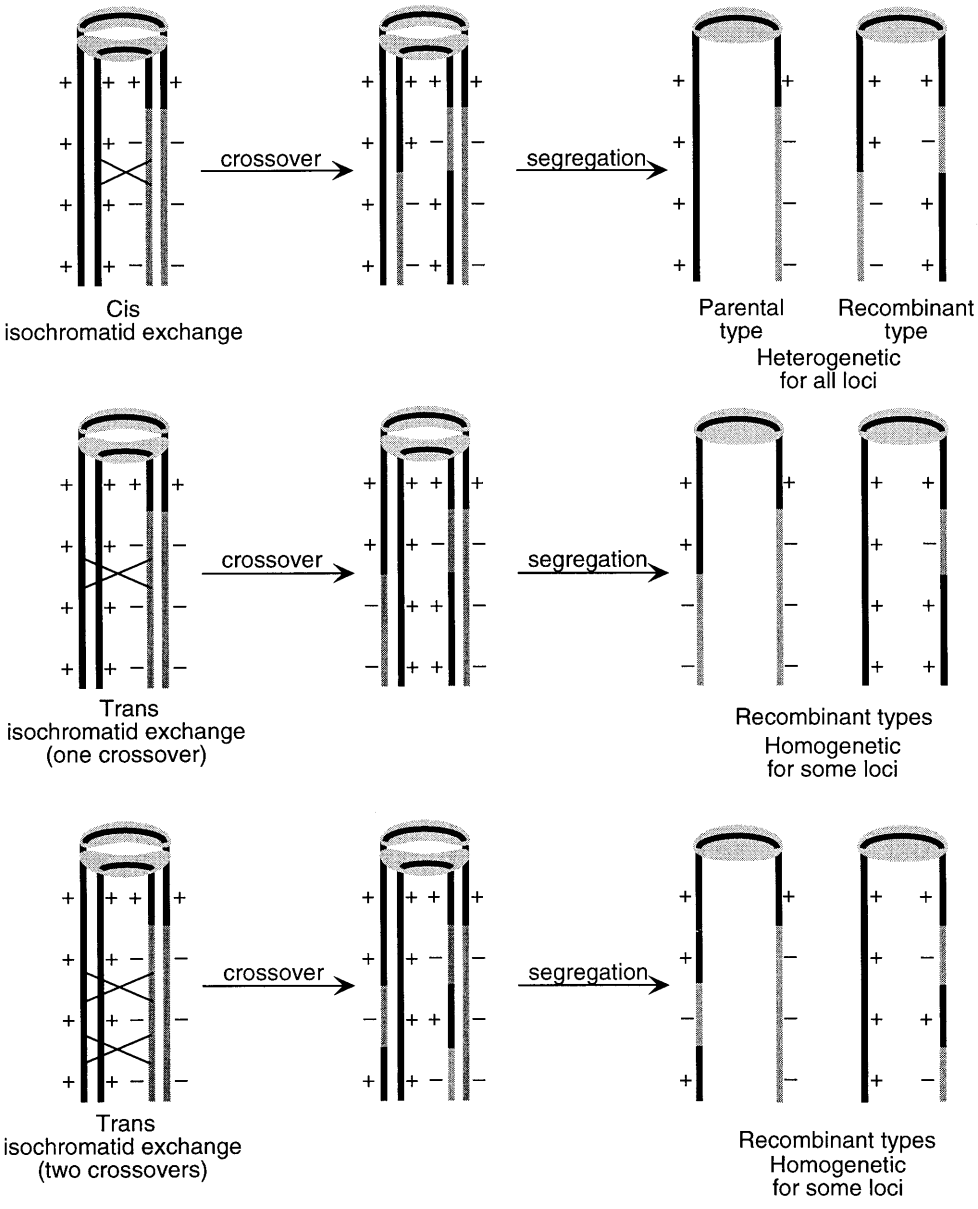

Fig. 4 Consequences of *cis* and *trans* isochromatid exchanges in i5BL^{rec}. Only *trans* isochromatid exchanges lead to some homozygous loci, whereas *cis* isochromatid exchanges lead to the parental pattern with heterozygousity at all loci. The *black lines* represent the CS chromatids, and *gray* lines those of *T. dicoccoides*

Meiotic pairing of the i5BL^{rec}

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 pollen mother cells (PMCs) analyzed. The i5BL^{rec} was unpaired and formed a rod univalent in the 13.2% PMCs. The i5BL^{CS} control formed a ring univalent in 95% (38 out of 40 analyzed PMCs). The frequency of ring univalents formed by i5BL^{rec} was not significantly (P=0.05) different from the control i5BL^{CS}.

Trans isochromatid exchanges detected by RFLP markers

The meiotic metaphase I pairing analysis revealed that the isochromosome i5BL^{rec} preferentially paired as a ring univalent, allowing for genetic recombination between the 5BL arms of CS and *T. dicoccoides* in a single cycle of meiosis. The homozygous i5BL^{rec} loci observed in derived progenies result from *trans* isochromatid exchanges. *Cis* isochromatid exchanges lead to heterozygous loci similar to the parental type (Fig. 4). Homozygous i5BL^{rec} loci were detected in 22 of the 98 plants analyzed (Figs. 5, 6). Thus, the average crossover frequency based on recombination resulting from *trans* isochromatid exchanges was 0.29.

Physical distribution of crossovers

The physical position of the RFLP markers used to assay recombination is known from previous studies (Gill KS et al. 1996; Faris et al. 2000). Combined physical-genet-

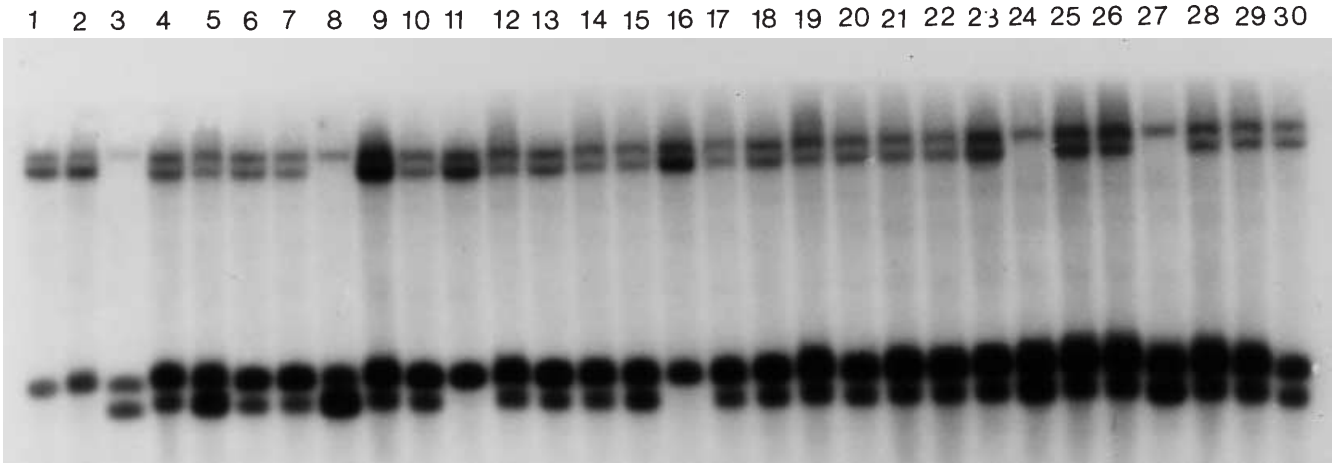

Fig. 5 Southern hybridization of probe PSR580 to *Dra*I-digested genomic DNA. *Lane 1* CS, *lane 2* Di5BL^{CS}, *lane 3* DS5B$^{T.\ dic}$ (5B^{CS}), *lane 4* Mi5BL^{rec}Tri5D; *lanes 5–30* part of the i5BL^{rec} mapping population, all with i5BL^{rec} but lacking 5B^{CS}. Plants in *lanes 8*, *24*, and *27* are homozygous for the *T. dicoccoides* allele, and plants in *lanes 11* and *16* are homozygous for the CS-type allele. All these plants resulted from a *trans* isochromatid exchange (see Fig. 4)

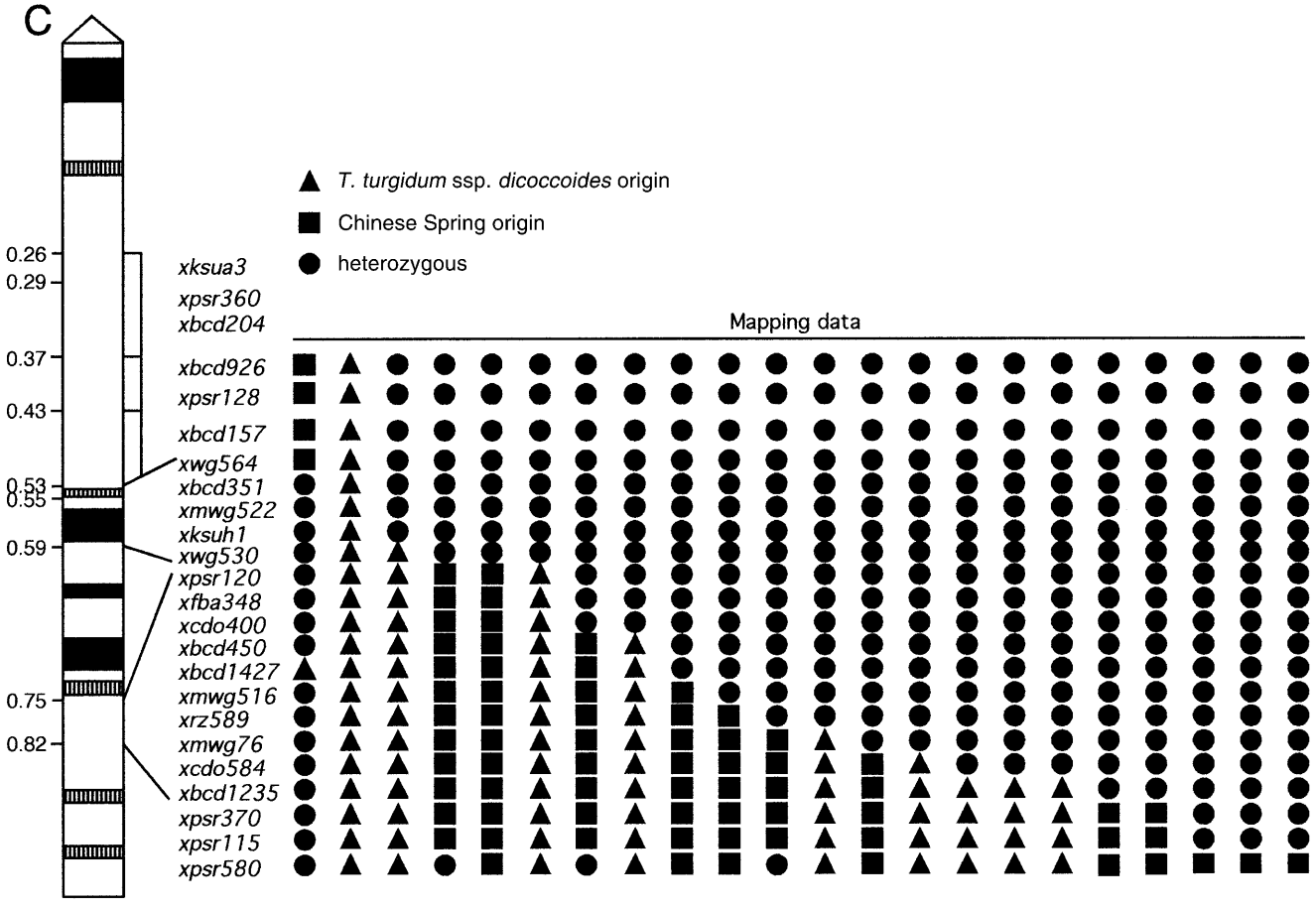

Fig. 6 Distribution of crossovers along the physical length of chromosome arm 5BL. The physical map of chromosome arm 5BL showing the deletion breakpoint intervals and the restriction fragment length polymorphism (RFLP) loci located in these intervals are shown on the *left*. The mapping data of the i5BL^{rec}population showing the distribution of crossovers are given on the *right*. Homozygous loci were detected by RFLP markers in 22 plants. A *triangle* represents the *T. dicoccoides* type, a *square* represents the CS type, and a *circle* represents a heterozygote. The C-banding pattern of 5BL was taken from Gill BS et al. (1991). The orders of markers on the physical map of 5BL is according to Gill KS et al. (1996) and Faris et al. (2000). Note that most crossovers are distributed in the RFLP marker-rich region (deletion interval 0.75–0.82)s

ic mapping and haplotype analysis were used to calculate the number of crossovers per meiosis and to determine the physical distribution of crossovers (Fig. 6). Eighteen plants had one crossover, three plants had two crossovers, and one plant (31-71) had four crossovers during meiosis (Fig. 6). Of the 28 total recombination events detected, 24 crossovers were in the distal 25% of the arm (FL 0.75–1.00) and only four crossovers occurred in the middle (38%) of the arm (FL 0.37–0.75). More than half (16/28) of the crossovers occurred in the FL 0.75–0.82 interval and about one-third in the distal FL 0.82–1.00 interval of 5BL. Three double crossovers occurred in the distal FL 0.75–1.00 interval. One plant (column 1 in Fig. 6) had one double crossover in the FL 0.37–0.59 interval, and one gene conversion-type recombination event was detected by probe *Xbcd1427* in the FL 0.75–0.82 interval, where the alternate allele was detected by only one marker.

Discussion

Distinction between *cis* and *trans* isochromatid exchanges

Genetic recombination results from nonsister chromatid exchanges at meiotic prophase. In a recombined isochromosome, recombination can result either from *cis* or *trans* isochromatid exchanges as shown in Fig. 4. Only *trans* isochromatid exchanges lead to homozygousity at some loci, whereas *cis* isochromatid exchanges result in parental patterns with heterozygousity at all loci (Fig. 4). Our combined meiotic metaphase I pairing and molecular mapping data indicate that crossover occurs preferentially between *cis* rather than *trans* isochromatids. There was an average of 0.87 chiasmata for i5BL^{rec} based on metaphase I pairing data. If *trans* and *cis* isochromatid exchanges occur at equal frequencies, as expected, half of the chiasmata (0.44) were contributed by *trans* isochromatid exchanges. In this case, one chiasma represents one crossover because *trans* isochromatid exchanges in i5BL^{rec} produce only recombinant gametes (Fig. 4). Of 98 plants tested by RFLP markers in the present study, 28 recombination events arising from *trans* isochromatid exchanges were detected in i5BL^{rec}. The average crossover frequency based on recombination was 0.29 for i5BL^{rec}, which is significantly ($P<0.01$) less than 0.44 crossovers estimated from chiasmata counts. At present, it is not clear why there is a preference for *cis* over *trans* isochromatid exchanges. Two mutually nonexclusive possibilities can be suggested. One could be steric hindrance that makes alignment and pairing more difficult between *trans* isochromatids as they are attached to different half-centromeres. The second possibility could be that, in addition to steric hindrance, centromere activity promotes *cis* isochromatid recombination.

Physical distribution of crossovers

Both physical and genetic linkage maps of chromosome 5B have been constructed in *T. aestivum* (Gill KS et al. 1996; Faris et al. 2000). The data indicate that most of the DNA markers in the long arm of 5B occupy four distinct locations in regions between FLs 0.26–0.37, 0.55–0.59, 0.75–0.79, and 0.82-telomere. Marker-rich regions apparently account for most of the recombination in the 5B long arm (Gill KS et al. 1996; Faris et al. 2000). Our data further reinforce these results. Of the 21 informative RFLP markers, 10 were mapped in the physically small interval of FL 0.75–0.82, which also accounted for more than 50% of the recombination events. This supports the hypothesis that recombination is mainly restricted to gene-rich regions (Schnable et al. 1996).

Singletons, where the switch in data type is only for one marker, have been observed in molecular mapping exercises in several crop plants (for review see Gill BS et al. 1997) and are thought to be artifacts or arise from novel recombination mechanisms that may involve gene-conversion type events. We observed one definite singleton involving marker *Xbcd1427* in the high-recombination FL 0.75–0.82 interval. This particular plant had two other recombination events in the proximal region also. Sybenga (1996) has hypothesized that because highly divergent parents were used in mapping, high heterozygosity may lead to breakdown of interference and, hence, abnormal patterns of recombination. However, as pointed out earlier, if recombination is restricted to genes then high rates of recombination including singletons would appear as chromosome regions where interference has broken down.

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References

Caldecott RA, Smith L (1952) A study of X-ray-induced chromosomal aberrations in barley. Cytologia 17:224–242

Delaney DE, Nasuda S, Endo TR, Gill BS, Hulbert SH (1995a) Cytologically based physical maps of the group-2 chromosomes of wheat. Theor Appl Genet 91:568–573

Delaney DE, Nasuda S, Endo TR, Gill BS (1995b) Cytologically based physical maps of the group-3 chromosomes of wheat. Theor Appl Genet 91:780–782

Driscoll CJ, Darvey NL (1970) Chromosome pairing effect of colchicine on an isochromosome. Science 169:290–291

Endo TR, Gill B S (1996) The deletion stocks of common wheat. J Hered 87:295–307

Faris JD, Haen KM, Gill BS (2000) Saturation mapping of gene-rich recombination hot spot region in wheat. Genetics 154: 823–835

Feldman M (1966) The effect of chromosomes 5B, 5D, and 5A on chromosomal pairing in *Triticum aestivum*. Proc Natl Acad Sci USA 55:1447–1453

Frary A, Presting GG, Tanksley SD (1996) Molecular mapping of the centromeres of tomato chromosomes 7 and 9. Mol Gen Genet 250:295–304

Gill BS, Kimber G (1974) Giemsa C-banding and the evolution of wheat. Proc Natl Acad Sci U S A 71:4086 – 4090

Gill BS, Friebe B, Endo TR (1991) Standard karyotype and nomenclature system for description of chromosome bands and structural aberrations in wheat (*Triticum aestivum*). Genome 34:830–839

Gill BS, Gill KS, Friebe B, Endo TR (1997) Expanding genetic maps: reevaluation of the relationship between chiasmata and crossovers. In: Henriques-Gil N, Parker JS, Puertas MS (eds) Chromosomes today, vol 12, Chapman & Hall, London, pp 283–398

Gill KS, Lubbers EL, Raupp WJ, Cox TS, Gill BS (1991) A genetic linkage map of *Triticum tauschii* (DD) and its relationship to the D genome of bread wheat (AABBDD). Genome 34:362–374

Gill KS, Gill BS, Endo TR (1993a) A chromosome region-specific-mapping strategy reveals gene-rich telomeric ends in wheat. Chromosoma 102:374–381

Gill KS, Gill BS, Endo TR, Mukai Y (1993b) Fine physical mapping of Ph1, a chromosome pairing regulator gene in polyploid wheat. Genetics 134:1231–1236

Gill KS, Gill BS, Endo TR, Boyko EV (1996) Identification and high-density mapping of gene-rich regions in chromosome group 5 of wheat. Genetics 143:1001–1012

Hohmann U, Endo TR, Gill KS, Gill BS (1994) Comparison of genetic and physical maps of group-7 chromosomes from *Triticum aestivum.* Mol Gen Genet 245:644–653

Kaszás É, Birchler JA (1996) Misdivision analysis of centromere structure in maize. EMBO J 15:5246–5255

Kato T, Yamagata H (1982) Effect of 3B chromosome deficiency on the meiotic pairing between the arm of an isochromosome in common wheat. Jpn J Genet 57:403–406

Kota RS, Gill KS, Endo TR, Gill BS (1993) Construction of a cytogenetically based physical map of chromosome 1B of common wheat. Genome 36:548–554

Lukaszewski AJ (1997) The development and meiotic behavior of asymmetrical isochromosomes in wheat. Genetics 145:1155–1160

Mickelson-Young L, Endo TR, Gill BS (1995) A cytogenetic ladder-map of the wheat homoeologous group-4 chromosomes. Theor Appl Genet 90:1007–1011

Ott RL (1993) An introduction to statistical methods and data analysis. Duxbury Press, Belmont, Calif, pp 380–385

Qi LL, Wang SL, Chen PD, Liu DJ, Friebe B, Gill BS (1997) Molecular cytogenetic analysis of *Leymus racemosus* chromosomes added to wheat. Theor Appl Genet 95:1084–1091

Schnable PS, Xu X, Civardi L, Xia Y, Hsia AP, Zhang L, Nikolau BJ (1996) The role of meiotic recombination in generating novel genetic variability. In: Sobral BWS (ed) The impact of plant molecular genetics, Birkhäuser, Boston, pp 103–110

Schreerman MC, Lee WS, Doyle G, Weber DF (1998) RFLP mapping of the centromere of chromosome 4 in maize using isochromosomes for 4S. Theor Appl Genet 96:361–366

Sears ER (1954) The aneuploids of common wheat. Missouri Agric Exp Station Res Bull 572, pp 58

Singh K, Ishii T, Parco A, Huang N, Brar DS, Khush GS (1996) Centromere mapping and orientation of the molecular linkage map of rice (*Oryza sativa* L.). Proc Natl Acad Sci USA 93: 6163–6168

Sybenga J (1996) Recombination and chiasmata: few but intriguing discrepancies. Genome 39:473–484

Werner JE, Endo TR, Gill BS (1992) Toward a cytogenetically based physical map of the wheat genome. Proc Natl Acad Sci USA 89:11307–11311