Saturation Mapping of a Gene-Rich Recombination Hot Spot Region in Wheat

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ABSTRACT

Physical mapping of wheat chromosomes has revealed small chromosome segments of high gene density and frequent recombination interspersed with relatively large regions of low gene density and infrequent recombination. We constructed a detailed genetic and physical map of one highly recombinant region on the long arm of chromosome *5B*. This distally located region accounts for 4% of the physical size of the long arm and at least 30% of the recombination along the entire chromosome. Multiple crossovers occurred within this region, and the degree of recombination is at least 11-fold greater than the genomic average. Characteristics of the region such as gene order and frequency of recombination appear to be conserved throughout the evolution of the Triticeae. The region is more prone to chromosome breakage by gametocidal gene action than gene-poor regions, and evidence for genomic instability was implied by loss of gene collinearity for six loci among the homeologous regions. These data suggest that a unique level of chromatin organization exists within gene-rich recombination hot spots. The many agronomically important genes in this region should be accessible by positional cloning.

THE polyploid nature of wheat (*Triticum aestivum* L. emend. Thell., $2n = 6x = 42$, AABBDD genomes) allows it to tolerate, and transmit through gametes, a certain degree of aneuploidy. Over 400 chromosome deletion lines covering the entire wheat genome are now available (Endo and Gill 1996). The deletion stocks are powerful tools for constructing physical maps as they eliminate the requirement for intragenomic polymorphism and can be used to localize agronomically important genes to relatively small chromosomal regions. Physical maps have been constructed for each of the 21 chromosomes of wheat using molecular markers (Gill *et al.* 1993, 1996a,b; Hohmann *et al.* 1994; Delaney *et al.* 1995a,b; Mickelson-Young *et al.* 1995). On the physical maps, most markers were tightly clustered in small-sized physical segments. These markers were identified primarily with cDNA probes and represent expressed genes. Furthermore, Gill *et al.* (1996a,b) compared physical maps with recombination-based maps and found that these gene-rich regions undergo recombination much more frequently than do gene-poor regions. Kilobase pair per centimorgan (cM) estimates ranged from 118 kb for gene-rich regions to 22,000 kb for gene-poor regions (Gill *et al.* 1996a). Other estimates of a gene-rich region on the short arm of chromosome *1D* indicate that 1 cM may constitute as little as 20 kb (W. Spielmeyer, personal communication).

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Physical distribution of recombination events is nonrandom in other plant species as well (Rick 1971). In tomato, recombination is commonly suppressed near the centromeres (Tanksley *et al.* 1992). An extensive study of chromosome *4* of Arabidopsis by Schmidt *et al.* (1995) identified regions in which the base pair to centimorgan ratios ranged from 30 to >550 kb/cM.

Recombination usually results in reciprocal exchange between two nonsister chromatids or gene conversion. Thurieaux (1977) postulated that recombination is confined to coding regions because different eukaryotic organisms have essentially the same number of genes, and the number of map units per genome is relatively constant even though the physical sizes of the genomes vary. Meiotic recombination seems to occur preferentially at defined sites, termed hot spots, along chromosomes of various eukaryotic organisms (Shiroishi *et al.* 1993; Smith 1994; Lichten and Goldman 1995). In maize, genes *per se* are recombination hot spots (Civardi *et al.* 1994; Xu *et al.* 1995). Recombination frequencies at the maize loci *a1* (Brown and Sundaresan 1991; Civardi *et al.* 1994), *adh1* (Freeling 1978), *b* (Patterson *et al.* 1995), *wx* (Nelson 1968), *gl1* (Salamini and Lorenzoni 1970), *r* (Dooner and Kermicle 1986), and *bz1* (Dooner 1986; Dooner and Kermicle 1986) are about 2 orders of magnitude higher than the average rate of recombination per kilobase for the whole genome.

Meiotic recombination in the yeast *Saccharomyces cerevisiae* is initiated by the formation of meiosis-specific DNA double-strand breaks at hot spots (Nicolas *et al.* 1989; Sun *et al.* 1989; Cao *et al.* 1990; Zenvirth *et al.* 1992; de Massey and Nicolas 1993; Goyon and Lich-

ten 1993; Nag and Petes 1993; Wu and Lichten 1994). The double-strand breaks occur preferentially at nucleosome-free regions that show hypersensitivity to nucleases (Ohta *et al.* 1994; Wu and Lichten 1994; Fan and Petes 1996). Therefore, chromatin structure that allows DNA accessibility seems essential for hot spot activity. Much less is known about the mechanisms of recombination in plants, but at least some aspects of meiotic recombination in yeast can be extended to plants. In both, the mechanism for meiotic recombination seems to follow the double-strand break model (Xu *et al.* 1995).

The objectives of this research were to (1) saturate a physically small gene-rich region of wheat chromosome *5B* with molecular markers, (2) assess the degree of recombination that occurs within the region, (3) compare the collinearity of markers in the region among the physical maps of homeologous group *5* chromosomes, and (4) compare levels of recombination among homologous regions of wheat, barley, rice, and *Aegilops tauschii* chromosomes.

MATERIALS AND METHODS

Plant materials: We used 36 lines of "Chinese Spring" (CS) with terminal chromosomal deletions in the long arms of group *5* chromosomes (Endo and Gill 1996) for physical mapping. The number of deletion lines for each chromosome was 16, 12, and 8 for *5AL*, *5BL*, and *5DL*, respectively. Of these deletion lines, 28 are either homozygous or hemizygous (monosomic) for the deletion chromosome, and 8 (*5AL*-16, *5AL*-19, *5BL*-3, *5BL*-7, *5BL*-15, *5DL*-7, *5DL*-8, and *5DL*-10) are heterozygous for the deletion chromosome.

A mapping population consisting of 117 recombinant substitution lines (RSLs) was generated from the cross of CS with CS that had a pair of *Triticum dicoccoides 5B* chromosomes substituted for the native *5B* chromosomes (Gill *et al.* 1996a). Briefly, the F_1 plant was crossed as a male with a monotelosomic *5BL* plant. Plants possessing 40 normal wheat chromosomes and a *5B* chromosome (lacking the *5BL* telosome) were selected and allowed to self. From the progeny of each plant, a 42-chromosome plant having the *5B* recombinant chromosome in the disomic condition was selected and used for mapping.

RFLP analysis: Leaf tissue (~5 g) was collected from 3- to 4-wk-old plants, frozen in liquid nitrogen, ground with a mortar and pestle, and transferred to 50-ml polypropylene tubes. Sodium bisulfite (3.8 g liter^{-1}) was added to the extraction buffer [0.5 m NaCl, 0.1 m Tris-HCl, pH 8.0, 50 mm EDTA, 0.84% (w/v) SDS], and the pH was adjusted to 8.0 with NaOH. Extraction buffer (10–15 ml) was heated to 65°, added to frozen tissue, and incubated at 65° for 30–45 min. A 24:1 solution of chloroform:isoamyl alcohol was added, mixed vigorously, and centrifuged at 8000 × *g* for 15 min. The upper phase was removed, and the DNA was precipitated with 1.5 volumes of cold 95% (v/v) ethanol, rinsed in 70% (v/v) ethanol, dried, dissolved in TE buffer, and quantified on a 0.9% agarose gel.

A total of 20 μg of DNA was digested with 40 units of endonuclease (*Eco*RI, *Eco*RV, *Dra*I, *Hin*dIII, or *Xba*I) in the presence of the appropriate buffer in a final volume of 35 μl. After 16 hr at 37°, the reactions were stopped by adding 8 μl of gel loading buffer [7.6 m glycerol, 0.5× neutral electrophoresis buffer (NEB) (1× NEB: 0.1 m Tris, 1 mm EDTA, 12.5 mm sodium acetate·3H_2O, pH 8.1), 0.02 mm EDTA, 0.2% (w/v) SDS, and 6 g liter^{-1} bromphenol blue]. The resulting mix was loaded on a 0.9% agarose gel made using 1× NEB and was run for 16 hr at 22 V in a horizontal gel apparatus. Gels were stained with ethidium bromide, rinsed in distilled water, and photographed.

DNA was transferred from gels to Hybond N^+ membranes (Amersham, Arlington Heights, IL) according to manufacturer's instructions, except that a large sponge soaked in 0.4 m NaOH served as the base of the blot.

The prehybridization and hybridization solutions were as described in Kam-Morgan *et al.* (1989). Probes were labeled by the random hexamer method with [^{32}P]dCTP (Feinberg and Vogelstein 1983), purified through spun columns containing Sephadex G50, denatured by boiling for 2 min, added to the membranes, and allowed to hybridize for 18–22 hr. Membranes were washed at 65° for 20 min each in 2× SSC and 1× SSC followed by 1 hr in 0.5× SSC (1× SSC: 0.15 m NaCl plus 0.015 m sodium citrate). All washing solutions also contained 0.1% (w/v) SDS. Membranes were placed in plastic sheets and were exposed to X-ray film for 3–7 days.

Microsatellite analysis: Three *5B* microsatellite markers (*Xgwm371*, *Xgwm408*, and *Xgwm499*) were selected on the basis of the map positions determined by Röder *et al.* (1998b). PCR reactions were performed as described in Röder *et al.* (1998a). Amplified products were run on a 2.3% agarose gel made with 1× NEB at 57 V for 4 hr. Gels were stained with ethidium bromide, visualized under UV light, and photographed.

Clone selection and sources: We used 135 RFLP clones that could hybridize to the physical segment of wheat chromosome *5B* that is flanked by fraction breakpoints 0.75 and 0.79. The descriptions of the clone libraries and their sources are given in Table 1. Most of the clones had been localized previously to chromosome group *5* of wheat (Xie *et al.* 1993; Ogihara *et al.* 1994; Devos *et al.* 1995; Nelson *et al.* 1995; Faris *et al.* 1996; Gill *et al.* 1996a; Kojima and Ogihara 1998; Li *et al.* 1999).

We selected many candidate markers from barley *5H* maps (Graner *et al.* 1991; Heun *et al.* 1991; Kleinhofs *et al.* 1993) because the order and constitution of genes and markers on barley chromosome *5H* is highly conserved with that of wheat group *5* chromosomes. Other candidate clones were selected from conserved regions of rice chromosomes *3* and *9* (Causse *et al.* 1994), oat linkage group *E* (O'Donoughue *et al.* 1992; Rayapati *et al.* 1994), and *Ae. tauschii* chromosome *5D* (Kam-Morgan *et al.* 1989; Gill *et al.* 1991; Boyko *et al.* 1999; Li *et al.* 1999).

Mapping and calculations: The computer program MAPMAKER (Lander *et al.* 1987) V2.0 for Macintosh was used to calculate linkage distances using the Kosambi mapping function (Kosambi 1944) and a LOD of 3.00. There are several methods used to calculate crossover interference along a chromosome, and there are limitations to each of them (for review see Ott 1997). We estimated crossover interference by calculating the actual number of crossovers within each marker interval. We divided the chromosome map into four marker intervals using the most informative loci separated by 10- to 15-cM intervals, which is the intermarker distance optimal for detecting interference (Ott 1991). Each adjacent pair of intervals was tested for interference by calculating the coefficient of coincidence for the interval pair.

RESULTS

Physical mapping: The previous chromosome group *5* long arm physical maps consisted of 155 markers of which 44, 54, and 57 loci were on *5AL*, *5BL*, and *5DL*,

TABLE 1

Clone library symbols, descriptions, sources, and their suppliers

Clone library	Description	Source	Marker designation	Supplier
ABC	cDNA	Barley	*Xabc_*	A. Kleinhofs
ABG	Genomic DNA	Barley	*Xabg_*	A. Kleinhofs
BCD	cDNA	Barley	*Xbcd_*	M. Sorrells
CDO	cDNA	Oat	*Xcdo_*	M. Sorrells
CMWG	cDNA	Barley	*Xcmwg_*	A. Graner
FBA	Genomic DNA	Wheat	*Xfba_*	P. Leroy
FBB	Genomic DNA	Wheat	*Xfbb_*	P. Leroy
KSUA,D,E,F,G,H,M	Genomic DNA	*Ae. tauschii*	*Xksu__*	B. Gill
KSUP,Q	cDNA	Wheat	*Xksu__*	B. Gill
LPX[a]	Lipoxygenase gene	Maize	*XLpx*	T. Musket
MGB	cDNA	Durum	*Xmgb_*	A. Blanco
MYBC1[a]	myb protein *c1*	Maize	*Xmybc1*	T. Musket
MWG	Genomic DNA	Barley	*Xmwg_*	A. Graner
OXO1[a]	Oxalate oxidase	Barley	*Xoxo1*	D. Collinge
PR1[a]	*Pr1* gene	Maize	*Xpr1*	S. Morris
PR1B[a]	*Pr1b* gene	Barley	*Xpr1b*	D. Collinge
PSR	Genomic DNA	Wheat	*Xpsr_*	M. Gale
RZ	cDNA	Rice	*Xrz_*	S. McCouch
TAG	Genomic DNA	Wheat	*Xtag_*	K. Tsunewaki
UBP	cDNA	Durum	*Xubp_*	A. Blanco
WG	Genomic DNA	Wheat	*Xwg_*	M. Sorrells

[a] Not clone library designations. These are DNA clones that represent defense-response genes and were described in Li *et al.* (1999).

respectively (Gill *et al.* 1996a). Of the 135 low-copy RFLP clones used in this research, 93 (69%) hybridized to the long arms of group *5* chromosomes resulting in 85, 82, and 78 additional loci on *5AL*, *5BL*, and *5DL*, respectively (Figure 1). The group *5* long arm physical maps now consist of 129 loci on *5AL*, 139 loci (including three microsatellites) on *5BL*, and 135 loci on *5DL*, for a total of 403 loci.

Of the 93 probes that detected group *5* loci, 57 (61%) detected loci on all three homeologous group *5* chromosomes (Table 2). A total of 7 probes was specific to chromosome *5A*, but 3 of these mapped within the chromosome *4A* translocation segment at the tip of *5AL* (Mickelson-Young *et al.* 1995; Nelson *et al.* 1995). A total of 8 probes was specific to *5B*, with 1 of these (*Xbcd307*) detecting two loci; no probes were specific to *5D*. A total of 5 probes detected loci on *5A* and *5B*, but not *5D*. A total of 8 probes hybridized to fragments on *5A* and *5D*, but not *5B*, and another 8 probes detected loci on *5B* and *5D*, but not *5A*. A total of 39 probes was specific to group *5* chromosomes and did not hybridize to any other chromosome.

The three microsatellite markers were specific to chromosome *5B*. Two of these markers mapped within the 0.55–0.59 interval, and one mapped within the 0.75–0.79 interval.

Previously, Gill *et al.* (1996a) identified 12 markers within the deletion interval *5BL* 0.75–0.79. Of the 93 RFLP clones and three microsatellites mapping to group *5* chromosomes in this experiment, 64 probes and one microsatellite mapped within the targeted deletion interval. Combining these 65 markers with the 12 mapped by Gill *et al.* (1996a), this small deletion interval now consists of 77 markers (Figures 1 and 2). We also identified 16 markers in addition to those previously mapped by Gill *et al.* (1996a) within the interval flanked by fraction breakpoints 0.55 and 0.59, and 3 additional markers were mapped within the interval flanked by fraction breakpoints 0.59 and 0.75.

We constructed a consensus physical map of the *5BL* 0.75–0.79 region using 58 markers that were also present on *5AL* and *5DL* (Figure 2). By comparing deletion breakpoints and the markers mapping within deletion intervals on the three homeologues, we constructed a physical map that consists of nine deletion intervals defined by 10 breakpoints. The order of markers across the three homeologous chromosomes agreed relatively well with 52 (90%) of the 58 markers showing a conserved order. Markers *Xtag644* and *Xbcd1734* were more proximal on *5A* and *5B*, but they mapped in different, more distal intervals on *5D*. The location of *Xcdo87* on *5A* and *5D* agreed with each other, but it mapped more proximal on *5B*. Similarly, the locations of *Xmwg900* and *dhn2* agreed with each other on *5B* and *5D*, but they were more distal on *5A*. *Xabg473* had a more proximal location on *5A* than *5D*, but the location of *Xabg473* on *5B* could not be determined relative to *5A* and *5D*.

Genetic mapping: We tested probes that hybridized within interval *5BL* 0.75–0.79 on the physical map for polymorphism between the parents of the mapping pop-

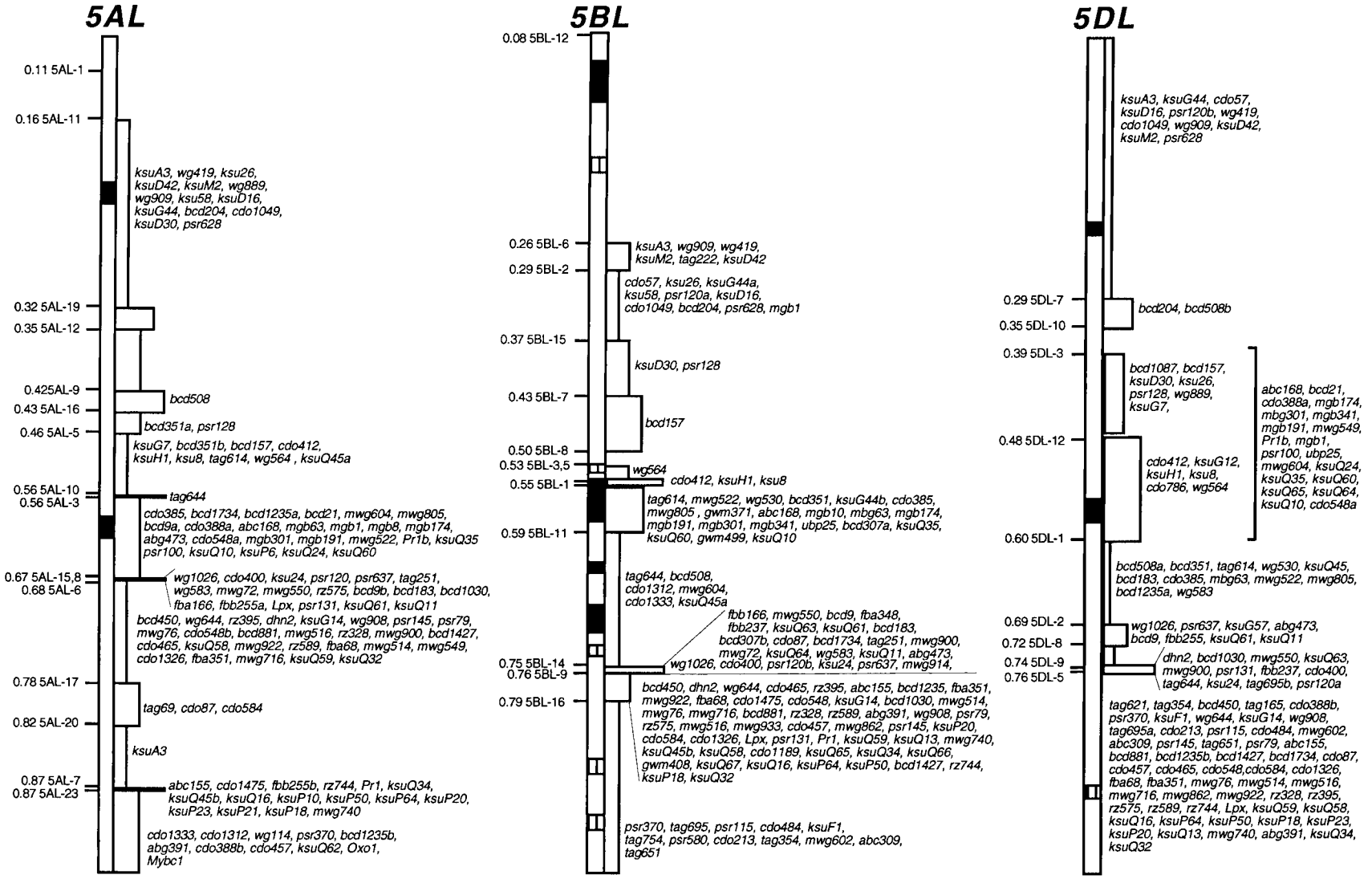

Figure 1.—Physical maps of the long arms of wheat group 5 chromosomes. Fraction breakpoints and the corresponding line numbers are indicated to the left of each chromosome. Boxes to the right of chromosomes indicate deletion intervals. Markers and the intervals in which they map are indicated to the right. The *X* symbol preceding the names of DNA markers according to the wheat genetics nomenclature is omitted to conserve space. Dark regions of the chromosomes represent prominent C-bands, and hatched regions represent lightly stained C-bands that are observed cytologically.

TABLE 2

RFLP clones, the restriction enzymes used to map them, the number of loci each clone detected on group *5* chromosomes, and their specificity to group *5* chromosomes

Probe	Enzyme	No. loci detected on: 5A	5B	5D	Specific to group 5
ABC155	*Eco*RI	1	1	1	Yes
ABC168	*Eco*RI	1	1	1	Yes
ABG391	*Eco*RI	1	1	1	Yes
ABG473	*Hin*dIII	1	1	1	No
BCD9	*Eco*RI	1	1	1	Yes
BCD21	*Hin*dIII	1	0	1	No
BCD183	*Eco*RI	1	1	1	Yes
BCD307	*Eco*RI	0	2	0	No
BCD881	*Eco*RV	1	1	1	No
BCD1030	*Hin*dIII	1	1	1	Yes
BCD1235	*Dra*I	2	1	2	Yes
BCD1427	*Hin*dIII	1	1	1	Yes
BCD1734	*Hin*dIII	1	1	1	No
CDO87	*Hin*dIII	1	1	1	No
CDO385	*Hin*dIII	1	1	1	No
CDO388	*Eco*RV	2	0	2	No
CDO457	*Eco*RV	1	1	1	Yes
CDO465	*Dra*I	1	1	1	Yes
CDO548	*Eco*RV	2	1	2	Yes
CDO584	*Eco*RI	1	1	1	No
CDO1189	*Eco*RI	0	1	0	No
CDO1326	*Hin*dIII	1	1	1	No
CDO1333	*Eco*RI	1	1	0	No
CDO1475	*Eco*RV	1	1	0	Yes
FBA68	*Hin*dIII	1	1	1	No
FBA166	*Eco*RI	1	1	0	Yes
FBA237	*Eco*RI	0	1	1	No
FBA348	*Hin*dIII	0	1	0	Yes
FBA351	*Hin*dIII	1	1	1	No
FBB255	*Dra*I	2	0	1	No
KSUG14	*Hin*dIII	1	1	1	Yes
KSUP6	*Dra*I	1	0	0	No
KSUP10	*Hin*dIII	1	0	0	No
KSUP18	*Dra*I	1	1	1	Yes
KSUP20	*Eco*RV	1	1	1	Yes
KSUP21	*Eco*RI	1	0	0	No
KSUP23	*Eco*RI	1	0	1	Yes
KSUP50	*Eco*RV	1	1	1	No
KSUP64	*Eco*RI	1	1	1	No
KSUQ10	*Dra*I	1	1	1	Yes
KSUQ11	*Dra*I	1	1	1	Yes
KSUQ13	*Eco*RV	0	1	1	No
KSUQ16	*Dra*I	1	1	1	Yes
KSUQ24	*Eco*RI	1	0	1	No
KSUQ32	*Dra*I	1	1	1	Yes
KSUQ34	*Eco*RI	1	1	1	Yes
KSUQ35	*Dra*I	1	1	1	Yes
KSUQ45	*Eco*RV	2	2	1	No
KSUQ58	*Hin*dIII	1	1	1	Yes
KSUQ59	*Dra*I	1	1	1	Yes
KSUQ60	*Eco*RI	1	1	1	No
KSUQ61	*Hin*dIII	1	1	1	No
KSUQ62	*Dra*I	1	0	0	No
KSUQ63	*Eco*RI	0	1	1	Yes
KSUQ64	*Hin*dIII	0	1	1	No
KSUQ65	*Eco*RI	0	1	1	No
KSUQ66	*Eco*RV	0	1	0	No
KSUQ67	*Eco*RI	0	1	0	No
LPX	*Eco*RI	1	1	1	No
MGB1	*Dra*I	1	1	1	Yes
MGB8	*Eco*RV	1	0	0	No
MGB10	*Hin*dIII	0	1	0	No
MGB63	*Eco*RV	1	1	1	Yes
MGB174	*Dra*I	1	1	1	No
MGB301	*Eco*RI	1	1	1	No
MGB341	*Hin*dIII	0	1	1	No
MWG72	*Hin*dIII	1	1	0	No
MWG76	*Hin*dIII	1	1	1	Yes
MWG514	*Eco*RV	1	1	1	Yes
MWG516	*Eco*RI	1	1	1	Yes
MWG549	*Hin*dIII	1	0	1	No
MWG550	*Eco*RI	1	1	1	No
MWG604	*Eco*RI	1	1	1	Yes
MWG740	*Eco*RI	1	1	1	Yes
MWG805	*Eco*RV	1	1	1	Yes
MWG862	*Hin*dIII	0	1	1	No
MWG900	*Eco*RI	1	1	1	Yes
MWG914	*Eco*RV	0	1	0	Yes
MWG922	*Dra*I	1	1	1	No
MWG933	*Eco*RI	0	1	0	No
MYBC1	*Hin*dIII	1	0	0	No
OXO1	*Eco*RI	1	0	0	No
PR1	*Hin*dIII	1	1	0	No
PR1B	*Eco*RV	1	0	1	No
PSR100	*Eco*RV	1	0	1	No
PSR131	*Eco*RI	1	1	1	No
RZ328	*Hin*dIII	1	1	1	No
RZ395	*Eco*RV	1	1	1	No
RZ575	*Hin*dIII	1	1	1	Yes
RZ589	*Eco*RV	1	1	1	No
RZ744	*Hin*dIII	1	1	1	Yes
TAG251	*Hin*dIII	1	1	1	No
UBP25	*Hin*dIII	0	1	1	No

(*continued*)

TABLE 2

(Continued)

Information on probes not mapped in this experiment was presented in Gill *et al.* (1996a).

ulation. The microsatellite marker and 41 of the 76 RFLP probes were polymorphic. The resulting map has a genetic length of 50 cM (Figure 2).

With one exception, the order of the markers on the recombination-based map was the same as that of the *5BL* physical map and the consensus physical map. Markers in deletion interval *5BL* 0.75–0.76 on the physical map were at the proximal end of the recombination-

based and consensus physical maps, and markers in deletion interval *5BL* 0.76–0.79 on the physical map were on the distal end of the recombination-based and consensus physical maps (Figure 2). The exception to the collinearity, *Xmwg900*, was placed at different locations on the *5AL*, *5BL*, and *5DL* physical maps. The location of *Xmwg900* in the *5B* recombination-based map corresponds most closely to its location on the *5AL* physical map.

Only two markers (*XksuQ11* and *Xabc155*) mapped at a LOD <3.00, and two markers (*Xcdo400* and *Xbcd183*) did not fit a 1:1 segregation ratio (Figure 2). Neighboring markers appeared to have slightly skewed segregation ratios as well but were not significant at $P < 0.05$. Faris *et al.* (1998) reported a segregation distortion locus within the homeologous region of chromosome *5D* in *Ae. tauschii*. It is likely that a *5B* homeoallele of the distortion factor is the cause of the skewed ratios observed for these two markers in this population.

Much of the recombination within the targeted interval *5BL* 0.75–0.79 occurred toward the distal end. Of the 50 cM on the genetic map, 22 cM is accounted for by the five most distal markers. We determined the number of crossovers that occurred in each member of the mapping population. Of the 117 RSLs, 45 had no crossovers in this region, 41 had a single crossover, 28 had double crossovers, and 3 had triple crossovers. In the RSLs with multiple crossovers, none of the crossover pairs flanked single marker loci, but one RSL had a double crossover within a distance of 3.6 cM where one crossover occurred between *Xmwg516* and *Xrz328*/*Xrz589*/*Xbcd881*, and the second crossover occurred between *Xwg908*/*Xcdo548* and *Xmwg900*.

Coefficient of coincidence values for the interval pairs 1, 2 and 3, 4 suggested positive crossover interference. Slight negative interference was observed between intervals 2 and 3 where the coefficient of coincidence was 1.05.

We compared our *5B* genetic map with the corresponding region of chromosome *5H* of barley, chromosome *5D* of *Ae. tauschii*, durum chromosome *5B* from "Langdon" (*T. turgidum*) × Langdon/*T. dicoccoides 5B* disomic substitution, chromosome *5B* of wheat from W7984 (synthetic) × "Opata 85" and W7976 (synthetic) × "Kulm," and rice chromosome *3* (Figure 3). With the exception of the durum and rice maps, a higher degree of recombination was observed in all of these maps with respect to the CS × CS/*T. dicoccoides 5B* map.

The barley chromosome *5H* map is 197 cM, and the region corresponding to the 0.75–0.79 deletion interval on wheat *5B* is 74 cM. Therefore, this region accounts for 38% of the recombination on barley chromosome *5H* in the cross "Proctor" × "Nudinka." The *Ae. tauschii* chromosome *5D* map is 429 cM and the corresponding region is 94 cM, accounting for ~22% of the total genetic length.

The marker interval lengths on the corresponding regions of *5B* maps developed from W7984 × Opata 85 and W7976 × Kulm were similar to each other. The region of the *5B* map from W7984 × Opata 85 corresponding to the 0.75–0.79 deletion interval is ~60 cM, and the length of the entire *5B* map from this cross is ~150 cM. Therefore, this region accounts for ~40% of the recombination along chromosome *5B* in this population. Fewer markers were mapped in the W7976 × Kulm population, and the map is 36 cM. However, the genetic distance between markers *Xmwg914* and *Xbcd450* is ~30 cM in the *5B* maps from both W7984 × Opata 85 and W7976 × Kulm, indicating a similar degree of recombination within this region between these two populations.

Recombination appeared to be suppressed in the Langdon × Langdon/*T. dicoccoides 5B* population. All of the markers on the map showed a high level ($P < 0.005$) of segregation distortion (data not shown). Faris *et al.* (1998) reported a segregation distortion factor (*QSd.ksu.3-5D*) in the homeologous region of chromosome *5D* in *Ae. tauschii* when the F_1 was used as the male parent in a backcross. It is likely that a homeoallele of the distortion factor is active in this cross and it may have an effect on the degree of recombination. Alternatively, the suppressed recombination may be due to a lack of homology between Langdon *5B* and *T. dicoccoides 5B*.

Comparison with the genetic linkage maps of rice (Causse *et al.* 1994) indicated a segment of rice chromosome *3* having some homology with wheat chromosome *5B* and there were five markers in common. However, the order of the markers on the rice map differed from that of wheat, which was probably due to the more distant evolutionary relatedness of rice to species of the Triticeae.

With one exception, the order of markers along the CS × CS/*T. dicoccoides 5B* map is in complete agreement with the order of markers on the compared maps (excluding the rice map; Figure 3). There appears to be an inversion within a small segment at the proximal region of the CS × CS/*T. dicoccoides 5B* map involving several closely linked markers.

DISCUSSION

Physical mapping: The first physical maps constructed using deletion lines indicated that certain regions of the chromosomes had high gene density (Gill *et al.* 1993, 1996a,b; Hohmann *et al.* 1994; Delaney *et al.* 1995a,b; Mickelson-Young *et al.* 1995), and when compared to recombination-based maps, the gene-rich regions preferentially participated in recombination (Gill *et al.* 1996a,b). We attempted to saturate a gene-rich region with molecular markers and to assess the degree of recombination. We chose the region on the long arm of chromosome *5B* flanked by fraction

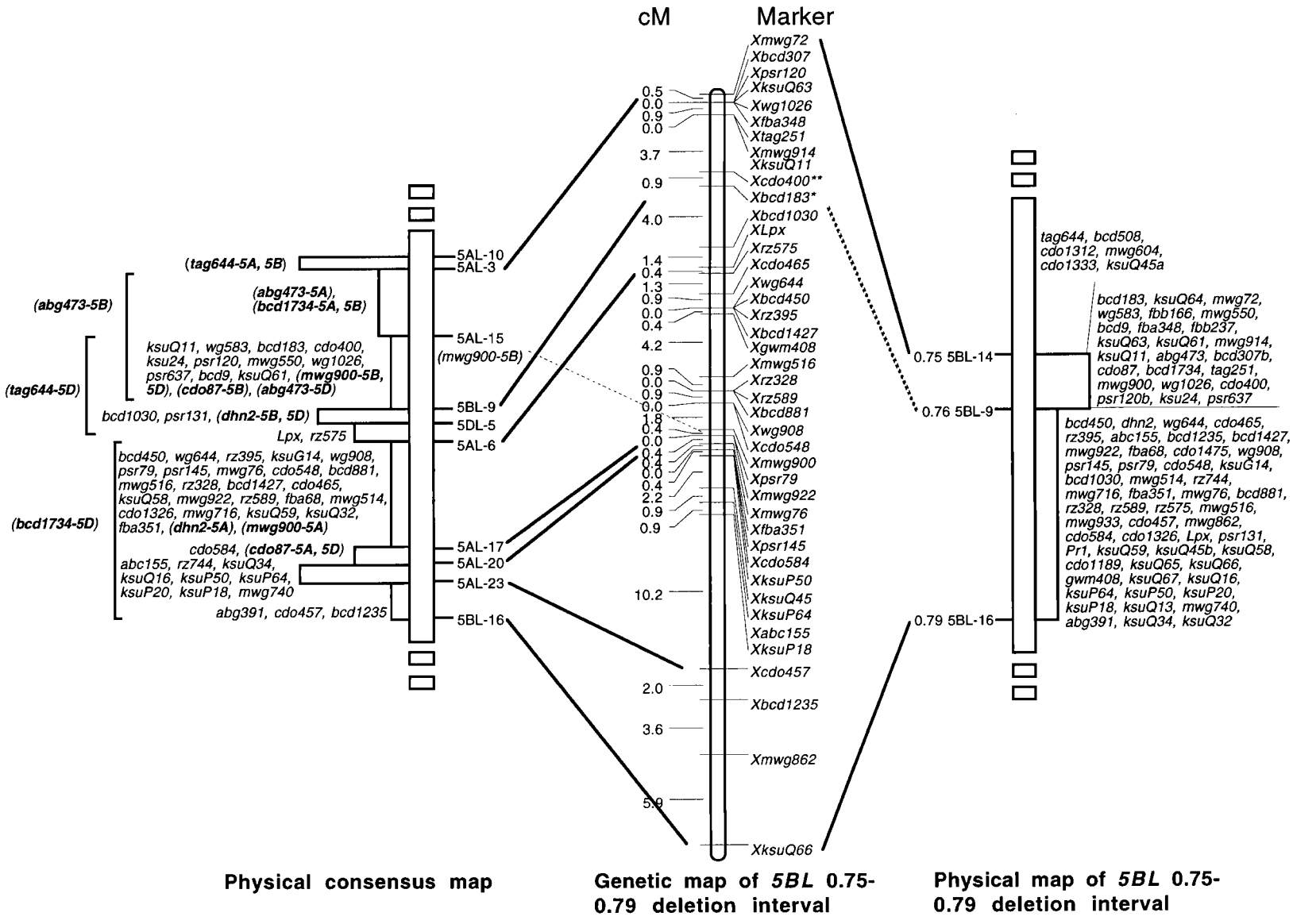

Figure 2.—The physical consensus map, recombination-based map, and physical map of the chromosomal region that corresponds to the physical segment on *5BL* flanked by fraction breakpoints 0.75 and 0.79. On the recombination-based map, centimorgan distances are indicated on the left and marker names are indicated on the right. Recombination was calculated for markers mapping at a LOD >3.00, and markers mapping at a LOD <3.00 (*XksuQ11* and *Xabc155*) are presented in their most likely intervals. Asterisks indicate skewed segregation of markers (*$P < 0.05$, **$P < 0.01$). The description for the physical map and the physical consensus map is the same as for Figure 1. The physical consensus map was constructed on the basis of comparisons of deletion breakpoints and marker orders within the regions of *5AL* and *5DL* that are homeologous to the targeted region on *5BL*. Markers along the consensus physical map that showed discrepant locations among *5AL*, *5BL*, and *5DL* are indicated in parentheses and in boldface type.

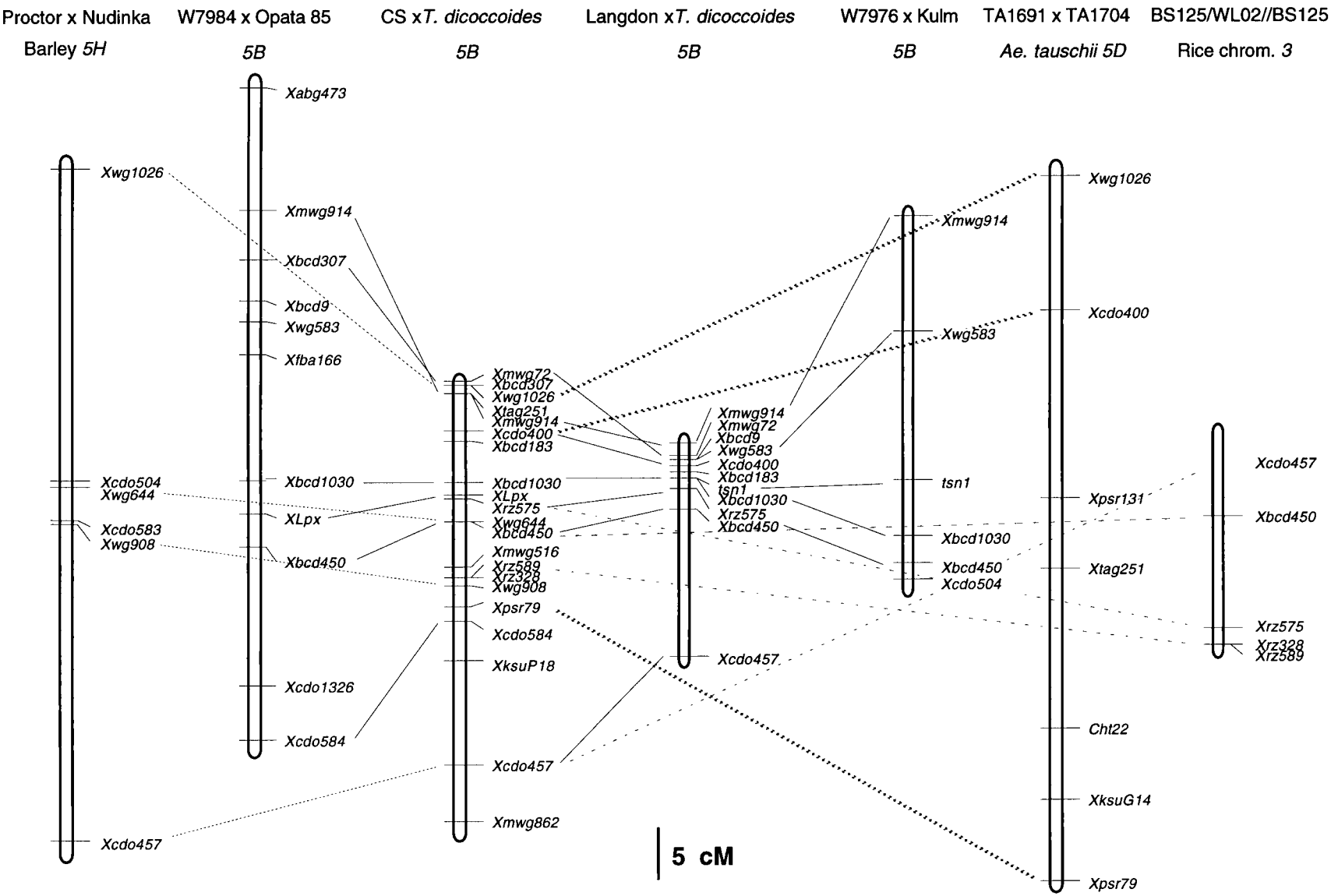

Figure 3.—The comparison of homologous and homeologous regions of group 5 maps. The regions presented correspond to the physical segment of wheat chromosome *5B* flanked by deletion breakpoints 0.75 and 0.79. The maps are barley chromosome *5H* from Proctor × Nudinka (Heun *et al.* 1991), wheat chromosome *5B* from W7984 (synthetic) × Opata 85 (Nelson *et al.* 1995), wheat chromosome *5B* from CS × CS/*T. dicoccoides* disomic *5B* substitution (this experiment), durum chromosome *5B* from Langdon × Langdon/*T. dicoccoides* disomic *5B* substitution (K. M. Haen, J. D. Faris and B. S. Gill, unpublished observations), wheat chromosome *5B* from W7976 (synthetic) × Kulm (Faris *et al.* 1996), *Ae. tauschii* chromosome *5D* from TA1691 × TA1704 (Boyko *et al.* 1999), and rice chromosome *3* from BS125 (*Oryza sativa*)/WL02(*Oryza longistaminata*)//BS125 (Causse *et al.* 1994).

breakpoints 0.75 and 0.79 because it is small (~4% of the long arm), apparently gene rich (Gill *et al.* 1996a), and carries agronomically important genes such as the *Pyrenophora tritici-repentis* toxin resistance gene *tsn1* (Faris *et al.* 1996).

We identified 248 loci on the long arms of group *5* chromosomes in addition to the 155 identified by Gill *et al.* (1996a). Deletion mapping in wheat requires polymorphism only between genomes, but it is possible that some group *5* loci were not identified due to the lack of intergenomic polymorphism. Hybridizing the probe to DNA digested with alternate restriction enzymes probably would remedy this problem. Also, some probes detected more than one fragment mapping in the same deletion interval. In these cases, we cannot determine if each fragment corresponds to a separate locus, or if the presence of multiple bands is due to a restriction site(s) within a single locus. Therefore, it is possible that we detected more loci than we are reporting here. The use of alternate restriction enzymes, the identification of additional deletion lines, or sequencing could solve this problem.

The collinearity of wheat homeologous chromosomes allowed us to use markers that map to all three homeologous group *5* chromosomes to derive a consensus map of the targeted region (Figure 2). The physical collinearity of these markers among the three group *5* chromosomes is generally conserved. The locations of six markers were not consistent across the three chromosomes. If the deletion stocks were characterized incorrectly, we would expect to observe a group of markers in disagreement. But the discrepancies observed here consist of mainly single markers scattered across the region. It appears that these inconsistencies resulted from small rearrangements and lack of microcollinearity among the three homeologues; however, it is possible that these probes detect multiple loci on the individual chromosomes, but were not detected with the restriction enzymes used. Higher-resolution mapping and eventually sequencing of this region will be required to provide definitive answers.

Genetic mapping: The linkage map corresponding to the *5BL* 0.75–0.79 deletion interval has a genetic length of 50 cM (Figure 2). Our results agree with those of Lukaszewski and Curtis (1993) who determined that most of the recombination on long arms of *B*-genome chromosomes occurred within the distal 20–30% of the arm. Our data indicated that 31 (26%) of the 117 RSLs had more than one crossover in the targeted region, which lies in the distal 25% of the chromosome and accounts for only ~4% of the long arm.

Nelson *et al.* (1995) and Xie *et al.* (1993) constructed genetic maps of chromosome *5B* that were ~150 cM in length. Therefore, the physical region marked by deletion breakpoints 0.75 and 0.79 accounts for as much as one-third of the recombination that occurs on chromosome *5B.* This evidence is consistent with previous reports of uneven distribution of recombination in wheat (Dvorak and Chen 1984; Curtis and Lukaszewski 1991; Werner *et al.* 1992; Gill *et al.* 1993, 1996a,b; Kota *et al.* 1993). Uneven distribution of recombination has also been observed in other plants (Rick 1971; Ganal *et al.* 1989) and animals (Steinmetz *et al.* 1987; Bollag *et al.* 1989), and there is much evidence to support the notion that recombination hot spots occur within or near genes (reviewed by Schnable *et al.* 1998). Intragenic recombination frequencies may be influenced by various factors including transposon insertions (Dooner 1986; Xu *et al.* 1995), *trans*-acting factors (Timmermans *et al.* 1997), and the presence of small base pair heterologies between allelic combinations (Borts and Haber 1989; Dooner and Martinez-Ferez 1997). In yeast, specific short DNA sequences required for recombination hot spot activity have been identified (reviewed by Smith 1994). But the activity of these hot spot sequences seems to depend on binding-specific transcription factors and/or chromatin structure that allows hypersensitivity to nucleases (Fox *et al.* 1997; Mizuno *et al.* 1997).

It seems logical that in wheat, a specific higher-order chromatin structure that allows DNA accessibility to *trans*-acting factors and other recombination machinery is required for a recombination hot spot. Gene-rich regions are expected to be highly decondensed to allow accessibility to transcription machinery, while heterochromatic regions and long stretches of highly repetitive sequences are highly condensed and, therefore, less accessible to recombination factors.

The chromosome deletion lines were produced by introducing a gametocidal (*Gc*) chromosome into wheat by interspecific hybridization and backcrossing with related Aegilops species. Plants monosomic for the *Gc* chromosome produce two types of gametes. Only those gametes possessing the *Gc* chromosome are normal. Gametes lacking the *Gc* chromosome undergo structural chromosome aberrations and, in most cases, are nonfunctional. However, if the damage caused by the chromosome breakage is not sufficient to kill the gamete, it may still function and be transmitted to the offspring. The gene-rich regions not only undergo frequent recombination, but most of the deletion breakpoints occurred within these regions as well (Gill *et al.* 1996a). Although the mechanism of the *Gc* gene is not yet understood (Nasuda *et al.* 1998), it seems plausible that regions accessible to recombination factors may also be prone to *Gc* gene action.

Typically, it has been assumed that homologous synapsis precedes and restricts crossing over to sequences in similar positions on homologous chromosomes. Small regions of heterology between allelic combinations may suppress recombination (Borts and Haber 1989; Dooner and Martinez-Ferez 1997). Recombination in the targeted region of chromosome *5B* in the interspecific crosses between CS and *T. dicoccoides* and be-

tween Langdon durum and *T. dicoccoides* appears to be suppressed compared with recombination in the crosses that utilized synthetic parents. Less heterology along chromosome *5B* is expected in the synthetic crosses than in the crosses that utilize *T. dicoccoides* chromosome *5B*. In addition, an inversion was observed in the proximal region of the CS × CS/*T. dicoccoides 5B* map compared with the others. If *T. dicoccoides* carries the inversion, then the F_1 was heterozygous for the inversion, which would reduce the frequency of recombination within the inverted segment. Therefore, reduced recombination in the CS × CS/*T. dicoccoides* population compared with the synthetic wheat × cultivar populations is expected.

The paradigm of a 1:1 relationship between chiasmata and genetic crossovers has long been accepted, but has been challenged recently (reviewed by Sybenga 1996). The increased use of molecular markers has inflated map lengths compared with those generated from chiasma counts (Nilsson *et al.* 1993; Nilsson 1994). One of the reasons for map inflation has been attributed to experimental error in classifying marker data. In our case, this is an unlikely source of error as we used only unambiguous data.

Another source of map inflation can be attributed to the Kosambi mapping function (Kosambi 1944), which does not consider variation in interference or crossover localization and places markers in the most plausible positions on the basis of statistical, not biological, probability. It seems unlikely that the marker order of our map would be incorrect due to the fact that we used a LOD of 3.00, a fairly stringent parameter. If the marker order were incorrect, then the degree of recombination within the targeted interval should have been much greater than what we found, and we would have observed several single-allele exchanges that would resemble gene conversion-type events.

There are also cytological explanations for chiasma counts not agreeing with the number of crossovers. The difficulty arises when it is impossible to distinguish between a single chiasma and two closely apposed chiasmata (Sybenga 1975). Therefore, if a double crossover occurs within a submicroscopic region, multiple chiasmata would not be resolved. In our case, 28 RSLs had double crossovers and 3 had triple crossovers in a very small physical segment of the chromosome. Multiple chiasmata probably could not be resolved microscopically, and chiasma counts would underestimate recombination.

It is also possible that recombination may occur without the formation of chiasmata. Observations of recombination in interspecific hybrids of plants indicate that, occasionally, recombination occurs in the absence of chiasmata (Gill *et al.* 1995). Similar exchanges of short interstitial segments that are not recovered as chiasmata have been found in interspecific rice hybrids (Jena *et al.* 1992; Ishii *et al.* 1994).

Genetic distance is defined on the basis of the assumption that recombination occurs randomly along the chromosome, but the occurrence of one crossover is thought to inhibit the formation of another nearby. This phenomenon is referred to as positive crossover interference and has been observed widely in many organisms. Interference has been thought to result from some steric chromosomal feature such as stiffness (Haldane 1919), but little is known about the causes of interference. Negative interference occurs when one crossover is more likely to be associated with the formation of another nearby. Negative interference is seldom reported in plant and animal species but is observed frequently in some species of yeast and fungi (Olson *et al.* 1978; Kohli and Bahler 1994). Lukaszewski and Curtis (1993) studied interference along *B*-genome chromosomes and found coefficients of coincidence ranging from 0 to 1.08 with an average of 0.19. Our data for the small segment of wheat chromosome *5B* ranges from positive (coefficient of coincidence = 0.46) to no, or slightly negative (coefficient of coincidence = 1.05), interference. Significant deviation from 1 is evidence for the existence of interference. Our values do not significantly deviate from 1 probably because of the small population size. Under optimal interval lengths of ~15 cM, >800 fully informative meioses are required to detect Kosambi-level interference with a power of 80% at a significance level of 0.05 (Ott 1991).

Collinearity of markers along the genetic maps of chromosome *5B* of wheat, *5H* of barley, and *5D* of *Ae. tauschii* is highly conserved. However, collinearity of markers along these maps was not well conserved with a homologous region of rice chromosome *3* (Figure 3). Comparative mapping studies between rice and other cereals have indicated sets of linked genes on rice chromosomes, known as linkage blocks, that contain homology to cereal chromosomes (Ahn *et al.* 1993; Moore *et al.* 1995a,b; van Deynze *et al.* 1995). A segment of rice chromosome *3* was found to have homology to a segment of the long arm of group *5* chromosomes of the Triticeae. Although linkage blocks of chromosome regions may show homology between wheat and rice, in-depth studies of specific genomic regions have indicated that the level of microcollinearity between wheat, barley, and rice is limited (Foote *et al.* 1997; Kilian *et al.* 1997; Moore *et al.* 1997; Gallego *et al.* 1998).

Künzel *et al.* (2000) constructed physical maps of barley chromosomes using microdissected translocation chromosomes. They found, just as in wheat, that recombination is confined to a few small regions that have high gene density. The barley region corresponding to the *5BL* 0.75–0.79 interval was very small in physical size, but it accounted for most of the recombination on chromosome *5H*. They estimated that recombination frequency was 4.4 Mb cM^{-1} at the whole genome level, but was reduced to <1.0 Mb cM^{-1} in recombination hot spots.

The entire map of the wheat genome constructed in the W7984 × Opata 85 population consists of ~3700 cM, and the wheat haploid genome consists of 1.6×10^{10} bp. This translates into a recombination frequency of 4.4 Mb cM^{-1} for the whole genome. We do not know the exact physical size of the chromosome region flanked by fraction breakpoints 0.75 and 0.79, but cytological experiments indicate that it consists of ~4% of the long arm. If we assume that each chromosome is of equal size and that the long arm of chromosome *5B* accounts for two-thirds of the chromosome, then the segment would consist of ~20 Mb. We now have 77 markers for this region, or at least 1 marker per 260 kb. As more markers are identified, this ratio will become smaller. The recombination-based map of this region is ~50 cM, so the recombination frequency is ~400 kb cM^{-1}, an 11-fold increase in recombination compared to the genomic average.

Our data indicate that recombination is somewhat suppressed in the CS × CS/*T. dicoccoides* population compared to the W7984 × Opata 85 and W7976 × Kulm populations. For example, the distances between the markers *Xmwg914* and *Xcdo584* are 22 and 50 cM for CS × CS/*T. dicoccoides* and W7984 × Opata 85, respectively. This result could mean that recombination in the CS × CS/*T. dicoccoides* population is only 44% of that in the W7984 × Opata 85 population. If this estimate of the degree of recombination suppression in the CS × CS/*T. dicoccoides* population is accurate, then the recombination frequency is <200 kb cM^{-1}.

Due to the large genome of wheat, molecular manipulations of the genome and attempts to perform techniques such as map-based cloning have been avoided. But our study suggests that the gene-rich regions in the wheat genome may be as amenable to molecular manipulations as are the smaller genomes of plants such as rice.

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