Effect of the Pairing Gene *Ph1* on Centromere Misdivision in Common Wheat

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ABSTRACT

The cytologically diploid-like meiotic behavior of hexaploid wheat (*i.e.*, exclusive bivalent pairing of homologues) is largely controlled by the pairing homoeologous gene *Ph1*. This gene suppresses pairing between homoeologous (partially homologous) chromosomes of the three closely related genomes that compose the hexaploid wheat complement. It has been previously proposed that *Ph1* regulates meiotic pairing by determining the pattern of premeiotic arrangement of homologous and homoeologous chromosomes. We therefore assume that *Ph1* action may be targeted at the interaction of centromeres with spindle microtubules—an interaction that is critical for movement of chromosomes to their specific interphase positions. Using monosomic lines of common wheat, we studied the effect of this gene on types and rates of centromere division of univalents at meiosis. In the presence of the normal two doses of *Ph1*, the frequency of transverse breakage (misdivision) of the centromere of univalent chromosomes was high in both first and second meiotic divisions; whereas with zero dose of the gene, this frequency was drastically reduced. The results suggest that *Ph1* is a *trans*-acting gene affecting centromere-microtubules interaction. The findings are discussed in the context of the effect of *Ph1* on interphase chromosome arrangement.

PAIRING of chromosomes at meiosis is essential for proper segregation of homologous chromosomes and reciprocal exchange of genetic information. Although the mechanism that restricts chromosome pairing exclusively to homologues is understood only in general terms (reviewed by Loidl 1990; Kleckner 1996), it is becoming clear that the primary recognition of homology precedes the process of pairing itself (Hawley and Arbel 1993; Kleckner and Weiner 1993). Indeed, it has been shown in yeast that homologous chromosomes are already associated via multiple interstitial interactions at premeiotic stages (Weiner and Kleckner 1994).

In allopolyploid species having two or more related genomes, chromosome pairing has to overcome an additional problem because, solely on the basis of homology, each chromosome has more than one potential pairing partner. Yet pairing usually occurs in such species between homologous chromosomes and not between homoeologues (partially homologous chromosomes of the different genomes). It has been recently suggested (Feldman *et al.* 1997) that exclusive homologous pairing in allopolyploids is facilitated by two complementary systems: (1) further differentiation of homoeologous chromosomes brought about by elimination of DNA sequences in the initial allopolyploids and (2) subsequent evolution of gene systems that determine exclusive bivalent pairing of homologues by suppressing pairing between homoeologues. Indeed, such genes were identified in several allopolyploid plant species, *e.g.*, *Triticum aestivum* (reviewed by Sears 1976), *Avena sativa* (Gauthier and McGinnis 1968; Rajhathy and Thomas 1972), *Festuca arundinacea* (Jauhar 1975), *Lolium multiflorum* (Evans and Aung 1985), and *Verbena oubleita* (Khoshoo and Arora 1969). The best-characterized at the cytological level is the *Ph1* gene of common (hexaploid) wheat, *T. æstivum*. This gene, located on the long arm of chromosome *5B* (*5BL*; Okamoto 1957; Riley 1960), suppresses the pairing of homoeologous chromosomes of the three closely related genomes that compose the hexaploid complement—thereby, because of strict homologous pairing, inducing exclusive bivalent formation (reviewed by Sears 1976; Feldman 1993). In the absence of *Ph1*, multivalent formation between homoeologous chromosomes is observed (Riley and Kempanna 1963).

During the years, two main hypotheses have been proposed to account for the mode of action of *Ph1*: the presynaptic and the synaptic. The presynaptic hypothesis (Feldman 1966, 1993; Feldman and Avivi 1988) assumes that pairing occurs in at least two stages. In the first stage, homologous chromosomes approach each other during premeiotic interphase, leading to their premeiotic alignment; and in the second stage, they pair at zygotene. Accordingly, only chromosomes that are premeiotically aligned will pair at meiotic prophase; failure of this alignment results in univalents at first metaphase. The presynaptic hypothesis suggests that *Ph1* determines the pattern of premeiotic alignment of

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homologous and homoeologous chromosomes by spatially separating genomes—and hence homoeologues from homologues—thus leading to exclusive homologous pairing. In the absence of the gene, both homologues and homoeologues lie near each other in the premeiotic nucleus, resulting in some multivalent pairing at first meiotic prophase and metaphase. The synaptic hypothesis (Holm and Wang 1988; Dubcovsky *et al.* 1995; Luo *et al.* 1996) assumes that *Ph1* affects the stringency of both synapsis and crossing over at meiotic prophase. In the presence of *Ph1*, synapsis occurs with a high stringency, and crossing over is confined to homologous chromosomes. The absence of the gene leads to decreased stringency, permitting crossing over and recombination between partially homologous chromosomes.

According to the presynaptic hypothesis, *Ph1* controls chromosome arrangement in the nucleus, somatic as well as premeiotic, by operating on the subcellular elements that are involved in chromosome positioning: microtubules and centromeres. These elements hold the chromosomes at anaphase and telophase, the stages when the spatial arrangement of chromosomes in the daughter cells might be determined. Evidence that *Ph1* affects the dynamics of the microtubule system came from a series of experiments with antimicrotubule drugs (Avivi *et al.* 1970a,b; Avivi and Feldman 1973; Ceoloni *et al.* 1984; Gualandi *et al.* 1984). The microtubule system of plants deficient for *Ph1* is more sensitive to colchicine and vinblastine than that of plants carrying the gene. Evidence that *Ph1* affects centromere behavior came from the work of Shimada *et al.* (1974), who found telocentric chromosomes in callus tissue of common wheat plants deficient for *Ph1*, while callus from normal plants had no telocentric chromosomes. In agreement with this, M. Feldman (unpublished results) observed a decrease in the frequency of broken centromeres in root-tip cells of common wheat as the dose of *Ph1* was elevated from zero to four. These results indicate that this gene might confer a greater stability on the centromere and the microtubule system.

To elucidate further the mode of action of *Ph1*, we have studied centromere behavior of univalents at meiosis of monosomic lines in the presence and absence of the gene. In common wheat, the centromere of unpaired chromosomes may undergo precocious division at first anaphase or telophase (Sears 1952). This division is either (1) longitudinal, leading to the formation of two sister chromosomes, each consisting of one chromatid, or (2) transverse (misdivision), leading to the formation of telocentric chromosomes and isochromosomes. Transverse division of the centromere of one-chromatid chromosomes may also occur at second anaphase. In the presence of *Ph1*, the frequency of centromere misdivision in both first and second meiotic divisions is much higher than in the absence of the gene. This finding suggests a role for *Ph1* in the interaction between kinetochores and microtubules at anaphase.

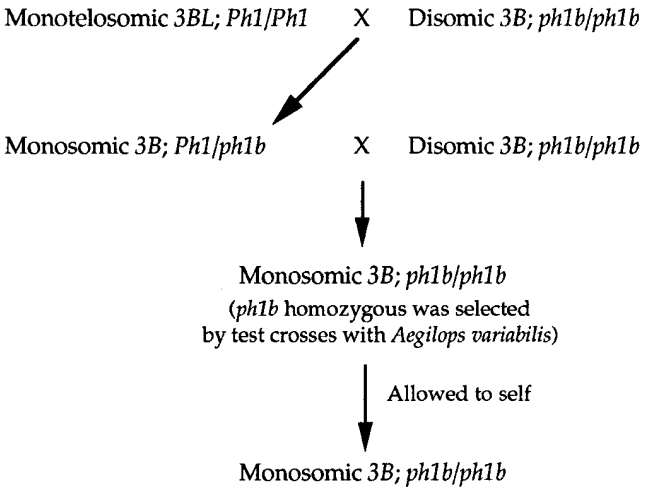

Figure 1.—Production of a monosomic *3B* line homozygous for the *ph1b* deletion.

Preliminary data from this study were published in Vega and Feldman (1993).

MATERIALS AND METHODS

The standard laboratory cultivar Chinese Spring (CS) of common wheat, *T. aestivum* ($2n = 6x = 42$; genomes *AABBDD*) has a complete series of aneuploid lines that facilitate studies on the single-chromosome level. To investigate the effect of *Ph1* on centromere behavior, we used monosomic lines ($2n = 2n - 1$) having either zero or two doses of the *5BL*-chromosomal segment carrying the gene. We selected monosomic lines for either chromosome *3B* or chromosome *5A* because in both cases the different lengths of the short and long arms (*3B* arm ratio = 1.3; *5A* arm ratio = 1.8; Gill 1987) make it possible to distinguish between longitudinal and transverse division of the centromere (Sears 1952; Steinitz-Sears 1966, 1973). For two doses of *Ph1*, we used the *3B* monosomic and *5A* monosomic lines, developed by the late E. R. Sears. These lines are disomic for chromosome *5B*, which carries *Ph1* on its long arm. The mutant line *ph1b/ph1b* (Sears 1977) was used for the production of *3B* or *5A* monosomic plants with zero dose of *Ph1*. This line is deficient for the gene because of an interstitial deletion in the critical chromosomal region (Gill and Gill 1991; Gill *et al.* 1993). The production of monosomic lines with zero dose of *Ph1* is illustrated in Figure 1 for chromosome *3B*. Since the *ph1b/ph1b* line carries several translocations due to homoeologous recombination (Naranjo *et al.* 1988), both the putative heterozygotes *Ph1/ph1b* and homozygotes *ph1b/ph1b* that we obtained in our cross showed multivalents at meiosis. The homozygous *ph1b/ph1b* plants were identified by test-crossing with *Aegilops variabilis* ($2n = 4x = 28$; genomes *UUSS*; Sears 1977). With *Ph1* missing, a high level of homoeologous pairing, involving on the average 25 or more of the 35 chromosomes, is observed in the F_1 pentaploid hybrid. In the presence of *Ph1*, the average amount of pairing in this hybrid is only about one bivalent per meiocyte. If the plant crossed with *Ae. variabilis* is heterozygous *Ph1/ph1b*, the hybrid progeny segregate into low and high-level pairing. Ten hybrids of each progeny were analyzed, and only when all of them showed high pairing was the tester considered to be deficient for the *Ph1* gene.

Plants were grown in a greenhouse at 20 ± 5°. Spikes were fixed in a mixture of three parts absolute alcohol to one part glacial acetic acid. Male meiocytes were analyzed on semi-

permanent slides sealed with a gelatine-acetic-acid medium. The data were analyzed using a contingency χ^2 test.

The *ph1/ph1b* line is partially asynaptic, and therefore univalents additional to that of the monosomic chromosome were occasionally observed. To ensure that the data would derive exclusively from the scoring of *3B* and *5A* univalents, only meiocytes that had one univalent were analyzed. In all cases, *3B* and *5A* univalent chromosomes were clearly identified because of their distinct heterobrachial appearance. Moreover, the identification of *5A* was confirmed by the presence of a minor constriction around the middle of its long arm, which was often bent when the chromosome or chromosome arm migrated to one of the poles (Figure 3).

RESULTS

***Ph1* effect on centromere misdivision of univalent chromosomes during first meiotic division:** In monosomic lines, the single chromosome appears as a univalent at first meiotic metaphase. The univalent lies either at the periphery of the equatorial plate or between this periphery and the polar area. At first anaphase, while bivalents separate normally (with each homologue moving to opposite poles) the univalent may exhibit one of two kinds of behavior: it either moves undivided to one pole, synchronously with the half-bivalents or shortly behind, or it remains at or near the equatorial plane and divides precociously. The centromere of this lagging univalent divides longitudinally (normal division) or transversally (misdivision). Figure 2 shows the observed patterns and frequencies of centromere division of a single *3B* or *5A* chromosome in monosomic *3B* or *5A* lines, respectively, having zero or two doses of *Ph1*, at first meiotic anaphase and telophase. A detailed description of these patterns follows.

Longitudinal division is the division of the centromere along the long axis of the chromosome into two daughter centromeres. This division results from sister centromeres being pulled to opposite poles. At first telophase it leads to the formation of two sister chromosomes that are directed to opposite poles (Figure 2a) or, rarely, to the same pole (Figure 2b).

Misdivision of one chromatid is a transverse division of one of the sister centromeres simultaneously with or subsequently to the longitudinal division of the whole centromere. These divisions lead to two telochromosomes and a whole sister chromosome (Figure 2, c–i). Misdivision of one chromatid that is accompanied by a partial longitudinal division of the centromere gives rise to three arms attached to one daughter centromere and one arm to the other daughter centromere (Figure 2, j–m).

Misdivision of both chromatids is a transverse division extended over both sister centromeres, resulting in two isochromosomes, one for the long arm and one for the short one (Figure 2, n–p). Misdivision of both chromatids followed by longitudinal division of one of the isochromosomes gives rise to one isochromosome and two telochromosomes (Figure 2, q–w).

In some of these patterns of centromere division, one of the resulting iso- or telochromosomes lies on the equatorial plane with no polar orientation (shaded areas in Figure 2). This suggests that the centromere misdivided asymmetrically so that one of the products was left without kinetic activity.

The frequency of precocious centromere division of the univalent *5A* chromosome at first meiotic anaphase and telophase is presented in Table 1. This frequency was similar in plants having zero or two doses of *Ph1* ($\chi^2 = 2.98^{ns}$; d.f. = 1). The percentage of cells showing a lagging chromosome was somewhat higher in the presence of two doses of *Ph1* than in the absence of the gene because some of the scored anthers were at earlier stages. This lagging chromosome was at the equatorial plane and most probably would have divided at a later stage.

Since the frequency of *5A* univalents that migrated to one pole without dividing was very similar in the two *Ph1* genotypes (20.9% in *ph1b/ph1b* and 22.0% in *Ph1/Ph1*, Table 1), we decided to focus on the univalents that divided precociously. Cells with a *3B* or *5A* single chromosome precociously dividing at first meiotic anaphase and telophase were classified into those showing longitudinal division and those showing misdivision (Table 2). The rate of misdivision of the centromere of the *3B* univalent in the presence of two doses of *Ph1* was 27.0%. The percentage of misdivision of this centromere was reduced to 15.0% in the absence of the gene. The χ^2 contingency test for misdivision frequency showed significant differences between the two lines ($\chi^2 = 4.34^*$; d.f. = 1). The misdivision frequency of the centromere of the *5A* univalent in the presence of two doses of *Ph1* was 51.0%. This percentage was reduced to 8.4% in the absence of the *Ph1* gene ($\chi^2 = 62.27^{***}$; d.f. = 1).

Irrespective of *Ph1* dose, the frequency of misdivisions affecting both chromatids was much higher in monosomic *5A* than in monosomic *3B*; in the former it almost equaled that affecting one chromatid (Table 2, Figure 2).

***Ph1* effect on centromere misdivision of a single-chromatid chromosome during second meiotic division:** Depending on the behavior of its centromere at first meiotic division, the univalent chromosome enters into the second division either as a complete chromosome (if it passed undivided to one pole), as a single chromatid (if it went through a longitudinal division), or as telo- or isochromosome (if it misdivided).

The *5A* single-chromatid chromosome resulting from longitudinal division at the first division passed during the second division undivided to one pole, either synchronously with the dividing chromosomes or shortly behind (Figure 3A), or remained at the spindle equator (Figure 3B). In the latter case, it appears that the centromere of the single-chromatid chromosome reached an equilibrium position by orientation of microtubule-binding sites to opposite poles. At early telophase the centromere became elongated (Figure 3C) and eventually broke transversely (misdivided; Figure 3D).

Cells with a *5A* single-chromatid chromosome at second meiotic anaphase and telophase were classified into those showing the *5A* derivative misdividing or not divid-

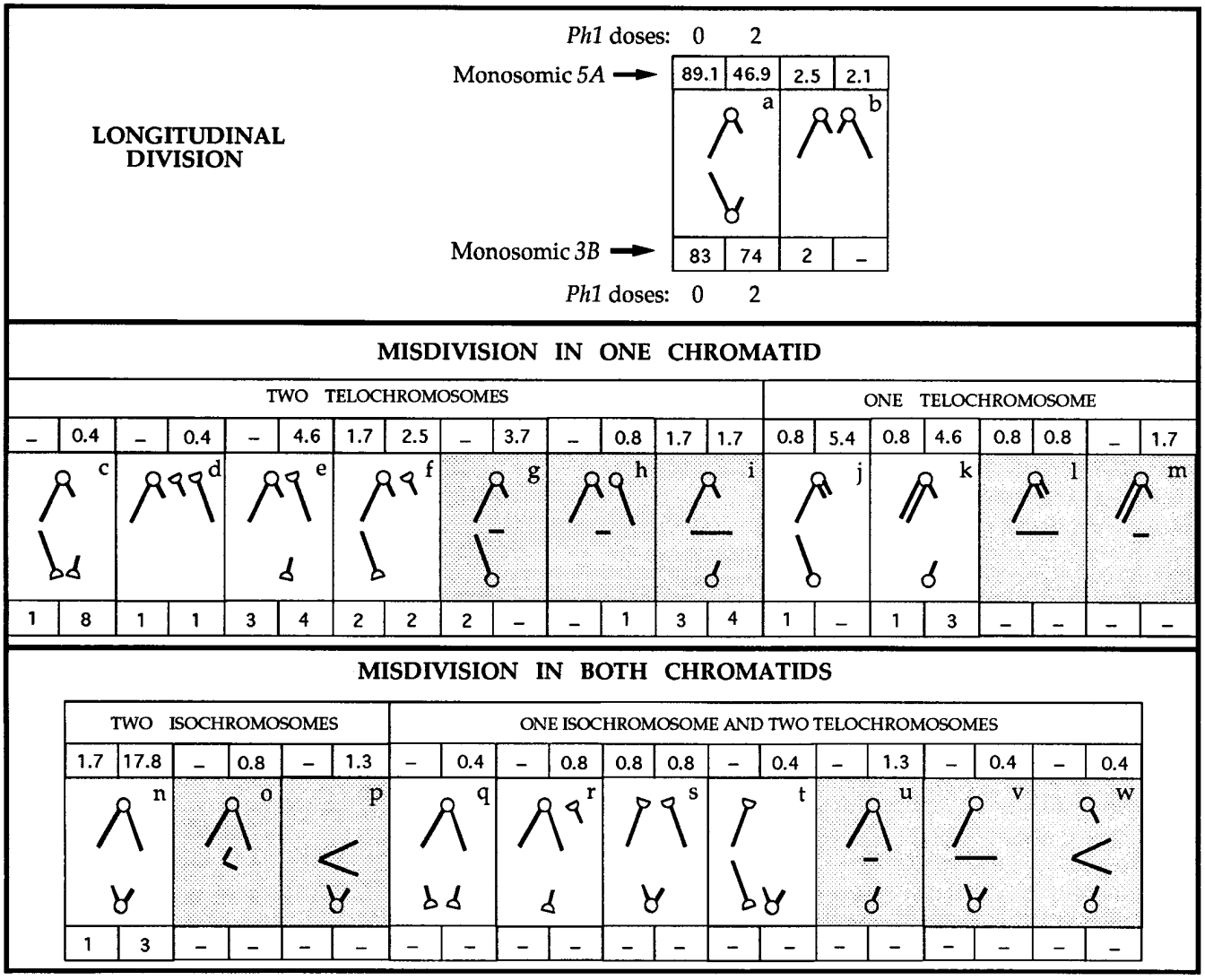

Figure 2.—Types of centromere division of a single *3B* or *5A* chromosome at first meiotic anaphase and telophase and the frequency (%) of each type in the presence or absence of the *Ph1* gene. One hundred cells were analyzed in each monosomic *3B* line; 119 cells were analyzed in monosomic *5A* with zero dose of *Ph1* and 241 cells in monosomic *5A* with two doses of *Ph1*. The frequency values for each type of orientation are given below the diagrams for *3B* and above the diagrams for *5A*. For each monosomic, the frequency to the left corresponds to the line having zero dose of *Ph1*, and the frequency to the right corresponds to the line having two doses. Centromeres are represented by circles, parts of centromeres by half-circles. The long- and short-armed chromatids are diagrammed accordingly. Shaded areas indicate those cases where one of the misdivision products lay on the equatorial plane with no polar orientation.

ing (Table 3). The frequency of misdivision in the presence of two doses of *Ph1* was 50%. This percentage decreased to 28% in the absence of the gene (χ^2 = 10.34**; d.f. = 1). The level of significance is the same even if we consider the possibility that all the laggards at the equatorial plane would have misdivided. Therefore, the decrease in misdivision in the absence of the gene was mainly due to an increase in the frequency of chromosomes segregating without dividing.

DISCUSSION

Centromere behavior in univalent chromosomes during meiosis: In aneuploid lines of wheat and in wheat hybrids, most univalents divide precociously during first anaphase or telophase. Studying univalent movement in wheat hybrids, Wagenaar and Bray (1973) referred to the behavior of kinetochores—highly differentiated structures at the centromeres that serve as microtubule attachment sites. They noticed that at first metaphase, the two sister kinetochores of a univalent chromosome are located adjacent to each other as in normal bivalents (Figure 4A), then move to become oriented in a typical mitotic configuration: sister kinetochores on opposite faces of the chromosome interacting with microtubules of opposite poles (Figure 4B). During this shift in orientation, one of the sister kinetochores is attached, simultaneously, to microtubules originating from both polar

TABLE 1

Frequency (%) of precocious centromere division of chromosome *5A* at first meiotic anaphase and telophase in monosomic *5A* plants carrying zero or two doses of *Ph1*

Ph1 dose	No. of cells	Dividing[a]	Not dividing	
			Not lagging[b]	Lagging[c]
0	129	72.9	20.9	6.2
2	349	64.5	22.0	13.5

Only cells in which the *5A* chromosome behaved like a univalent were analyzed. In the absence of the *Ph1* gene the *5A* chromosome was involved in a trivalent at first metaphase in 12% of the cells; thus, it is assumed that in these cases it segregated without dividing to one of the poles at first anaphase. Those cells were not included in the analysis.

[a] *5A* univalent going through longitudinal division or misdivision.

[b] *5A* univalent with poleward movement without division.

[c] *5A* univalent oriented at the equator.

regions (Figure 4C), suggesting not only the presence of several microtubule-binding sites within a given sister kinetochore but also the independent activities of these sites. The idea of multiple microtubule-binding sites on each sister kinetochore is supported by observations (we made) using light microscopy: a pair of parallel fibers protruding perpendicularly from each sister centromere was observed in dividing univalent chromosomes (J. M. Vega and M. Feldman, unpublished data). We designated these centromeric structures "subunits of a sister kinetochore" (Figure 4A) because they are stretched poleward, indicating their direct interaction with microtubules. This is in agreement with the observations of Zinkowski *et al.* (1991) that multiple fragments resulting from detached mammalian kinetochores still progress through mitosis. These authors suggested that the kinetochore is formed by the folding of a linear DNA fiber consisting of tandemly repeated subunits interspersed with DNA linkers. The fact that most misdivision events give rise to two products that are still capable of moving poleward (Figure 2) and can be transmitted regularly indicates that not only the kinetochore but also the centromere is a repetitive structure. Recently, Kaszás and Birchler (1996) obtained molecular evidence from the analysis of misdivision derivatives that the maize centromere is composed of repeat DNA units that can be changed in copy number without a change in function.

A kinetochore composed of several subunits that interact independently with microtubules could result in two possible orientations of the univalent at the equator at early first meiotic anaphase. First, the subunits of the two sister kinetochores interact with opposite poles (Figure 4B). In this case, the centromere would divide longitudinally at late first meiotic anaphase. In the second orientation, the subunits within one or both sister kinetochores (Figure 4, C and D) interact with microtubules from opposite poles, leading to misdivision in one sister centromere or in both, respectively. At the second meiotic metaphase the kinetochore subunits of the single-chromatid chromosome can again interact independently with microtubules coming from opposite poles, resulting in the chromosome reaching an equilibrium at the equatorial plane (Figure 3B). Ultimately, the centromere breaks transversely because of forces exerted by the spindle in opposite directions (Figure 3, C and D). In common wheat, the frequency of misdivision as well as the relative frequency of one-chromatid and two-chromatid misdivisions is chromosome specific and is affected by the genetic background (Sears 1952; Steinitz-Sears 1973; Makino *et al.* 1977; Morris *et al.* 1977). In our observations, 27% of the precociously dividing *3B* univalents and 51% of the precociously dividing *5A* univalents misdivided at first meiotic anaphase and telophase; 50% of the *5A* single-chromatid chromosomes misdivided at second anaphase and telophase. The frequency of misdivision of wheat chromosomes is high if one considers that it involves a transversal breakage of the centromere through the differentiated kinetochore and the densely packed heterochromatin.

Effect of *Ph1* on centromere division of univalents at meiosis: Our observations indicate that *Ph1* does not affect the frequency of cells with a precociously dividing

TABLE 2

Frequency (%) of longitudinal division and misdivision of the centromere of the single *3B* or *5A* chromosome at first meiotic anaphase and telophase in lines carrying zero or two doses of *Ph1*

Monosomic line	*Ph1* dose	No. of cells	Longitudinal division	Misdivision	Misdivision types	
					One-chromatid type	Two-chromatid type
3B	0	100	85.0	15.0	14.0	1.0
	2	100	73.0	27.0*	24.0	3.0
5A	0	119	91.6	8.4	5.9	2.5
	2	241	49.0	51.0***	26.6	24.4

Only cells with single *3B* or *5A* chromosomes dividing precociously were scored.

*$P < 0.05$, significantly higher than the other *3B* genotype.

***$P < 0.001$, significantly higher than the other *5A* genotype.

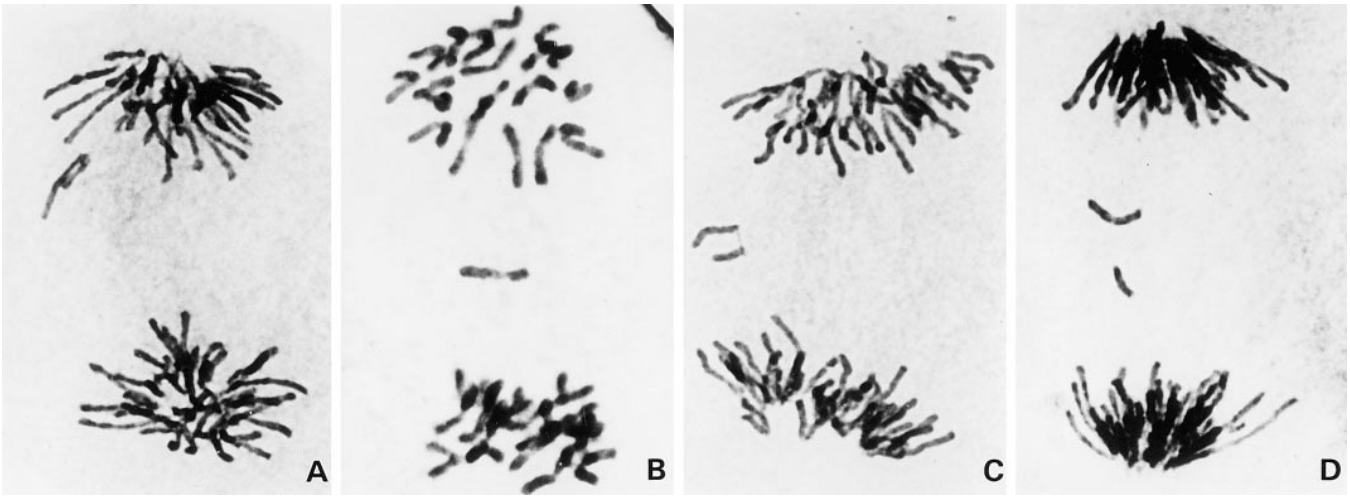

Figure 3.—Types of centromere behavior in derivatives of a single *5A* chromosome at second meiotic anaphase and telophase of a monosomic *5A* line with two doses of *Ph1*. (A) Whole chromatid segregation. (B) Lagging chromatid at the equatorial plane. (C and D) Two cells showing a sequence of chromatid misdivision with telochromosomes going separately to each pole.

univalent (Table 1), but that it does increase the frequency of centromere misdivision at both first and second meiotic division. Transverse centromere division could be favored when duplication of the centromere region has not yet been completed or when sister-chromatid cohesion along the arms and at the centromere region is maintained. These two factors, however, apparently do not play a major role in misdivision, as *Ph1* still causes an increase in misdivision frequency at the second meiotic division even though the chromosome is composed of a single chromatid. These results can be explained in terms of the effect of *Ph1* on the interaction of kinetochore subunits with microtubules.

Kinetochore microtubules (kMts), the spindle microtubules that link the kinetochore to the poles, display the dynamic instability characteristic of microtubule assembly (Mitchison and Kirschner 1984). Several studies identified the kinetochore as the major site of polymerization-depolymerization dynamics during elongation and shortening of kMts (Mitchison *et al.* 1986; Cassimeris *et al.* 1988, 1990; Mitchison and Salmon 1992; Skibbens *et al.* 1993). Another aspect demonstrating the dynamic structure of the kMts is the transient nature of the kinetochore-microtubule interaction. The data support cycles of capture and release of kMts by the kinetochore (Cassimeris *et al.* 1988; Wise *et al.* 1991; Zhai *et al.* 1995). As suggested by Cassimeris *et al.* (1990), detachment of kMts is probably important for the events of chromosome reorientation involved in sister (mitosis) and homologous (meiosis) kinetochores becoming attached to polar microtubules from opposite poles. Recurrent detachments and reattachments of the univalent chromosome at the equator could also resolve the univalent orientations when different subunits within a kinetochore interact with opposite poles (Figure 4, C and D). The centromeric region offers resistance to transverse breakage (misdivision) by making use of the dynamics of microtubule attachment, which favor polarizations ending up in a longitudinal division. *Ph1* may affect the equilibrium between alternative orientations of the univalent at the equator by stabilizing the kinetochore-microtubule interaction. It may do so by encoding or modifying one of the kinetochore proteins or a component of the microtubule system involved in the interaction of kinetochores and microtubules. Assuming that in the absence of *Ph1* the attachment of the microtubules to the kinetochore is relatively labile, then those cases where subunits within a sister kinetochore interact with microtubules coming from opposite poles (Figure 4, C and D) could be corrected by reattachment to the same pole (Figure 4B). On the other hand, in the presence of two doses of *Ph1*, the increased stability in the kinetochore-microtubule interaction hampers these corrections, resulting in an increased rate of misdivision. Increased stability of kinetochore-microtubule interaction by *Ph1* is in accord with previous findings that this gene increases the resistance of microtubules to various anti-

TABLE 3

Frequency (%) of misdivision of the centromere of the *5A* single-chromatid chromosome at second meiotic anaphase and telophase in lines carrying zero or two doses of *Ph1*

Ph1 dose	No. of cells	Misdividing	Not dividing	
			Not lagging[a]	Lagging[b]
0	75	28.0	29.4	42.6
2	180	50.0**	15.0	35.0

** $P < 0.01$, significantly higher than the other genotype.
[a] *5A* univalent with poleward movement without division.
[b] *5A* univalent oriented at the equator.

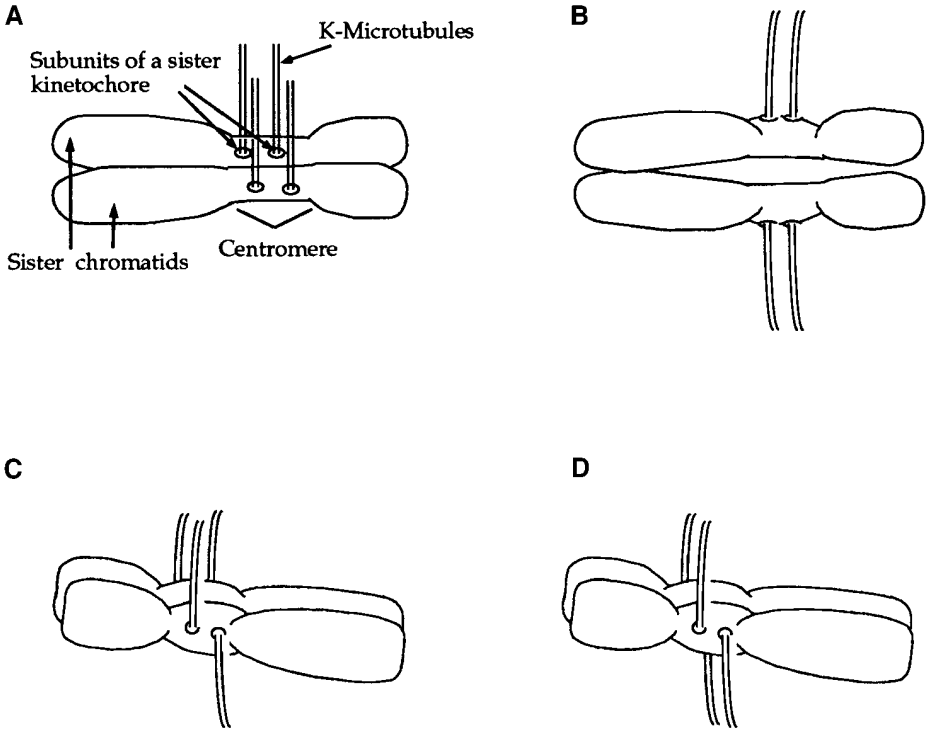

Figure 4.—Schematic diagram of sister kinetochores' orientations in univalent chromosomes at first meiotic division. (A) Adjacent sister kinetochores on one univalent side at early metaphase. (B–D) Sister kinetochores facing opposite centromere sides in univalents oriented at the equatorial plane at early anaphase. (B) The subunits of a kinetochore interact with one pole, and the subunits of the sister kinetochore interact with the opposite pole. (C) The subunits within one sister kinetochore interact with microtubules from opposite poles. (D) The subunits within both sister kinetochores interact with microtubules from opposite poles. k-mts, kinetochore microtubules.

microtubule drugs (Avivi *et al.* 1970a,b; Avivi and Feldman 1973; Ceoloni *et al.* 1984; Gualandi *et al.* 1984).

The *ph1b* phenotype results from a submicroscopic interstitial deletion on *5BL* that includes the *Ph1* locus (Sears 1977; Gill and Gill 1991). Since the estimated size of the deletion is somewhat less than 3 Mb (Gill *et al.* 1993), it may contain a number of genes, in addition to *Ph1*, that might affect centromere-microtubule interaction. However, the fact that premeiotic treatments with colchicine an other antimicrotubule drugs phenocopy the effect of extra doses of *Ph1* on the pattern of chromosome pairing (Feldman and Avivi 1988) indicates that the effect on pairing and on centromere-microtubule interaction is caused by *Ph1*.

An alternative interpretation is that homoeologous pairing in the *ph1b/ph1b* plants might reduce the rate of misdivision of the monosomic chromosome. We found that the *5A* monosome was involved in a trivalent in 12% of the cells at first metaphase. Those cells were not included in the analysis because it was assumed that in these cases *5A* segregated undivided to one of the poles at first anaphase. We therefore analyzed only those cells whose monosome behaved like univalent. Nevertheless, there is still the possibility that the monosome had paired and not crossed over, causing it to appear as a univalent at first metaphase. In this case, it might be that pairing itself would have some effect on the rate of misdivision of that univalent. However, there is no evidence that single chromosomes in the *ph1b/ph1b* genotype pair and desynapse before first metaphase. Moreover, it seems unlikely that pairing at first meiotic prophase could affect not only the frequency of misdivision at first meiotic anaphase but also the frequency of misdivision at second meiotic division.

Studies in callus tissues (Okamoto *et al.* 1973; Shimada *et al.* 1974) and root-tips (M. Feldman, unpublished results) of common wheat showed an increased frequency of telocentric chromosomes in the absence of chromosome *5B*. It is not clear whether the telocentric chromosomes in somatic cells were produced by centromere misdivision. In any case, the discrepancy between the observations in meiotic cells and those in somatic cells may be explained by the different structural and mechanistic aspects of centromere behavior during mitosis and meiosis.

The finding of a kinetochore mutant, *ctf13-30*, in *Saccharomyces cerevisiae*, which shows an increase in the stability of a linear dicentric chromosome (Doheny *et al.* 1993), supports our hypothesis that *Ph1* is a *trans*-acting factor affecting the frequency of misdivision through the stability of the kinetochore-microtubule interaction. The *S. cerevisiae* kinetochore binds a single microtubule

and thus could be considered the simplest ancestral unit of the eukaryotic kinetochore. If a chromosome has two centromeres, kinetochores on the same chromatid may become attached to opposite poles of the mitotic spindle, causing the DNA molecule to break and the dicentric chromosome to become rapidly lost or rearranged to a stable form (Haber and Thorburn 1984). The kinetochore mutant was visually assessed by a reduction in the rate of loss of a marker gene, present on the artificial dicentric chromosome, when compared with the wild-type strain (Doheny *et al.* 1993). These authors bring genetic and biochemical data indicating that CTF13 is an essential kinetochore protein, and believe that this kinetochore mutant might assemble kinetochores in which the attachment to microtubules is weakened. This could lead to microtubule detachment before chromatid breakage, resulting in stabilization of the dicentric chromosome.

Centromeres and *Ph1* action: The presynaptic hypothesis concerning the mode of action of *Ph1* (Feldman 1966; Feldman *et al.* 1966; Avivi *et al.* 1969; Feldman and Avivi 1988; Feldman 1993) assumes that a nonrandom chromosomal distribution in interphase is brought about at the end of every anaphase through movement of centromeres to specific sites in each polar area. This chromosomal arrangement is maintained throughout interphase by physical attachment of centromeres to the polar sites, which by then have become incorporated into the reconstructed nuclear membrane. *Ph1* regulates the distribution of homologous and homoeologous centromeres in both somatic and premeiotic cells. The closer association of homologues, compared with homoeologues, at premeiotic stages leads to exclusive homologous pairing at meiosis (Feldman 1966).

The results presented here indicate that *Ph1* plays a role in the centromere-microtubule interaction at anaphase and telophase, the stages when a distinct, nonrandom arrangement of chromosomes is assumed to be brought about through this interaction. Various lines of evidence support the involvement of the centromere in chromosomal arrangement. In wheat, at somatic and meiotic metaphases, telocentrics for the opposite arms of the same chromosome were found to be significantly closer to each other than expected on a random basis (Feldman *et al.* 1966; Mello-Sampayo 1973; Yacobi *et al.* 1985). Since the centromere is apparently the main homologous part shared by two complementary telocentric chromosomes, and since homologous telocentrics were found closer to each other than homoeologous telocentrics (Feldman *et al.* 1972), it is most likely that the centromere is the chromosomal region determining the nonrandom position of homologues in wheat. In humans, homologues are positioned on opposite sides of prometaphase chromosome rosettes, with the chromosomes aligned toward each other at the level of their centromeres at the hub of the rosette (Nagele *et al.* 1995). These authors raise the possibility that this precise chromosome order in human cells is achieved by the chromosomes' being attached permanently to one another at the level of their centromeric domains. There is also direct evidence for nonrandom distribution of centromeres in the interphase nucleus. Using antikinetochore antibodies in interphase nuclei of rat-kangaroo and Indian muntjac, Hadlaczky *et al.* (1986) observed that centromeres were arranged in pairs. In a similar experiment, half the expected number of prekinetochores were detected at interphase in *Vicia faba* (Houben *et al.* 1995). These results indicate at least transient association of homologous centromeres at interphase.

In summary, the data available in the literature suggest that chromosomes may occupy specific positions relative to each other throughout the cell cycle, and that the centromeres may be involved in maintaining this chromosomal order within the nucleus. In fact, the existence of chromosomal domains was first hypothesized by Rabl (1885). During anaphase, centromeres become clustered at one pole, with the telomeres oriented toward the opposite pole of the nucleus. Because similar centromeric and telomeric patterns were found in interphase nuclei of some plants, Rabl pointed out that chromosomes maintain their anaphase-telophase orientation throughout the cell cycle. A Rabl orientation at premeiotic interphase might facilitate chromosome pairing at meiosis (Fussell 1987). Having the centromeres in a defined nuclear area, with alignment of centromeres to centromeres and telomeres to telomeres would ease homologous chromosomes' interactions during homologue search (Weiner and Kleckner 1994; Scherthan *et al.* 1996). This would result in a presynaptic alignment of homologues along a substantial amount of their length. Once the recognition of homologues is completed, the cells enter into meiosis with a shift from centromere clustering (Rabl orientation) to telomere clustering (bouquet stage); this new arrangement, with telomeres attached to the inner nuclear membrane, would facilitate side-by-side contacts and synapsis.

As suggested by the presynaptic hypothesis, each pair of homologous centromeres has affinity to a specific polar site at anaphase, which in turn would define a specific pattern of chromosome arrangement at every cell cycle (Avivi and Feldman 1980). This model also requires that nonhomologous centromeres have particular characteristics that allow them to interact with specific polar sites. Indeed, the different frequencies and types of misdivision among wheat chromosomes found in this study and others (Sears 1952; Steinitz-Sears 1973; Makino *et al.* 1977; Morris *et al.* 1977) are an indication of differences in centromere composition or structure. Consistent with this view, alpha satellite DNA is organized in a highly chromosome-specific manner at the centromere region of each human chromosome (Willard and Waye 1987). This chromosome specific-

ity reflects modes of genome evolution that are largely limited to homologous chromosomes (Willard 1991). We speculate that chromosome-specific sequences or chromosome-specific organization of common sequences at the centromere may mediate the proximity of homologous centromeres, compared with homoeologues, at anaphase. The isolation of sequences located at the centromeres of cereal chromosomes (Aragón-Alcaide *et al.* 1996; Jiang *et al.* 1996) may be instrumental in the characterization of the centromeres of homologous and homoeologous wheat chromosomes.

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