Chromatid and Chromosome Type Breakage-Fusion-Bridge Cycles in Wheat (*Triticum aestivum* L.)

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ABSTRACT

During the development of disomic additions of rye (*Secale cereale* L.) chromosomes to wheat (*Triticum aestivum* L.), two reverse tandem duplications on wheat chromosomes *3D* and *4A* were isolated. By virtue of their meiotic pairing, the reverse tandem duplications initiated the chromatid type of the breakage-fusion-bridge (BFB) cycle. This BFB cycle continued through pollen mitoses and in the early endosperm divisions, but no clear evidence of its presence in embryo mitoses was found. The chromosome type of BFB cycle was initiated by fusion of two broken chromosome ends resulting in a dicentric or a ring chromosome. Chromosome type BFB cycles were detected in embryo mitoses and in root tips, but they did not persist until the next meiosis and were not transmitted to the progeny. Active BFB cycles induced breakage of other wheat chromosomes that resulted in additional reverse tandem duplications and dicentric and ring chromosomes. Four loci, on chromosome arms *2BS, 3DS, 4AL,* and most likely on *7DL,* were particularly susceptible to breakage. The BFB cycles produced high frequency of variegation for pigmentation of the aleurone layer of kernels and somatic chimeras for a morphological marker. With the exception of low mutation rate, the observed phenomena are consistent with the activity of a *Ds*-like element. However, it is not clear whether such an element, if indeed present, was of wheat or rye origin.

McCLINTOCK (1938a, 1941a,b, 1951) extensively studied the behavior of broken chromosome ends in maize (*Zea mays* L.) and described two types of breakage-fusion-bridge (BFB) cycles. In the chromatid type of BFB cycle, a newly broken end of a chromosome, following a fusion of sister chromatids at the point of breakage, produces an acentric fragment and a chromatid bridge in mitotic anaphase. The bridge is broken and each daughter nucleus receives one broken chromosome end. Upon chromosome replication, the sister chromatids fuse again at the new point of breakage, and the cycle continues until the broken ends are healed. In maize, the chromatid-type BFB cycle continues in the gametophyte and in the endosperm, but it almost always ceases in the zygote.

When two chromosomes with broken ends are introduced into the zygote through both gametes, the broken ends fuse forming a dicentric chromosome. Depending on the orientation of the centromeres, a dicentric chromosome may produce a two-chromatid bridge in mitotic anaphase, thereby initiating a chromosome-type BFB cycle. Careful observations of the progenies of maize plants with active BFB cycles demonstrated that the chromatid type and the chromosome type BFB cycles may alter the structure of chromosomes other than the ones directly involved in the cycle. This led to the discovery of the *Ac-Ds* and other transposition systems (McCLINTOCK 1951, 1978).

It has been demonstrated in recent years that when present in the genetic background of wheat (*Triticum aestivum* L.), some chromosomes of *Aegilops speltoides* Tausch, *Ae. sharonensis* Eig, *Ae. longissima* Scheing & Maschl. or *Ae. cylindrica* Host are capable of inducing extensive chromosome breakage (TSUJIMOTO and TSUNEWAKI 1985; ENDO 1988; KOTA and DVORAK 1988). Similarly, during the development of sets of disomic additions of rye (*Secale cereale* L.) chromosomes to wheat (LUKASZEWSKI 1988), it became evident that breakage of wheat chromosomes occurred. Among several different types of aberrations observed were chromosomes with reverse tandem duplications (RTDs) and corresponding deficient chromosomes. By virtue of their meiotic pairing pattern, RTDs are capable of initiating the chromatid type of the BFB cycle. This article summarizes several years of observations of wheat plants with RTDs and their progenies, and the effects on the wheat karyotype of the chromatid and chromosome types of BFB cycles.

MATERIALS AND METHODS

Standard designations of wheat chromosomes are followed in this article. C-band nomenclature of wheat chromosomes is that of GILL *et al.* (1991). The terminology used to describe the BFB cycles is that of McCLINTOCK (1951), and the description of chromosomes with missing segments is by BRIDGES (1917). According to BRIDGES (1917), deficiency is a "structural change of a chromosome resulting in the loss of a terminal acentric chromosome-, chromatid-, or subchromatid segment. . . ." On the other hand, the term deletion will be used in reference to the loss of an interstitial segment of a chromosome.

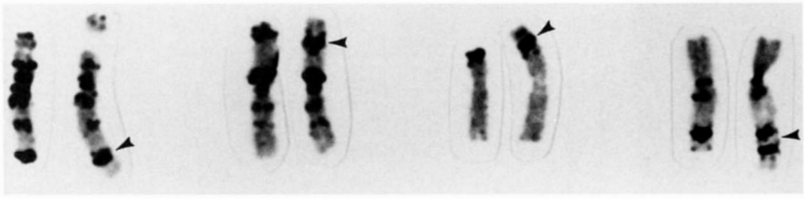

FIGURE 1.—C-banded chromosomes *1B, 2B, 3D* and *4A* of Chinese Spring wheat (on the left in each pair) and their counterparts with the RTDs. Locations of the original breakpoints in the RTDs are indicated by arrowheads.

All observations described in this article were made in hexaploid bread wheat (*T. aestivum* L.) cv. "Chinese Spring" and its hybrids. Original stocks of Chinese Spring, euploid and aneuploid were obtained from the late Dr. E. R. SEARS. Seed samples of rye (*S. cereale* L.) cultivars "Blanco" and "Dakold" and of the blue aleurone line H83–952–1 of hexaploid bread wheat were kindly provided by Dr. R. J. METZGER, Corvallis, Oregon. Line H83–952–1 is a disomic addition ($2n = 42 + 2$) of an *Agropyron elongatum* chromosome with a gene for blue aleurone (R. J. METZGER, personal communication). This chromosome appears homoeologous to group-4 chromosomes of wheat. The expression of blue pigmentation in the aleurone layer is dosage-dependent. Two or more doses of the Agropyron chromosome in the aleurone layer result in a blue-colored kernel; one copy of the chromosome produces white or red kernel color depending on the genetic background. Under normal growing conditions and for all practical purposes, kernels with a single dose of the Agropyron chromosome cannot be distinguished from kernels without the chromosome.

During the development of disomic alien addition lines of chromosomes of rye cultivars Blanco and Dakold to Chinese Spring wheat, chromosome *3D* and *4A* with RTDs were identified (Figure 1). Plants with these duplications were crossed and backcrossed to euploid Chinese Spring or to corresponding Chinese Spring monosomics, and all rye chromatin originally present was eliminated. Eventually, lines homozygous and heterozygous for the two duplications were selected. Among the progenies of plants with the two original RTDs, other chromosome aberrations were observed. Some of those were retained and included in the experiment, as explained in detail in the text. All progenies of crosses, backcrosses, and self-pollinations were always screened by C-banding to identify plants with the duplications and other chromosome aberrations. In all generations, spikes were bagged before anthesis to avoid uncontrolled outcrossing.

Analyses of chromosome behavior in meiosis and in pollen mitoses were performed on squashes of anthers. For this purpose, the stages of the divisions in the pollen mother cells were determined on acetocarmine squashes of one fresh anther per flower; if the desired stage was present, the remaining two anthers were fixed in a mixture of three parts of absolute alcohol to one part of glacial acetic acid and refrigerated for various periods, from several days to several months. For cytological analyses, fixed anthers were hydrolyzed in 1 N HCl at 60° for 8–11 min, stained in Feulgen stain, and often counterstained with acetocarmine.

For the analyses of early embryo and endosperm development, heads of RTD homozygotes and heterozygotes were emasculated and pollinated with pollen from euploid Chinese Spring, and the time of pollination was noted. One, two, or three days after pollination, entire heads were fixed in a 3:1 mixture of absolute alcohol and glacial acetic acid and refrigerated. Staining of the ovules and the embryo and endosperm preparative techniques were those of BENNETT *et al.* (1975).

To test for the possibility of the RTD-induced variegation of kernel pigmentation, the blue-aleurone line H83–952–1 was reciprocally crossed to the RTD homozygotes and/or heterozygotes; the F_1 was C-banded to ascertain the presence of the duplications, grown and self-pollinated. Seed samples from the original crosses of the H83–952–1 to RTD homozygotes and the F_2 seed produced by the blue aleurone/RTD F_1 heterozygotes were screened visually and under a dissecting microscope for the distribution of pigmentation in the aleurone layer. Seeds obtained from crosses of euploid Chinese Spring to H83–952–1 and from the plants from crosses of the RTD heterozygotes to H83–952–1, which did not have the duplications, served as controls.

RESULTS

The origin and structure of the RTDs: RTDs of the type described in this article very likely arise as a product of the chromatid type aberration, as described by MCCLINTOCK (1951). If a chromatid is broken by physical or genetic means in the G1 stage of the cell cycle, the acentric fragment distal to the breakpoint is lost, and upon replication two sister chromatids fuse at the point of the breakage into a chromatid loop. This loop produces a chromatid bridge in the first upcoming mitotic anaphase. The bridge is ruptured, most likely in a random place, with one of the two daughter nuclei receiving a chromatid with a deficiency, and the other receiving a chromatid with a deficiency (for the segment lost after the original break) and a duplication (Figure 2). Because both duplicated segments are next to each other and one is in an inverted orientation, the term "reverse tandem duplication" applies following the generally accepted terminology (SYBENGA 1992).

The first two RTDs identified here were on the short arm of chromosome *3D* and on the long arm of chromosome *4A*. The initial break that produced the $3D_{rtd}$ was located very close to the telomere, in the euchromatic terminal region distal to the 1.9 C-band. This region is too short to be visible in a normal C-banded chromosome *3D* of Chinese Spring in which the distal C-band 1.9 is of considerable size. However, this euchromatic segment is clearly visible in chromosomes *3D* of other cultivars in which the 1.9 C-band is smaller and in the duplication (Figure 1). Cytologically, the length of the deficiency in the distal segment of the $3D_{rtd}$ chromosome was negligible. The second break delimiting the duplicated-inverted segment was located in the 1.4 region of the arm. The length of the duplicated-inverted segment in the original chromosome $3D_{rtd}$ was about one-half of the arm. However, the length of that segment may change from generation to generation.

The initial break that produced the $4A_{rtd}$ chromo-

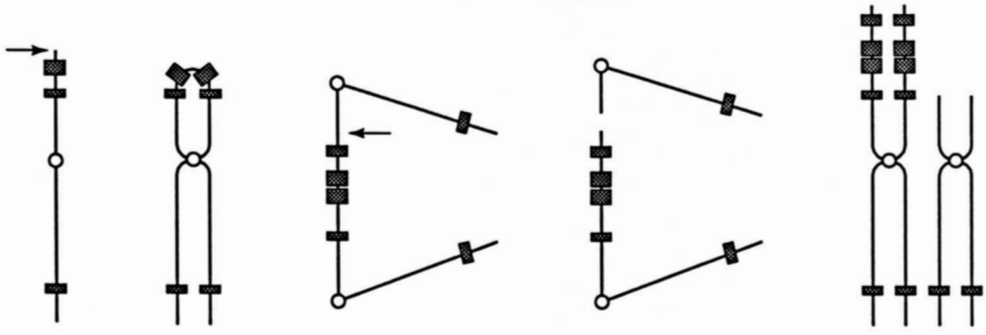

FIGURE 2.—Schematic diagram of the origin of RTDs in bread wheat. C-banding pattern is that of chromosome *3D* of Chinese Spring. From left to right, breakage of the chromosome at the arrow results in a chromatid loop. This loop produces a bridge in mitotic anaphase. Rupture of the bridge at the arrow results in a deficiency and a RTD.

some was located in the 2.7 region of the long arm, ~6–8% of the relative arm length proximal to the telomere. The second break delimiting the length of the duplicated-inverted segment in the original $4A_{rtd}$ chromosome was located in the 2.1 region of the long arm, ~55% of the relative arm length from the centromere. Consequently, the original $4A_{rtd}$ chromosome was deficient for the distal ~6–8% of the long arm, and the duplicated inverted segment was equal in length to ~38–40% of the normal long arm (Figure 1).

Chromosome $3D_{rtd}$, described here and used in the experiments below, was identified among the progeny of a plant with chromosome constitution 21″ + *1R*′ + *5R*′ + *7R*′ obtained from a cross and backcross of a Chinese Spring/Dakold rye amphiploid to Chinese Spring (genomically AABBDDR × AABBDD). Chromosome $4A_{rtd}$ was isolated in the progeny obtained from the same type of a cross but involving Blanco rye. In addition to the $4A_{rtd}$, another $3D_{rtd}$ and several deficient and dicentric chromosomes were identified in the Blanco series. All plants with chromosome aberrations originated from a 48-chromosome F_1 plant (21″ + 6′R) with rye chromosome *3R* missing. The two $3D_{rtd}$ chromosomes, one recovered in the Dakold series and the other in the Blanco series, were cytologically indistinguishable.

RTDs are capable of initiating chromatid type BFB cycles. Among several types of aberrant chromosomes found among progenies of plants with active BFB cycles, new RTDs were identified. Of those, one RTD on the long arm of chromosome *1B* and another one on the short arm of chromosome *2B* (Figure 1) were included in the experiments and their behavior was studied in some detail. The original breakpoint in the $1B_{rtd}$ was in the heterochromatic telomeric band 2.8 on the long arm, and the secondary breakpoint was in the 2.5 region. Duplicated and inverted was distal ~25% of the long arm. The original breakpoint in the $2B_{rtd}$ was in the 2.6 region of the short arm, and the secondary breakpoint was located in the vicinity of the 2.2 C-band. Consequently, ~45% of the short arm was duplicated and inverted.

Meiotic behavior of the RTDs: Depending on the position and the number of chiasmata and the number and structure of the pairing partners, a RTD can be involved in several different pairing configurations in meiosis (SYBENGA, 1992). In RTD heterozygotes, a segment of the chromosome is present in three copies: two in normal and one in inverted orientation. For simplicity, only configurations based on one chiasma per RTD arm in heterozygotes (two strand single cross over) and no exchange of pairing partners are considered. The presence of four copies of the same chromosome segment in RTD homozygotes (two in normal and two in inverted orientations) increases the number of possible pairing configurations.

In a duplication heterozygote, the original segment of a RTD chromosome may pair with the corresponding segment of the normal chromosome (Figure 3a). This would result in unequal chromatids in anaphase I (Figure 3d) and segregation of the normal and duplication chromatids to tetrads without any structural changes. The duplicated-inverted segment of a RTD chromosome may pair with the corresponding segment of a normal chromosome (Figures 4 and 3b), resulting in a chromatid bridge and an acentric fragment configuration in anaphase I (Figure 3e). Lastly, the duplicated-inverted segment of a RTD may fold back and pair with the corresponding segment in the original orientation (Figures 5 and 3, b and c). This mode of pairing may be intra- or interchromatid (involving one or both chromatids, respectively). In the intrachromatid fold-back pairing, the order of segments in the loop distal to the crossover will be reversed with no detectable consequences. On the other hand, in the interchromatid fold-back pairing, an acentric fragment will be present in anaphase I (Figure 3f) and a chromatid bridge will be present in anaphase II (Figure 3g). In the event that more than one chiasma is formed, combinations of several features described above are possible.

In the heterozygotes of $1B_{rtd}$ and $3D_{rtd}$, 20–25% of the anaphase I pollen mother cells had the bridge + fragment configuration and a slightly higher proportion had the fragment configuration (Table 1). This

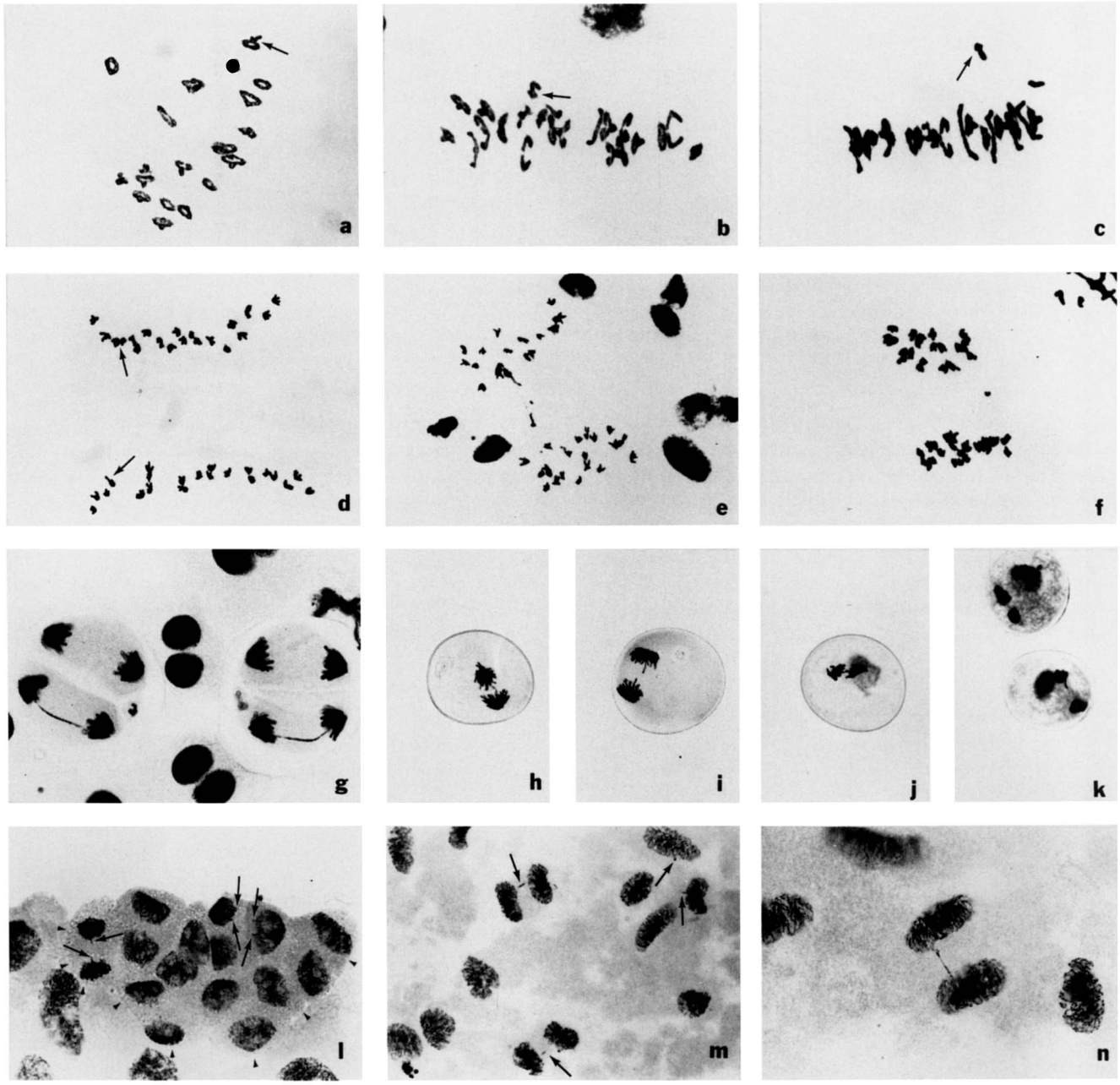

FIGURE 3.—Behavior of chromosome $4A_{rtd}$ in meiosis, in the anaphases of pollen mitoses, and in the early endosperm and embryo divisions in bread wheat. (a) Pairing of the original segment in the RTD chromosome with the corresponding segment of the normal chromosome (arrowed) resulting in unequal chromatids in anaphase I (d). (b) A combination of foldback pairing and pairing of the duplicated inverted segment of the RTD with the corresponding segment in the normal chromosome (arrowed) resulting in a bridge + fragment configuration in AI (e). (c) Foldback pairing in a univalent $4A_{rtd}$ resulting in a fragment configuration in anaphase I (f) and a bridge configuration in anaphase II (g). (h and i) Anaphase of the first pollen mitosis; chromatid bridge in h and protruding chromatids, perhaps remnants of a bridge, in i. (j and k) Anaphase of the second pollen mitosis with a chromatid bridge in j and a protruding chromatid in k. (l) 16-cell embyro, its boundary indicated by arrowheads. Arrows point to the remnants of anaphase bridges. (m and n) Telophases in the endosperm divisions. Arrows point to the chromatid bridges and their remnants.

indicates that the duplicated inverted segments of the two duplications paired with roughly equal frequencies in the interchromatid foldback fashion, as with the corresponding segment of a normal chromosome. In the $2B_{rtd}$ heterozygotes, the frequency of the anaphase I bridge + fragment configuration was much lower than of the fragment configuration, indicating a preference for the fold-back pairing. This reduction of pairing frequency of the duplicated-inverted segment in $2B_{rtd}$ with a corresponding segment of a normal *2B* may be related to the length of the deficiency in this chromosome. Both chromosomes $1B_{rtd}$ and $3D_{rtd}$ are deficient for very short segments; $4A_{rtd}$ is deficient for ~6–8%. On the other hand, chromosome $2B_{rtd}$ is deficient for ~12–

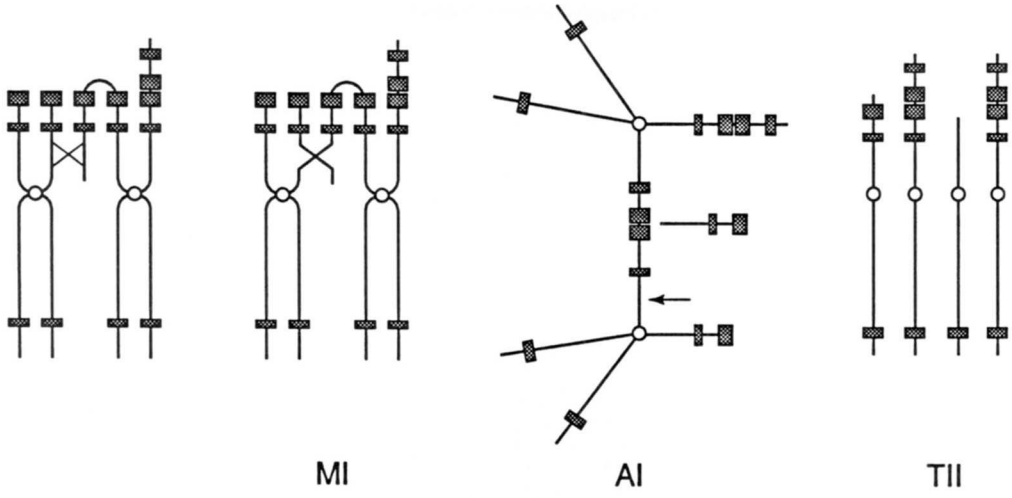

FIGURE 4.—Pairing configuration in the $3D_{rtd}$ heterozygote in bread wheat. Crossing over (indicated by x) involving the duplicated inverted segment of the RTD and the corresponding segment of a normal chromosome results in an acentric fragment and a chromatid bridge in anaphase I. Among four resulting chromatids two have stable ends (normal *3D* and the original $3D_{rtd}$), and two have broken ends (a new duplication and a deficiency). The latter two chromatids will continue the chromatid type BFB cycle.

13% of the short arm. Another chromosome $4A_{rtd}$, deficient for ~18% of the long arm, showed only 8.9% frequency of the anaphase I bridge + fragment configuration compared to 22.4% of the AI fragment configuration (data not shown). It has been observed in wheat that heterozygosity for even small deficiencies dramatically reduces meiotic pairing frequencies (CURTIS *et al.* 1991). Apparently, also in a RTD, a deficiency for a longer segment reduces the probability of pairing with a normal chromosome, thus reducing the frequency of the bridge + fragment configuration in anaphase I, but does not affect the foldback pairing frequency and so the frequency of the anaphase I fragment and anaphase II bridge configurations remains normal.

In all combinations analyzed, low frequencies of anaphase I configurations two fragments, two bridges + two fragments, or one bridge + two fragments were observed. These indicate that more complicated pairing configurations than those assumed here were also present, albeit with low frequency. For simplicity, the configurations with more than one fragment or bridge were included in the totals of the corresponding fragment or bridge + fragment configurations in Table 1. However, these data indicate that RTD could be used for detailed studies of chiasma frequencies and distribution in wheat.

In three of four instances, the frequencies of the anaphase II bridge configuration were higher than of the anaphase I fragment configuration. In the fourth case ($2B_{rtd}$), the two frequencies were within the expected chance deviation (Table 1). This suggests that the presence of acentric fragments in anaphase I may not be always detectable.

Pollen mitoses in RTD homozygotes and heterozy-

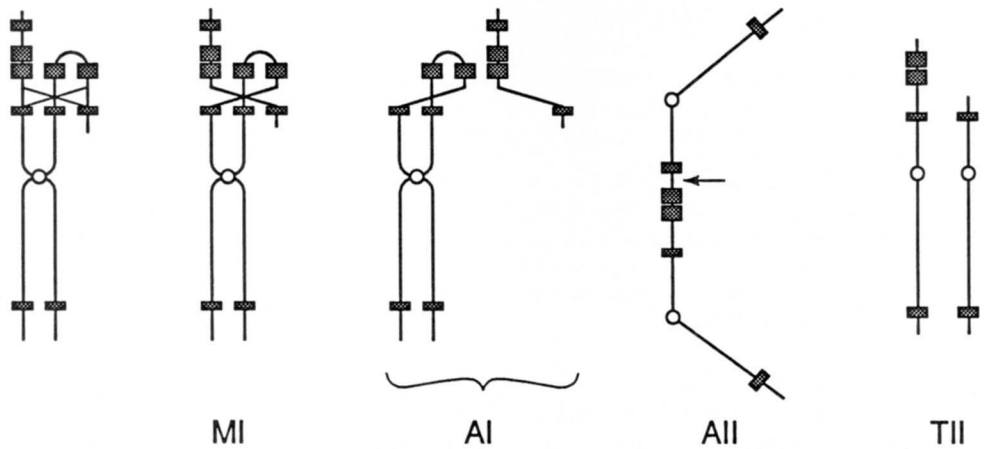

FIGURE 5.—Pairing configuration in the $3D_{rtd}$ heterozygote in bread wheat. Crossing over (indicated by x) involving the duplicated inverted segment of the RTD and the original segment in noninverted position of the second chromatid (fold-back pairing) results in an acentric fragment in anaphase I and a chromatid bridge in anaphase II that, when broken at the arrow, results in a chromatid with a duplication shorter than the original RTD and a deficiency.

TABLE 1

Meiotic behavior of the reverse tandem duplications *1B*, *2B*, *3D* and *4A* in Chinese Spring wheat, and the frequencies of anaphase bridges in mitoses in the pollen, embryos, and the coenocytic endosperm

Division/configuration	Control (CS)	RTD on chromosome			
		1B heterozygote	*2B* heterozygote	*3D* heterozygote	*4A* homozygote
Anaphase I					
Bridge + fragment	0 (53)	25.2 (492)	14.1 (306)	20.2 (376)	25.0 (64)
Fragment	0	27.8	24.5	23.4	28.1
Anaphase II, bridge	0 (83)	30.4 (562)	22.7 (264)	31.7 (400)	42.5 (87)
1st pollen mitosis	0 (250)	6.7 (30)	1.7 (412)	2.9 (102)	5.2 (446)
2nd pollen mitosis	0 (800)	0.5 (208)	0.2 (1718)	0.6 (176)	0.4 (700)
Coenocytic endosperm	8.0 (25)	20.8 (130)	22.5 (40)	22.2 (27)	31.5 (130)
Embryo mitoses	0 (16)	4.6 (65)	0.0 (32)	0.0 (16)	17.3 (104)

Frequencies are percentages; number of anaphases observed in parentheses.

gotes: The frequencies of chromatid bridges in both meiotic anaphases indicated that, on the average, ~40–50% of the microspores received a broken chromosome end. Such broken ends should fuse upon replication and produce chromatid bridges in the anaphases of pollen mitoses. However, among ~1200 anaphases of the first pollen mitosis analyzed, only 1.7–6.7% showed the presence of a chromatid bridge (Table 1, Figure 3h), and this frequency was even lower in the anaphase of the second pollen mitosis (Table 1, Figure 3j). These frequencies represent the actual bridges observed. However, frequently, chromatids protruding from late anaphase or telophase nuclei were observed (Figure 3, i and k), but it was never clear whether these represented remnants of broken anaphase bridges or were a normal feature of pollen mitoses. No chromatid bridges were observed in the anaphases of pollen mitoses in normal Chinese Spring, but, occasionally, ends of chromatids protruding from late anaphase or telophase nuclei were visible. It is, therefore, possible that the frequencies of the anaphase bridges in pollen mitoses in plants with RTDs were underestimated.

Early embryo and endosperm divisions: Single or multi-chromatid bridges in mitotic anaphases of the coenocytic phase of the endosperm development in wheat occur with low frequency (KALTSIKES *et al.* 1975). In triticale, this phenomenon was related to the underreplication of the heterochromatic telomeres of rye chromosomes (BENNETT 1977). There seems to be no simple explanation for the presence of such bridges in the early endosperm divisions in wheat. Quite often the chromatid bridges in coenocytic endosperm do not break but the connected nuclei continue undergoing consecutive rounds of the DNA replication. This leads to the formation of large polyploid nuclei.

The frequencies of anaphase bridges in the endosperm divisions in Chinese Spring plants with the RTDs were 2.5–4 times higher than in Chinese Spring plants without the duplications (Table 1). Most of the bridges appeared to break in late anaphase or in early telophase, so that in late telophase and even in interphase, only small remnants of these bridges protruding from the nuclei could be seen (Figure 3, m and n). In the endosperms of the $4A_{rtd}$ line, the chromatid bridges, and at least one of each pair of bridge remnants, appeared to involve a block of higher-density chromatin (Figure 3, m and n). This corresponds to the location of the 2.4 C-band on the *4AL* arm and would appear to strengthen the impression that most of the anaphase bridges in the endosperm of this line involved the long arm of the $4A_{rtd}$ chromosome. On the other hand, the frequencies of large, polyploid endosperm nuclei resulting from the spontaneous formation of anaphase bridges followed by repeated rounds of DNA replication were similar in normal Chinese Spring and in Chinese Spring plants with the RTDs (data not shown).

The embryos collected 1–3 days post pollination rarely exceeded the size of 25 cells, and anaphases were few and not always accessible to observation. Consequently, the presence of bridges in mitotic anaphases of embryo divisions was ascertained on a much more limited scale than in the endosperm divisions. However, observations of 217 anaphase figures made it clear that the BFB cycle was present in some embryos (Figure 3l), although it was not certain at all whether the cycle was the chromatid or chromosome type. In at least several anaphase-telophase cells, up to three bridges connecting two daughter nuclei, or remnants of more than one bridge per pair of sister nuclei, were observed (Figure 3l), suggesting that these might have been instances of the chromosome type BFB cycles. Dicentric and ring chromosomes were observed in 2.8% of the progeny of plants with RTDs (Table 2).

Transmission of the RTDs through the gametes: No large-scale test crosses to study the gametic transmission rates of the RTD chromosomes were performed. However, in all cross-progenies analyzed, the female transmission of such chromosomes approached 50% (when deficient and dicentric chromosomes were included). Among the progenies obtained after self pollination of

TABLE 2

Recovery rates of RTDs and various aberrant chromosomes in the progenies obtained from self-pollination of Chinese Spring *1B*, *2B*, *3D* and *4A* RTD homozygotes and heterozygotes

Original RTD on chromosome	Number of progeny analyzed	Numbers of recovered			
		RTDS	Deficient chromosomes	Dicentrics/rings	Other broken chromosomes
$1B_{rtd}/1B$*	307	221	27	8	14
$2B''_{rtd}$**	100	180	16	1	1
$2B_{rtd}/2B$	38	27	4	0	0
$3D''_{rtd}$**	210	303	92	9	8
$3D_{rtd}/3D$	84	50	10	6	7
$4A_{rtd}$-1″	128	205	35	6	16
$4A_{rtd}$-1/*4A*	295	169	44	3	12
$4A_{rtd}$-2/*4A*	21	7	11	0	0
Total					
Homozygotes	438	688	143	16	25
Heterozygotes	745	474	96	17	33

*, heterozygotes; **, homozygotes.

heterozygotes, the frequency of the RTD chromosomes (including aberrations) was 38.9%. It appears, therefore, that the deficit of the RTD chromosomes in progenies obtained from self-pollinations of heterozygotes reflects their reduced competitiveness relative to normal chromosomes on the male side. Also, among the progenies of homozygotes, a 3.1% deficit of the RTD chromosomes was observed (Table 2). This deficit reflects the frequency of plants monosomic for the RTD chromosome, most likely resulting from a failure of the chromatid bridge to rupture, producing a lagging dicentric chromosome in one of the anaphases of meiosis or of pollen mitoses. Such lagging chromosomes were occasionally observed, particularly in anaphase II and in the first pollen mitosis.

Among the progenies of RTD homozygotes and heterozygotes, 16.6% of the RTD chromosomes recovered had modified structure (Table 2). These modifications resulted from the chromatid type BFB cycle initiated in meiosis. Considerable variation in the frequency of modifications for different duplications was observed, ranging from 3.2% for $2B_{rtd}$ to 22.8% for the $3D_{rtd}$ heterozygotes. These frequencies of structural alteration of the RTD chromosomes were substantially lower than expected on the basis of the anaphase I and II bridge frequencies. The frequencies of anaphase bridges indicated that ~50% of gametes received a broken chromosome end. The discrepancy in the frequency of gametes receiving broken ends of chromosomes and the frequency of modified RTD chromosomes in the progeny suggests that in a considerable proportion of cases the RTD-produced chromatid bridges broke in the same spots resulting in RTD chromosomes with unaltered structure.

Chromosome type of the BFB cycles: Fusion of the broken ends of two chromosome arms produces a dicentric or a ring chromosome. Depending on the attachment of the two centromeres in a dicentric chromosome to the mitotic spindle, the dicentric chromosome may undergo normal separation of chromatids in anaphase, or it may form a double chromatid bridge (Figure 6). Rupture of the bridges contributes two newly broken chromosome ends to each of the daughter nuclei. Apparently, these two broken ends fuse quickly and the cycle continues. A ring chromosome may also undergo a normal separation in anaphase or, after sister

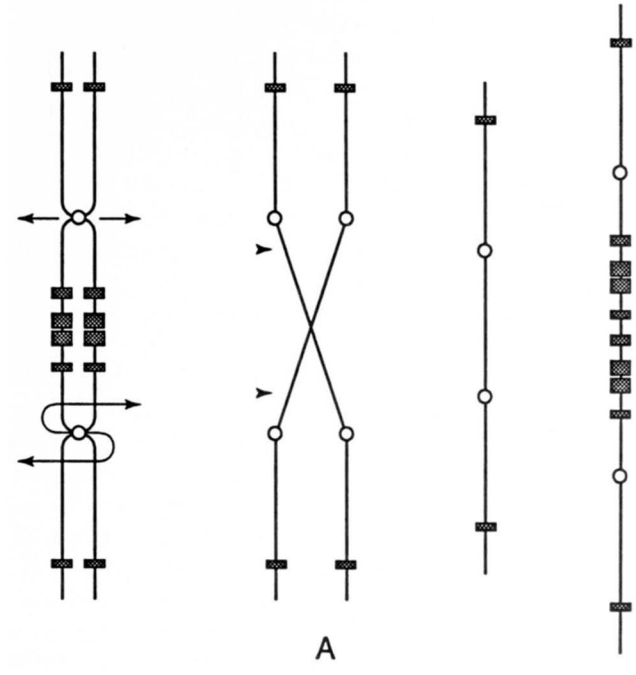

FIGURE 6.—Initiation of the chromosome type BFB cycle by a dicentric chromosome in bread wheat. If the two centromeres on the same chromatid are oriented to the opposing poles in mitotic anaphase (on the left, arrows), a two-chromatid bridge is formed. After breakage at arrowheads, each daughter nucleus receives two broken chromosome ends that fuse, producing new dicentric chromosomes.

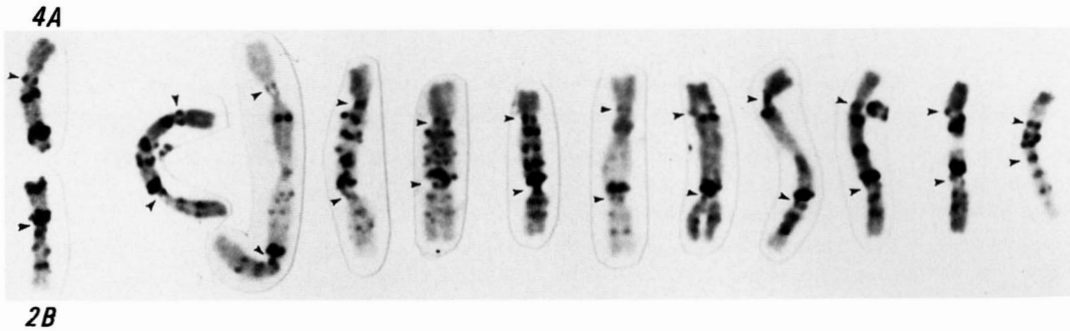

FIGURE 7.—Permutations of a dicentric chromosome *2B-4A* in bread wheat. Normal chromosomes *2B* and *4A* are on the left, labeled. The long arm of chromosome *4A* was broken in the chromatid type BFB cycle initiated in meiosis by the RTD. Active BFB cycle apparently induced a breakage of the short arm of chromosome *2B* and the two broken monocentric chromosomes fused into a dicentric chromosome. All photographs are from a preparation of a single root tip. Positions of centromeres indicated by arrowheads.

chromatid exchange, will form a double bridge in anaphase, initiating the chromosome-type BFB cycle. Both types of the chromosome-type BFB cycles, dicentric and ring chromosomes, were observed among the progenies of the plants with the chromatid type of the BFB cycle.

Dicentric chromosomes were of three classes. The most frequent class resulted from fusions of broken arms of chromosomes that were not originally involved in the chromatid type of the BFB cycle, with the broken chromatids of the RTD chromosomes (Figure 7). The second class of dicentric chromosomes resulted from fusion of two broken chromatids of pairs of RTD chromosomes, most likely introduced into the zygote through both gametes. However, nondisjunction, or failure of an RTD-produced chromatid bridge to break in any of the meiotic anaphases or pollen mitoses, could also lead to the formation of a gamete with a two-centromere chromatid. Sporadically, such lagging dicentric chromatids were observed in this material, particularly in AII and in the anaphase of the first pollen mitosis.

The least frequent class of dicentric chromosomes represented fusions of broken ends of the RTD chromosomes with broken centromeres of other chromosomes. These dicentrics were more frequent in the early stages of the experiment and most often involved rye chromosomes. Rye chromosomes were present as monosomic additions, hence did not have pairing partners and were susceptible to centric fission (misdivision). Once rye chromosomes were eliminated from the material, the frequency of this class of dicentric chromosomes dropped considerably but they were still evident.

Of the several spontaneous ring chromosomes encountered, most involved chromosomes *3D* or *1B* and must have resulted from a breakage of the chromosome arm without the RTD (*3DL* and *1BS*), followed by fusion of the two broken chromosome ends. All wheat ring chromosomes were small in size with hardly discernible banding patterns. This did not permit the observations of their permutations in consecutive mitoses. Such permutations were clear, however, in a monosomic ring chromosome *7R* of rye that was identified among the progeny of a Chinese Spring plant with the $3D_{rtd}$ chromosome. Observations of the banding patterns of the ring *7R* suggested that a segment of the original chromosome (here a C-band in the long-arm telomere) could be repeated up to 12 times in a ring chromosome (Figure 8).

Dicentric and ring chromosomes underwent considerable permutations in consecutive mitoses, as described by McCLINTOCK (1938b, 1941b, 1951). Proge-

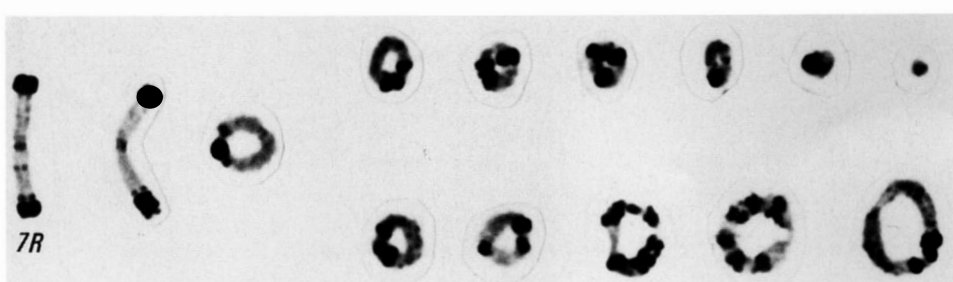

FIGURE 8.—Permutations of a ring chromosome *7R* of rye (on the left, labeled) in Chinese Spring bread wheat heterozygous for a $3D_{rtd}$. As far as it is possible to reconstruct the events, the first break in the long arm of *7R* resulted in a RTD (second chromosome from left), and the second break, in the short arm proximal to the telomeric C-band, resulted in a ring chromosome. After sister chromatid exchange, the ring chromosome formed a double bridge in mitotic anaphase, which ruptured and contributed two broken chromosome ends to each daughter nucleus. Random breakage reduced (upper row) and increased (lower row) the size of the ring. All photographs are from a preparation of a single root tip.

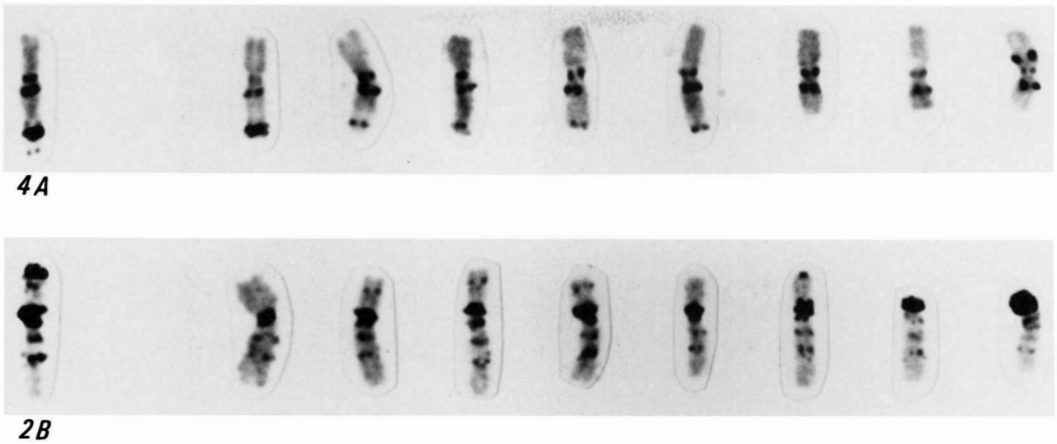

FIGURE 9.—Structural rearrangements of chromosomes *2B* and *4A* after one generation of the chromosome type BFB cycle (dicentric *2B-4A*, Figure 7) in bread wheat. Normal chromosomes *4A* and *2B* are on the left, labeled.

nies of several plants with the chromosome-type aberrations were screened by C-banding. Among the progenies of plants with dicentric chromosomes *2B-4A*, substantially rearranged chromosome arms *2BS* and *4AL* were observed, including one completely heterochromatic short arm of *2B* (Figure 9). Among 45 viable progeny obtained from the plant with ring chromosome *7R*, only eight chromosomes *7R* were found, all of which were monocentric. Based on the C-banding patterns, seven of these appeared to be simple deficiencies of one of the arms, and one chromosome very likely was a combination of a deficiency and a pericentric inversion (Figure 10). The C-banding patterns of chromosomes that have undergone the chromosome type of BFB cycle indicated substantial structural rearrangements including deficiencies, deletions, inversions, and changes in the amount of constitutive heterochromatin. Quite likely, these cytological changes are accompanied by transfers of segments from one chromosome arm to another.

Meiotic chromosome pairing was analyzed in several plants in which chromosome type BFB cycles (dicentrics *4A-2B* and ring *7R*) were observed in the root tips. In no instance was the presence of a dicentric or ring chromosome evident in meiosis, and the original dicentric or ring chromosomes were never found among the progeny of such plants. This indicates that in wheat the chromosome type BFB cycle initiated in the preceding meiosis or at fertilization does not continue indefinitely in the sporophyte and is not transmitted to the progeny.

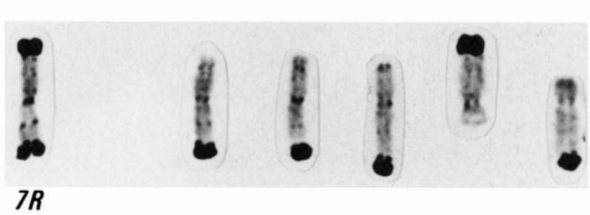

FIGURE 10.—Structural rearrangements of rye chromosome *7R* in bread wheat resulting from one generation as a ring. Normal chromosome *7R* is on the left, labeled; C-banding pattern of the first chromosome on the right suggests it may be a deficiency/inversion combination.

Aberrations of chromosomes not originally involved in the BFB cycles: The presence of the chromatid type of the BFB cycle induced breakage of chromosome arms of the complement other than the ones involved in the BFB itself. Among 1183 progeny of RTD homozygotes and heterozygotes, 58 instances of such secondary breakage have been noted, for an average frequency of 4.9%. The presence of the chromosome type BFB cycle also induced breakage in chromosomes not originally involved in the aberration. Among 226 progeny of plants with ring or dicentric chromosomes screened by C-banding, 14 such secondary breaks were identified, for a frequency of 6.2%.

The secondary chromosome breaks identified among the progenies of plants with BFB cycles resulted in dicentric and ring chromosomes, new RTDs, and deficient chromosomes. Most of the chromosomes involved were wheat chromosomes, but in several instances, rye chromosomes, when they were still present in the early stages of the experiment, were also involved. Among the progeny of a plant with a dicentric chromosome *5R-5B*, RTDs $1B_{rtd}$ and $2B_{rtd}$ (Figure 1) were isolated; $1B_{rtd}$ in the first generation after selfing and $2B_{rtd}$ in the second generation. Dicentric chromosome *5R-5B* was formed by a fusion of the short arm of rye chromosome *5R* after centromeric fission, with the distal end of the short arm of wheat chromosome *5B* after a noncentric breakage. In turn, the *5R-5B* dicentric chromosome was identified among the progeny of a $3D_{rtd}$ heterozygote. The second $4A_{rtd}$ chromosome (the one with 18% deficiency, as mentioned earlier) was found among the progeny of a plant with *4R-4A* dicentric chromosome. This *4R-4A* dicentric resulted from a fusion of the broken centromere of *4R* with the long arm of *4AL* in the RTD. Chromosome $4A_{rtd}$-2 may be a permutation of the original $4A_{rtd}$, but it may also be a result of a new breakage event of the second normal *4A* in the plant with the *4R-4A* dicentric.

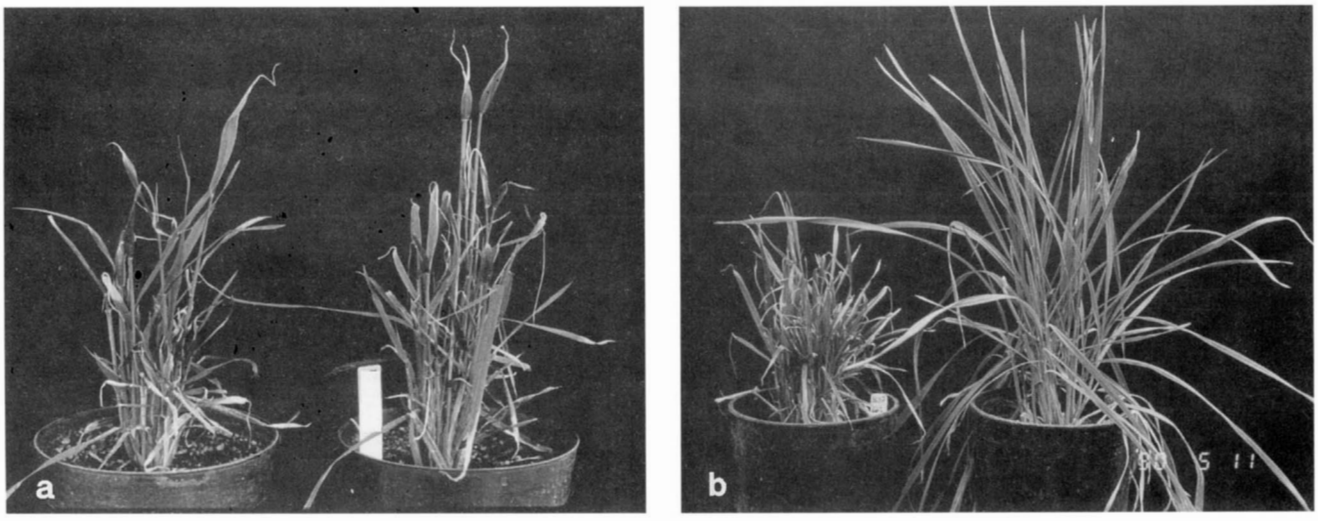

FIGURE 11.—Necrosis in Chinese Spring wheat associated with four doses of the *Dne* locus on the long arm of chromosome *4A*. In each figure, the plant on left is a $4A_{rtd}$ homozygote, the plant on the right is tetrasomic *4A* grown during the fall (a) and grown in the spring (b).

The 72 detected instances of the secondary chromosome breakage involved a total of 20 chromosome arms in 15 chromosomes of wheat and two arms of two chromosomes of rye. The frequencies with which individual chromosome arms were involved in the aberrations did not appear to be random. The short arm of chromosome *2B* was the most frequently involved in the secondary breaks (16 instances or 22.2%), followed by chromosome *7D* (most likely *7DL*, nine instances or 12.5%) and chromosome *4AL* (six instances, 8.3%). Chromosome arms *1BL*, *2AL*, and *5BS* were involved in four breaks each; the remaining 14 chromosome arms of wheat: *1AS*, *1BS*, *3BS*, *3DS*, *3DL*, *4BS*, *4BL*, *5AS*, *5BL*, *5DL*, *6BL*, *7A*(?), *7BS*, *7BL* were involved in 1–3 breaks each. Chromosome arm location of the two breaks in chromosome *7A* were not determined.

Somatic variegation and the morphological offtypes: A locus on the long arm of chromosome *4A* of Chinese Spring wheat, when present in four and higher doses, causes a form of necrosis. Necrotic lesions form across the leaf blades at some point during the leaf development and the part of the leaf distal to the lesion emerges from the sheath completely necrotic and drops off. Consequently, plants with four doses of the locus have very short and erect leaves as opposed to long and droopy leaves of normal Chinese Spring (Figure 11). In addition, the necrosis stunts plant growth so that they require ~6–8 months from planting to maturity, compared to ~4 months for normal Chinese Spring. Seed set of the necrotic plants is reduced and, under less favorable conditions, they are completely sterile. This phenotype is typical for all aneuploid lines of Chinese Spring with four doses of the long arm of chromosome *4A* (tetrasomic, nulli-tetrasomic, di-isosomic).

Observations of plants with $4A_{rtd}$ chromosomes, in which the length of the duplicated inverted segment varied, have shown that the locus responsible for the necrotic phenotype was located in the 2.3 region of the *4AL* arm, adjacent and proximal to the large C-band 2.4. Following a suggestion of Dr. G. HART, this locus will be referred to as *Dne* (dosage necrosis). The *Dne* locus is present in two doses in both $4A_{rtd}$ chromosomes and in four doses in $4A_{rtd}$ homozygotes. Consequently, $4A_{rtd}$ homozygotes exhibit the necrotic phenotype. Plants with higher-than-four doses of the locus, several of which have been produced, died in the three-leaf stage.

The expression of the necrosis is strongly influenced by the environment and by the distribution of the *Dne* loci. Under unfavorable conditions (winter greenhouse growing season), tetrasomics *4A* (including the nulli-tetrasomic combinations), di-isosomics *4AL*, and $4A_{rtd}$ homozygotes are strongly necrotic and usually set very few seeds, if any at all. However, with higher temperatures and higher light intensities, as during the spring greenhouse growing season in Riverside, California, the effects of necrosis are mitigated to a considerable extent. Tetrasomics *4A* planted late in spring exhibit only variable levels of chlorosis and are practically free of the necrotic lesions, so that their leaves are of normal length (Figure 11). Also, their rate of development, while somewhat slower than that of normal Chinese Spring, is considerably faster than during the winter growing season. Under the same growing conditions, plants homozygous for the $4A_{rtd}$ as shown on Figure 1 still exhibit the typical phenotype of four doses of the *Dne* locus but usually complete their life cycle in ~5 months.

The severity of necrosis in $4A_{rtd}$ homozygotes appears to be inversely related to the distance between the pairs of the *Dne* loci on each chromosome. Plants with $4A_{rtd}$ chromosomes in which segments between the *Dne* loci were deleted (during the chromatid type BFB cycle initiated in meiosis) show milder symptoms of necrosis

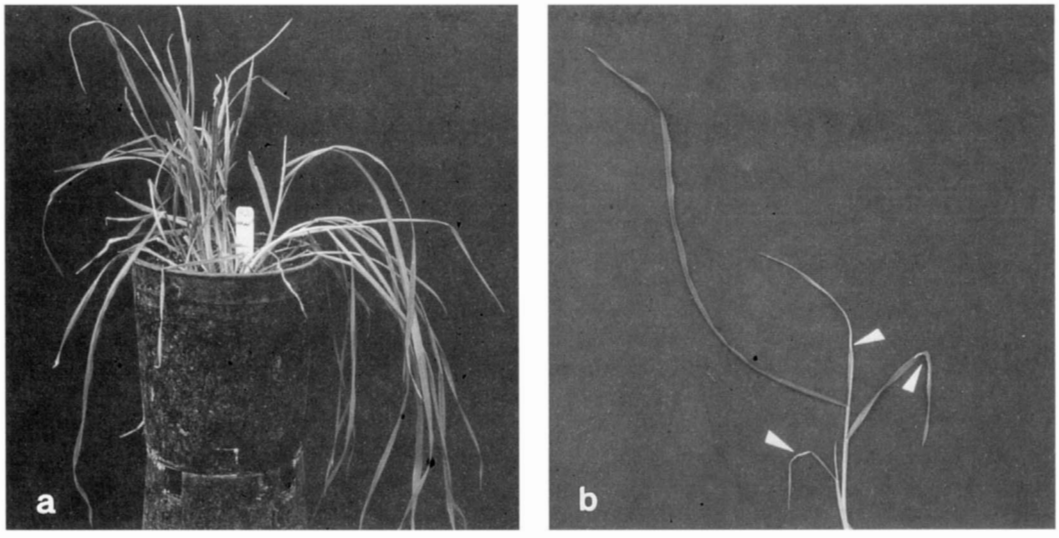

FIGURE 12.—Chimerism for necrosis in a Chinese Spring wheat homozygote $4A_{rtd}$. (a) The erect part of the plant on the left is necrotic; the droopy part on the right is nonnecrotic. The tiller between the two sectors is chimeric: the first and the fourth leaves from the bottom are necrotic, the second and the third leaves are normal. (b) A chimeric tiller of a $4A_{rtd}$ homozygote. The first, second and the fourth leaves from the bottom have necrotic lesions (arrowheads); the third leaf is normal.

than plants homozygous for the standard $4A_{rtd}$ or diisosomics *4AL*. These changes in the severity of necrosis depending on the physical location of the *Dne* copies suggest a position effect.

Each time $4A_{rtd}$ homozygotes were grown somatic chimeras for the *Dne* necrosis appeared (Figure 12). Usually ~10% of the $4A_{rtd}$ homozygotes show normal nonnecrotic sectors at some point during development. On two occasions, the same necrotic plant produced more than one normal sector. Such nonnecrotic sectors begin to appear ~6–8 weeks after planting and may continue appearing through flowering of the first heads of the necrotic sector, in the seventh month after planting. Because the nonnecrotic sectors develop very rapidly, they quickly outcompete the necrotic sectors that die without producing seed. After the initial observation of the phenomenon, necrotic and nonnecrotic sectors of chimeras are routinely separated and grown in different pots to maturity and harvested separately.

Progeny harvested from necrotic and nonnecrotic sectors of the same plants were karyotyped by C-banding. In all instances studied, the progeny obtained from the necrotic sectors had a high frequency (80–90%) of the original $4A_{rtd}$ chromosomes, with the remaining $4A_{rtd}$ chromosomes having modified structure resulting from breakage in the anaphases of meiosis or pollen mitoses. With one exception, progeny from the normal nonnecrotic sectors of the chimeras had ~65–70% frequency of the original $4A_{rtd}$ chromosome and ~30–35% frequency of broken $4A_{rtd}$ chromosomes. The points of breakage in many of these chromosomes were in, or proximal to, the distal of the two 2.4 C-bands. The karyotype of a normal sector of one $4A_{rtd}$ homozygote was analyzed in meiosis, and it was found that one of the two $4A_{rtd}$ chromosomes was broken through the distal of the two 2.4 C-bands. Since the *Dne* locus in a normal chromosome *4A* is located in the 2.3 region, proximally to the 2.4 C-band, and, consequently, the second copy of the locus in a $4A_{rtd}$ chromosome must be located distally to the distal of the two 2.4 C-bands, elimination of this region in at least one $4A_{rtd}$ chromosome reduced the number of *Dne* loci to three. These observations indicate that the appearance of normal sectors in necrotic $4A_{rtd}$ homozygotes resulted from somatic breakage of at least one $4A_{rtd}$ chromosome and a loss of a segment of the chromosome with at least one copy of the *Dne* locus. In one exception to this rule, there was no difference in the frequency of $4A_{rtd}$ chromosomes in the progenies of the necrotic and normal sectors of a chimera, with both having 85% frequency of the original chromosome, typical of normal segregation from $4A_{rtd}$ homozygotes. The $4A_{rtd}$ homozygous progenies grown from both the necrotic and nonnecrotic sectors of the chimera were necrotic and so, the genetic basis for the appearance of this *Dne* chimera was never established.

Conversely to the normal sectors in necrotic $4A_{rtd}$ homozygotes, among $4A_{rtd}$ heterozygotes streaks of necrotic tissue on one or more leaves, but usually only on the flag leaf, were frequently observed (Figure 13). The $4A_{rtd}$ heterozygotes have three doses of the *Dne* locus: two on the RTD chromosome and one on normal *4A*. It is possible that these necrotic streaks occur in sectors in which the *Dne* dosage was increased to four. However, because of lack of competitiveness of the necrotic sectors in otherwise normal plants, it was never possible to isolate such sectors, grow them to maturity and analyze their progeny.

Among the progenies of plants with RTDs, series of deficient chromosomes were recovered (CURTIS *et al.*

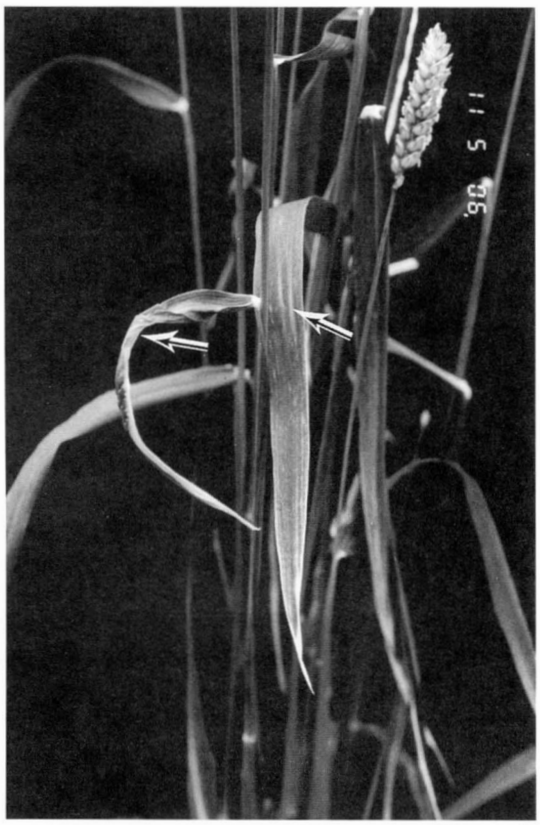

FIGURE 13.—Necrotic sectors (arrowed) on flag leaves of $4A_{rtd}$ heterozygote of Chinese Spring wheat.

1991; A. J. LUKASZEWSKI, unpublished data). Among 10 homozygotes for a 17% deficiency of the long arm of chromosome *4A* selected in the progeny of a heterozygote, nine plants had normal morphology and one plant was much shorter with a compact head (Figure 14). Measurements indicated that the reduction in height and the compactness of the head resulted from a progressive shortening of the internode lengths, starting from the second internode up. Consequently, this character was termed reduced internode length (*ril*). Monosomic analysis indicated that *ril* was recessive and

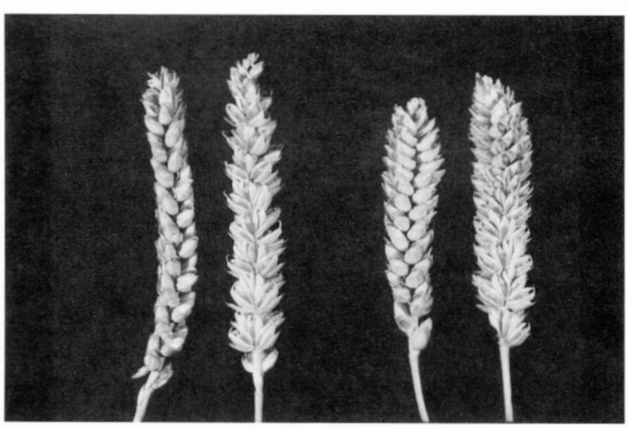

FIGURE 14.—Pairs of spikes of normal Chinese Spring wheat (on the left) and of the reduced internode length phenotype (on the right).

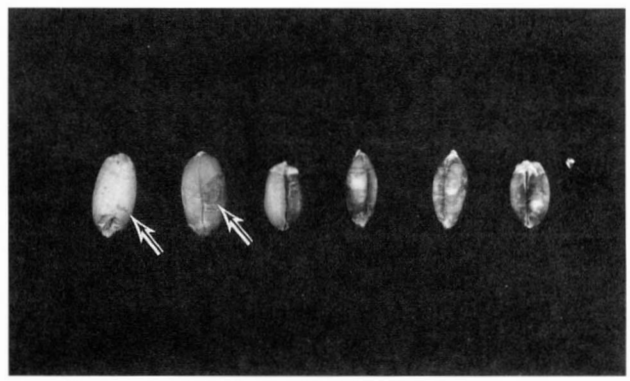

FIGURE 15.—Variegation for the pigmentation of the aleurone layer in kernels harvested from bread wheat plants monosomic for an *Agropyron elongatum* chromosome with the gene for blue aleurone and heterozygous for the $3D_{rtd}$. Blue sectors in nonblue kernels are arrowed.

located on chromosome *4A*. Because ditelosomic line *4AL* of Chinese Spring has normal phenotype and ditelosomic *4AS* line is very short and has a compact head, *ril* must be located on the long arm of chromosome *4A*. Because only one of 10 homozygotes for the 17% deficiency of *4AL* exhibited the character, it must be located proximally to the 17% deficiency breakpoint. As a matter of fact, homozygotes for the deficiency for the distal 23% of the long arm of *4A* did not exhibit the *ril* phenotype.

Variegation of seed pigmentation: Among 892 kernels of line H83–952–1 screened under a dissecting microscope, four showed variegation for the presence of pigment in the aleurone layer. Three of these kernels were also shrivelled; the fourth was normally developed. Among 1001 kernels from reciprocal crosses of Chinese Spring to H83–952–1 and their F_2, two were variegated for the aleurone pigmentation. On the other hand, among 2139 F_1 and F_2 kernels of hybrids involving the $1B_{rtd}$ chromosome, and among 1257 F_1 and F_2 kernels involving the $3D_{rtd}$ chromosome with line H83–952–1, 65 and 69 kernels, respectively, exhibited variegation for the aleurone pigmentation (Table 3, Figure 15). This indicated that the presence of RTDs $1B_{rtd}$ and $3D_{rtd}$ affected the distribution of the Agropyron chromosome with the gene for the blue aleurone pigmentation in different cell lineages of the aleurone layer. The actual frequencies of variegation must have been higher than the data indicate. In the F_1 hybrids the Agropyron chromosome was monosomic and as such did not segregate in the 1:2:1 ratio. Results from test crosses of a monosomic addition of the chromosome have indicated that it was transmitted through the female gametes with ~23% frequency. On the basis of the female transmission rate and the proportions of dark blue and light blue kernels (with three and two doses of the blue aleurone gene, respectively) among the progeny of self-pollinated monosomic additions of the chromosome, the frequency of the Agropyron chromosome in the F_2

TABLE 3

Frequencies of variegated kernels produced by hybrids of a blue aleurone line H83-952-1 with Chinese Spring (CS), and Chinese Spring *1B*$_{rtd}$ and *3D*$_{rtd}$ RTD heterozygotes

Origin of kernels	No. of kernels scored	No. of variegated kernels	Frequency of variegated kernels (%)
H83-952-1	892	4	0.45
H83-952-1 × CS, F_1	135	0	0.0
CS × H83-952-1, F_1	129	0	0.0
H83-952-1 × CS, F_2	737	2	0.27
Total control	1001	2	0.20
CS *1B*$_{rtd}$ × H83-952-1 F_1	239	7	2.93
H83-952-1 × CS *1B*$_{rtd}$ F_1	287	12	4.18
H83-952-1 × CS *1B*$_{rtd}$ F_2	1613	46	2.85
Total *1B*$_{rtd}$	2139	65	3.03
CS *3D*$_{rtd}$ × H83-952-1 F_1	33	3	9.09
H83-952-1 × CS *3D*$_{rtd}$ F_1	36	3	8.33
H83-952-1 × CS *3D*$_{rtd}$ F_2	1188	63	5.30
Total *3D*$_{rtd}$	1257	69	5.49

generation was estimated at ~28–30%. Variegation of the pigmentation of the aleurone layer could only be detected in seeds that had at least one dose of the chromosome.

DISCUSSION

The RTDs studied in this project appeared among the progenies of wheat-rye hybrids. Originally, three such aberrations were identified, but their number grew as a wider group of materials was screened. The mechanism that lead to the formation of the original duplications was never clear, but obviously the duplications must have been formed after a breakage of a chromatid, fusion of the sister chromatids at the point of breakage, followed by at least one round of the chromatid type of the BFB cycle as described by McCLINTOCK (1941a,b).

Over the years of observations described in this manuscript, several thousand plants of bread wheat were screened cytologically, mostly of cultivars Chinese Spring and Pavon but also of other cultivars and different ploidy levels. Among them there were euploids and aneuploids, plants completely unrelated and directly related to those described here as their normal sibs. Among these plants several deficient chromosomes were found but not a single RTD. On the other hand, during the same period, and apart from the *1B*$_{rtd}$, *2B*$_{rtd}$, two *3D*$_{rtd}$ and two *4A*$_{rtd}$ described in this article, in a roughly similar-sized sample of various wide hybrids of wheat and their direct descendants, another nine RTDs were identified, all apparently independently derived. Among those nine, there were three each *2B*$_{rtd}$ and *3D*$_{rtd}$, two *4A*$_{rtd}$ and one *4D*$_{rtd}$. The original breakpoints in these new duplications on *2B*, *3D* and *4A* were in the same regions of chromosome arms as in the duplications described here. Two independently derived RTDs on the long arm of rye chromosome *7R* were found in hexaploid triticale with the breakpoints in the telomeric C-band on the long arm, as in the *7R*$_{rtd}$ shown on Figure 8. In addition, there were three aberrations on *1BS*, one each on *1RL*, *5BL*, and *6BS* that appeared to be RTDs but are yet to be confirmed as such. This brings the total number of RTDs or suspected RTDs to 23, all of which appeared in wide hybrids of wheat (mostly with rye) *vs.* none in euploid and aneuploid wheat. Clearly, wide hybridization of wheat induced breakage of wheat chromosomes, which led to the formation of the RTDs.

Extensive breakage of wheat chromosomes caused by chromosomes of alien species like *Ae. speltoides*, *Ae. cylindrica*, *Ae. sharonensis*, *Ae. longissima*, has been reported (ENDO 1988; KOTA and DVORAK 1988; TSUJIMOTO and TSUNEWAKI 1985, 1988; TSUJIMOTO and NODA 1989). These chromosomes were called gametocidal because often their action rendered gametes inviable. Very large numbers of aberrant chromosomes resulting from the action of the gametocidal chromosomes were observed, and hundreds were isolated, most often with aid of C-banding. However, not one RTD has ever been reported among the aberrations. Moreover, with the exception of the breakage caused by chromosome *6* of *Ae. speltoides* (KOTA and DVORAK 1988), higher-order rearrangements of chromosomes like translocations, duplications, deletions or inversions are rarely mentioned. The reports on the genetic screening of deficient chromosomes resulting from the action of the gametocidal chromosomes do not present evidence for higher-order rearrangements and reinforce the opinion that the aberrations result from single-step chromosome breaks (KOTA *et al.* 1993; WERNER *et al.* 1992). This implies that the broken chromosome ends resulting from the action

of gametocidal chromosomes may be more stable, hence their capacity for fusion with other broken chromosome ends is low.

Broken chromosome ends observed in this study showed a high tendency for fusion with other broken chromosome ends whenever such ends were present and irrespective of the manner in which they were produced. Chromosome ends broken in the chromatid type of BFB cycle fused with broken chromosome ends resulting from centromeric fission of univalents and of chromosomes that had pairing partners but, nevertheless, still misdivided in the centromeres or with broken ends produced by unknown means in the interstitial positions. Fusion of such broken ends produced dicentric or ring chromosomes. When a second broken chromosome end in a cell was not available for fusion, a deficient version of the original RTD chromosome was produced. So, while large numbers of deficient chromosomes *1B, 2B, 3D* and *4A* were observed, very few other deficient chromosomes were found. Most of the secondary breaks observed here were detected not by the presence of a deficiency, as is the case after the action of gametocidal chromosomes, but by the presence of a dicentric or a ring chromosome. Occasionally a new RTD chromosome was found, and while over the years a fair number of these were observed, the numbers of dicentric and ring chromosomes were far greater. All of this demonstrates clearly that the BFB cycle-induced chromosome breakage in wheat creates sticky chromosome ends that behave in the same fashion as those observed in maize (McCLINTOCK 1941a,b, 1950, 1951). It is possible, therefore, that the mechanisms involved in the original breakage of chromosomes *3D* and *4A* that resulted in RTDs and the chromosome breakage induced by active BFB cycles, either of the chromatid or chromosome type, may be different from the mechanisms responsible for the chromosome breakage induced by gametocidal chromosomes. However, it is not entirely clear if the mechanisms were the same as those detected in maize by McCLINTOCK (1951).

It has never been established which rye chromosome was responsible for the breakage that led to the formation of the first two RTDs, $3D_{rtd}$ and $4A_{rtd}$, and the associated deficient chromosomes *3D* and *4A*. Chromosome *3R* is a plausible candidate as it was missing in the original F_1 hybrid from which the plants with the RTDs were derived. In this sense, it would resemble the action of gametocidal chromosomes that cause breakage in the gametes from which they are absent. Chromosome *3* of *Ae. triuncialis* and chromosomes *2* and *6* of *Ae. speltoides* were responsible for chromosome breakage in wheat (ENDO 1988; KOTA and DVORAK 1988; TSUJIMOTO and TSUNEWAKI 1985). Chromosome *3R* of rye is partially homoeologous to group 3 and 6 chromosomes of wheat (NARANJO and FERNANDEZ-RUEDA 1991). If the gene in rye responsible for chromosome breakage belongs to the same class as the chromosome-breaking genes in the Aegilops species, they could be homoeoalleles.

The original possible cause of chromosome breakage in this study, rye chromatin, was quickly eliminated. RTDs were retained as means of initiating the BFB cycles. While it appeared that the frequency of the secondary chromosome breakage, that is, breakage of chromosomes not originally involved in the BFB cycle, dropped after the elimination of rye chromatin, there was no doubt that, as in maize (McCLINTOCK 1950, 1951, 1978), these secondary chromosome breaks did occur even several generations after rye chromatin was eliminated. These secondary breaks were present only among the progenies of plants with active BFB cycles. No chromosome breakage was found among the progenies of their normal sibs. Therefore, it appears reasonable to conclude that in wheat, as in maize, active BFB cycles induce secondary chromosome breakage.

The distribution of the secondary breakpoints throughout the karyotype of the Chinese Spring wheat was not random. Among 72 such breakpoints identified, 16, or 22%, were in the short arm of chromosome *2B*. As far as could be ascertained, and this was not possible in dicentric chromosomes, the breakpoints in chromosome *2B* were confined to the 2.6 region of the short arm, the subtelomeric heterochromatic band. In addition to the frequent involvement of *2BS* in the secondary breaks, and in addition to the $2B_{rtd}$ studied here, all other observed RTDs on the *2BS* arm had the original breakpoint in the same 2.6 region. The second chromosome particularly susceptible to breakage was *7D*. However, because of problems with reliable identification of this chromosome or its arms, the frequency of breakage listed here probably represents only the possible minimum. Another defined area particularly susceptible to breakage appeared to be located in the euchromatic 2.7 region of the long arm of chromosome *4A*. Chromosome *4A* broken in this area was observed in 8.3% of all secondary breakages and in several independently derived RTDs. It needs to be pointed out that progenies of plants with $4A_{rtd}$ or with dicentric chromosomes involving at least one chromosome *4A* constituted more than $^1/_3$ of all progenies analyzed in this study. Consequently, the actual frequency of breakage in the 2.7 region of *4A* must have been at least $^1/_3$ higher.

The two chromosome regions, on *2B* and *4A*, that were particularly susceptible to breakage reacted by breakage to wide hybridization and to the BFB cycles initiated by the RTDs. The characteristics of the breakage-susceptible site on chromosome *7D* were not clear. Because of the identification problems, the actual breakage frequency of this chromosome was not determined, and duplications, even if present, very likely would not be identified. On the other hand, the breakage-susceptible site in the 1.9 region of short arm of chromosome *3D* reacted frequently to wide hybridization by forming RTDs, but it did not react to the BFB

cycles. Only one secondary break in *3DS* was detected among the analyzed populations.

In addition to the loci particularly predisposed to breakage, it also appeared that the entire B genome was more predisposed to breakage than the A and the D genomes. Of the 72 secondary breaks observed, A- and D-genome chromosomes combined accounted for 25 breaks, with the remainder involving the B-genome chromosomes. Additionally, every B-genome chromosome was observed involved in aberrations in one form or another at least once, while five A-genome chromosomes and only three D-genome chromosomes were observed to break. Increased predisposition of the B-genome chromosomes to breakage was observed after the action of the gametocidal chromosome *6* of *Ae. speltoides* (KOTA and DVORAK 1988) and that of *Ae. cylindrica* (ENDO 1988). However, some, but not all, of the increase in the aberration frequency of the B-genome chromosomes relative to the A- of D-genome chromosomes may be related to much clearer and better defined C-banding patterns that make the detection of aberrations easier and perhaps also to the differences in chromosome length.

The frequencies of chromatid bridges in meiotic anaphases indicated that the RTDs initiated the BFB cycle in about one-half of the gametophytes. However, among the progenies obtained either by self-pollination or by controlled hybridization with normal Chinese Spring, the frequencies of RTD chromosomes with unchanged original structure, as judged by C-banding patterns, usually exceeded 80%. Only $3D_{rtd}$ consistently showed higher than 20% frequency of modification of the duplication structure from one generation to the next. This suggests that in a majority of cases, chromatid bridges in meiotic anaphases and in both pollen mitoses must have broken at the point of the original break and fusion (which produced the duplication) or in its general vicinity (within the resolution of light microscope and C-banding patterns). A similar tendency to repeated breaks at the point of the original fusion was observed in maize (MCCLINTOCK 1941a).

The frequencies of chromatid bridges in meiotic anaphases (Table 1) indicated that about one-half of the microspores received a broken chromosome end. However, the frequencies of chromatid bridges in the anaphases of both pollen mitoses were considerably lower compared to those in meiotic anaphases (by a factor of about ten for each division). It is not clear at this point whether the actual frequencies of the chromatid bridges in the anaphases of pollen mitoses were underestimated, or whether the chromatid type BFB cycle has ceased in large proportions of the spores, perhaps due to the healing of the broken chromosome ends. No such healing was reported for the microspores in maize (MCCLINTOCK 1941a,b) where the BFB cycles initiated in meiosis continued through the endosperm divisions and only ceased in the zygote. On the other hand, the fusion points of previous breaks could be physically weaker and break much earlier in anaphase and hence, the BFB cycle would be less detectable cytologically. High frequency of variegation of pigmentation in the aleurone layer in hybrids between the $1B_{rtd}$ and $3D_{rtd}$ lines and the blue aleurone line observed here suggests that the chromatid type BFB cycle continued in a substantially higher proportion of the microspores than the observed frequencies of anaphase bridges in pollen mitoses.

The variegation of the pigmentation in the aleurone layer of kernels must have resulted from the dosage changes of the gene for blue aleurone. It was observed in several instances, but could not be clearly photographed, that in light blue kernels twin sectors were present: a sector with higher intensity of the aleurone pigmentation adjacent to a sector without aleurone pigmentation. The light blue kernels must have had two doses of the blue aleurone gene (received through the egg). The dark patches in twin sectors would then contain an increased dose of the blue aleurone gene, while the patches without the pigment would likely have one or zero doses of the gene. Loss or gain of the blue aleurone gene might have resulted from events affecting either the entire Agropyron chromosome or only the chromosome segment containing the gene. Changes in the chromosome dosage could have resulted from nondisjunction of chromatids in mitotic anaphase, with one daughter nucleus receiving both chromatids and the other receiving none. Changes in the dosage of only the segment of the chromosome with the blue aleurone gene might have resulted from breakage of the chromosome leading to the formation of an anaphase chromatid bridge. Such a bridge, when broken, contributed a duplication to one daughter nucleus and a deficiency to the other. Considering that no evidence for nondisjunction was observed in this study (except for rare lagging dicentric chromatids of RTD chromosomes), and that breakage of wheat and rye chromosomes induced by active BFB cycles was observed in many plants, it would seem more likely that the variegation in pigmentation of the aleurone layer observed here resulted from the breakage of the Agropyron chromosome, brought about by the continuation in the endosperm tissue of the BFB cycle initiated in meiosis by the RTDs. On the other hand, no apparent modifications of the Agropyron chromosome were observed among the karyotyped F_1 and F_2 hybrids involving the RTDs, suggesting that the BFB cycle did not continue in the zygote. Low frequency of anaphase bridges was observed in embryo divisions (Figure 3l, Table 1). However, in a majority of instances, it appeared that more than one bridge was present per cell, suggesting that these might have resulted from the chromosome type BFB cycle.

Apparently spontaneous variegation of pigmentation has been observed in kernels of normal wheat (HARE

1992) and in blue-aleurone lines of wheat (METTIN *et al.* 1991 and references therein). However, the frequencies of variegated kernels were always very low. Among the exceptions known to the author were hybrids of the blue aleurone line H83–952–1 used in this study with two wheat cultivars Omar and Roedel that consistently produced ~1% of variegated kernels (R. J. METZGER, personal communication). In this experiment, low frequency of variegation was observed in the H83–952–1 line itself and among the siblings that inherited the Agropyron chromosome but not a RTD (Table 3). This level of variegation, while higher than that reported by METTIN *et al.* (1991), can be considered as background. It probably resulted from the anaphase bridges in mitoses in the coenocytic stage of the endosperm development that are common in normal wheat (KALTSIKES *et al.* 1975). By chance alone, any chromosome of the complement, including the Agropyron chromosome with the blue aleurone gene, may become involved in the aberration resulting in sectors with different intensity of pigmentation. However, when the Agropyron chromosome was combined with the RTD-induced BFB cycles, the frequency of variegation increased dramatically, up to 9% (Table 3). The actual frequency must have been even higher. Only a fraction of the F_2 kernels scored for the presence of pigment variegation received a copy of the Agropyron chromosome. Additionally, even among kernels with the Agropyron chromosome present, only some aberrations could have been detected because the reduction in dosage of the blue aleurone gene from one to zero would not be apparent. Consequently, the actual frequency of aberrations involving the Agropyron chromosome in the endosperm divisions must have been much higher than the data in Table 3 indicate. This would make the Agropyron chromosome in line H83–952–1 particularly susceptible to breakage.

A similar effect of somatic variegation for a morphological character was repeatedly observed among the $4A_{rtd}$ homozygotes. There is little doubt that the appearance of normal sectors in necrotic plants was related to somatic elimination of at least one copy of the *Dne* locus. With one exception, the frequencies of $4A_{rtd}$ chromosomes with altered structure among the progeny grown from seed collected from self-pollinated normal sectors of the $4A_{rtd}$ homozygotes were substantially higher than the typical ~15% observed among the progeny grown from seed harvested from the self-pollinated necrotic sectors. Karyotyping in meiosis in one such normal sector of a chimera revealed the presence of one broken $4A_{rtd}$ chromosome. Acentric fragments of chromosome *4A* were observed on several occasions in small sectors of root tip preparations of the $4A_{rtd}$ homo- and heterozygotes.

While it appears plausible that somatic breakage of the $4A_{rtd}$ chromosome was responsible for the appearance of the somatic chimeras, the mechanism responsible for such breakage was not clear. If it were a natural chromosome breakage that could be expected to occur with low frequencies in somatic tissues, tetrasomics *4A* and di-isosomics *4AL* should also produce nonnecrotic sectors with frequencies similar to the $4A_{rtd}$ homozygotes, but they do not. Nonnecrotic sectors in $4A_{rtd}$ homozygotes appeared quite late in plant development so it seems unlikely that the BFB cycles initiated in the preceding meiosis were responsible. First, no active chromatid type BFB cycles have ever been observed in the root tip squashes, reinforcing the observation that this type of the BFB cycle continues in the gametophyte and the endosperm but not in the sporophyte. Second, the seedlings in which the chromosome type BFB cycle involving at least one chromosome $4A_{rtd}$ was observed in the root tip squashes never produced necrotic plants. Apparently, cells or sectors of tissue with less than four doses of the *Dne* locus have such selective advantage over those with four doses that the necrotic sectors never develop into a plant. On the other hand, observations of the permutations of dicentric chromosomes suggest that these generate a wide enough range of altered chromosome types, so that there is plenty of opportunity for the establishment of cell lines with less than four copies of the *Dne* locus. Therefore, it seems reasonable to suspect that the appearance of the normal sectors in the necrotic $4A_{rtd}$ homozygotes, and perhaps also of short necrotic streaks on upper leaves of plants with three doses of the locus, resulted from the initiation of new cycles of breakage of the $4A_{rtd}$ chromosomes.

In conclusion, it appears that the nature of the chromosome breakage observed in this study was quite different from that caused by the gametocidal chromosomes (ENDO 1988; KOTA and DVORAK 1988; TSUJIMOTO and TSUNEWAKI 1985). The difference was primarily in the fact that the broken chromosome ends observed here showed high tendency for fusion with other broken chromosome ends. This generated the first RTDs, which, by the nature of their meiotic behavior, initiated the breakage fusion bridge cycles. In turn, these BFB cycles induced breakage of other chromosomes, most often in defined loci. In this sense, the observed karyotypic consequences of the BFB cycles in wheat meet the criteria MCCLINTOCK (1951) used for the detection of the *Ds* element on chromosome *9* in maize. At least three clearly defined regions on wheat chromosomes were detected that showed high relative frequency of breakage: the 2.6 region on *2BS*, the 1.9 region on *3DS* and the 2.7 region on *4AL*. Chromosome *7D* also showed a high frequency of breakage but precise location of the breakpoints could not be determined. The nature of the locus on *3DS* appeared somewhat different than those on the remaining two chromosomes as it reacted only to the presence of alien chromatin (rye and *Ae. speltoides*) but not to the BFB cycles initiated by wheat chromosomes. The frequently breaking loci on

chromosome arm *2BS* and *4AL*, however, reacted by breakage to the active BFB cycles initiated by other chromosomes.

It is possible that a *Ds*- or *X*-like element (McCLINTOCK 1978) was responsible for the observed phenomena. Such an element could be a native wheat element induced to activity by the genetic stress associated with wide hybridization or the presence of BFB cycles. On the other hand, a *Ds*-like element of rye origin might have invaded wheat after the initial hybridization. The existence in wheat of several localized loci highly susceptible to breakage supports the suspicion of a native wheat element, while the drop in the frequency of chromosome breakage after the removal of rye chromatin suggests that rye origin of the factor is a clear possibility.

While the cytological observations on chromosome breakage suggest that a *Ds*-like element may be responsible, the genetic evidence to support such hypothesis is rather tenuous at the moment. The BFB cycles observed here did not appear to be associated with the high mutation rate expected for a transposable element. However, accumulation of sufficient genetic evidence on the action of transposable elements may always be difficult in wheat because of its polyploid nature and scarcity of simply inherited morphological markers that make the identification of mutation difficult.

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