Chromosome Research **9**: 137–146, 2001.

Chromosome healing by addition of telomeric repeats in wheat occurs during the first mitotic divisions of the sporophyte and is a gradual process

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Received 9 October 2000; received in revised form and accepted for publication by Pat Heslop-Harrison 14 December 2000

Key words: breakage–fusion–bridge cycle, chromosome healing, gametocidal factor, telomere, *Triticum aestivum*

Abstract

Alien gametocidal chromosomes cause extensive chromosome breakage prior to S-phase in the first mitotic division of gametophytes lacking the alien chromosome. The broken chromosomes may be healed either by addition of telomeric repeats in the gametophyte or undergo fusions to form dicentric or translocation chromosomes. We show that dicentric chromosomes undergo breakage–fusion–bridge (BFB) cycles in the first few mitotic divisions of the sporophyte, are partially healed before the germ line differentiation regimen, and are healed completely in the ensuing gametophytic stage. The gametocidal factor on chromosome 4Mg of *Aegilops geniculata* was used to induce dicentrics involving the satellite chromosomes1B and 6B of wheat, *Triticum aestivum*. The dicentrics 1BS · 1BL-2AL · 2AS and 6BS · 6BL-4BL · 4BS initiated BFB cycles that ceased 2 to 4 weeks after seed germination. At the end of the BFB cycles, we observed deficient 1B and 6B chromosomes with breakpoints in proximal regions of the 1BL and 6BL arms. The process of chromosome healing was analyzed in root tip meristems, at meiotic metaphase I, and in the derived progenies by fluorescence *in-situ* hybridization analysis using a telomeric probe pAtT4. The results show that chromosome healing in wheat occurs during very early mitotic divisions in the sporophyte by *de-novo* addition of telomeric repeats and is a gradual process. Broken chromosome ends have to pass through several cell divisions in the sporophyte to acquire the full telomeric repeat length.

Introduction

McClintock (1938, 1941, 1942, 1984) analyzed the behavior of broken chromosome ends in maize (*Zea mays* L.) and reported two types of BFB cycles. The chromatid-type BFB cycle results from the fusion of broken sister chromatids, which form an acentric fragment and a chromatid bridge at anaphase. After the chromatid bridge breaks, each of the two daughter nuclei receive one broken chromosome end. Following DNA replication, the sister chromatids fuse again at the new breakpoints and the cycle continues. If the broken ends of different chromosomes fuse, translocation

chromosomes are formed and, depending on where the fusion occurs, these chromosomes have either one or two centromeres. The orientation of the centromeres during mitotic anaphase influences whether the dicentric chromosomes are transmitted normally to the daughter nuclei or form a double-chromatid bridge and initiate the chromosome-type BFB cycle. A ring chromosome is formed if two broken ends from opposite arms of one chromosome fuse. Ring chromosomes show normal anaphase segregation or produce double-chromatid bridges at mitotic anaphase depending on whether or not sister-chromatid exchanges have occurred. Thus, ring chromosomes are also capable of initiating the chromosome-type BFB cycle. The BFB cycle continues until the broken chromosome ends are healed by the addition of a functional telomere at the breakpoint.

The healing of broken chromosomes in wheat is achieved either by the fusion of broken ends resulting in cytologically stable terminal translocation chromosomes (Endo 1988) or by the addition of a functional telomere to the broken termini to form stable chromosome deficiencies (Werner *et al.* 1992, Tsujimoto 1993a). Telomeres (Muller 1940) are protective protein–DNA caps that maintain the integrity of linear eukaryotic chromosomes during cell proliferation and inheritance. The enzyme telomerase, an RNA-primed DNA-polymerase with reverse transcriptase activity is involved in the replication of telomeres (for review, see Blackburn 1991, Wellinger & Sen 1997). Telomeres consist of a tandemly repeated, simple-sequence DNA and are highly conserved among plants and animals (Kipling 1995). In many plants, the repeat unit is TTTAGGG (Richards & Ausubel 1988) Telomeres guard against exonucleolytic degradation (for review see Zakian 1989, 1995) and also ensure complete replication of the DNA at the 5′-end, which otherwise would have a primer-originated gap from semi-conservative replication (Lingner *et al.* 1995, 1997). Tsujimoto *et al.* (1997, 1999) characterized gametocidal (Gc) gene-induced wheat *Triticum aestivum* L.) deficiencies and reported that the breakpoints were healed by the *de-novo* addition of telomeric repeats.

We used the Gc gene located on chromosome $4M^g$ of *Aegilops geniculata* Roth to induce dicentric chromosomes in common wheat involving chromosomes 1B and 6B. These chromosomes are easy to follow because of a prominent cytological structure called satellite (SAT) that is separated by a secondary constriction. The fate of the dicentrics and the healing of broken ends by the addition of telomeric repeats was analyzed by fluorescence *in-situ* hybridization (FISH) in root tip meristems, at meiotic metaphase, and in the derived progenies. Restriction fragment length polymorphism (RFLP) analysis was used to further characterize the SAT chromosome deficiencies.

Materials and methods

The Gc gene of chromosome $4M^g$ of *Ae. geniculata* was used to induce dicentric chromosomes in 'Chinese Spring' wheat. Chromosome $4M^g$ is known to induce a moderate level of chromosome breaks in the first postmeiotic interphase mainly in the gametophytes lacking it (Kynast *et al.* 2000). The progeny of a plant monosomic for chromosome $4M^g$ were screened by phase-contrast and C-banding analyses for the presence of dicentric chromosomes. Chromosome identification was according to Gill & Kimber (1974) and Gill *et al.* (1991).

The process of chromosome healing was analyzed in root tip meristems of first generation plants, in pollen mother cells (PMCs) at meiotic metaphase I, and in derived progenies (second generation plants) by FISH using the telomeric probe pAtT4 isolated from *Arabidopsis thaliana* (Richards & Ausubel 1988) and the rDNA probe pTa71 isolated form *T. aestivum* (Gerlach & Bedbrook 1979) according to Kynast *et al.* (2000). Briefly, 1 μg of pAtT4 DNA and 1 μg of pTa71 DNA were labeled with biotin-14-dATP (Life Technologies) or tetramethylrhodamine-5-dUTP (Roche Molecular Biochemical) and fluorescein-11-dUTP (Amersham Pharmacia Biotech) by nick translation. The hybridization solution containing 50% deionized formamide, 2 × SSC, 10% dextran sulfate, 0.1% SDS, 1 mg/ml of sheared salmon testes DNA and 1.5 μg/ml of the labeled probe DNA was denatured by heating in a boiling water bath for 7 min. Thirty μl of the denatured hybridization mixture was added to each slide. Slides with the hybridization solution were kept

at 80°C for 10 min and allowed to cool to 37°C in an incubator. Hybridizations were made in a humidity chamber at 37°C for 6 h. Post-hybridization washes were performed at 42°C, twice in 2 × SSC for 5 min, once in 50% formamide/2 × SSC for 10 min, and twice in 2 × SSC for 5 min. The biotin-labeled probes were detected with fluorescein (FITC) anti-biotin (Vector Laboratories). Chromosomes were counterstained with propidium iodide (PI) or 4′,6-diamidino-2-phenylindole (DAPI) and mounted in antifade solution (Vector).

Four clones were selected for RFLP analysis. Clones AGB20 (barley gDNA) and WG223 (wheat gDNA) previously were mapped physically in the proximal heterochromatin of the 6BL arm, and clones KSUG58 (*Ae. tauschii* gDNA) and CDO772 (oat cDNA) mapped distal to AGB20 and WG223 in the adjacent euchromatic region of the 6BL arm (Gill *et al.* 1993, Weng *et al.* 2000). Clones WG223 and CDO772 were kindly provided by Dr. M.E. Sorrells (Cornell University, Ithaca, NY, USA) and clone AGB20 was kindly provided by Dr. A. Kleinhofs (Washington State University, Pullman, WA, USA). Clone KSUG58 is maintained by the Wheat Genetics Resource Center at Kansas State University, Manhattan, KS, USA. DNA extraction, restriction enzyme digestion, DNA hybridization, probe labeling, and hybridization conditions were as described by Qi *et al.* (1997).

Microphotographs of C-banded chromosomes were taken with a Zeiss photomicroscope III using Kodak Imagelink HQ microfilm 1461. Signals were visualized using a Zeiss Axioplan microscope equipped for phase contrast and epifluorescence. Images were captured with a SPOT CCD camera using the appropriate SPOT 2.1 software (Diagnostics Instruments, Inc., Sterling Heights, Michigan, USA) and processed with PhotoShop 4.0 software (Adobe Systems Inc., San Jose, California, USA). Images were printed on a Kodak ds 8650 PS Color Printer.

Results

Generation of dicentric chromosomes

Seventy-three self-progenies derived from a plant monosomic for chromosome $4M^g$ were screened in phase-contrast for the presence of dicentric chromosomes. Dicentrics were identified in 16 plants, and one plant had a ring chromosome. The presence of a secondary constriction indicated that two of the dicentrics involved SAT chromosomes. C-banding analysis identified the dicentrics as dic6BS · 6BL-4BL · 4BS (dic6B-4B) and dic1BS · 1BL-2AL · 2AS (dic1B-2A) (Figures 1c & 2c).

Fate of the dic6B-4B chromosome

Root tips from the plant with the dic6B-4B were collected 2, 3, 4, and 7 weeks after seed germination. The dic6B-4B underwent BFB cycles that persisted until 3 weeks after germination. Different cells of the same root-tip meristem had different permutations of the dicentric (Figure 1). The BFB cycles ceased 4 weeks after germination. At this time, none of the root-tips of this plant had the dicentric but all had an acrocentric SAT chromosome identified as a 6BL deficiency (def6BL) with the breakpoint near the centromere in the long arm (Figure 1). FISH analysis using pAtT4 as a probe was performed 7 weeks after germination on root tip meristems of the plant with def6BL. FISH sites of pAtT4 were observed at the telomeres of all normal wheat chromosomes. The def6BL chromosome was identified easily as an acrocentric SAT chromosome by the presence of a secondary constriction at the nucleolus organizer region (NOR). Nine def6BL chromosomes were analyzed and all had pAtT4 FISH sites at the telomere in the non-deficient SAT arm. Seven chromosomes lacked detectable pAtT4 FISH sites at the broken termini (Figure 3a-1), whereas the remaining two had small but distinct pAtT4 FISH sites at the breakpoints in the 6BL arm (Figure 3a-2 and 3a-3).

The chromosomal constitution of this plant also was observed at meiotic metaphase I in PMCs of three different spikes. The 6BL deficiency was present in each spike, indicating that the plant was not chimeric and paired as a heteromorphic rod bivalent with its normal 6B homolog in the intact short arm (Figure 1). FISH analysis with pAtT4, detected by red rhodamine fluorescence, and pTa71, detected by yellow–green FITC fluorescence, was performed at meiotic metaphase

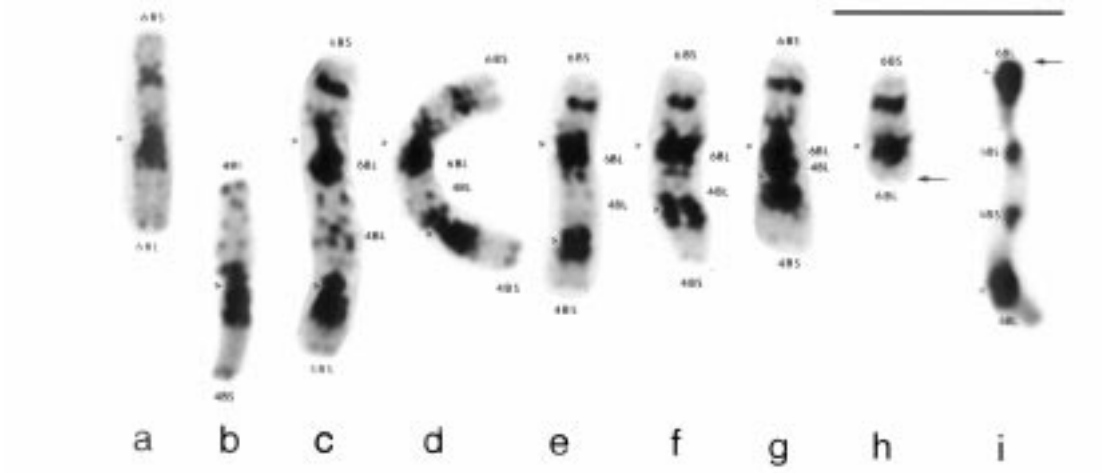

Figure 1. Permutations of a 6BS · 6BL-4BL · 4BS dicentric chromosome induced by the gametocidal gene on chromosome $4M^g$ of *Aegilops geniculata* observed in root tip meristems and at meiotic metaphase I. Arrowheads indicate the position of centromeres and arrows point to the stabilized breakpoint. (**a**) Normal chromosome 6B; note the secondary constriction and distal satellite in the short arm; (**b**) normal chromosome 4B; (**c–g**) permutations of the dicentric 6BS · 6BL-4BL · 4BS chromosome with decreasing sizes of the 6BL-4BL segment; (**h**) 6BL deficiency stabilized 4 weeks after seed germination; (**i**) 6B/def6BL heteromorphic rod bivalent at meiotic metaphase I with chiasmate association in the non-deleted 6BS arm. Scale bar = 10 μm.

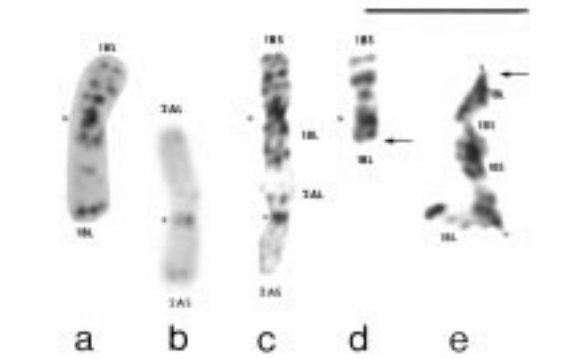

Figure 2. Fate of a 1BS · 1BL-2AL · 2AS dicentric chromosome induced by the gametocidal gene on chromosome $4M^g$ of *Aegilops geniculata* observed in root tip meristems and at meiotic metaphase I. Arrowheads indicate the position of centromeres and arrows point to the stabilized breakpoint. (**a**) Normal chromosome 1B; (**b**) normal chromosome 2A; (**c**) dic1BS · 1BL-2AL · 2AS; (**d**) 1BL deficiency stabilized 2 weeks after seed germination; (**e**) 1B/def1BL heteromorphic rod bivalent at meiotic metaphase I with chiasmate association in the non-deleted 1BS arm. Scale bar = 10 μm.

I on the same plant. The 6BL deficiency formed chiasmate associations with the normal 6B chromosome in the non-deficient SAT arm as indicated by pTa71 FISH sites at the NORs. Thirteen 6B/def6BL rod bivalents were analyzed, and each one had a pAtT4 FISH site at the broken terminus of the 6BL arm. The pAtT4 FISH sites at the breakpoints were smaller than those at the telomere of the non-deficient arm (Figure 3a-4 & d).

The transmission of the 6BL deficiency was determined in 53 self-progenies by C-banding. Nineteen plants were homozygous for chromosome 6B, seven plants were homozygous for def6BL, 26 plants were heterozygous for 6B and def6BL, and one plant had an isochromosome consisting of the short arms of chromosome 6B (i6BS). FISH analysis on root-tip meristems of self-progenies derived from this plant revealed pAtT4 FISH sites at the broken termini of the 6BL arm in all the def6BL chromosomes analyzed (Figure 3a-5).

RFLP analysis was performed on three progeny plants that were homozygous for the 6BL deficiency and that were derived from different spikes of the original plant that had the dic6B-4B chromosome. All homozygous 6BL deficiency plants had the same RFLP pattern (data not shown). The ABG20 and WG23 probe-specific fragments were observed in euploid Chinese Spring and in all plants with the 6BL deficiencies. The KSUG58 and CDO772 probe-specific

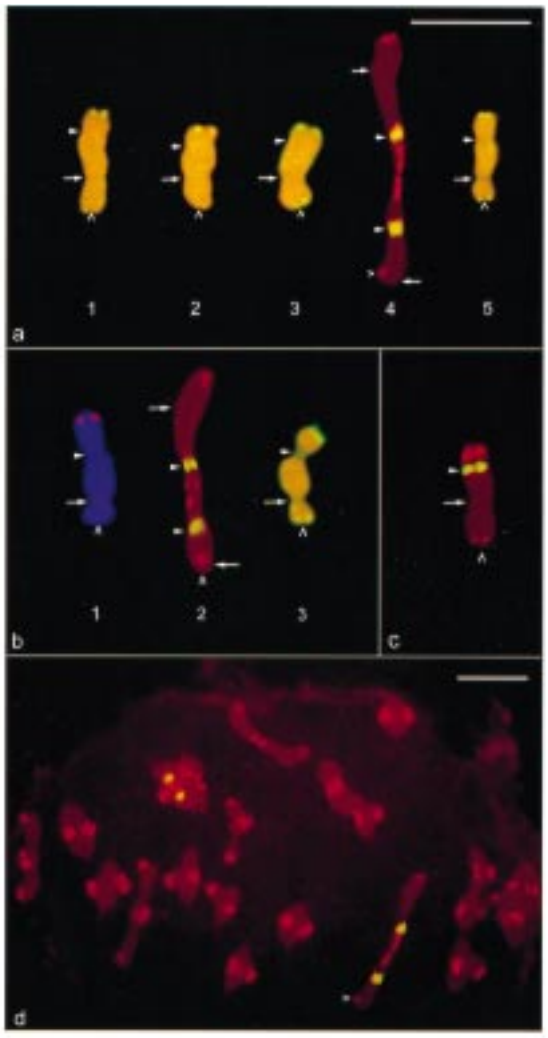

Figure 3. FISH with the telomeric probe pAtT4 and the rDNA probe pTa71 to newly broken SAT chromosomes of wheat (arrows point to the centromeres, solid arrowheads point to the NORs, and open arrowheads point to the broken termini). The pAtT4 FISH sites are visualized by yellow–green FITC fluorescence and counterstained with propidium iodide (a-1–a-3 and a-5) and by red rhodamine fluorescence (a-4); pTa71 sites (a-4) are visualized by yellow–green FITC fluorescence. (**a**) Fate of the dic6BS · 6BL-4BL · 4BS: 6B deficiencies in a root-tip meristem 7 weeks after germination (a-1–a-3), (a-4) a 6B/def6BLheteromorphic rod bivalent at meiotic metaphase I paired in the unbroken 6BS arm, note the presence of pAtT4 FISH sites at the broken end; and (a-5) a 6BL deficiency recovered in the self-progeny of this plant, note the absence of pAtT4 FISH sites in (a-1) and presence of small (a-2) and larger (a-3, to a-5) pAtT4 FISH sites at the broken termini. (**b**) Fate of the dic1BS · 1BL-2AL · 2AS, pAtT4 FISH sites are visualized by red rhodamine (b-1 and b-2) and yellow–green FITC fluorescence (b-3) and counterstained with DAPI (b-1) and propidium iodide (b-3), pTa71 sites (b-2) are visualized by yellow–green FITC fluorescence; (b-1) a 1B deficiency in a root tip meristem 3 weeks after germination, note the absence of pAtT4 FISH sites at the broken terminus; (b-2) a 1B/def1BL heteromorphic rod bivalent at meiotic metaphase I paired in the complete 1BS arm, note the presence of pAtT4 FISH sites at the broken terminus; and (b-3) a 1B deficiency recovered in the self-progeny of this plant, note the presence of pAtT4 FISH sites at the broken end. (**c**) A first-generation 1B deficiency with the breakpoint in the long arm, pAtT4 FISH sites are visualized by red rhodamine and pTa71 FISH sites by yellow–green FITC fluorescence; note the small but distinct telomeric signals at the broken terminus. (**d**) Meiotic metaphase I of the plant with the dic6BS6 · BL-4BL · 4BS using pAtT4 (red rhodamine fluorescence) and pTa71 (yellow–green FITC fluorescence); note that the broken terminus in the long arm has a small, but distinct, pAtT4 FISH site. Scale bar = 10 μm.

fragments were present in euploid Chinese Spring but absent in the 6BL deficiencies. Probes ABG20 and WG23 were previously mapped in the proximal heterochromatic region of the 6BL arm, whereas KSUG58 and CDO772 were mapped in the distal adjacent euchromatic 6BL region (Gill *et al.* 1993, Weng *et al.* 2000). These data suggest that the fusion of the 6BL and 4BL arm occurred in the heterochromatic region of the 6BL arm distal to probes ABG20 and WG23 and proximal to probes KSUG58 and CDO772. Absence of KSUG58 and CDO772 probe-specific fragments in all the 6BL deficiencies suggests that the terminal euchromatic segment in the broken 6BL arm was probably derived from the 4BL arm involved in the dic6BS·6BL-4BL·4BS dicentric chromosome. Thus, the 6BL deficiency is actually a deficient translocation chromosome where the distal euchromatic terminal region of the long arm probably was derived from the 4BL arm.

Fate of the dic1B-2A chromosome

None of the root tips collected from the plant 2, 3, and 4 weeks after seed germination had the dicentric chromosome, but all had an acrocentric SAT chromosome that was identified by C-banding as a 1BL deficiency with the breakpoint in the proximal region of the long arm (Figure 2). FISH was made on root-tip meristems collected 2 and 3 weeks after germination of the plant with def1BL. Four of the five def1BL chromosomes available for this analysis lacked pAtT4 FISH sites at the broken termini (Figure 3b-1) but a small signal was observed at the breakpoint in the 1BL arm in the fifth (data not shown).

This plant was very weak and produced only a few tillers. The chromosome constitution was determined in PMCs at meiotic metaphase I in one spike. The 1BL deficiency was present and paired with a normal 1B chromosome in the short arm as a heteromorphic rod bivalent. FISH with pAtT4 and pTa71 as probes at meiotic metaphase of this plant revealed small pAtT4 FISH sites at the 1BL breakpoints in all seven 1BL·1BS//1BS·def1BL rod bivalents analyzed (Figure 3b-2).

Only six viable seeds were obtained from this plant after selfing. Two plants were homozygous for 1B, one plant was homozygous for def1BL, and the remaining three plants were heterozygous. All 1BL deficiencies recovered in the self-progeny of this plant had pAtT4 FISH sites at their broken termini (Figure 3b-3).

First-generation SAT deficiencies

Apart from dicentrics involving SAT chromosomes, three of the 73 self-progenies derived from the plant monosomic for chromosome $4M^g$ had first-generation deficiencies in the long arms of the wheat SAT chromosomes. The resulting acrocentric SAT chromosomes were identified by C-banding analysis as two 1BL and one 6BL deficiencies. None of these plants survived, but root-tip meristems from one plant with a 1B deficiency were available for FISH analysis. FISH analysis using pAtT4 and pTa71 as probes also was performed on root-tip meristems of one plant with a first-generation deficiency in the long arm of chromosome 1B. Data were taken on 10 chromosomes and all had small but distinct pAtT4 FISH sites at their broken termini in the long arms (Figure 3c).

Discussion

Three different mechanisms of chromosome healing by telomere addition have been reported. Wang & Zakian (1990) reported that acquisition of telomeres can occur by telomere–telomere recombination in yeast. An alternative method to heal broken chromosomes was observed in a malignant melanoma. Meltzer *et al.* (1993) demonstrated that several apparent terminal deletions are, in fact, subtelomeric translocations. The broken chromosomes were stabilized by telomere capture. In addition to telomere–telomere recombination and telomere capture, broken chromosomes are healed by the *de-novo* addition of telomeric repeats to the broken ends, as in *Tetrahymena* (Harrington & Greidler 1991), humans (Lamb *et al.* 1993, Wilkie *et al.* 1990), and plants (Werner *et al*. 1992, Wang *et al.* 1993, Tsujimoto 1993a, 1993b, Tsujimoto *et al*. 1997, 1999, Gill & Friebe 1998, Kynast *et al.* 2000).

Wheat deficiencies induced by the Gc gene on chromosome $2C^c$ of *Ae. cylindrica* Host. that have passed through six generations of backcrossing had telomeric repeats at their broken ends that were visualized by *in-situ* hybridization analysis (Werner *et al*. 1992). Newly induced first-generation wheat deficiencies recovered in progenies of monosomic $2C^c$ addition plants also had cytologically detectable telomeric repeats at their broken termini (Werner *et al*. 1992, Gill & Friebe 1998). Similarly, the first-generation 1BL deficiency identified in this study had distinct pAtT4 FISH sites at the broken end. On the contrary, Tsujimoto (1993b) reported that first-generation deficiencies either were lacking or had fewer telomeric repeats at the broken ends, implying that the process of chromosome healing occurs gradually and that the deficiencies have to pass through several cell cycles to acquire the entire repeat length. Similarly, Kynast *et al.* (2000) reported that acrocentric and telocentric chromosomes derived from chromosome-type BFB cycles induced by the gametocidal gene on the *Ae. geniculata* chromosome $4M^g$ lacked cytologically detectable telomeric repeats at their broken ends. Because morphologically similar rearrangements were detected in different cells of the same root tip meristem, it was speculated that chromosome healing by addition of telomeric repeats occurs gradually. However, unambiguous evidence was lacking.

First-generation deficiencies induced by Gc genes during gametogenesis have already passed through several mitotic cell cycles before they are detected in root tip meristems. Another system to analyze the fate of newly broken chromosomes is provided by di- and multicentric chromosomes that initiate chromosome-type BFB cycles. The chromosome rearrangements derived from BFB cycles were either produced in the telophase of the last preceding mitosis or might have passed through only a few cell cycles. This approach allows the identification of marker chromosomes that can be followed easily in root meristems and at meiotic metaphase I of first-generation sporophytes and in the derived self-progenies of second generation sporophytes. New insights into the mechanism of chromosome healing can be obtained by analyzing the fate of these marker chromosomes.

In the present study, the Gc gene on chromosome $4M^g$ was used to induce dicentric chromosomes in common wheat. The frequency of Gc-induced dicentric and ring chromosomes observed (23%) is similar to that reported by Kynast *et al.* (2000) using the same Gc system (27%). Two of the dicentrics involved wheat SAT chromosomes 1B and 6B. The dicentrics initiated chromosome-type BFB cycles that ceased 2–4 weeks after seed germination. Both dicentrics were stabilized as deficiencies with breakpoints in proximal regions of the long arms of chromosomes 1B and 6B. At that time, all observed mitotic metaphases of these plants had the same 1BL and 6BL deficiencies, as indicated by C-banding analysis. The presence of the same deficiency in different cells of the same plant suggests that the broken chromosome ends were healed.

At the resolution of the FISH technique, telomeric repeats were not detected at the broken termini in four of the five def1BL and seven of the nine def6BL chromosomes analyzed. The remaining three chromosomes had small but distinct telomeric loci at their broken termini. At meiotic metaphase I of those plants, all def1BL and def6BL chromosomes analyzed had small pAtT4 sites at the broken ends. Similarly, all def1BL and def6BL chromosomes recovered in self-progenies of these plants had telomeric repeats at their broken ends.

The lack of detectable telomeric repeats in the majority of the broken ends of def1BL and def6BL shortly after they were stabilized probably is caused by the limited sensitivity of the FISH technique. Because these deficiencies were transmitted stably through mitotic cell divisions, a small number of telomeric repeats may have been added at the broken ends, but their detection is beyond the sensivity of the FISH technique. The few 1BL and 6BL deficiencies with telomeric repeats at the broken termini may already have passed through several mitotic divisions and, thus, may have acquired a larger number of telomeric repeats. At meiotic metaphase I and in the derived progenies, the 1BL and 6BL deficiencies had passed through a larger number of cell divisions and all had cytologically detectable telomeric repeats at their broken ends. Our data unambiguously show that chromosome healing in wheat by the *de-novo* addition of telomeric repeats is a

gradual process and that the broken ends have to pass through several cell cycles to acquire the full repeat length.

Kilian *et al.* (1995) reported that meristematic tissues had longer telomeres compared with chromosomes in differentiated leaf tissues and speculated that telomerase activity is repressed in differentiated plant tissues. Heller *et al.* (1996) recently confirmed this fact by using a modified telomeric repeat amplification protocol (TRAP) and showing that several tissue extracts of barley had telomerase activity including embryo, anther, and carpel tissues as well as immature seeds but not in the leaf tissue.

Previous studies have shown that Gc genes induce chromosome breaks prior to the S-phase of the first postmeiotic interphase mainly in male gametophytes lacking them (Finch *et al.* 1984, Nasuda *et al.* 1998). Although a large number of Gc-induced chromosome fragments were observed at ana-/telophase of the first pollen mitosis, fewer aberrant ana-/telophases were observed at the second pollen mitosis, suggesting that some of the broken chromosomes were healed (Nasuda *et al*. 1998). The recovery of cytologically stable first-generation deficiencies in the progenies of plants monosomic for a Gc gene suggests that the broken chromosome ends also were healed during either gametogenesis and/or early zygote development. Some Gc genes are known to cause chromosome breakage also during early embryo and endosperm development (King & Laurie 1993). Our study showed that chromosome healing by addition of telomeric repeats also occurs during mitotic cell divisions in the sporophyte. Recently, Zheng *et al.* (1999) analyzed the fate of dicentric chromosomes during the first 10 weeks after germination in root-tip meristems of maize. This study showed that chromosome-type BFB cycles ceased about 10 weeks after germination as a result of chromosome healing. Similar observations also were made in wheat (Kynast *et al*. 2000) and suggest that chromosome healing at least in wheat and in maize can occur both in gametophytic and sporophytic tissues.

The process of chromosome healing was analyzed in root tip meristems of first-generation sporophyte. We did not expect the same stabilized rearrangement at meiotic metaphase I and in all the derived progenies of these plants. Although the stabilized chromosomal rearrangements appeared to be cytologically identical, breakpoints located in different positions at the molecular level are possible. To verify this hypothesis, four RFLP probes were mapped on the def6BL chromosomes derived from three different tillers of the original dic6BS · 6BL-4BL · 4BS plant. All plants had similar RFLP patterns, suggesting that the 6BL deficiency most likely is a deficient T6BS · 6BL-4BL translocation chromosome where the terminal euchromatic region of the long arm probably was derived from 4BL. Because all the plants also had two normal 4B chromosomes, we could not determine whether all the T6BS · 6BL-4B chromosomes had breakpoints in the same position.

The presence of the same stabilized rearrangement in different meristems can be explained by different mechanisms. One explanation is that there are predetermined hot spots along the chromosomes for breakage and/or telomere addition. The structure of the dicentric determines its ana–telophase orientation and, therefore, always results in the same rearrangement. However, Endo & Gill (1996) recovered a series of 6BL deficiencies that span the entire 6BL arm, which makes this hypothesis unlikely.

The most likely explanation is that the dicentric chromosome was present in the zygote but chromosome healing already had occurred during the first few divisions. Presence of the same deficient chromosome in both root tip meristems and in different tillers of the same plant suggests that the healing occurred before germ cell line differentiation. As a result, the same rearrangement was transmitted to all offspring. First, the initial cells that still had the dicentric chromosome produced roots, then, at a later stage, the root meristems were derived from initial cells that had the healed rearrangement.

Acknowledgements

Contribution No. 01-9-J from the Kansas Agricultural Experiment Station, Kansas State University, Manhattan, Kansas. We thank W. John Raupp and Duane Wilson for excellent assistance. This research was supported in part by a

USDA-ARS competitive grant No. 96-3501-3149 and by a grant from the Kansas Wheat Commission.

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