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De novo synthesis of telomere sequences at the healed breakpoints of wheat deletion chromosomes

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Abstract When chromosomes are broken, the breakpoints become highly unstable and acquire the ability to fuse with other broken ends. The breakpoints are, however, eventually stabilized, and, therefore, the broken chromosomes are transmitted to the daughter cells without further morphological change. This phenomenon, known as "healing of breakpoints", involves the addition of repetitive telomere sequences at the breakpoints by telomerase, the enzyme that normally synthesizes the telomere sequence at normal chromosome terminals. In many higher organisms, however, this property has not been well investigated. In this study, we examined the telomere sequences in wheat deletion lines with breakpoints on chromosome 1B. Lines that had breakpoints around the nucleolar organizer region were first selected on the basis of cytological observations, and the precise breakpoints were determined by mapping a fragment of rDNA and RFLP markers. In three lines – in addition to one previously reported – the DNA fragments encompassing the breakpoints were amplified by PCR using primers located in the rDNA and in telomere sequences. The DNA sequences provide insight into the properties of the telomerase activity at the breakpoints. The telomere sequences initiated from 2- to 4-nucleotide motifs in the original ribosomal DNA sequence which are also found in the repeat unit characteristic of telomere sequences. No specific sequences or structures were observed at or around the breakpoints. At all of the four breakpoints investigated, the newly synthesized telomere sequences contained considerable numbers of atypical telomere sequence units, particularly TTAGGG, which is the common unit of mammalian telomere sequences. Based on these results, we discuss the ability of plant telomerase to initiate the de novo synthesis of telomere sequences at internal breakpoints.

Key words *Triticum aestivum* · Telomerase · Chromosome healing · Gametocidal gene · Chromosome breakage

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Introduction

Eukaryote chromosomes are made up of linear forms of DNA. Because DNA polymerase cannot complete replication at the 3′ ends of linear DNA molecules, the chromosomes are shortened by about 50 bp in every cell cycle (Harley et al. 1990; Levy et al. 1992). In many organisms the loss of DNA due to this "end-replication problem" is compensated for by the presence of repetitive telomere sequences at the ends. The enzyme telomerase synthesizes these sequences independently of the ordinary semi-conservative replication by DNA polymerase (see review Greider 1990; Blackburn 1992). The telomerase consists of several proteins and an RNA molecule that includes a sequence complementary to the repetitive telomere sequences. Some of the components of the telomerase have been purified from unicellular organisms (Greider and Blackburn 1987, 1989; reviewed by Kipling 1995) and humans (Nakayama et al. 1997). However, the plant telomerase has not been isolated and its detailed characteristics remain unknown, although telomerase activity has been detected in the germline of barley (Shippen and McKnight 1998).

Telomerase also plays an important role in the healing of broken chromosome ends. During development of the macronucleus of ciliates, the chromosomes are fragmented. At this stage the telomerase synthesizes telomere sequences at the ends of each fragment. In most organisms, however, chromosomes are broken acciden-

tally. The broken ends become "sticky" and tend to fuse with other sticky ends because of their lack of telomere sequences, thus forming aberrant chromosomes. Such aberrant forms as ring and dicentric chromosomes cause further chromosome breakage through repeated breakage-fusion-bridge (BFB) cycles (McClintock 1941). The ends are, however, eventually healed and thereafter the BFB cycle ceases. The healing process is now thought to involve the addition of repetitive telomere sequences synthesized by telomerase. In wheat, the healed broken ends of deletion chromosomes have been shown to hybridize with telomere sequences (Werner et al. 1992; Tsujimoto 1993). The telomere sequences are synthesized soon after the initial chromosome breakage in embryogenesis (Tsujimoto 1993).

Tsujimoto et al. (1997) cloned the DNA sequence of a healed broken end and found indications that BFB cycles had occurred after the initial chromosome breakage. The telomere repeats initiated from a 3-bp chromosomal sequence that is also found in the telomere repeat unit. Various telomere units were included in the synthesized telomere repeats. In this paper, we report the DNA sequence of three other broken ends that were produced by independent breakage events. We elucidated the mechanism of healing and the mode of de novo synthesis of the repetitive telomere sequences at broken chromosome ends.

Materials and methods

Plant materials and their evaluation

We used 15 chromosome deletion lines of chromosome 1B. Cytological observation showed that the breakpoints of these lines were located within the nucleolus organizer region (NOR), which consists of thousands of tandem repeats of 18S and 26S rRNA gene (rDNA) units. Eight of the lines were made by Endo and Gill (1996) (line nos. 1BS-2, 1BS-5, 1BS-6, 1BS-8, 1BS-16, 1BS-18, 1BS-20, and 1BS-21) and seven by Tsujimoto et al. (unpublished; line nos. Kdel1B-53B, Kdel1B-64, Kdel1B-65, Kdel1B-66, Kdel1B-67, Kdel1B-93, and Kdel1B-110). These lines were produced in the genetic background of common wheat (*Triticum aestivum* L.) cv. Chinese Spring (CS, $2n = 6x = 42$) by using the gametocidal gene of *Aegilops cylindrica* Host to induce chromosome breakage (Endo 1988). As controls, normal CS, nullisomic 1B tetrasomic 1A, and nullisomic 6B tetrasomic 6D lines were used (Sears 1965). In the last two lines a pair of whole chromosomes 1B and 6B were absent, respectively.

The breakpoints of these lines were first analyzed by Southern hybridization with six RFLP probes located around the NOR. The probes used were ksuE19, abc156, bcd98, psr596a, cdo1173, and cdo534 (Gill et al. 1991; Anderson et al. 1992; Kleinhofs et al. 1993; Gale et al. 1995). The RFLP probes were kindly provided by the Wheat DNA Repository of Japan. Common wheat carries three major rDNA loci on chromosomes 1B, 6B and 5D. To distinguish the rDNA locus on chromosome 1B from others, the 2.8-kb *Taq*I fragment of rDNA spacer region was subcloned from pTa71, which carries a full-length rDNA repeat unit (Gerlach and Bedbrook 1979). This clone was named pWrdnTaq2.8. The method used for Southern hybridization was that described by Ogihara et al. (1994).

Amplification of broken ends

Fragments encompassing breakpoints were amplified by PCR using primers specific for rDNA and telomere repeats (Tsujimoto et al. 1997). We designed seven primers for rDNA in addition to the ten primers previously reported (Tsujimoto et al. 1997). These primers have the following sequences: RDN-N1 (GCCAACCGGCTCAAAACCAG), RDN-N2 (AAGTGATCCTCCCGTGCTAT), RDN-N3 (TTGGGTATAGGGGCGAAAGA), RDN-N4 (CTTCGGGGGCTTTCCCCGAG), RDN-NR1 (TTCCCGGACCTCTCATACCC), RDN-NR2 (TGTCACACCAGCAGCAATAA), RDN-NR3 (CGCTCAGACATAGTTCACCA). Because the sequence of the rDNA has not been completely determined in wheat, RDN-N3, RDN-N4 and RDN-NR3 were based on the sequence from rice (Sugiura et al. 1985; Barker et al. 1988; Kiss 1989a, 1989b; Hamby et al., unpublished results). The primers RDN-NR1, RDN-NR2 and RDN-NR3 were designed from the complementary sequence of rRNA. The conditions for PCR were the same as reported previously (Tsujimoto et al. 1997).

Cloning and sequencing

The fragments of PCR that were specifically amplified from a given deletion line with rDNA and telomere primers were purified by ethanol precipitation. The fragments may possess a projecting single-stranded 3′ end because the telomere primer (Telo20, see Tsujimoto et al. 1997) can anneal at any position in the telomere repeats. In order to increase the cloning efficiency, the projecting end of the PCR product was blunted using T4 DNA polymerase. After purification by phenol extraction, the fragments were cloned into the *Eco*RV site of the vector pBluescript II SK (−). *Escherichia coli* strain DH5α was transformed with this DNA. The methods for DNA isolation and sequencing were as reported previously (Tsujimoto et al. 1997).

Results

Location of break points

The breakpoints of the 15 deletion lines were localized using RFLP markers that map near the NOR of chromosome 1B (Tsujimoto et al. 1998). The locations of the DNA markers were defined using the deletion lines and the breakpoints of the deletion lines were defined by the DNA markers (Table 1). Four lines (1BS-2, 1BS-16, 1BS-20 and Kdel1B-110) did not show any rDNA signal on chromosome 1B, indicating that the breakpoints in these lines lie proximal to the NOR. Two lines (Kdel1B-64 and Kdel1B-53B) that carried RFLP markers on the satellite of chromosome 1B appeared to have the breakpoints within the satellite. In the other nine lines, the breakpoints were likely to lie within the NOR itself.

The pWrdnTaq 2.8 insert used as a probe includes the intergenic spacer region of rDNA, and revealed five clear bands in *Taq*I-digested DNAs of wild-type plants. The 3.1- and 3.9-kb bands were derived from chromosome 1B because they were not detectable in a line that lacks chromosome 1B (nullisomic 1B tetrasomic 1A). Of the lines that appeared to have breakpoints within the NOR, four lines (1BS-5, 1BS-8, Kdel1B-65, and Kdel1B-66) showed only the 3.1-kb band (Fig. 1, Table 1). This fact clearly indicated that the NOR of chromosome 1B consists of two subregions that can be distinguished on the basis of the size of the intergenic spacer, and implied that the rDNA unit including the 3.1-kb *Taq*I fragment was situated at a more proximal

Table 1 Localization of breakpoints on the 1B deletion chromosomes using RFLP markers

Line	Probe[a]							
	κsuE19	abc156	bcd98	3.9 kb	3.1 kb	psr596a	cdo1173	cdo534
1BS-2	–	–	–	–	–	–	–	+
1BS-16	–	–	–	–	–	+	+	+
1BS-20	–	–	–	–	–	+	+	+
Kdel1B-110	–	–	–	–	–	+	+	+
1BS-5	–	–	–	–	+	+	+	+
1BS-8	–	–	–	–	+	+	+	+
Kdel1B-65	–	–	–	–	+	+	+	+
Kdel1B-66	–	–	–	–	+	+	+	+
1BS-6	–	–	–	+–	+	+	+	+
1BS-18	–	–	–	+	+	+	+	+
1BS-21	–	–	–	+	+	+	+	+
Kdel1B-67	–	–	–	+	+	ND	+	+
Kdel1B-93	–	ND	ND	+	+	ND	+	–
Kdel1B-64	–	–	+	–	+	+	+	+
Kdel1B-53B	–	+	+	ND	ND	+	+	+

[a] The indicated RFLP probes were used for Southern hybridization to DNAs from the indicated deletion lines. + and – indicate the presence and absence, respectively, of a hybridization signal from chromosome 1B. The 3.9 and 3.1 kb signals were detected using pWrdnTaq2.8 as the probe

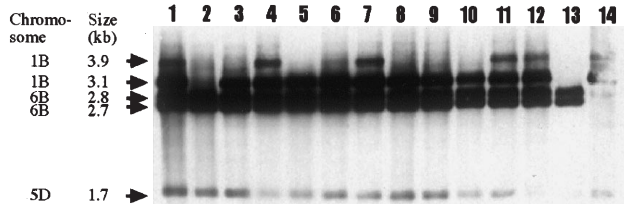

Fig. 1 Southern analysis of *Taq*I-digested DNA from deletion lines using pWrdnTaq2.8 as probe. The sizes and chromosome locations of the bands are indicated. Lane 1, normal CS; 2, Nulli 1B tetra 1 A; 3, Kdel1B-64; 4, 1BS-18; 5, 1BS-8; 6, 1BS-6; 7, 1BS-21; 8, 1BS-5; 9, Kdel1B-66; 10, Kdel1B-65; 11, Kdel1B-67; 12, Kdel1B-93; 13, Kdel1B-110; 14, Nulli 6B tetra 6D

position than the other type of rDNA repeat in this NOR. The line 1BS-6 showed both bands, but the 3.9-kb band was significantly fainter than in the normal line, suggesting that in this line the chromosome was broken within the sub-region of the NOR that includes repeat units containing the 3.9-kb *Taq*I fragment.

Specific amplification of breakpoints

Using primers for rDNA and telomere sequences, we amplified by PCR the sequences that encompass the breakpoints of chromosome 1B, and obtained three breakpoints in addition to that previously characterized in Kdel1B-65 (Tsujimoto et al. 1997).

With a combination of the primers Telo20 and RDN-1, the deletion line Kdel1B-66 showed a smear extending from 50 bp to 20 kb. This pattern did not appear in the other lines. The other lines, as well as the normal line, showed instead three discrete fragments at 500, 650 and 1100 bp (Fig. 2A) that may be derived from interstitial sequences. Likewise, the primer combination Telo20 and RDN-2 produced a specific smear pattern around

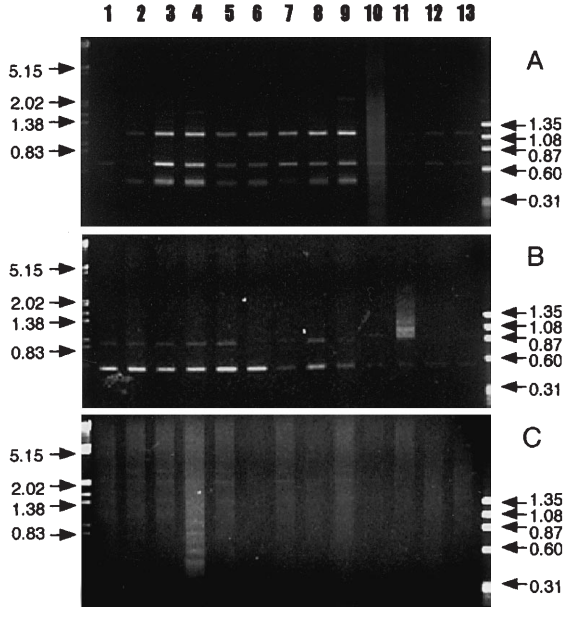

Fig. 2A–C Amplification of DNA fragments by PCR with the telomere primer Telo20 and rDNA primer RDN-1 (**A**), RDN-2 (**B**), or RDN-NR3 (**C**). **A**, **B** Lane 1, CS (control); 2, 1BS-2, 3; 1BS-5; 4, 1BS-6; 5, 1BS-8; 6, 1BS-16; 7, Kdel1B-53B; 8, Kdel1B-64; 9, Kdel1B-65; 10, Kdel1B-66; 11, Kdel1B-67; 12, Kdel1B-93; 13, Kdel1B-110. **C** Lanes 1, 1BS-2; 2, 1BS-5; 3, 1BS-6; 4, 1BS-8; 5, 1BS-16; 6, Kdel1B-53B; 7, Kdel1B-64; 8, Kdel1B-65; 9, Kdel1B-66; 10, Kdel1B-110; 11, 1BS-18; 12, 1BS-20; 13, 1BS-21. The size marker on the *left* is lambda DNA digested with *Eco*RI and *Hin*dIII, that on the right ϕX174 DNA digested with *Hae*III. Sizes are given in kb

0.9–1.1 kb in the deletion line Kdel1B-67. No such amplification was observed with this primer pair in the other lines (Fig. 2B). The deletion line 1BS-8 showed a specific smear pattern around 0.2–2.0 kb after PCR with the primers Telo20 and RDN-NR3. In the other deletion and normal lines no such pattern appeared (Fig. 2C).

The line-specific fragments from these three deletion lines were cloned and sequenced. All sequences consisted of part of an rDNA unit fused to telomere sequences (Fig. 3). Although the three clones from Kdel1B-66 had telomere sequences that differed in size, they had the same rDNA sequence, because the Telo20 could bind to any portion of the repeat array during the annealing step of PCR. The 3′ ends of these clone differed in sequence from Telo20, suggesting that the telomere sequences had been partly truncated, probably during the cloning step. Nonetheless, these clones could have originated from the same location on the chromosome, because the positions of the atypical telomere units (described below) and of the rDNA sequence were identical in all three clones. The clones had 51 nucleotides of rDNA amplified from RDN-1 which were homologous (94.1%) to the sequence of intergenic spacer region of wheat (Barker et al. 1988). No remarkable sequence rearrangements were observed at the junction between rDNA and telomere sequences. The telomere sequences initiated from a 4-nucleotide motif in rDNA (GGTT), which was also present in the telomere unit (Fig. 3). The longest clone had 32 telomere repeat units, seven of which were different from the typical repetitive telomere sequence in plants, TTTAGGG.

The fragment from Kdel1B-67 was 914 bp long (Fig. 3). The ends carried the expected sequences of Telo20 and RDN-2. The rDNA sequence was 832 bp long and homologous (96.2%) to the intergenic spacer region of wheat and was located about 1.7 kb upstream of the Kdel1B-66 sequence. The telomere sequences initiated from a 2-nucleotide motif (GG) in the rDNA sequence. These two nucleotides also form part of the telomere unit (Fig. 3).

In 1BS-8 three sequences were determined. In these clones, the 3′ ends conformed to the sequences expected from the telomere primer. The rDNA sequence and the identical positions of the atypical telomere units indicated that these clones all came from the same location on the chromosome. The clones had 235 bp of rDNA from RDN-NR3, which is homologous to the sequence of 25S rDNA of rice (93.8%). The telomere sequences had initiated from 2 nucleotides (GG) in the rDNA, as at the breakpoint of Kdel1B-67. The longest clone included ten telomere repeat units, four of which were atypical (Table 2).

Table 2 Frequency of variation in telomere repeats acquired at four healed broken ends of chromosomes

Sequence	Deletion line				Total (%)
	Kdel1B-66	Kdel1B-67	1BS-8	Kdel1B-65	
TTTAGGG	25	5	6	60	96 (75.6)
TTAGGG	5	1	4	17	27 (21.3)
TTTGGGG	–	–	–	1	1 (0.8)
TTTTAGGG	1	–	–	–	1 (0.8)
TATATA	–	–	–	1	1 (0.8)
TTA	1	–	–	–	1 (0.8)
Total	32	6	10	79	127

Discussion

Initiation of telomere sequences at broken ends

In humans, yeast and *Tetrahymena*, the 3′ ends of broken chromosomes that exhibit telomeric sequences or include sequences within about 30 bp from the ends are efficiently healed by the addition of telomere sequences (Murray et al. 1988; Harrington and Greider 1991; Morin 1991; Barnett et al. 1993). In the breakpoints discussed here, from wheat, the telomere sequences initiated from 2-4-nucleotide motifs that are also found in the telomere repeat unit in higher plants. No telomere or TG-rich sequences were present within 30 bp of the ends of these chromosomes. In addition, the breakpoints were located at various positions in the rDNA sequence. This indicates that any short sequence that matches part of the telomere repeat can act as a primer for telomerase. Consequently, the wheat telomerase recognizes short sequences as primers for initiation of telomere sequences. The critical short sequences of rDNA apparently interacted with the template RNA sequence of telomerase and the sequences were synthesized normally. The initial nucleotide recognized by the telomerase is unknown, although all of the short primer sequences included at least one G.

Variation in telomere units and their distribution in the repeat array

In many higher plants, the telomere sequence repeat is TTTAGGG (Richards and Ausubel 1988). This sequence also appeared most frequently in the arrays of the telomere sequence at the broken chromosome ends described here (Table 2). A variant, TTAGGG, was included in all of the broken ends with similar frequency. This finding may indicate the presence of novel telomere RNA molecules in the genomes of wheat. Common wheat is hexaploid, and thus at least three kinds of genes for telomere

Fig. 3 DNA sequences at the breakpoints of the deletion chromosomes in Kdel1B-66, Kdel1B-67 and 1BS-8. The sequences that are homologous to rDNA are *underlined*. The primer of telomere repeat synthesis at the junction is *boxed*. Atypical telomere sequences are in *italics*. These sequences have been deposited in the GenBank database under Accession Nos. AB026895–AB026897

Kdel1B-66 AGTGATCCTCCCGTGCTCTGCGCGGGCGGAGGCTTGGTTTAGGG(TTTAGGG)$_2$*TTAGGG*TTTAGGG

Kdel1B-67 ACGGGCTGTACGAGGACACGGGAAAAAAGTGCCCGACGGTTTAGGGTTTAGGG*TTAGGG*(TTTAGGG)$_3$

1BS-8 GCAGCGCAGTGCCCCGAGGGACCACCTTTCGGCGCGCGGG(TTTAGGG)$_4$*TTAGGG*(TTTAGGG)$_2$

Kdel1B-65 CTGTTTGAGGCTATATACTCGTTGAATACATCAGTGTAGGG(TTTAGGG)$_2$(*TTAGGG*)$_3$*TTTGGGG*

RNA should be present. The appearance of this variant in all our material may indicate that one of the genes is altered. On the other hand, the other variants that appeared once in the sequences may have been produced accidentally during reverse transcription by telomerase.

The telomerase of Tetrahymena synthesizes the telomere repeats in a processive manner in vivo (Greider 1991). Once synthesis has started, an enzyme molecule will continue to extend the chain rather than dissociate from it, giving rise to an array of uniform repeats. However, in vivo, the telomere sequences may be synthesized in a non-processive manner, because a Tetrahymena cell that expresses both wild-type and mutant template RNAs produces arrays in which both wild-type and mutant units are mixed at random. Likewise, in the present cases, the variant TTAGGG was distributed at random positions throughout the arrays, although it tended to repeat two or three times. This finding indicates that several telomerase molecules took part in the process of healing of broken chromosome ends in wheat.

The structure of healed broken ends

Tsujimoto et al. (1997) previously reported an inverted repeat sequence at the broken ends of Kdel1B-65, and suggested that at least one cycle of breakage-fusion-bridge (BFB) had taken place after the initial chromosome breakage by the *Gc* gene. The three breakpoints discussed here did not show such a structural rearrangement. The telomere sequences simply initiated from the 2–4 nucleotides of rDNA. This, however, does not mean that the BFB cycle did not occur after the initial chromosome breakage. The PCR method used here to obtain the breakpoints cannot detect all of the chromosome rearrangements at this point. If replication and fusion follow the breakage of a chromatid, a dicentric chromosome that is symmetrical about the breakpoint would be the result. In the following anaphase the dicentric chromosome is mechanically broken. Only one of the daughter cells will obtain the chromosome carrying the initial breakpoint with an inverted repeat. The other chromosome will carry only a simple breakpoint. In addition, if the break in anaphase happens to occur very far from the initial breakpoint, it would be impossible to amplify the fragment including the initial breakpoint by PCR. In this case, however, the rDNA would be inverted, and, consequently, the reverse primer for rDNA could amplify the breakpoint in combination with the telomere primer. Of the four breakpoints determined, one (1BS-8) was amplified by the reverse primer, suggesting the presence of a large inverted repeat caused by the BFB cycle.

The breakpoints of deletion lines

In this study we used 15 chromosome deletion lines broken at or near the NOR. In four lines, we were successful in amplifying the breakpoints. Three (1BS-8, Kdel1B-65, Kdel1B-66) of the lines showed only a 3.1-kb band upon Southern hybridization using pWrdnTaq2.8 (Fig. 1 and Table 1). This result confirmed that the breakpoints in chromosome 1B in these lines are obviously located within the NOR. One line, Kdel1B-67, showed both 3.1- and 3.9-kb *Taq*I fragments. In this line the breakpoint was located at a position more distal than those in the above three lines. Based on the result of the Southern analysis, the breakpoint in at least two other lines (1BS-5 and 1BS-6) should have been amplifiable. However, we did not find any specific amplification in these lines. At the breakpoint of Kdel1B-65, an insertion of an unidentified 8-bp sequence was found (Tsujimoto et al. 1997). If an insertion is very large, the distance between rDNA and telomere primers may exceed the size that can be amplified by PCR.

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References

Anderson JA, Ogihara Y, Sorrels ME, Tanksley SD (1992) Development of a chromosomal arm map for wheat based on RFLP markers. Theor Appl Genet 83:1035–1043

Barker RF, Harberd NP, Jarvis MG, Flavell RB (1988) Structure and evolution of the intergenic region in a ribosomal DNA repeat unit of wheat. J Mol Biol 201:1–17

Barnett MA, Buckle VJ, Evans EP, Porter ACG, Rout D, Smith AG, Brown WRA (1993) Telomere-directed fragmentation of mammalian chromosomes. Nucleic Acids Res 21:27–36

Blackburn EH (1992) Telomerases. Annu Rev Biochem 61:113–129

Endo TR (1988) Induction of chromosomal structural changes by a chromosome of *Aegilops cylindrica* L. in common wheat. J Hered 79:366–370

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

Gale MD, Atkinson MD, Chinoy CN, Harcourt RL, Jia J, Li QY, Devos KM (1995) Genetic maps of hexaploid wheat. In: Li ZS, Xin ZY (eds) Proceedings of Eighth International Wheat Genetics Symposium, Beijing, pp 29–40

Gerlach WL, Bedbrook JR (1979) Cloning and characterisation of ribosomal RNA gene from wheat and barley. Nucleic Acids Res 7:1869–1886

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

Greider CW (1990) Telomeres, telomerase and senescence. Bioessays 12:363–369

Greider CW (1991) Telomerase is processive. Mol Cell Biol 11:4572–4580

Greider CW, Blackburn (1987) The telomere terminal transferase of *Tetrahymena* is a ribonucleoprotein enzyme with two kinds of primer specificity. Cell 51:887–898

Greider CW, Blackburn (1989) A telomeric sequence in the RNA of *Tetrahymena* telomerase required for telomere repeat synthesis. Nature 337:331–337

Harley CB, Futcher AB, Greider CW (1990) Telomeres shorten during aging of human fibroblasts. Nature 345:275–282

Harrington LA, Greider CW (1991) Telomerase primer specificity and chromosome healing. Nature 353:451–454

Kipling D (1995) The telomere. Oxford University Press

Kiss T, Kiss M, Solymosy F (1989a) Nucleotide sequence of a 25S rRNA gene from tomato. Nucleic Acids Res 17:796

Kiss T, Szkukalek A, Solymosy F (1989b) Nucleotide sequence of a 17S (18S) rRNA gene from tomato. Nucleic Acids Res 17:2127

Kleinhofs A, Kilian A, Saghai Maroof MA, Biyashev RM, Hayes P, Chen FQ, Lapitan N, Fenwick A, Blake TK, Kanazin V, Ananiev E, Dahleen L, Kudrna D, Bollinger JD, Hoffman D, Skadsen R, Steffenson BJ (1993) A molecular, isozyme and morphological map of the barley (*Hordeum vulgare*) genome. Theor Appl Genet 86:705–712

Levy MZ, Allsopp RC, Futcher AB, Greider CW, Harley CB (1992) The telomere end-replication problem and cell aging. J Mol Biol 225:951–960

McClintock B (1941) The stability of broken ends of chromosomes in *Zea mays*. Genetics 26:234–282

Morin (1991) Recognition of a chromosome truncation site associated with α-thalassaemia by human telomerase. Nature 353:454–456

Murray AW, Claus TE, Szostak JW (1988) Characterization of two telomeric DNA processing reactions in *Saccharomyces cerevisiae*. Mol Cell Biol 8:4642–4650

Nakayama J, Saito M, Nakamura H, Matsuura, A, Ishikawa F (1997) *TLP*: a gene encoding a protein component of mammalian telomerase is novel member of the WD repeat family. Cell 88:875–884

Ogihara Y, Hasegawa K, Tsujimoto H (1994) High-resolution cytological mapping of the long arm of chromosome 5A in common wheat using a series of deletion lines induced by gametocidal (*Gc*) genes of *Aegilops speltoides*. Mol Gen Genet 244:253–259

Richards EJ, Ausubel FM (1988) Isolation of a higher eukaryotic telomere from *Arabidopsis thaliana*. Cell 53:127–136

Sears ER (1965) Nullisomic-tetrasomic combinations in hexaploid wheat. In: Riley R, Lewis KR (eds) Chromosome manipulations and plant genetics. Oliver and Boyd, Edinburgh and London, pp 29–45

Shippen DE, McKnight TD (1998) Telomeres, telomerase and plant development. Trends Plant Sci 3:126–130

Sugiura M, Iida Y, Oono K, Takaiwa F (1985) The complete nucleotide sequence of a rice 25S rRNA gene. Gene 37:255–259

Tsujimoto H (1993) Molecular cytological evidence for gradual telomere synthesis at the broken chromosome ends in wheat. J Plant Res 106:239–244

Tsujimoto H, Yamada T, Sasakuma T (1997) Molecular structure of a wheat chromosome end healed after gametocidal gene-induced breakage. Proc Natl Acad Sci USA 94:3140–3144

Tsujimoto H, Hasegawa K, Usami N, Yamada T, Kojima T, Endo TR, Ogihara Y, Sasakuma T (1998) Use of chromosome 1B deletion lines in wheat for high-resolution mapping and genetic analysis on the break points. In: Slinkard AE (ed) Proceedings of Ninth International Wheat Genetics Symposium. University Extension Press, University of Saskatchewan, Saskatoon, pp 123–126

Werner JE, Kota RS, Gill BS, Endo TR (1992) Distribution of telomeric repeats and their role in the healing of broken chromosome ends in wheat. Genome 35:844–848