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Identification of Healed Terminal DNA Fragments in Linear Minichromosomes of *Schizosaccharomyces pombe*

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The minichromosome Ch16 of the fission yeast *Schizosaccharomyces pombe* is derived from the centromeric region of chromosome III. We show that Ch16 and a shorter derivative, Ch12, made by gamma-ray cleavage, are linear molecules of 530 and 280 kilobases, respectively. Each minichromosome has two novel telomeres, as shown by genomic Southern hybridization with an *S. pombe* telomere probe. Comparison by hybridization of the minichromosomes and their chromosomal counterparts showed no sign of gross rearrangement. Cosmid clones covering the ends of the long arms of Ch16 and Ch12 were isolated, and subcloned fragments that contained the breakage sites were identified. They are apparently unique in the genome. By hybridization and *Bal* 31 digestion, the ends appear to consist of the broken-end sequences directly associated with short stretches (about 300 base pairs) of new DNA that hybridizes to a cloned *S. pombe* telomere. They do not contain the telomere-adjacent repeated sequences that are present in the normal chromosomes. The sizes of the short telomeric stretches are roughly the same as those of the normal chromosomes. Our results show that broken chromosomal ends in *S. pombe* can be healed by the de novo addition of the short telomeric repeats. The formation of Ch16 must have required two breakage-healing events, whereas a single cleavage-healing event in the long arm of Ch16 yielded Ch12.

Broken ends of chromosomes formed by a variety of physical and chemical means behave differently from normal telomeres. They are mitotically unstable and can become "healed," that is, mitotically stable (18, 19; reviewed in reference 2). At least three different kinds of healing events have been proposed (Fig. 1). In the budding yeast *Saccharomyces cerevisiae*, the majority of healing events involve recombination between a broken chromosome end and its intact homologous chromosome. In other organisms such as maize, chromosomal rearrangements allow a broken chromosome to acquire a new telomere by a translocation involving another, nonhomologous chromosome. Alternatively, the de novo formation of a telomere may take place at a broken end. An enzyme responsible for the de novo formation of telomeres has been identified and characterized in *Tetrahymena* species (10). Although these healing events have been supported by genetic evidence, little is known about the healed terminal structures of *S. cerevisiae* and maize at the nucleotide sequence level.

Gamma irradiation of a highly unstable aneuploid disome for chromosome III of the fission yeast *Schizosaccharomyces pombe* produced a stable partial aneuploid which contained a minichromosome (designated Ch16) in addition to the three normal chromosomes (24). Ch16 is stably inherited mitotically but is impaired in meiotic bivalent formation. During pulsed field gradient (PFG) gel electrophoresis (26) and orthogonal field alternation gel electrophoresis (4), which allow separation of large DNAs, Ch16 DNA migrates as a single electrophoretic band corresponding to a size of approximately 500 kilobases (kb). Genetic analysis showed that three pericentric genetic markers, known to encompass the centromere of chromosome III, are present on Ch16. The minichromosome band in PFG gel electrophoresis also hybridized to a 4-kb sequence common to the centromere regions of all three normal chromosomes (22). Ch16, therefore, retains the centromere region of chromosome III. In this paper, we examine the origin of the telomeres of the minichromosomes. A priori, the termini might consist of the telomeres of the same or other chromosomes or telomeric ends formed de novo. Alternatively, the minichromosome might have been circular, although previous circumstantial evidence (24) suggested that this was unlikely. We report the identification of telomeric sequences on the two minichromosomes, Ch16 and Ch12. We also present evidence that both minichromosomes are linear, ending in termini formed de novo.

MATERIALS AND METHODS

Strains and media. The following *S. pombe* strains were used: haploid wild type, 972 (h^-); HM348 (h^+ *leu1 fur1-1 ade6-210 tps16-112*) containing the minichromosome Ch16, which carries the alleles *fur1*$^+$, *ade6-216*, and *tps16*$^+$; and HM361 (h^+ *leu1 ade6-210 fur1-1*) containing the minichromosome Ch12 (*fur1*$^+$). The alleles *ade6-216* and *ade6-210* complement intragenically, providing a selection for retention of the minichromosomes. h^- and h^+ are mating-type alleles. A tester strain, HM343 (h^+ *leu1 ade6-210 fur1-1 tps16-112*), was used for mapping. *S. cerevisiae* AH22 chromosomes were size markers for PFG gel electrophoresis. Complete YPD and minimal SD media were used (24). Auxotrophic markers were determined on SD supplemented with appropriate nutrients as described previously (24).

Minichromosomes. Strain HM248 contains the minichromosome Ch16 and has been described previously (24). Strain HM348 was obtained by crossing HM248 with marker strains. Ch12 is a shorter derivative of Ch16. Its generation will be described in detail elsewhere. Briefly, HM348 was

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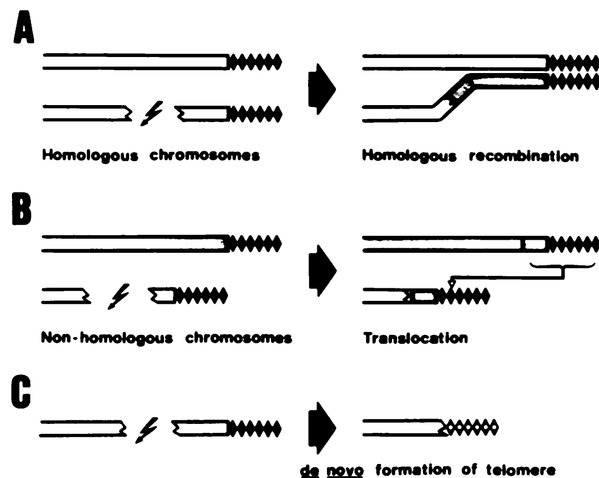

FIG. 1. Three possible healing events for the broken end of a chromosome.

irradiated with gamma rays, followed by screening for Ade$^-$ colonies (the deletion of the *ade6* locus in Ch16 causes red colonies). Chromosomal DNA from a series of such strains was examined by PFG gel electrophoresis, and the size of the minichromosome was estimated. About 20 strains containing a minichromosome of reduced size were isolated. These minichromosomes had lost both *ade6* and *tps16*. Ch12 is the shortest minichromosome obtained so far.

Plasmids and transformation. pSPT16 contains the telomeric sequence of *S. pombe* (N. Sugawara and J. W. Szostak, manuscript in preparation). pNS1 contains the gene for *ade6*$^+$ (24). pUC18 (31) and YIp5 (3) were used for subcloning the terminal DNA sequences. The integration vector YIp32 consists of the *S. cerevisiae LEU2* gene inserted into pBR322 (3) and was used for the integration of cloned sequences into *S. pombe* chromosomes by homologous recombination (24, 30). The transformation procedure of Ito et al. (14) was followed.

Preparation of genomic DNA of *S. pombe*. High-molecular-weight genomic DNA of *S. pombe* was isolated as follows. Cells from a 1-liter log-phase YPD culture were harvested and washed once with 30 ml of 20 mM citrate-phosphate buffer (pH 5.6) and then suspended in 30 ml of the same buffer containing 50 mM EDTA, 1 M sorbitol, 0.2 mg of Zymolyase-100T (Kirin Brewery, Tokyo, Japan) per ml, and 5 mg of Novo Enzyme SP234 per ml (1). The cell walls were digested at 30°C for 2 h, and the resulting spheroplasts were lysed by suspending them in 30 ml of 50 mM EDTA–50 mM Tris chloride (pH 7.5)–1% (vol/vol) Triton X-100. The lysed cells were centrifuged at 300 × *g* for 10 min, and the supernatant was centrifuged at 8,000 × *g* at 0°C for 20 min. The pellets, containing the nuclei, were suspended in 10 ml of 300 mM NaCl–50 mM EDTA to 50 mM Tris chloride (pH 7.5), followed by the addition of sodium dodecyl sulfate (final concentration, 1% [wt/vol]) and proteinase K (final concentrations, 100 μg/ml). The suspension was incubated at 50°C for 5 h, extracted twice with phenol, and precipitated with 2 volumes of ethanol. The pellet was rinsed with 70% (vol/vol) ethanol and suspended in 10 mM Tris chloride (pH 8.0)–1 mM EDTA. The genomic DNA thus obtained was treated with RNase A (20 μg/ml) and extracted with phenol. DNA was precipitated with ethanol and dissolved in 10 mM Tris chloride (pH 8.0)–1 mM EDTA. The material obtained was 200 to 300 kb long as determined by PFG gel electrophoresis. The yield of DNA from a 1-liter culture was 200 to 400 μg.

Isolation of cosmid clones. The *S. cerevisiae LEU2* gene was inserted into cosmid vector pHC79 (13). The resulting cosmid, pSS10 (22), was used for construction of a genomic library of *S. pombe* DNA. Purification of *S. pombe* genomic DNA, partial restriction with *Sau*IIIA1, and fractionation of digested genomic DNA by a linear sucrose gradient centrifugation were done as previously described (22). Purified DNA fragments were cloned into the *Bam*HI site of pSS10.

***Bal* 31 digestion.** *S. pombe* genomic DNA (final concentration, about 50 μg/ml) was mixed with *Bal* 31 reaction buffer containing 12 mM $CaCl_2$, 12 mM $MgCl_2$, 20 mM Tris chloride (pH 8.0), 200 mM NaCl, and 1 mM EDTA. *Bal* 31 enzyme (Takara Shuzo Co.) was added to 0.4 U/μg of DNA. The reaction mixture was incubated at 30°C for 0 to 90 min, samples were taken at intervals, and the reaction was terminated by adding phenol, followed by extraction and ethanol precipitation of the DNA.

Colony hybridization and Southern blot hybridization. Standard procedures described by Maniatis et al. (17) were followed. Hybridization was done in 5 × SSPE–0.3% sodium dodecyl sulfate containing 100 μg of salmon sperm DNA per ml at 65°C.

PFG gel electrophoresis. The procedures described by Schwartz and Cantor (26) and Carle and Olson (4) were followed. Preparation of *S. pombe* chromosomal DNAs for PFG electrophoresis was as described previously (24). To calibrate the size of minichromosome DNA, the *S. cerevisiae* chromosome DNAs (5, 26) and concatemeric λDNA (28) were used.

RESULTS

Sizes of minichromosomes. The minichromosome Ch16 contains the pericentric region of chromosome III (24). It carries three known markers, namely, *fur1*, *ade6*, and *tps16* (11, 15), that encompass the centromere (Fig. 2a). To understand the relationship between the structure and behavior of chromosomes in *S. pombe*, we undertook to make shorter derivatives of Ch16 as described in Materials and Methods. One strain, HM361, contained a minichromosome (designated Ch12) greatly reduced in length (see below). Genetic analysis of HM361 by crossing with marker strains showed that Ch12 lacked the *ade6* and *tps16* alleles but contained *fur1* (data not shown), indicating that the breakage yielding Ch12 occurred centromere proximal to *ade6* (Fig. 2a). Ch12 was stably inherited in the mitotic cell cycle but not during meiosis. The detailed behavior of Ch12 and other derivatives in mitosis and meiosis will be described elsewhere.

By PFG gel electrophoresis of chromosomal DNA from HM348 and HM361 (Fig. 2b), Ch16 (lanes 2 and 4) and Ch12 (lanes 3 and 5) were estimated to be 530 and 280 kb long, respectively, by using the chromosomes of *S. cerevisiae* (lanes 1 and 6) as size markers. The same values were obtained by calibrating their sizes with ladders of concatemeric λ DNAs (data not shown). Among more than 20 different minichromosomes examined, Ch12 is the shortest obtained so far. The normal chromosomes of *S. pombe* are much longer (3,000 to 9,000 kb) (29) and formed a thick, smeared band migrating only slightly from the wells under the experimental conditions employed (Fig. 2b).

Hybridization of minichromosome bands with marker sequences. To determine whether any of the marker sequences were present in Ch16 and Ch12, minichromosomal DNAs of HM348 and HM361 were run in PFG gel electrophoresis,

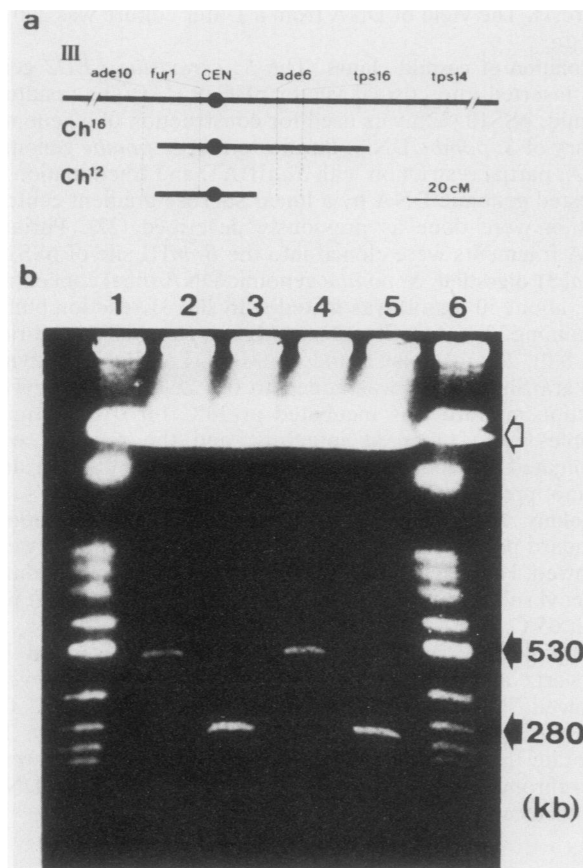

FIG. 2. (a) Linkage map of the centromeric region of chromosome III. Minichromosomes Ch16 and Ch12 are also shown. Symbol: ●, Approximate location of the centromere (CEN). (b) Ethidium bromide-stained PFG gel electrophoretic pattern of chromosomal DNAs from HM348, containing Ch16 (lanes 2 and 4), and HM361, containing Ch12 (lanes 3 and 5). Symbols: ⧫, minichromosome bands, with the estimated size in kb; ◊, normal chromosomes. *S. cerevisiae* chromosomal DNAs are shown in lanes 1 and 6.

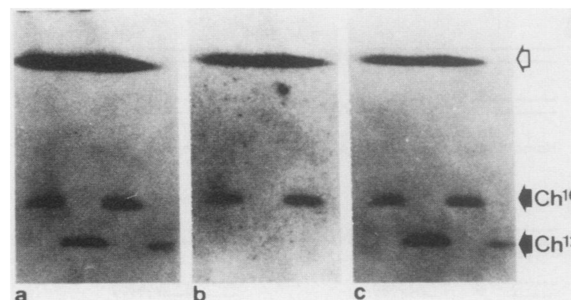

FIG. 3. Blot hybridization patterns of the *S. pombe* minichromosome bands probed with ^{32}P-labeled sequences. (a) Probed with pSPT16, a plasmid that contains an *S. pombe* telomere; (b) probed with the cloned *ade6*$^+$ gene; and (c) probed with the cloned *fur1*$^+$ gene. The positions of the minichromosome bands are indicated by the arrows. The upper hybridizing band represents the normal chromosomes.

transferred to nylon filters, and hybridized with various ^{32}P-labeled DNA probes. In Fig. 3a, plasmid pSPT16, containing an *S. pombe* telomere sequence consisting of a short repeated sequence (Sugawara and Szostak, in preparation), was used as the probe. Both the Ch16 and Ch12 bands hybridized strongly with pSPT16, indicating that they contain sequences homologous to the telomeric ends of normal chromosomes. The centromeric sequences dg (22) and dh (23) were also used as probes, and both Ch16 and Ch12 hybridized strongly (data not shown), suggesting that the centromeric region is retained in the minichromosomes. In Fig. 3b, the cloned *ade6*$^+$ gene was used as the probe; it hybridized with Ch16 but not Ch12. Two other sequences located between *ade6* and *tps16* also hybridized with Ch16 but not with Ch12 (data not shown). In Fig. 3c, the cloned *fur1*$^+$ gene was used as the probe; it hybridized with both Ch16 and Ch12. These results are consistent with the genetic results (Fig. 2a); the cleavage that produced Ch12 must have occurred in Ch16 centromere proximal to *ade6*. Ch12 seems to have a deletion of about 250 kb of the right arm of Ch16.

Hybridization of minichromosome ends with pSPT16. Genomic DNAs were isolated from the wild-type, HM348, and HM361 strains, digested with *Eco*RI, run in 0.7% agarose gel, transferred to nitrocellulose paper, and probed with ^{32}P-labeled pSPT16 (Fig. 4). A single diffuse band was observed in the wild type (lane 1) at approximately 0.8 kb (Sugawara and Szostak, in preparation), whereas two additional bands were seen in HM348 (lane 2) and HM361 (lane 3). They were at 4.4 and 2.3 kb for HM348 (lane 2) and at 4.8 and 2.3 kb for HM361 (lane 3). These additional bands were thought to represent the terminal DNA fragments of the minichromosome.

Isolation of cosmid clones covering a terminal fragment of Ch12. We had previously made a small library of minichromosome sequences by extracting Ch16 DNA from an agarose gel band after PFG gel electrophoresis, digesting the DNA with *Hin*dIII, and cloning the DNA in the chromosome integration vector YIp32 (24). Sixty independent clones containing different fragments of Ch16 were obtained, and 11 of them were integrated into chromosome III by homologous

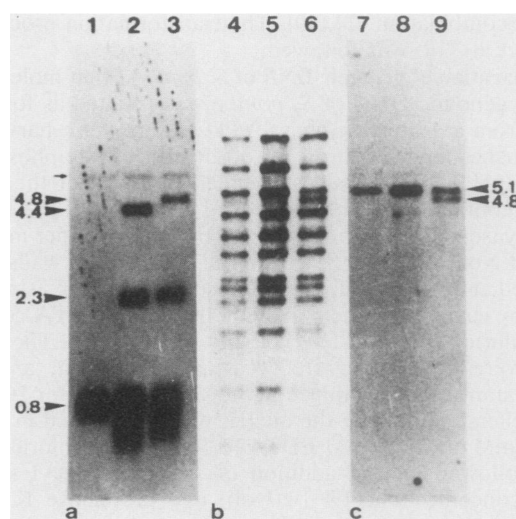

FIG. 4. Genomic Southern hybridization of wild-type (lanes 1, 4, and 7), HM348 (lanes 2, 5, and 8), and HM361 (lanes 3, 6, and 9) DNAs digested with *Eco*RI and probed with (a) pSPT16, (b) cosmid clone cosm3, or (c) pEE105 (a subclone of cosm3 that contains a 5.1-kb *Eco*RI fragment). The numbers indicate fragment length in kb. A faint band in lanes 1, 2, and 3 (arrow) seems to be due to weak hybridization of pSPT16 with nontelomeric DNA. These weak bands were apparently nontelomeric DNA and were not reproducibly seen in different experiments and hybridization conditions.

TABLE 1. Chromosome mapping of integrated pSAm3 and pSAm187[a]

Marker pair	PD	NPD	TT	Genetic distance (cM)
furl-pSAm3	119	0	12	4.6
ade6-pSAm3	120	0	12	4.5
tps16-pSAm3	109	0	23	8.7
tps14-pSAm187	53	0	14	10.4
ade6-pSAm187	34	0	33	24.6
tps16-pSAm187	15	0	9	18.8
pSAm3-pSAm187	40	0	32	22.2

[a] Plasmids pSAm3 and pSAm187 were employed to transform a host strain, HM123 (h^- *leul*). The integration sites of the plasmids were determined by crossing the transformants with tester strains. The tetrad data obtained are shown. Abbreviations: PD, parental ditype; NPD, nonparental ditype; TT, tetratype.

recombination. Their map locations were determined by tetrad analyses. One clone (pSAm3) integrated at a position approximately 4 cM centromere proximal to *ade6*, so that of the 11 it was the closest to the right-arm terminus of Ch12. Since only 20 tetrads had been dissected previously, we examined more than 100 additional tetrads to obtain a more precise map location for the integrated pSAm3. It was located 4.5 cM from *ade6* and 4.6 cM from *furl* (Table 1).

We used pSAm3 as a probe in screening a cosmid library of *S. pombe* wild-type genomic DNA (not containing minichromosome DNA) and obtained five hybridizing clones (Fig. 5a). Cosmid clone cosm3 was ^{32}P labeled and used as a probe for Southern blot hybridization to *Eco*RI-digested genomic DNAs (Fig. 4, lanes 4 through 6). If the cosmid insert had not been rearranged during cloning and contained no repeated sequences, the hybridization patterns should be identical to the ethidium bromide-stained restriction patterns of the cosmid DNA except for bands containing vector sequences. If the cosmid insert covered a terminus of a minichromosome, however, one additional band representing the terminal fragment might appear in strains containing the minichromosome. As expected, the hybridizing bands in *Eco*RI-digested wild-type (Fig. 4, lane 4) and HM348 (lane 5) DNAs were identical to each other and to the ethidium-stained bands of cosm3. One additional hybridizing band, however, was observed at 4.8 kb with HM361 DNA (lane 6). This band is identical in length to one of the two presumed terminal fragments of Ch12 (lane 3). Therefore, this additional 4.8-kb *Eco*RI band most likely represents the *ade6*-proximal terminus of Ch12.

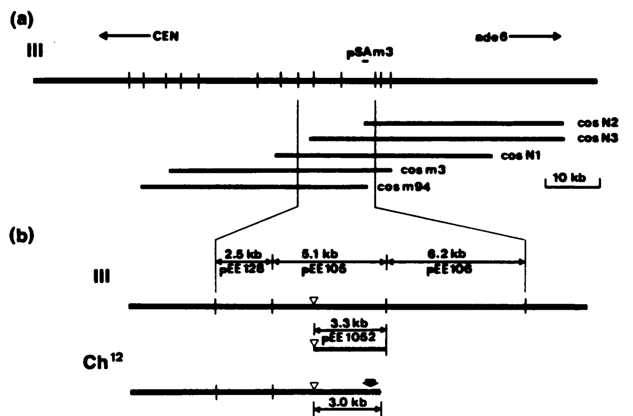

FIG. 5. Restriction map in the vicinity of the pSAm3 sequence. (a) *Eco*RI sites are shown with the five cosmid clones obtained by hybridization with pSAm3. The directions of the centromere (CEN) and the *ade6* gene are indicated. (b) The subcloned fragments in the vicinity of the breakage site. Symbols: ◆, breakage site; ▽, *Hin*dIII sites; ◆◆◆, telomere sequence in Ch12.

Subcloning the terminal fragment. *Eco*RI restriction sites in cosm3 were mapped (Fig. 5b), and three *Eco*RI fragments were subcloned into YIp5. In genomic Southern blot hybridization (Fig. 4c), the plasmid (pEE105) that contained the 5.1-kb *Eco*RI fragment was found to produce two hybridizing bands (5.1 and 4.8 kb in length) in HM361, whereas the wild type and HM348 produced only one self-hybridizing band; the sequence in the 5.1-kb fragment was unique in the genome. The additional band was again 4.8 kb long, identical in length to one of the two telomere-hybridizing fragments of Ch12 (lane 3). The results strongly suggested that the 5.1-kb fragment in pEE105 covers the *ade6*-proximal terminus of Ch12.

Hybridization of the minichromosome bands in PFG gel electrophoresis with probe sequences adjacent to pEE105 supported the above conclusion. pEE128, which contains the 2.5-kb *Eco*RI fragment adjacent to the pEE105 fragment, hybridized with both the Ch16 and Ch12 bands (Fig. 6a). In contrast, pEE106, which contains the 6.2-kb *Eco*RI fragment that is also adjacent to the pEE105 insert but in the opposite direction, hybridized with Ch16 but not with Ch12 (Fig. 6b). The pSAm3 sequence also did not hybridize with Ch12 but did with Ch16. Therefore, we concluded that the pEE128 insert is centromere proximal to the right-arm end of Ch12, whereas the pEE106 insert is distal to the terminus and not present in Ch12. The *ade6*-proximal chromosomal DNA end of Ch12 must be located within the 4.8-kb terminal fragment. The approximate breakage site in the fragment could be assigned if the size of the associated telomeric sequence were known.

Size of the telomeric sequence in Ch12. When genomic DNA is digested with an exonuclease and then with a restriction endonuclease, telomeric sequences are shortened, whereas internal chromosomal sequences remain the same length. We identified the terminal chromosomal sequence and measured the length of the telomeric sequence by *Bal* 31 exonuclease digestion followed by Southern blot hybridization. Genomic DNA of HM361 containing Ch12 DNA was digested with exonuclease *Bal* 31 (0.4 U/μg) at 30°C for 0 to 90 min followed by digestion with *Eco*RI and *Hin*dIII. Figure 7a shows the Southern blot hybridization pattern after probing with pEE1052, a subclone of pEE105 containing the 3.3-kb *Eco*RI-*Hin*dIII fragment (Fig. 5b). Two hybridizing bands (one chromosomal at 3.3 kb and the other minichromosomal end at 3.0 kb) were initially seen, and only

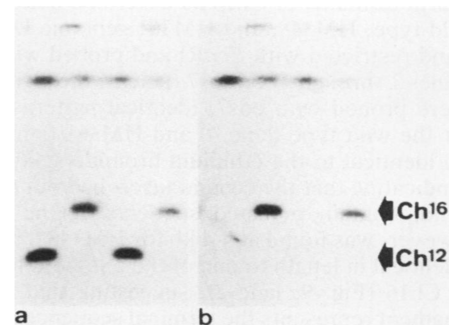

FIG. 6. Blot hybridization of the Ch16 and Ch12 bands in PFG gel electrophoresis probed with pEE128 (a) and pEE106 (b). (See the text.)

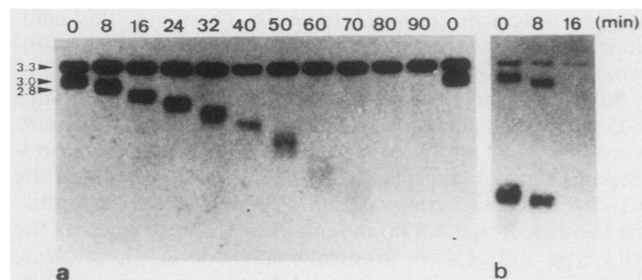

FIG. 7. Southern blot hybridization of genomic DNA containing a minichromosome digested with *Bal* 31 and probed with pEE1052 (a) and pSPT16 (b). Isolated genomic DNA of HM361 (containing Ch12) was digested with *Bal* 31 exonuclease (0.4 U/μg) at 30°C for 0 to 90 min, followed by double digestion with *Eco*RI and *Hin*dIII. Digested DNAs were electrophoresed in an agarose gel, transferred to a nylon membrane, and probed with pEE1052 (a) or pSPT16 (b). The numbers indicate the lengths of fragments in kb.

the band initially at 3.0 kb decreased in size during incubation with *Bal* 31. Therefore, the 3.0-kb fragment must be the terminal sequence.

When the same blot membrane was probed with pSPT16 (Fig. 7b), the band initially at 3.0 kb was shortened to 2.8 kb at 8 min. At 16 min, however, no band was seen. This suggested that the telomeric sequence was longer than 200 base pairs (bp) but probably shorter than 400 bp. Thus, a roughly 300-bp-long telomeric sequence seemed to be associated with the broken-end chromosomal sequence that is present in the 3.3-kb *Eco*RI fragment. The breakage site could be assigned to a position approximately 2.7 kb from the *Hin*dIII site (Fig. 5b). The telomeric sequences of the normal chromosomes, seen at 0.8 kb, appeared to be similarly digested with *Bal* 31 exonuclease and approximately 300 bp long.

Identification of a terminal DNA fragment in Ch16. The chromosomal DNA that covers a long-arm terminus of Ch16 was identified by procedures similar to those described above. Genetic analyses indicated that the end was distal to *tps16* but proximal to *tps14* (Fig. 2a) (24). The plasmid pSAm187, carrying the 0.8-kb *Hin*dIII fragment of Ch16, was integrated between *tps16* and *tps14* on chromosome III by homologous recombination (24); the integration site was mapped by tetrad analysis to a position 10.4 cM proximal to *tps14* (Table 1). The distance between *tps16* and *tps14* is 40 cM (15). Four cosmid clones that hybridized with pSAm187 were obtained, and their restriction maps were determined (Fig. 8). The sequence pSAm187 was found to be present within a 4.2-kb *Eco*RI fragment which turned out to cover the right-arm terminus of Ch16 as described below.

The wild-type, HM348, and HM361 genomic DNAs were isolated and restricted with *Eco*RI and probed with pSPT16 (Fig. 9, lanes 1 through 3) or cos7 (lanes 4 through 6). When DNAs were probed with cos7, identical patterns were obtained for the wild type (lane 4) and HM361 (lane 6). They were also identical to the ethidium bromide-stained pattern of cos7, indicating that the cosmid insert had not rearranged and did not contain repeated sequences. One additional band, however, was found at 4.4 kb for HM348 (lane 5). This band is identical in length to one of the telomere-hybridizing bands of Ch16 (Fig. 9, lane 2), suggesting that the 4.4-kb *Eco*RI fragment represents the terminal sequence of the right arm of Ch16. By subcloning and subsequent hybridization, we identified a subclone, the 2.2-kb *Hin*dIII-*Eco*RI fragment shown in Fig. 8b, that covered the terminus; a genomic

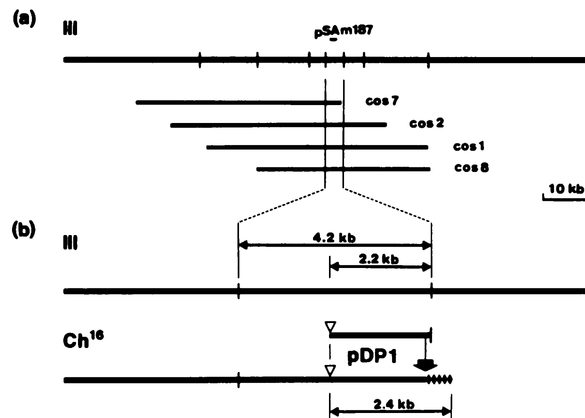

FIG. 8. Restriction map near the long-arm terminus of Ch16. (a) *Eco*RI restriction sites are shown in four cosmid clones (cos7, cos2, cos1, and cos8) isolated by hybridization with a fragment of pSAm187, which is close to the terminus of Ch16. (b) Restriction sites and subcloned plasmids containing the 4.2-kb *Eco*RI fragment covering the broken-end sequence of Ch16 (◆).

Southern blot probed with the 2.2-kb subclone showed one band at 2.2 kb for the wild type (Fig. 9, lane 7) and HM361 (lane 9) but two bands at 2.2 and 2.4 kb for HM348 (lane 8). Therefore, the 2.4-kb band should represent the terminal fragment. The reason that the terminal fragment of Ch16 is larger than the chromosomal counterpart is probably due to the associated telomeric sequence. If it were 300 bp long, the breakage site would be 100 bp proximal to the *Eco*RI site of the 2.2-kb fragment (Fig. 8).

Absence of the telomere-adjacent sequences in Ch16 and Ch12. The *S. pombe* telomere-adjacent region contains several different classes of repeating sequences common to all telomeres (Sugawara and Szostak, in preparation). pNSU31 contains such sequences derived from one chromosomal telomere but not the extreme telomeric sequence.

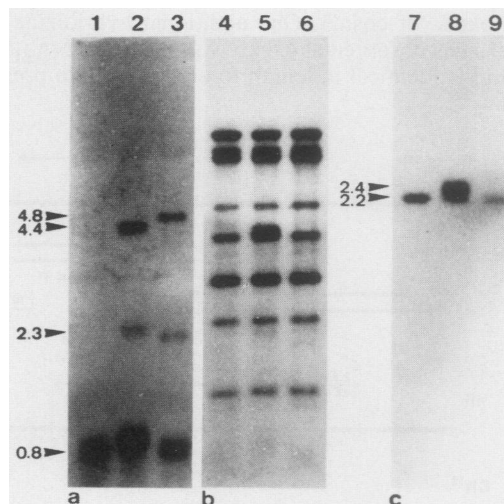

FIG. 9. Genomic Southern hybridization of the wild type, HM348, and HM361 probed with (a) pSPT16, (b) cos7, and (c) the 2.2-kb *Hin*dIII-*Eco*RI fragment in pDP1. Genomic DNAs of the wild type (lanes 1, 4, and 7), HM348 (lanes 2, 5 and 8) and HM361 (lanes 3, 6 and 9) were isolated and digested with *Eco*RI (lanes 1 through 6) or *Eco*RI-*Hin*dIII (lanes 7 through 9). The numbers indicate the sizes of the fragments in kb.

This plasmid was used as the probe for hybridization of PFG gel electrophoresis patterns of Ch16 and Ch12. No hybridization to Ch16 and Ch12 was observed (data not shown), indicating that these minichromosomes do not contain telomere-adjacent sequences.

DISCUSSION

We have investigated the structures of two different minichromosomes of *S. pombe*, namely, Ch16 and Ch12. Ch16 was obtained previously by gamma irradiation (24). It contains three centromere-linked markers of chromosome III and the centromere-specific repeating sequences (22), suggesting that at least the greater part of Ch16 consists of the pericentric region of chromosome III. We isolated a new minichromosome (Ch12) from Ch16 by gamma ray cleavage. As determined by PFG gel electrophoresis, Ch16 DNA was 530 kb long, approximately one-sixth of the size of normal chromosome III (27), whereas Ch12 was 280 kb long, roughly half the length of Ch16. Genetic analysis showed that Ch12 lacks two centromere-linked markers, *ade6* and *tps16*, in the long arm of chromosome III. These minichromosomes are mitotically stable and behave independently of the normal chromosomes, including chromosome III, in the mitotic and meiotic cell cycles. Their meiotic behavior was impaired (24; unpublished results). This might be due to their small size, their altered telomere structures, or the deletion of sequences responsible for meiotic pairing.

The terminal DNA sequences of Ch16 and Ch12 have been identified by cosmid cloning and hybridization. Our results showed that Ch12 and Ch16 are linear but that their ends differ considerably from those of the normal chromosomes. Evidence is presented that the minichromosomes were formed by chromosomal breakage, followed by healing of the broken ends by the de novo addition of the short telomeric sequences. Ch12 and Ch16 hybridized with the cloned telomeric sequence but not with the telomere-adjacent sequence. Only the extreme ends of the minichromosomes share homology with the telomere of the regular chromosomes. The telomeres of normal chromosomes contain a long region of complex repetitive sequences adjacent to the actual short telomere repeats (Sugawara and Szostak, in preparation). At the termini of the minichromosomes, roughly 300-bp telomeric sequences are present adjacent to the unique sequences of chromosome III. The size of the telomeric sequence in normal chromosomes is also about 300 bp. Cosmid clones derived from the pericentric chromosomal regions genetically mapped to the region of the termini of the minichromosomes were shown to overlap the breakpoints corresponding to the minichromosome telomeres. Comparison by hybridization between minichromosome sequences and their counterparts in chromosome III showed no sign of gross rearrangement. Two minichromosome ends analyzed have the 300-bp telomeric sequences directly associated with the unique pericentric sequences. Thus it is very unlikely that the new telomere was brought to the end of the minichromosomes by recombination or translocation or deletion. Instead, the de novo formation of a telomere is more likely; that is, the addition of the 300-bp telomeric sequences appears to have taken place directly on the broken ends of the minichromosomes. The formation of Ch16 must have required two such breakage-healing events, whereas a single cleavage-healing event in the long arm of Ch16 yielded Ch12. A simple model for the structure of the minichromosomes would thus be the pericentric chromosomal DNAs (identical to the counterparts in chromosome III) with the two broken ends capped by short telomeric sequences. The high mitotic stability of the minichromosomes suggests that the telomere-adjacent sequences are not essential for their maintenance.

It is not clear why shorter minichromosomes were not obtained in *S. pombe*. The centromere domain of *S. pombe* seems to be very large, roughly 60 kb in size (7, 22). Attempts to make synthetic circular minichromosomes such as those successfully made in *S. cerevisiae* (6, 8, 9, 12, 16, 20, 21, 29) have failed in *S. pombe*. Attempts to make minichromosomes from chromosomes I and II have not been successful. Because aneuploidy for the larger chromosomes I and II is lethal, but aneuploidy for chromosome III is not (25), stable partial aneuploidy (the production of a minichromosome) may take place more frequently for chromosome III than for chromosomes I and II.

The exact healing mechanism is unknown. The nucleotide sequences of the minichromosome ends are not known; comparison of the minichromosome terminal sequences and their chromosomal counterparts is necessary to identify the exact breakage sites. Homology comparison between the telomeric sequences of the minichromosomes and the normal chromosomes may help to show how the telomeres of the minichromosomes were formed. The telomeric sequence of the normal chromosomes has been sequenced (Sugawara and Szostak, in preparation); it consists of a repeating sequence of which the consensus is $C_{1-6}G_{0-1}T_{0-1}GTA_{1-2}$. A terminal transferaselike enzyme (10) is thought to be involved in the de novo synthesis of telomeres. The same enzyme may be involved in the healing of broken chromosomal ends.

Cosmid clones that cover the left-arm ends of Ch12 and Ch16 were not isolated, due to the lack of an integrated plasmid at a useful site. Both minichromosomes contained the *fur1* locus but not *ade10*. Therefore, the left ends of Ch12 and Ch16 must be located distal to *fur1* and proximal to *ade10*. The left ends of Ch12 and Ch16 are probably identical. When strains containing Ch12 and Ch16 were probed with the telomeric sequence in pSPT16, two additional hybridizing bands were seen; the bands with the same size are most likely to be derived from the left termini.

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