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Role for Telomere Cap Structure in Meiosis

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Telomeres, the natural ends of eukaryotic chromosomes, are essential for the protection of chromosomes from end-to-end fusions, recombination, and shortening. Here we explore their role in the process of meiotic division in the budding yeast, *Kluyveromyces lactis*. Telomerase RNA mutants that cause unusually long telomeres with deregulated structure led to severely defective meiosis. The severity of the meiotic phenotype of two mutants correlated with the degree of loss of binding of the telomere binding protein Rap1p. We show that telomere size and the extent of potential Rap1p binding to the entire telomere are irrelevant to the process of meiosis. Moreover, we demonstrate that extreme difference in telomere size between two homologous chromosomes is compatible with the normal function of telomeres during meiosis. In contrast, the structure of the most terminal telomeric repeats is critical for normal meiosis. Our results demonstrate that telomeres play a critical role during meiotic division and that their terminal cap structure is essential for this role.

INTRODUCTION

Telomeres are special DNA–protein structures at the natural ends of all eukaryotic chromosomes that cap and protect chromosome ends from fusions, recombination, and degradation. Telomeres are synthesized by the specialized enzyme telomerase, which adds new telomeric repeats by copying a short template sequence within its own RNA moiety (Greider and Blackburn, 1989; Shippen-Lentz and Blackburn, 1990). Mutations in the telomerase RNA template residues are incorporated into telomeres (Yu *et al.*, 1990; McEachern and Blackburn, 1995). Some mutations in the telomeric sequence cause drastic changes in telomere length and structure associated with many cellular phenotypes and in some cases with cell death (*Yu et al.*, 1990; McEachern and Blackburn, 1995; Kirk *et al.*, 1997). These changes were suggested to be caused by loss of binding of specialized proteins to telomeric DNA length (McEachern and Blackburn, 1995; Krauskopf and Blackburn, 1996).

Telomeres were shown to be involved in various cellular processes. Among these are chromosome maintenance, silencing of gene expression, signaling cellular senescence, and meiosis

Meiosis is a cell division process common to almost all eukaryotes. Meiosis initiates with DNA synthesis followed by prophase I, during which homologous chromosomes align side by side (homolog pairing) and then form a structure termed the synaptonemal complex (reviewed by Roeder, 1997; Zickler and Kleckner, 1999).

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In recent years, evidence has accumulated suggesting the involvement of telomeres in homolog pairing during meiosis. A distinguishing feature of meiotic telomere behavior of many organisms has been a configuration termed the bouquet arrangement in which the ends of most chromosomes are attached to a small region of the nuclear envelope during early prophase (reviewed by Dernberg *et al.*, 1995). Recently, the bouquet arrangement has also been observed in yeast (Trelles-Sticken *et al.*, 1999), suggesting that it is a highly conserved meiotic feature. Light microscopic and fluorescence in situ hybridization studies have established that the bouquet arrangement and telomere clustering overlap temporally with zygotene, the stage of prophase in which homolog pairing is first detected (Dernberg *et al.*, 1995; Scherthan *et al.*, 1996; Trelles-Sticken *et al.*, 1999; Bass *et al.*, 1997, 2000). These studies, as well as studies in *Schizosaccharomyces pombe* (Chikashige *et al.*, 1997), have also shown that telomeres cluster de novo during meiotic prophase and have ruled out the possibility of premeiotic clustering.

Studies of the striking telomere-mediated chromosome movement during the early meiotic prophase in *S. pombe* revealed the importance of telomere clustering for proper meiosis (Chikashige *et al.*, 1994; Scherthan *et al.*, 1994; Chikashige *et al.*, 1997).

In the past few years, the body of cytological evidence suggesting a role for telomeres in meiosis has been supported by several genetic studies. These studies, conducted in budding and fission yeast and in mice, examined the meiotic phenotypes caused by mutations in telomere-associated proteins and of cells harboring circular chromosomes. In *Saccharomyces cerevisiae*, a role for the Tam1p/Ndj1p-telomere–associated protein in homolog pairing and the sta-

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bilization of homology-dependent interactions was suggested (Chua and Roeder, 1997; Conrad *et al.*, 1997).

The *S. pombe* telomere binding protein Taz1p was also shown to be essential for spore viability and the characteristic telomere clustering at the spindle pole body (SPB) (Cooper *et al.*, 1998; Nimmo *et al.*, 1998). Proper telomere clustering thus appears to be required to facilitate pairing and recombination.

A study of meiotic kinetics in *S. cerevisiae* has revealed that in the absence of telomeres, the characteristic delay in meiotic prophase I indicative of pairing is lost (Rockmill and Roeder, 1998). Therefore, it was concluded that without telomeres, there was little or none homolog pairing.

S. pombe cells in which all three chromosomes were circular exhibited severe defects in meiosis, as evident from their greatly reduced spore viability (Naito *et al.*, 1998). This again indicated a crucial role for telomeres in meiosis.

To directly study the effect of telomere structure on meiosis, we have studied the meiotic phenotypes of telomeric mutants of the budding yeast, *Kluyveromyces lactis*, in which the structure of telomeres themselves is altered. In contrast to its relative budding yeast, *S. cerevisiae* (Prescott and Blackburn 1997), mutations in the template region of the RNA subunit of telomerase of *K. lactis* are precisely incorporated into new telomeric repeats and result in predictable and homogeneous repeats. Depending on the mutation, this results in alterations in telomere length and in the structure of the telomeric complex (McEachern and Blackburn, 1995). Therefore, any effect on meiosis can be attributed to defined changes in the telomeric structure and/or function, and direct evidence for the role of telomeres in meiosis can be obtained. Here we have used telomeric RNA (*TER1*) template mutations to study the role of telomeres in meiosis. Our findings demonstrate that *ter1* mutants with long and deregulated telomeres are severely defective in meiosis. By comparing the meiotic phenotypes of two *ter1* mutants, we show that general telomere size and the binding potential for Rap1p throughout the entire length of the telomere are insignificant for the process of meiosis. We also show that extreme heterogeneity in telomere size of homologous chromosomes has no effect on the normal function of telomeres during meiosis. In contrast, we demonstrate that the structure of the most terminal telomeric repeats is critical for normal meiosis.

MATERIALS AND METHODS

Plasmids

The following plasmids were a gift from M. McEachern (University of Georgia, Athens, GA):

pTER1-Acc: An integrative plasmid bearing a *URA3* marker and an ~4-kb *Bam*HI-XbaI fragment containing the *TER1* gene with the Acc substitution in the template region.

pTER1-Bsi: Same as pTER1-Acc but with the Bsi substitution in the template region.

pTER1-Bcl: Same as pTER1-Acc but with the Bcl substitution in the template region

Yeast Strains

All *K. lactis* strains used in this study are isogenic to CBS2359 and homothallic.

GG1929: *ade2-202 ura3-59 TER1*

GG1935: *ade1-201 ura3-59 TER1*

Acc-29: (GG1929 *ter1-Acc*). Constructed by integration of pTER1-Acc into GG1929 and selection of loop outs on 5-fluoroorotic acid (5-FOA). To screen for clones that retained *ter1-Acc* and lost *TER1*, we used primers outside the template region of *TER1* to polymerase chain reaction (PCR)-amplify a 360-bp region containing the template. PCR products were separated on gel, blotted, and probed with a probe designed to react only with the *ter1-Acc* template sequence and not with the wild-type sequence.

Acc-35: (GG1935 *ter1-Acc*). Selected as described for Acc-29

$$\text{Acc/Acc:}\ \frac{ade2\text{-}202}{ADE2}\ \frac{ura3\text{-}59}{ura3\text{-}59}\ \frac{ADE1}{adel\text{-}201}\ \frac{ter1\text{-}Acc}{ter1\text{-}Acc}\ \frac{MATa}{MAT\alpha}$$

TER1/ter1-Acc: Acc-29 crossed with GG1935

Bsi-29: (GG1929 *ter1-Bsi*). Constructed by integration of pTER1-Bsi into GG1929 and screening for cells that retained ter1-Bsi upon plating on 5-FOA by PCR and hybridization to a Bsi-specific probe, as described for Acc-29

Bsi-35: GG1935 but *ter1-Bsi*, constructed as described for Bsi-29

$$\text{Bsi/Bsi:}\ \frac{ade2\text{-}202}{ADE2}\ \frac{ura3\text{-}59}{ura3\text{-}59}\ \frac{ADE1}{adel\text{-}201}\ \frac{ter1\text{-}Bsi}{ter1\text{-}Bsi}\ \frac{MATa}{MAT\alpha}$$

TER1/ter1-Bsi: Bsi-29 crossed with GG1935

ter1-Bcl/ter1-Acc: *Acc/Acc* was transformed with pTER-Bcl resulting in a diploid stain with three copies of TER1: *ter1-Acc-URA3-ter1-Bcl* on one chromosome and ter*1-Acc* on the homologous chromosome, with capped telomeres. On selection on 5-FOA, cells that retained one copy of *ter1-Acc* and one copy of *ter1-bcl* were screened for by PCR and hybridization to a Bcl–specific probe, as described for Acc-29

TER1/ter1-Bcl ex-Acc: spore product of ter1-Bcl/ter1-Acc, which retained ter1-Bcl (ter1-Bcl ex-Acc) was crossed to GG1929 or GG1935

Oligonucleotides Used as Primers and Probes

PCR Primers.

TER1 936–952 5′-GCTATGACAACAATACC-3′

TER1 1301–1287 5′-AATGGAGCAAGGACG-3′

Telomeric Probes for Hybridization.

Wild-type 5′-GGATTTGATTAGGTATGT-3′

Acc specific 5′-GGTATGTGGTATACGGATTTGATTA-3′

Bsi specific 5′-GGTATGTGGCGTACGGATTTGATTA-3′

Bcl specific 5′-GGATTTGATCAGGTATGT-3′

Telomere Length Analysis

Southern Blots. Genomic DNA was prepared from saturated cultures with the use of a modified version of the zymolase method (Guthrie *et al.*, 1991). Pelleted cells were resuspended in 150 μl of SEB plus lyticase (1 M sorbitol, 0.1 M EDTA, 14 mM 2-mercaptoethanol, 200 ng/ml lyticase) and incubated at 37°C for 30 min at 100 rpm. After pelleting, cells were resuspended in 150 μl EDS (50 mM EDTA, 0.2% SDS) and incubated at 65°C for 15 min. Tubes were placed on ice and 75 μl of 8 M NH_4OAc were added for 30–60-min incubation at 4°C. Tubes were centrifuged at 14 krpm for 10 min and supernatants were precipitated with 135 μl of isopropanol. Genomic DNA was digested with *Eco*RI and resolved in 0.8% agarose gels and blotted onto Nylon membranes with the use of alkaline conditions. Membranes were UV cross-linked with a Stratalinker 1800 (Stratagene, La Jolla, CA). Hybridizations were carried out in SDS-Na_2PO_4 according to the procedure of Church and Gilbert (1984). Telomeric-specific probe was made by phosphorylation with T4 polynucleotide kinase of the oligonucleotide containing a G-strand *K. lactis* telomeric repeat. Hybridizations (1 h) and washes (total 15 min) were performed at appropriate temperature for each probe used.

Pulse-Field Gel Electrophoresis. Yeast genomic DNA (10 μl) was digested with restriction enzymes and subjected to gel electrophoresis in a CHEF-DRIII (Bio-Rad, Hercules, CA) device. Electrophoresis

parameters were 0.6% agarose, 0.5× Tris borate/EDTA, 14°C at 2 V with pulse 200-1800 s. Gels were run for 72 h. Blotting and hybridization were performed as described above for Southern blots

Meiosis Protocols

***K. lactis* Mating.** Yeast cells (3×10^8/ml) of two strains were combined in a 4-μl YPD drop on malt extract (2%) plate. After 48 h at 30°C cells were scraped off, resuspended in 50 μl of H_2O, and plated on selective plates (SD−Ade) at 30°C. As soon as colonies became visible (2 d) the resulting diploid colonies were transferred to YPD plates and incubated at 30°C for 2 d.

Sporulation. Cells were grown to ~10^8 cells/ml unless indicated differently, pelleted, washed twice in H_2O, and resuspended with 2 ml of sporulation medium and incubated while rolling at 25°C for 4–5 d.

Tetrad Dissection. Cells from sporulation cultures were incubated for 10 min with 0.5 μl (2 U/1 μl) glucoronidase in H_2O, 20 μl were spotted on a YPD plate, and tetrads were dissected with the use of a Singer micromanipulator. Spores were incubated at 30°C for 3 d for germination.

Kinetics of Meiosis. One milliliter of methanol was added to 0.5 ml of cells from sporulation culture. Cells were then washed in 1 ml of phosphate-buffered saline (PBS). After sonication (10 s), 1 μg/ml 4,6-diamidino-2-phenylindole (DAPI) was added, and cells were viewed in a fluorescent microscope. Experiments were performed at least two times and at each time point at least 200 cells were scored.

Fluorescence-Activated Cell Sorting (FACS) Analysis. One milliliter of 70% ethanol was added to 0.3 ml of cells from sporulation culture. Cell were then washed twice with 1 ml of PBS, resuspended in 0.5 ml of PBS + 1 mg/ml RNaseA and incubated overnight while rotating at 4°C. Twenty micrograms of proteinase K was added and samples were incubated at 55°C for 1 h. Cells were washed with 1 ml of PBS and resuspended in 0.5 ml (50 μg/ml) of propidium iodide for 1 h at room temperature and analyzed on a BD Biosciences FACSort (San Jose, CA). Ten thousand cells were analyzed at 580 nm for each histogram. MPLUS AV software (Phoenix Flow Systems, CA) was used to statistical analyses.

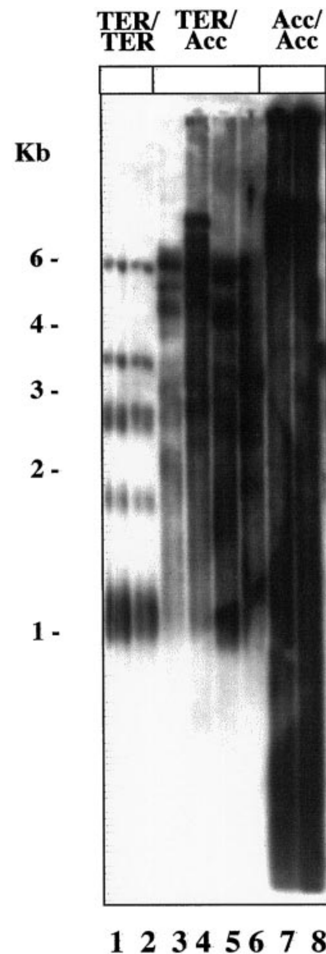

Figure 1. Telomere phenotype of *TER1/TER1* and *Acc/Acc* strains. Genomic DNA was digested with *Eco*RI and resolved on a gel. The gel was blotted and probed with a telomeric wild-type probe. Lanes 1 and 2, *TER1/TER1*; lanes 3–6, *TER1/Acc*; and lanes 7 and 8, *Acc/Acc*.

RESULTS

Severe Telomeric Phenotype of *Acc/Acc* Mutants Leads to Severe Defects in Meiosis

We reasoned that if a role for telomeres in meiosis existed, mutants whose telomeres are severely abnormal in length and/or structure would fail to undergo normal meiosis. We chose to study a *K. lactis* strain harboring a telomerase RNA template mutation termed *ter1-Acc*. This mutation consists of a G to A substitution in the template sequence of *TER1,* the gene coding for the RNA moiety of telomerase. This substitution is incorporated faithfully into all newly synthesized telomeric repeats (McEachern and Blackburn, 1995). The abnormal repeats contain an altered binding site for the protein Rap1p, reducing the in vitro binding affinity for Rap1p by 300-fold relative to the wild-type site (Krauskopf and Blackburn, 1996). Because Rap1p is essential for telomere length regulation, the *ter1-Acc* mutation leads to immediate deregulation of telomere length and increased mean telomere length in vivo (McEachern and Blackburn, 1995; Krauskopf and Blackburn, 1996). Three diploid strains were created: *TER1/TER1*, *TER1/ter1-Acc* (*TER1/Acc*), and *ter1-Acc/ter1-Acc* (*Acc/Acc*). First, their telomeric phenotypes were confirmed. Total genomic DNA digested with *Eco*RI was analyzed by a Southern blotting with the use of a probe specific for wild-type telomeric repeats (which exist in the internal part of the telomere in *Acc/Acc* mutants (McEachern and Blackburn, 1995). In the *TER1/TER1* strain, a typical telomeric pattern was obtained (Figure 1, lanes 1 and 2). The pattern of bands is due to the different locations of the *Eco*RI recognition sites in the 12 subtelomeric regions of the six *K. lactis* chromosomes. In contrast, and as previously seen in *ter1-Acc* haploid strains, mean telomere size was greatly elevated in the diploid *Acc/Acc* strains compared with the characteristic wild-type telomere length. Moreover, as previously seen in *ter1-Acc* haploid strains, the telomeric signal appeared as a smear. This loss of the characteristic pattern of bands is indicative of telomere length deregulation because the cell population now harbors a variety of telomere sizes (Figure 1, lanes 7 and 8). In the heterozygote *TER1/Acc* (Figure 1, lanes 3–6), where both *TER1* and *ter1-Acc* were

Table 1. Effect of Acc mutation on sporulation efficiency and spore viability

Cross	Sporulation efficiency (%)	Spore viability (%)	No. of tetrads dissected
TER1/TER1	43	95	42
TER1/ter1-Acc	50	98	53
ter1-Acc/ter1-Acc	3	22	115

Sporulation efficiency was determined by counting the fraction of cells forming asci after 4–5 d in sporulation medium. Spore viability was determined by tetrad dissection.

present in a single cell, distinct bands appeared in addition to a faint smear. This suggests, that once a wild-type repeat is added onto a telomere end by TER1, this telomere's chances to remain regulated are very high because it becomes inaccessible to telomerase. Residual smearing observed in the telomeric pattern of these strains may reflect occasional addition of telomeric repeats by *ter1-Acc* in individual cells, followed by incorporation of a wild-type repeat(s) by TER1.

To assess the mitotic phenotypes of the three strains a plating efficiency assay was performed. The efficiency of the *Acc/Acc* strain was 70% compared with that of the *TER1/TER1* strain, whereas the plating efficiency of the heterozygote strain *TER1/Acc* was similar to that of the *TER1/TER1* strain (our unpublished results).

To test the effect of the *ter1-Acc* mutation on meiosis, each of the diploid strains was induced to sporulate, and sporulation rate and spore viability were assessed. As seen in Table 1, sporulation rate was reduced by >10-fold in the *Acc/Acc* strain (3%) compared with the *TER1/TER1* strain (43%). This reduction in the number of meiotic products clearly indicated that the telomeric mutant cells were greatly affected in one (or more) stages in meiosis. In contrast, despite its semiregulated telomeric pattern, the heterozygote strain *TER1/Acc* did not show any reduction in sporulation rate, indicating that most *TER1/Acc* cells successfully completed all the crucial stages of meiosis.

In some cases, defects in meiosis result in the production of mature tetrads whose spores are genetically aberrant (reviewed by Kupiec *et al.*, 1997; Naito *et al.*, 1998). To test for the presence of such aberrations, spore viability was assessed. To measure spore viability, we counted the number of spores that germinated and produced colonies upon tetrad dissection. Nonviability of spores was defined as the inability to form visible colonies. As seen in Table 1, spores originating from the *Acc/Acc* diploid were more than fourfold (22%) less viable than spores from the *TER1/TER1* or *TER1/Acc* diploids (95 and 98%, respectively). This failure to produce viable products indicates that even in the rare event of full tetrad formation (3%), the meiotic process in *Acc/Acc* diploids is fundamentally defective. Spore nonviability could result from a physiological defect leading to inability to germinate. Alternatively, it could result from a genetic defect in the spore products themselves. In this case, spores would germinate but not be able to form full-grown colonies. We observed that nearly all the spores scored as nonviable actually germinated and divided to produce microcolonies of <10 cells (our unpublished results). This observation confirmed that the inviability of *Acc/Acc* spore products was due to a genetic defect rather than to a physiological defect in germination.

Kinetics of Meiosis in Acc/Acc Mutants

Our observations on the effects of the long and heterogeneous telomeres in the *Acc/Acc* strain on sporulation rate and spore viability confirmed that telomeres have an important role in meiosis. To identify the stage of meiosis, in which *Acc/Acc* are defective, we followed the meiotic process through its different stages in *TER1/TER1* and *Acc/Acc* strains. We first tested whether the mutant was able to enter meiosis and duplicate its DNA. Cultures were grown to saturation in rich medium and transferred to liquid sporulation medium to induce sporulation. Samples were taken from both cultures at consecutive time points, and DNA content was analyzed by FACS. As seen in Figure 2A, in both strains approximately two-thirds of the cells had gone through a cycle of DNA replication by 27 h after transfer to sporulation medium. Thus, the early meiotic event of DNA synthesis was not impaired in the *Acc/Acc* strain. To test at which stage of meiosis *Acc/Acc* cells were arrested, we compared the timing of appearance and the fractions of *TER1/TER1* and *Acc/Acc* cells with one, two, and four nuclei, corresponding, respectively, to cells before the first meiotic division, cells that were past the first but not the second division, and cells that completed both divisions. Samples were drawn at different time points after transfer to sporulation medium and were fixed and stained with DAPI to enable visualization of nuclei. The results of this experiment are presented graphically in Figure 2B. Two-nuclear cells were most abundant between 15 and 30 h after transfer to sporulation medium. During this time they averaged 7% in *TER1/TER1* and 3.5% in *Acc/Acc*. These values are probably an underestimate, because the three-dimensional orientation of two nuclei in a cell can cause their fluorescence signals to merge, and the two are then counted as one. Four-nuclear cells started to appear ~17 h after transfer to sporulation medium, and reached their maximal concentration ~30 h after transfer to sporulation medium. In the *TER1/TER1* strain they reached 60% of total cells, whereas in *Acc/Acc* this value was reduced to 5%. From these results we conclude that the majority of *Acc/Acc* cells duplicate their DNA as do *TER1/TER1* cells. However, in contrast to the latter, most of the mutant cells do not proceed to divide and arrest before MI. The minority of cells that do manage to continue beyond DNA synthesis are not delayed in timing of meiosis.

Meiotic Phenotype of Milder Telomeric Mutation ter1-Bsi

The meiotic phenotypes of the *Acc/Acc* strain clearly showed that long and deregulated telomeres, associated with reduced Rap1p binding affinity, were inhibitory to the meiotic process. This effect could be attributed to their increased size, the heterogeneity of telomere length within each cell, or the lack of Rap1p binding. To distinguish between these possibilities and further study the relation between telomere structure and meiosis, we next examined the meiotic phenotype of a milder telomere

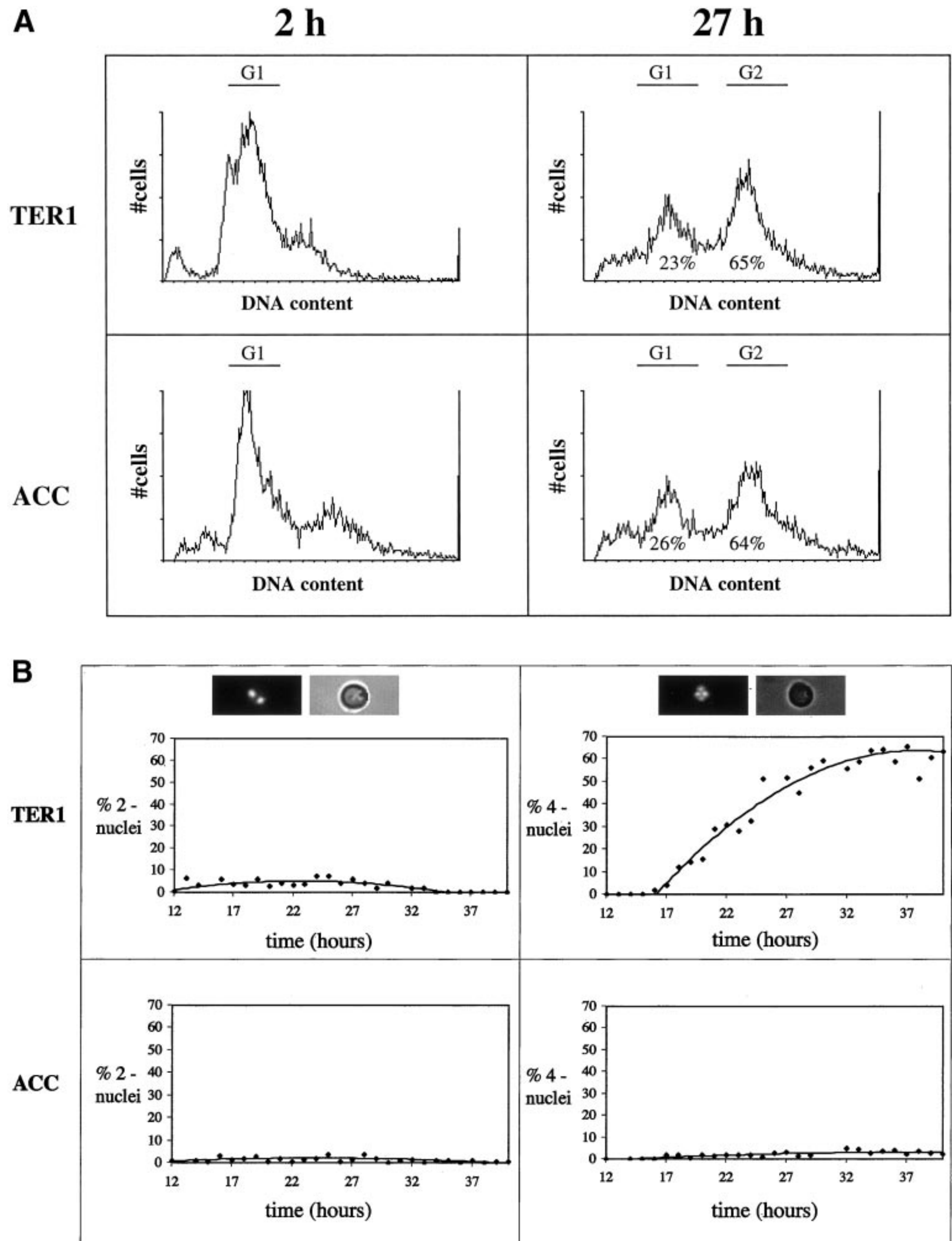

Figure 2. Progression of meiosis in *TER1/TER1* and *Acc/Acc* strains. (A) FACS analysis (DNA content) of *TER1/TER1* and *Acc/Acc* cells at 2 and 27 h after transfer to sporulation medium. The histograms represent number of cells versus 580-nm signal. G1 and G2 peaks and the proportion of the populations in t = 27 h are indicated. (B) Kinetics of meiotic nuclear division in *TER1/TER1* and *Acc/Acc*. The percentage of binucleate cells and tetranucleate cells was monitored by DAPI fluorescence microscopy during sporulation of *TER1/TER1* and *Acc/Acc*.

mutant. The *ter1-Bsi* mutation consists of a single template base substitution adjacent to the *ter1-Acc* substitution. Like the latter, the *Bsi* substitution falls within the Rap1p binding site, and in vitro, binding affinity of Rap1p to the mutated site is reduced 100-fold relative to the wild-type site and is three times higher than its affinity to *Acc* (Krauskopf and Blackburn, 1996). The characteristic phenotype of long and deregulated telomeres is observed in *ter1-Bsi* cells but is less immediate than in *ter1-Acc*, with telomeres reaching their maximal length and heterogeneity after 50–100 cell divisions (McEachern and Blackburn, 1995). To test the effect of the *ter1-Bsi* mutation on meiosis, the diploid strains *ter1-Bsi/ter1-Bsi* (*Bsi/Bsi*) and *TER1/ter1-Bsi* (*TER1/Bsi*) were created and their telomere phenotypes were confirmed, as described above for the *Acc/Acc* strains. As can be seen in Figure 3, lane 3, telomeres of the homozygote strain *Bsi/Bsi* were deregulated, as expected. As was the case with the *TER1/Acc* strain, the telomeres of the heterozygote *TER1/Bsi* (Figure 3, lane 2) were semi-regulated, because distinct bands were visible, in addition to a smear. The mitotic phenotypes of the three strains were assessed by a plating efficiency assay. The efficiency of the *Bsi/Bsi* strain was 80% compared with that of the *TER1/TER1* strain, whereas the plating efficiency of the

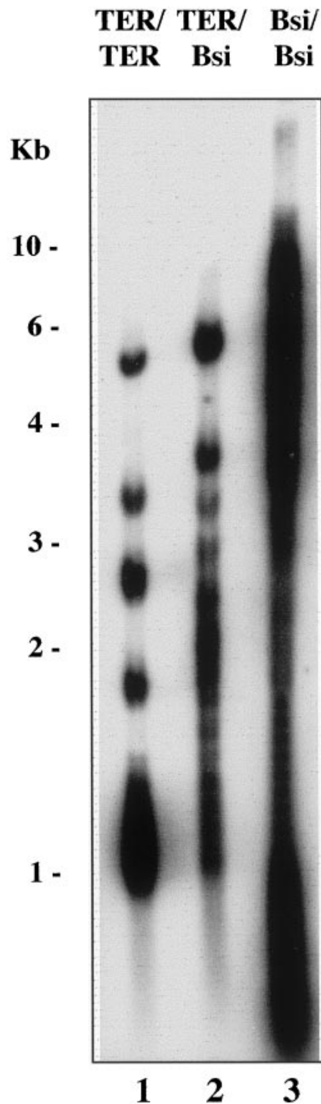

Figure 3. Telomere phenotype of *TER1/TER1* and *Bsi/Bsi* strains. Genomic DNA from the strains indicated above the lanes was digested with *Eco*RI and resolved on a gel. Gels were blotted and probed with a telomeric wild-type probe. Lane 1, *TER1/TER1*; lane 2, *TER1/Bsi*; and lane 3, *Bsi/Bsi*.

heterozygote strain, *TER1/Bsi* was similar to that of the *TER1/TER1* strain (our unpublished results).

To study the effect of this mutation on meiosis, *Bsi/Bsi* strains were induced to sporulate, and again, sporulation rate and spore viability were assessed. As seen in Table 2, the sporulation rate of the *Bsi/Bsi* strain (10%) was significantly lower than for *TER1/TER1* cells (43%). Thus, *Bsi/Bsi* cells, like *Acc/Acc* cells, are clearly impaired in their ability to complete some stage(s) of meiosis. The heterozygote *TER1/Bsi* had the same sporulation rate as *TER1/TER1*. Therefore, we concluded that it was able to successfully complete all crucial stages of meiosis. Although the sporulation rate of the *Bsi/Bsi* strain was substantially reduced compared with wild type, spore viability was less affected and reached 70%

Table 2. Effect of Bsi mutation on sporulation efficiency and spore viability

Cross	Sporulation efficiency (%)	Spore viability (%)	No. of tetrads dissected
TER1/TER1	43	95	42
TER1/ter1-Bsi	46	97	56
ter1-Bsi/ter1-Bsi	10	70	116

Sporulation efficiency was determined by counting the fraction of cells forming asci after 4–5 d in sporulation medium. Spore viability was determined by tetrad dissection.

(Table 2). The better viability of *Bsi/Bsi* spores indicated that in the 10% of *Bsi/Bsi* cells that succeeded to complete meiosis, in most cases, the meiotic mechanism itself was sufficiently intact to produce viable meiotic products. This contrasts with the situation in *Acc/Acc*, where, in most cases, the meiotic mechanism failed to produce viable products even if meiosis reached the tetrad stage.

Higher Resolution Analysis of Telomere Sizes Reveals Similarity in Telomere Sizes of Acc/Acc and Bsi/Bsi

Having seen that the meiotic phenotypes of *ter1* mutant strains correlated with the severity of their telomere phenotypes, we wanted to examine how this difference correlated with differences in several characteristics of telomeres in the two strains. We hypothesized that such a correlation would be indicative of the role of telomeres in meiosis.

The telomeric patterns of *Acc/Acc* and *Bsi/Bsi* strains showed that in both strains telomere length is deregulated, and mean length is greatly increased compared with wild type (Figures 1 and 3). One possible explanation for the difference between the severity of meiotic defects in *Bsi/Bsi* relative to *Acc/Acc* strains would be that the telomeres of the latter strain are in fact longer but that this difference was not apparent due to the limitations of resolution of the gel system used. The limit mobility of DNA in standard electrophoresis is only 15–20 kb. To better resolve long telomeric DNA fragments, we used pulse-field electrophoresis of *Eco*RI-digested genomic DNA in a CHEF apparatus to separate high-molecular weight fragments. Blotting and hybridization were performed as described for conventional telomere blots. As seen in Figure 4, in the diploid mutant strains *Acc/Acc* and *Bsi/Bsi* the mean telomere lengths were very similar to each other in the high range: the bulk of telomeric hybridizing fragments being on ~20- to ~50-kb fragments. The faint smear seen above 50 kb in the *Acc/Acc* strain may indicate that a very small fraction of telomeres in this strain was >50 kb in length. Therefore, it is highly unlikely that differences in telomere lengths per se accounted for the differences observed in the meiotic phenotypes of *Acc/Acc* and *Bsi/Bsi* strains.

Capping Suppresses Meiotic Defects in ter1 Mutants

As mentioned above, the in vitro binding affinity of Rap1p to Bsi telomeric repeats was 3 times higher than to Acc repeats

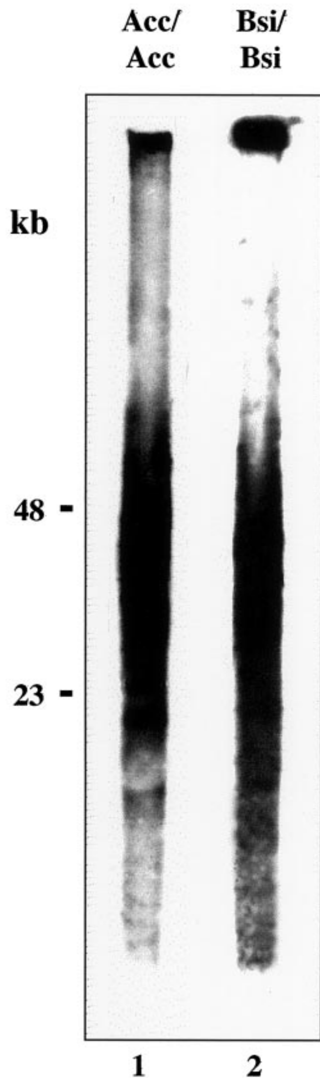

Figure 4. CHEF analysis of *Acc/Acc* and *Bsi/Bsi* telomeres. Genomic DNA from the strains indicated above the lanes was digested with *Eco*RI and separated by pulse-field electrophoresis. Lane 1, *Acc/Acc*; and lane 2, *Bsi/Bsi*.

(Krauskopf and Blackburn, 1996). Because telomere length was indistinguishable between diploid *Bsi/Bsi* and *Acc/Acc* strains, the observed differences in the severity of meiotic phenotypes between the two mutants might be accounted for by the difference in the number of Rap1p molecules associated with the entire telomere or with its most distal end. It is possible that Rap1p molecules bound throughout the entire telomere are important for a putative telomere meiotic function. Alternatively, it is conceivable that only the Rap1p bound to the most distal end of the telomere is important for such meiotic function. To distinguish between these possibilities, we analyzed the meiotic phenotypes of *Bsi/Bsi* or *Acc/Acc* strains whose mutant telomeres were capped by incorporation of a very few telomeric repeats capable of binding Rap1p onto the termini of their telomeres. We reasoned that if the number of Rap1p molecules bound throughout the telomere was important for meiosis then capping would not suppress the meiotic defects in either mutant strain. In addition, the mutant strains would still exhibit differences in the severity of their meiotic phenotypes compared with each other. In contrast, if normal binding of Rap1p to the most distal repeats of the telomere is both necessary and sufficient for meiosis and uncapping of the telomeric termini of *ter1* telomeres was the sole reason for the meiotic defects exhibited by *ter1* strains then recapping with normal repeats would suffice to override these meiotic defects. Moreover, in this case we would expect that meiosis would be rescued to a similar extent in both strains. We have previously reported that such capping of long and deregulated telomeres was sufficient to restore telomere length control. In these cases, telomere length, although regulated, became set at new sizes, often much longer than the normal length (Krauskopf and Blackburn, 1998; Smith and Blackburn, 1999). Therefore, we compared the ability of strains with long telomeres, capped or uncapped, to undergo meiosis. The *ter1-Bcl* mutation, which introduces a *Bcl1* restriction site into the newly incorporated telomeric repeats, is located outside the Rap1p binding site and was previously shown to have no effect on telomere length or cell phenotype (Krauskopf and Blackburn, 1998; Roy *et al.*, 1998). Therefore, it can be used to cap telomeres with marked but phenotypically silent telomeric repeats. *Acc/Acc* and *Bsi/Bsi* cells were transformed with an integrative copy of *ter1-Bcl*. Telomeric patterns were compared among the original *ter1* strains and those capped by *ter1-Bcl*. As seen in Figure 5A, lanes 3–6, upon introduction of the *ter1-Bcl* gene (Acc capped lanes), the telomeric patterns of four independent clones immediately exhibited discrete bands indicative of capping. This indicates that upon capping, the size of all telomeres of all cells emanating from the original "capped" cell are stably kept throughout hundreds of cell divisions. As previously seen, the newly capped telomeres were kept at sizes significantly longer than the wild-type telomeres (Figure 5A, lane 1) with mean length similar to that of the original *Acc/Acc* (Figure 5A, lane 2). This result showed that upon capping of deregulated telomeres in *Acc/Acc* strains by *Bcl* repeats, telomere length control was resumed, as expected. In contrast, the original *Acc/Acc* strain or a strain that lost the *ter1 Bcl* gene after 5-FOA (Acc ex-capped), exhibited a smear characteristic of deregulated telomeres (Figure 5A, lane 2 and lanes 7 and 8, respectively). To verify that capping required the addition of only a few Bcl repeats, the same genomic DNA samples were double digested with *Eco*RI and *Bcl1* (Figure 5A, lanes 9–16). *Eco*RI cuts in subtelomeric locations internal to the telomeric repeats (unique to each telomere), whereas *Bcl1* specifically cleaves off the marked Bcl telomeric repeats added onto the preexisting repeats of the telomeres. Therefore, the resulting telomeric fragment pattern reflects the lengths of the remaining repeat tracts located internally to the newly incorporated Bcl repeats. Comparing the telomeric pattern of the double-digested DNA with that resulting from restriction digest with *Eco*RI alone reflects the extent of incorporation of *Bcl1* repeats onto the ends of the telomeres. As can be seen in Figure 5A, lanes 11–14, upon double digestion with *Eco*RI and *Bcl1*, most of the telomeric bands were only slightly shorter than those bands seen after digestion with *Eco*RI alone (lanes 3–6). Some shorter fragments are also generated

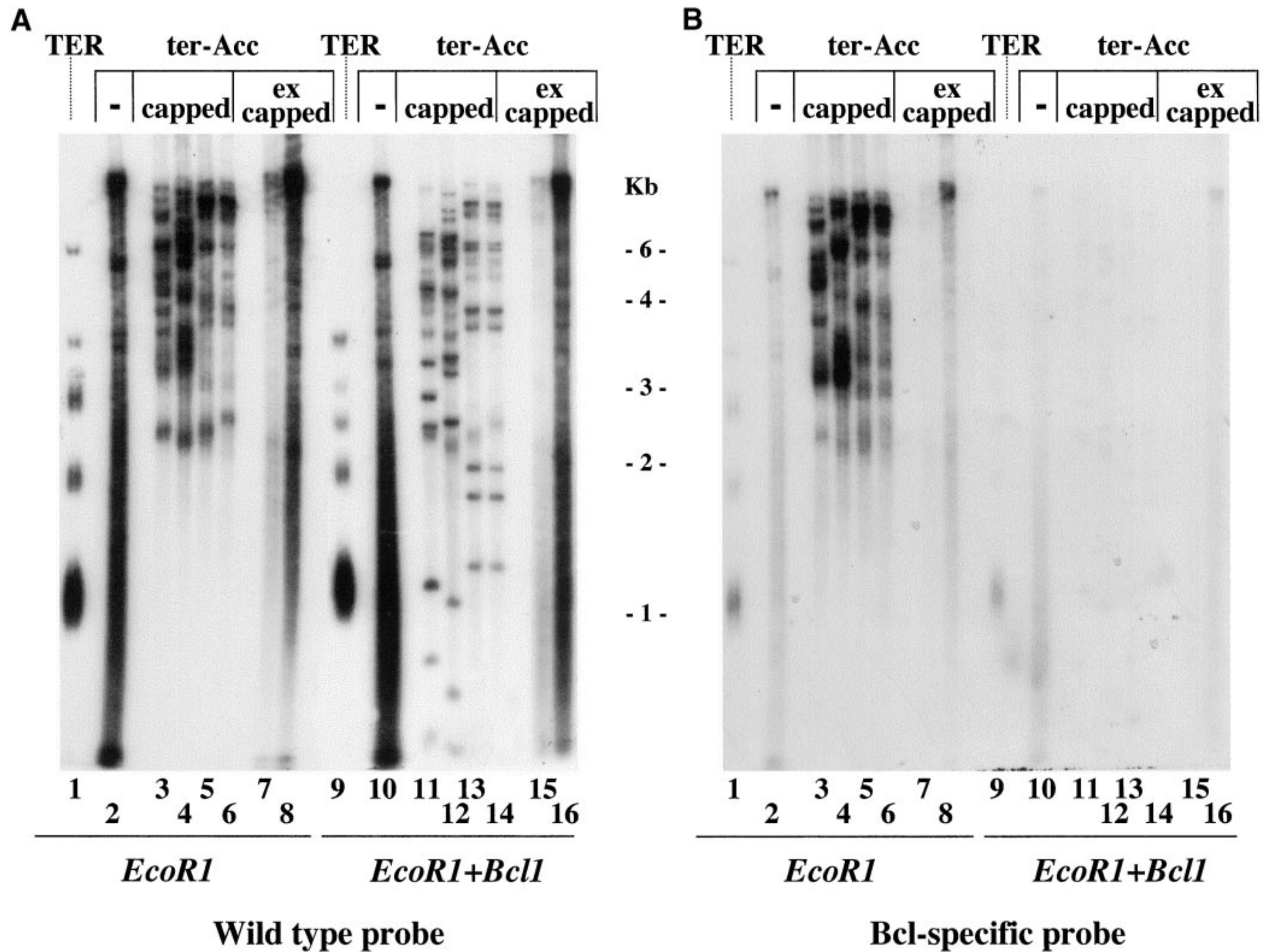

Figure 5. Telomere phenotype of *Acc/Acc* strains capped with *ter1-Bcl*. (A) Genomic DNA from the strains indicated above the lanes was digested with either *Eco*RI alone (lanes 1–8) or *Eco*RI and *Bcl*I (lanes 9–16). Lanes 1 and 9, *TER/TER1*; lanes 2 and 10, *Acc/Acc*; lanes 3–6 and lanes 11–14, *Acc/Acc* strains capped with *ter1-Bcl*, which upon selection on 5-FOA retained one copy of *ter1-Acc* and one copy of *ter1-Bcl*; and lanes 7 and 8 and lanes 15 and 16, *Acc/Acc* strains capped with *ter1-Bcl*, which upon selection on 5-FOA retained two copies of *ter1-Acc*. The blot was probed with a wild-type specific probe that hybridizes with the internal wild-type repeats of all strains. (B) Same blot was probed with a probe specific to Bcl repeats.

by cutting at *Bcl1* sites in certain subtelomeric locations. Hence, in most telomeres, only a very few *Bcl* repeats had been incorporated onto the chromosomal termini. To confirm that all *Bcl* repeats were indeed cleaved by *Bcl1*, the same blot was stripped and reprobed with a Bcl-specific probe under stringent conditions (Figure 5B). As seen in Figure 5B, lanes 11–14, after cleavage with *Bcl1* the telomeric fragments no longer hybridized with the Bcl-specific probe, whereas the telomeric fragments, which resulted from digestion with *Eco*RI alone (lanes 3–6), still hybridized. This confirmed that cleavage with *Bcl1* was complete. Capping was confirmed by the same manner for the capped *Bsi/Bsi* strains (our unpublished results).

To test the effect of capping on meiosis, the strains original *Acc/Acc*, *Bcl/Acc* capped, and *Acc/Acc* ex-capped, which retained two copies of *ter1-Acc* after selection on 5-FOA, as well as original *Bsi/Bsi*, *Bcl/Bsi* capped, *Bsi/Bsi* ex-capped, and *TER1/TER1* were induced to sporulate. As seen in Table 3, sporulation rates before capping were 5% in the original *Acc/Acc* strain, 1% in the ex-capped *Acc/Acc* strain, 4.5% in the original *Bsi/Bsi* strain, and 2.5% in the ex-capped *Bsi/Bsi* strain. In contrast, average sporulation rates of the capped strains were 49% (for *Bcl/Acc* capped) and 47% (for *Bcl/Bsi* capped), similar to their rate in the wild-type strain (*TER1/TER1*). The effect of capping on spore viability was also analyzed. Table 3 shows that although spore viability in the original *Acc/Acc* strain was only 16% in this experiment, the average viability of the capped *Acc/Acc* strains was 90%. Likewise, although spore viability in the original *Bsi/Bsi* strain was 61%, the average viability of the capped strains was 93%. In all cases, viabilities of the capped

Table 3. Effect of capping in ter1-Bsi and ter1-Acc on sporulation efficiency and spore viability

TER1	Sporulation efficiency (%)	Spore viability (%)	No. of tetrads dissected
TER1/TER1	52	93	32
ter1-Bsi/ter1-Bsi	4.5	61	31
ter1-Bcl/ter1-Bsi	47	93	153
	(47, 45, 46, 47, 47)	(98, 91, 99, 87, 97)	(24, 47, 24, 36, 22)
ter1-Bsi/ter1-Bsi excapped*	2.5		
ter1-Acc/ter1-Acc	5	16	32
ter1-Bcl/ter1-Acc	49	90	154
	(54, 44, 55, 50)	(96, 79, 93, 97)	(26, 48, 48, 32)
ter1-Acc/ter1-Acc ex-capped*	1		

Five independent capped *ter1-Bsi* clones (ter1-Bsi/ter1-Bcl) and four independent capped *ter1-Acc* clones (ter1-Acc/ter1-Bcl) were induced to sporulate. Sporulation efficiency was determined by counting the fraction of cells forming asci after 4–5 d in sporulation medium. Spore viability was determined by tetrad dissection. Average results are presented in bold and results obtained from each independent clone is presented in parentheses.

*The ex-capped are strains that retained the original *ter1* allele (ter1-Bsi or ter1-Acc) and lost the *ter1-Bcl* allele after passage through 5-FOA.

strains were similar to each other and resembled that of the wild-type *TER1/TER1* strain.

In summary, capping of *ter1* mutant telomeres, *Bsi/Bsi* or *Acc/Acc*, with a few normally-Rap1p binding repeats was sufficient to completely suppress the meiotic phenotypes of these strains. This was despite the fact that telomeric sizes were still abnormal, and most of the telomeric repeats in the capped strains were still mutant and hence only able to bind Rap1p with greatly reduced affinity.

Telomere Length Heterogeneity between Homologous Chromosomes Is Compatible with Normal Meiosis

Incorporation of Bcl repeats competent to bind Rap1p onto the most distal portion of the telomere caused the disappearance of the heterogeneity in telomere length characteristic of uncapped telomeres. This Bcl capping was thus evidenced by the substantial narrowing of telomere size range and disappearance of the smear observed in the original uncapped strains. This resulting pattern of discrete bands indicates that upon capping, the telomeres of a given cell are "captured" at the sizes they had at the moment of capping and that this structure is subsequently passed on to its progeny. This results in a uniform clonal cell population with respect to telomere sizes. In contrast, before capping, telomere length heterogeneity reflects a situation in which the telomeres within a cell can vary greatly in size, including telomeres of homologous chromosomes. Therefore, we tested two possibilities that could explain how capping rescues meiosis in *ter1*-capped strains. The first was that it is the binding of Rap1p to the most distal repeat(s) alone that is crucial for meiosis. The second was that it is the narrowing of telomere size range within the same cell, specifically that of homologous chromosomes, that is crucial for meiosis. To distinguish between these two possibilities, we directly tested the effect of telomere length heterogeneity on meiosis. We created diploid strains that were genotypically wild type but contained two distinct sets of chromosomes: one with long telomeres (capped and therefore regulated) and the other with wild-type–sized telomeres. These strains were created by sporulating the capped diploid strains, either *Acc/Bcl*-capped or *Bsi/Bcl*-capped. Spore clones that retained the ter1-Bcl allele had long but regulated telomeres (capped) and were genotypically wild type because the *Bcl* mutation is silent. Figure 6A shows representative telomeric patterns of spore clones of capped Acc strains (Figure 6A, lanes 3–5, TER ex-Acc) and capped Bsi strains (Figure 6B, lanes 3 and 4). These strains were mated with a "naïve" *TER1* strain with normal-sized telomeres. As can be seen in Figure 6A, lanes 6–11(TER ex-Acc × TER), and Figure 6B, lanes 5–8 (TER ex-Bsi × TER), in the resulting diploids, all the "input" telomeres remained unchanged in size or structure. This resulted in two sets of chromosomes in the same cell: one with short telomeric restriction fragments ranging from 1 to 5 kb (contributed by the *TER1* parent) and one with long telomeric restriction fragments ranging from 5 to >20 kb (apparently contributed by the capped *ter1-Acc* or *ter1-Bsi* parent, respectively). This contrasts with the situation in the corresponding heterozygous *TER1/ter1-Acc* or *TER1/Bsi* strains, which have intermediate telomere sizes and structure, as seen in Figures 1 and 3. Telomere sizes remained stable through 10 restreaks (~250 cell divisions; our unpublished results).

The ability of these strains to go through meiosis was tested in two independent crosses of each strain. Sporulation efficiency and spore viability were scored and compared with the original *ter1* strains. As seen in Table 4, despite the fact that telomeres of the two sets of chromosomes remained very different in size, average sporulation efficiency (59% in *TER1/ter1-Bcl ex Acc* and 50% in *TER1/ter1-Bcl ex Bsi*) and spore viability (98% in *TER1/ter1-Bcl ex Acc* and 98.5% in *TER1/ter1-Bcl ex Bsi*) were significantly improved relative to their values in the original *ter1* strains, reaching values comparable with those observed in *TER1/TER1* strains. We conclude that telomere size heterogeneity is compatible with normal meiosis and cannot account for the meiotic defects observed in *ter1* mutants.

DISCUSSION

Many aspects of telomere function and metabolism are still largely unknown. The rapid advances in telomere research

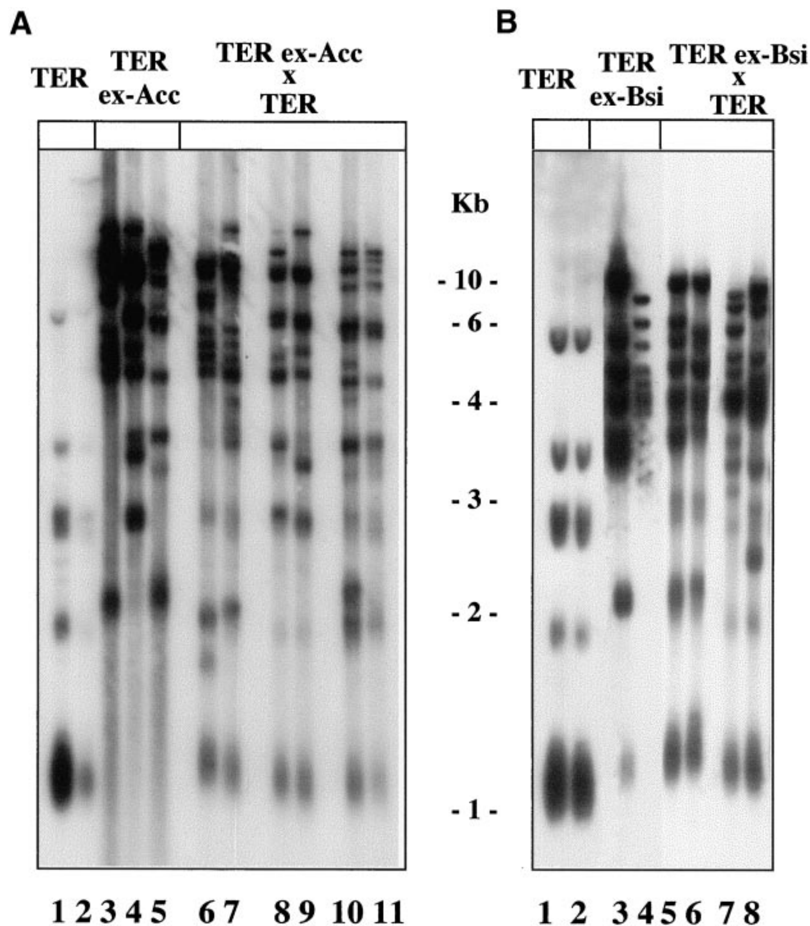

Figure 6. Telomere phenotype of *ter1-Acc*-capped– or *ter1-Bsi*–capped strains mated with *TER1*. (A) Genomic DNA from the strains indicated above the lanes was digested with *Eco*RI and resolved on a gel. Gels were blotted and probed with a telomeric wild-type probe. Lanes 1 and 2, *TER1*; lanes 3–5, spore products from *Acc/Acc* strains capped with *ter1-Bcl*, which retained the *ter1-Bcl* allele (TER1 ex-Acc); lanes 6 and7, spore clone shown in lane 3 mated with *TER1*; lanes 8 and 9, spore clone shown in lane 4 mated with *TER1*; and lanes 10 and 11, spore clone shown in lane 5, mated with *TER1*. (B) Genomic DNA from the strains indicated above the lanes was digested with *Eco*RI and resolved on a gel. Gels were blotted and probed with a telomeric wild-type probe. Lanes 1 and 2, *TER1*; lanes 3 and 4, spore products from *Bsi/Bsi* strains capped with *ter1-Bcl*, which retained the *ter1-Bcl* allele (TER1 ex-Bsi); lanes 5 and 6, spore clone shown in lane 3 mated with *TER1*; and lanes 7 and 8, spore clone shown in lane 4 mated with *TER1*.

during recent years have repeatedly demonstrated that telomeres perform complex, and sometimes unexpected, cellular functions. One of these functions may be their involvement in the meiotic process. The bouquet structure conserved among many organisms has led researchers to hypothesize that the ends of homologous chromosomes have an active role in spatially facilitating their pairing. More recently, this hypothesis was strengthened by genetic studies performed in mutants of telomere-associated proteins.

Table 4. Effect of heterogeneity in telomere length of homologous chromosomes on sporulation efficiency and spore viability

Cross	Sporulation efficiency (%)	Spore viability (%)	No. of tetrads dissected
TER1/TER1	60	95	42
TER1/ter1-Bcl ex-Bsi	49.5	98.5	113
	(53, 46)	(99, 98)	(47, 66)
TER1/ter1-Bcl ex-Acc	59	98	96
	(56, 56, 68)	(98, 98, 98)	(32, 32, 32)

Two independent spore clones resulting from sporulation of *ter1-Bcl/ter1-Bsi* (ter1-Bcl ex-Bsi) and three independent spore products resulting from sporulation of *ter1-Bcl/ter1-Acc* (ter1-Bcl exAcc) were mated with *TER1* and induced to sporulate. Sporulation efficiency was determined by counting the fraction of cells forming asci after 4–5 d in sporulation medium. Spore viability was determined by tetrad dissection. Average results are presented in bold and results obtained from each independent clone is presented in parentheses.

We set out to address the question of the role of telomeres in meiosis by investigating the meiotic behavior of telomeres with defined alterations in telomere size and structure. The experimental system used, the budding yeast, *K. lactis*, a close relative of the widely studied *S. cerevisiae*, combines the advantages of yeast as a well-characterized genetic system for the study of meiosis with considerable specific advantages for telomere study and manipulation.

Timing of Telomeric Involvement in Meiosis

The two *ter1* mutations we analyzed unambiguously caused a defective meiotic phenotype, thus providing direct evidence for a meiotic role for telomeres. First, the kinetics of progress of *Acc/Acc* cells through the meiotic process showed that *Acc/Acc* cells when induced to sporulate, like wild-type cells, initiate meiosis by going through one round of DNA replication. However, most *Acc/Acc* cells fail to proceed further to complete meiotic division I as indicated

by the fact that they remain with one nucleus. The minority of cells that do proceed beyond this stage continue through the two divisions to the completion of meiosis II with the same kinetics as wild-type cells. However, only 22% of their progeny are viable.

Telomeres may play an active role in meiosis such as ensuring proper segregation of chromosomes. In this case, it is possible that *Acc/Acc* cells that proceed beyond DNA synthesis encounter a catastrophe due to their inability to carry out this role properly. According to this "active" model, escapers, which somehow manage to proceed beyond meiosis I, are expected to give rise to aberrant products. Alternatively, telomeres might be envisioned to play a passive role in meiosis, such as being monitored by a meiotic checkpoint apparatus. In this case, after DNA replication, telomeres with Acc repeats may be perceived as abnormal and elicit a checkpoint signal to arrest progression beyond this stage. According to this "passive" model, escapers, which somehow evade the checkpoint surveillance system, are expected to give rise to normally viable products. Therefore, the fact that the few *Acc/Acc* cells that did manage to complete meiosis and produce tetrads gave rise to inviable spores, supports the active model. However, we cannot exclude the possibility that telomeres play a dual role, that of being monitored by a checkpoint apparatus and later on a more active role.

Telomeric Element Essential to Meiosis

What properties of *ter1* telomeres can make them incapable of fulfilling their normal meiotic role, according to a model of active involvement? Telomeres of *ter1* cells are abnormal in at least three respects: mean length, size uniformity, and Rap1p binding potential.

Excessive Telomere Length. Conceivably, excessive telomere length could mechanically hinder a function such as physical facilitation of homologous chromosome alignment. For example, a cluster of abnormally long telomeres may not be able to support chromosomal movements properly. This model is not supported by our results: *ter1-Bsi* telomeres are as long as *ter1-Acc* telomeres, yet the meiotic phenotype of *ter1-Bsi* is much less severe. Moreover, upon capping of *Acc/Acc* or *Bsi/Bsi*, telomeres remain very long, whereas their meiotic defect is completely suppressed.

Heterogeneity of Telomere Lengths. Wild-type *K. lactis* telomeres, as is the case in most organisms, are more or less uniform in length in a single cell. Theoretically, this uniformity may be necessary to carry out an active role in meiosis. For example, telomere-mediated chromosome alignment could be impaired if telomeres of homologous chromosomes were not the same length. To assess the effect of heterogeneity between two individual telomeres of homologs in the same cell, we constructed diploid strains containing one haploid set of chromosomes with normal-sized telomeres, and the other haploid set with very long telomeres. The fact that sporulation efficiency and spore viability were completely normal in these strains excludes the possibility that telomere size heterogeneity between clonal homologs per se impairs the meiotic function of telomeres.

Decreased Rap1p Binding Potential of the Entire Telomere. Rap1p itself may be the active mediator of telomeric involvement in meiosis. For example, it may interact with other proteins to initiate and maintain the bouquet formation. Taz1p and the recently reported *S. pombe* homolog of Rap1p are both required for proper meiosis and specifically for the bouquet-reminiscent "horsetail" movement (Cooper *et al.*, 1998; Hiraoka, 1998; Nimmo *et al.*, 1998). It was not shown, however, whether the factor directly active in *S. pombe* meiosis was the protein itself, or the telomeric structure that depends upon its regulatory activity.

Relatively very few Rap1p molecules are expected to be bound to the telomeric repeats in *ter1* strains. Therefore, it was conceivable that their number may be too small to fulfill a putative active role in meiosis. To test this model, we capped *ter1-Bsi* and *ter1-Acc* telomeres with a few Rap1p binding repeats. On capping, both meiotic parameters, sporulation efficiency and spore viability, were completely rescued. As shown by specific restriction digestion, the entire length of the telomeres contained mostly *Bsi* or *Acc* repeats in the respective strains. Therefore, in the capped strains, meiosis was normal despite the fact that most of the telomeric repeats within a given telomere had decreased Rap1p binding capability. Moreover, upon capping, no differences could be detected between *Acc/Acc* and *Bsi/Bsi* despite the fact that *Bsi* telomeric repeats are able to bind Rap1p 3 times better than *Acc* repeats. This further supports the conclusion that differences in Rap1p binding throughout the entire telomeric length are insignificant for the meiotic process.

Loss of Capping at Telomeres. A putative active role of telomeres may require their "cap" structure to be intact. Here we refer to capping in its most general form: a functional structure at the very end of the telomere. We have previously shown that telomere length regulation is particularly dependent on the most distal double-stranded repeats of the telomere. In view of their special importance for telomere functions in mitotic cells, the most distal telomeric repeats may also be important for carrying out a putative meiotic role. In *ter1* mutants the distal repeats are altered and the degree of the loss of Rap1p binding to the mutated repeats correlated with the severity of the meiotic phenotype of these mutants. Therefore, we speculated that this might underlie their meiotic phenotypes. The capping experiments referred to above show unambiguously that it is the impairment of this terminal cap structure that is responsible for the severe meiotic defects exhibited by *ter1* mutants. The fact that meiosis was not impaired in the heterozygote strains whose telomeres were capped further stresses the importance of the terminal cap for meiosis.

Although the mutations tested were single base substitutions within the Rap1p binding domain, the possibility that it is the impairment in binding of a telomere binding factor other than Rap1p that accounted for the observed meiotic defects in ter1 mutants, cannot be ruled out.

Role of Telomeric Cap Structure in Meiosis

What is the role of the cap structure in meiosis? As mentioned above, normal telomeres are required for passage through the first meiotic division and for viable spore pro-

duction. Because capping of mutant telomeres suppresses both phenotypes, we conclude that it is the very distal repeats that carry out those two putative functions, although not necessarily through the same mechanism.

Recently, it was shown that in the absence of the telomeric-associated meiosis-specific protein Ndj1p, telomeres are scattered throughout the nucleus and fail to form the perinuclear meiosis-specific distribution pattern characteristic of this stage. Because Rap1p and Ndj1p show extensive colocalization in pachytene nuclei (Chua and Roeder 1997.), it is possible that Ndj1p may function together with Rap1p to tether meiotic telomeres to the nuclear periphery (Trelles-Sticken *et al.*, 2000).

Evidence from *S. pombe* showed that the pairing of homologous chromosomes is impaired in several mutants that are defective in telomere clustering at the SPB. A mutant defective in kms1, a component of the SPB that functions specifically in meiosis, failed to form a telomere cluster due to the disintegration of the SPB structure, and exhibited a reduced rate of meiotic recombination (Shimanuki *et al.*, 1997). As mentioned above, in mutants of the telomere binding protein taz1, telomeres fail to cluster at the SPB during meiotic prophase, causing severe meiotic phenotype. Recently, it has been shown that telomeres of rodent spermatocytes are associated with the same telomere binding proteins that are associated with them in somatic cells (mouse Trf1, rat TRF2, and Rap1 at meiotic telomeres of both rodents) (Scherthan *et al.*, 2000)

Taken together, it is possible that in yeast, a telomere binding protein, most likely Rap1p, which is bound to the very end of the telomere, is able to anchor chromosomes by interacting with a meiosis-specific telomere binding protein(s), perhaps Ndj1p or other associated proteins. This anchorage may be essential to facilitate telomere localization and the unique chromosomal movements observed during meiosis.

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