

Meiotic telomere clustering requires actin for its formation and cohesin for its resolution

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In diploid organisms, meiosis reduces the chromosome number by half during the formation of haploid gametes. During meiotic prophase, telomeres transiently cluster at a limited sector of the nuclear envelope (bouquet stage) near the spindle pole body (SPB). Cohesin is a multisubunit complex that contributes to chromosome segregation in meiosis I and II divisions. In yeast meiosis, deficiency for Rec8 cohesin subunit induces telomere clustering to persist, whereas telomere cluster–SPB colocalization is defective. These defects are rescued by ex-

pressing the mitotic cohesin Scc1 in *rec8Δ* meiosis, whereas bouquet-stage exit is independent of Cdc5 pololike kinase. An analysis of living *Saccharomyces cerevisiae* meicytes revealed highly mobile telomeres from leptotene up to pachytene, with telomeres experiencing an actin- but not microtubule-dependent constraint of mobility during the bouquet stage. Our results suggest that cohesin is required for exit from actin polymerization-dependent telomere clustering and for linking the SPB to the telomere cluster in synaptic meiosis.

Introduction

The formation of haploid cells from diploid progenitors requires a tightly regulated series of differentiation steps. In meiosis, homologous chromosomes (homologues) undergo pairing and crossing over, which are prerequisites for the reductional segregation that compensates for genome doubling at fertilization. Specialized meiotic cohesin complexes are assembled along meiotic chromosomes and underlie the axial elements (AEs) of the synaptonemal complex (SC; for reviews see Jessberger, 2002; Page and Hawley, 2003). AEs consist of Rec8-containing meiotic cohesin complexes (Rec8 replaces mitotic Scc1/Mcd1/Rad21), STAG3 (instead of mitotic Scc3/SA1 or SA2; Klein et al., 1999; Watanabe and Nurse, 1999; Pezzi et al., 2000; Prieto et al., 2001, 2002; Siomos et al., 2001; James et al., 2002; Eijpe et al., 2003; Molnar et al., 2003; Pasierbek et al., 2003; Couteau et al., 2004), condensin (Yu and Koshland, 2003), and specific AE proteins such as mammalian SCP3 and SCP2 (Lammers et al., 1994; Offenberger et al., 1998; Yuan et al., 2000), or *Caenorhabditis elegans* Him3 (Zetka et al., 1999), *Arabidopsis*

thaliana and *Brassica oleracea* Asy1 (Armstrong et al., 2002), or *Saccharomyces cerevisiae* Red1 (Smith and Roeder, 1997).

The induction of meiosis causes telomeres to attach to the inner nuclear membrane. Once attached, they move along the inner nuclear envelope and transiently cluster in a minimal time window at the leptotene–zygotene transition to form a so-called chromosomal bouquet, which is highly conserved among eukaryotes (for reviews see Zickler and Kleckner, 1998; Scherthan, 2001). In leptotene, the transesterase Spo11 generates DNA double strand breaks (DSBs; Bergerat et al., 1997; Keeney et al., 1997; Mahadevaiah et al., 2001) that are instrumental in homology searching and in initiating crossing over (for review see Keeney, 2001; Lichten, 2001). The extent of homologue pairing increases during zygotene and is fortified by the SC, a ribbon-like structure that forms between homologues and regulates crossing over (von Wettstein et al., 1984; Bishop and Zickler, 2004; Page and Hawley, 2004). During diplotene, the nuclear envelope disintegrates, chromosomes condense, and sister kinetochores attach as a single unit to microtubules (MTs) from the same spindle pole in metaphase I (monopolar attachment). The subsequent release of arm cohesion mediates reductional segregation in the first meiotic division (for reviews see Page and Hawley, 2003; Petronczki et al., 2003).

The existence of a temporal overlap between homologue pairing and telomere clustering has led to the suggestion that telomere clustering supports homologue pairing (Loidl, 1990;

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Abbreviations used in this paper: AE, axial element; DSB, DNA double strand break; Lat B, latrunculin B; MT, microtubule; SC, synaptonemal complex; SPB, spindle pole body.

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Dernburg et al., 1995). Indeed, in the absence of telomere clustering in budding and fission yeast, homologue pairing is significantly delayed or perturbed (Cooper et al., 1998; Nimmo et al., 1998; Trelles-Sticken et al., 2000; Ding et al., 2004).

In vegetative cells of budding yeast, telomeres localize to a few clusters at the periphery of the nucleus (Klein et al., 1992; Gotta et al., 1996). After induction of meiosis, yeast telomeres disperse over the nuclear periphery and subsequently cluster at the spindle pole body (SPB; the centrosome equivalent of yeast; Trelles-Sticken et al., 1999). The meiotic telomere protein Ndj1/Tam1 (Chua and Roeder, 1997; Conrad et al., 1997), but not Spo11, Kar3, or Sir3, is required for telomere attachment and clustering (Trelles-Sticken et al., 2000, 2003). In asynaptic prophase I fission yeast, telomere clustering is regulated by the mating pheromone signaling pathway (Chikashige et al., 1997; Yamamoto et al., 2004). Telomere proteins Taz1 and Rap1 contribute to attachment and clustering (Cooper et al., 1998; Nimmo et al., 1998; Chikashige and Hiraoka, 2001; Kanoh and Ishikawa, 2001), which is maintained during much of prophase I when the nucleus undergoes sweeping movements (Chikashige et al., 1994). In the maize *pam1* mutant and in rye meocytes treated with the MT drug colchicine, telomere clustering fails (Cowan and Cande, 2002; Golubovskaya et al., 2002). However, telomere clustering in plants and fission yeast does not require cytoplasmic MTs (Chikashige et al., 1994; Ding et al., 1998; Cowan and Cande, 2002).

Because little is known about the requirements for and dynamics of telomere movements in species with synaptic meiosis, we probed the role of the cytoskeleton and cohesin in this process and investigated meiotic telomere clustering in live and fixed budding yeast meocytes.

Results

Meiotic telomere mobility is constrained at the bouquet stage

To investigate telomere dynamics in living meocytes, we created *S. cerevisiae* SK1 strains in which the endogenous Rap1 protein was replaced with a GFP-tagged version of the protein (see Materials and methods and Table S1, available at <http://www.jcb.org/cgi/content/full/jcb.200501042/DC1>). Rap1 is telomere associated in vegetative and meiotic cells (Longtine et al., 1989; Klein et al., 1992). This allowed us to track the general behavior of telomeres in live cells by using an approach similar to that of Hayashi et al. (1998). Rap1-GFP strains underwent meiotic divisions (as measured by the appearance of bi- and tetranucleate cells) with wild-type kinetics (unpublished data). We obtained a series of time-lapse images from individual live cells (see Materials and methods) and recorded Rap1-GFP signal dynamics over time periods of 7 or 44 min in prophase I. Nuclei were identified by diffuse GFP fluorescence and were staged by the alignment of observed telomeric Rap1-GFP signal distribution patterns with substage-specific patterns that were previously established by telomere FISH and Zip1 immunostaining. For instance, wild-type meocyte nuclei with a single telomere cluster were identified as being zygotene nuclei, as bouquet formation occurs during this substage of yeast meiosis (Trelles-Sticken et

al., 1999). Because small Rap1-GFP signals faded rapidly, we recorded a series of time-lapse images (at 0.5- and 5-s intervals) at a fixed focal plane and limited the analysis to the time period during which signals could be traced. This approach was also used successfully in a previous study (Heun et al., 2001). Taking images that were spaced apart by minutes or hours was not feasible for recording meiotic telomere dynamics/clustering in wild-type yeast meiosis (Hayashi et al., 1998).

Telomeres in living vegetative (premeiotic) cells were grossly immobile, as seen in videos lasting 7 (not depicted) and 44 min (Video 1, available at <http://www.jcb.org/cgi/content/full/jcb.200501042/DC1>), which is consistent with previous observations (Heun et al., 2001). The few vegetative perinuclear telomere signals underwent locally restricted movements (Fig. 1 A) with a mean velocity of $9.5 \pm 3.07 \mu\text{m}/\text{min}$ ($n = 29$ trajectories), which was determined by trajectory analysis (i.e., particle tracking; see Materials and methods) that revealed the velocity of telomere spot movement (Fig. 1 B). Meocytes arrested in premeiotic S-phase by hydroxyurea treatment also exhibited only a few peripheral telomere signal clusters that were similar to those of premeiotic cells (unpublished data), which, in agreement with previous FISH analysis (Trelles-Sticken et al., 1999, 2000), suggests that the resolution of peripheral vegetative telomere clusters occurs at the end of or shortly after premeiotic S-phase.

Entry into prophase I leads to a rimlike telomere distribution (Trelles-Sticken et al., 1999, 2000). Leptotene meocytes with a rimlike Rap1-GFP telomere distribution were also detected by live cell imaging and were characterized by rapidly moving, usually dim, and small perinuclear GFP telomere signals in videos that spanned 7 and 44 min (Fig. 1 A; and Video 2, available at <http://www.jcb.org/cgi/content/full/jcb.200501042/DC1>). Leptotene Rap1-GFP signals moved with a mean velocity of $12.6 \pm 3.89 \mu\text{m}/\text{min}$ ($n = 19$ trajectories; Fig. 1 B). The low intensity of individual Rap1-GFP telomere signals is likely to be a bleaching effect (Hayashi et al., 1998), which prevented the recording of videos with >500 images. The few, occasionally seen strong leptotene Rap1-GFP signals suggest that telomeres form miniclusters before congregating into the large cluster of the bouquet stage, which seems to be the case in plants (Carlton et al., 2003).

In the subsequent bouquet stage, telomere movements were restricted to a limited area of the nuclear periphery (Fig. 1 A and Video 3, available at <http://www.jcb.org/cgi/content/full/jcb.200501042/DC1>), which, in fixed cells, is represented by a single telomere signal at the SPB (Trelles-Sticken et al., 1999). In videos with frame rates of 0.5 s, telomeres moved in a restricted area of the nuclear periphery (Video 3). The meiotic telomere cluster repeatedly split and reformed, which indicates that telomeres are motile during this stage as well. The mean velocity of telomere GFP signals was $12.5 \pm 4.48 \mu\text{m}/\text{min}$ ($n = 20$ trajectories), which is similar to the mean velocity of leptotene telomeres (Fig. 1 B). It was not possible to record the formation of a tight telomere cluster in videos with 5-s exposure intervals, which underlines the transient nature of the SK1 bouquet stage and is consistent with earlier observations (Hayashi et al., 1998). A single, large telomere cluster in a favorable SK1 meocyte was observed to persist for ~ 30 s before it resolved

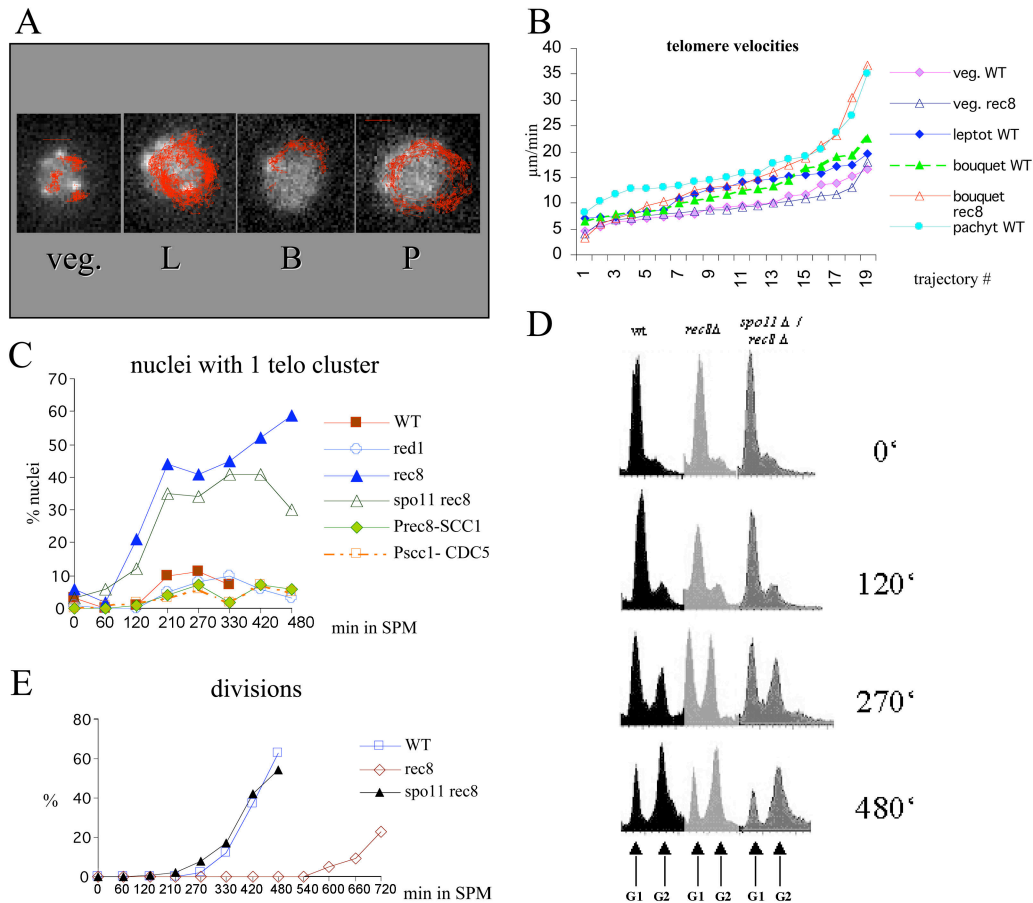


Figure 1. Meiotic telomere dynamics and exit from the bouquet stage require functional cohesin. (A) Projections of trajectories (red threads) of tracked Rap1-GFP telomere spots from a time-lapse image series displayed over an image frame of the respective nucleus. Vegetative telomere clusters (veg.) displaying locally accumulated trajectory projections as a result of constrained motility range. Leptotene telomeres (L) move across the nuclear periphery, which leads to a web of trajectories around the nucleus. Bouquet nucleus (B) with trajectories confined to a limited sector of the nuclear periphery, indicative of telomere clustering. Pachytene nucleus (P) with trajectories around the nucleus, consistent with unconstrained, peripheral telomere movements around the nucleus. Bar, 1 μm . (B) Cumulative plot displaying the velocities of single telomere signals derived from movements of tracked GFP-Rap1 signals in an image series captured every 0.5 s during 7 min. Each trajectory represents a traceable telomere signal through an image series (focal plane fixed at nuclear equator). The trajectories are ranked according to speed. It is evident that in the wild type, vegetative telomere movements are slower than leptotene/bouquet movements, whereas pachytene telomere movements are the most rapid ones. In *rec8* cells, vegetative telomere velocities match those of wild type, whereas telomere velocities in bouquet-like *rec8*Δ meiotic cells are variable. (C) Analysis of bouquet frequencies by telomere FISH to meiotic time courses. The wild-type frequency of nuclei with a single telomere cluster reaches a maximum after 210 min in sporulation medium (SPM). Wild-type time courses were shorter because meiotic divisions appeared after 270 min and rendered FISH patterns difficult to interpret. *rec8*Δ and *rec8*Δ *spo11*Δ nuclei maintain a telomere cluster up to meiosis I division (compare C with E). Telomere cluster frequency drops after 420 min in *rec8*Δ *spo11*Δ meiosis when meiotic divisions become abundant (E). Meiotic expression of the mitotic cohesin SCC1 ($P_{\text{rec8}}\text{-SCC1}$) restores bouquet-stage exit in a *rec8*Δ background, leading to low frequencies of nuclei with a single telomere cluster. Reduction of meiotic CDC5 expression by SCC1 promoter control in *REC8* meiosis ($P_{\text{sccl}}\text{-CDC5}$) does not increase telomere clustering. A delay in bouquet formation is evident in *red1*Δ, $P_{\text{rec8}}\text{-SCC1}$, and $P_{\text{sccl}}\text{-CDC5}$ meiosis. (D) Development of FACS profiles in wild-type (WT), *rec8*Δ, and *rec8*Δ *spo11*Δ sporulation indicates that all strains pass through S-phase. G1/2, cell-cycle stage gap 1/2. (E) Timing of meiotic divisions in wild-type, *rec8*Δ, and *rec8*Δ *spo11*Δ strains derived by scoring bi- and tetranucleate cells (100–200 per time point). Divisions were significantly delayed in *rec8*Δ meiosis and occurred at a maximum of 20% (even in longer time courses; not depicted), whereas the *spo11*Δ mutation eliminated the DSB-dependent prophase I arrest.

and formed again (Video 3), suggesting that telomeres in the bouquet stage are highly mobile and that several telomeres move together. During a longer time period (i.e., in the range of minutes), a single telomere cluster may repeatedly dissolve and reform, making a more detailed analysis of telomere dynamics during this time period impossible. To overcome this drawback of the fast-sporulating SK1 strain, we searched for a mutant with enduring telomere clustering (see next section).

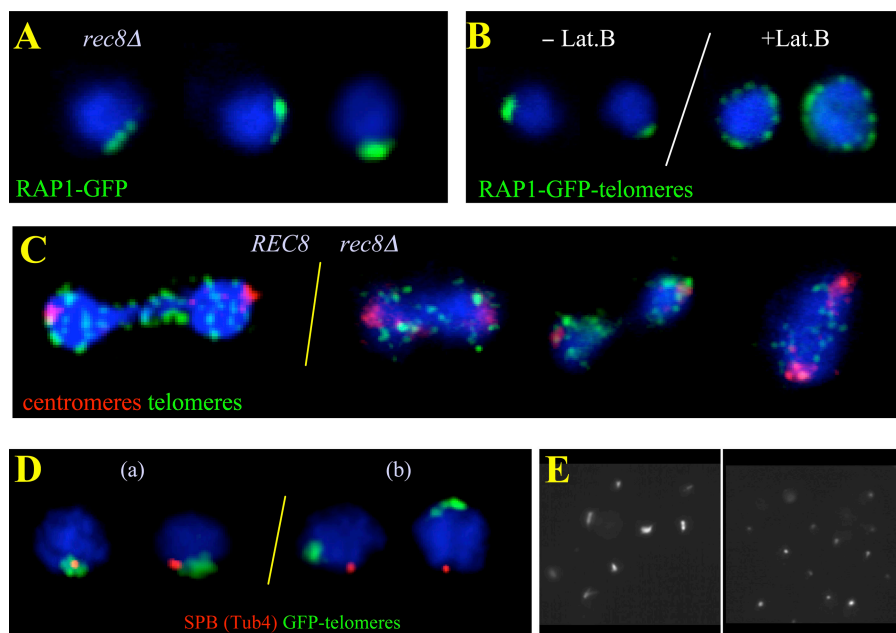
In postbouquet pachytene cells, GFP telomeres underwent rapidly oscillating movements over the entire nuclear periphery (Fig. 1 A) and had a mean velocity of $16.3 \pm 5.66 \mu\text{m}/\text{min}$

($n = 31$ trajectories). Rapid telomere movements (Fig. 1 B) were accompanied by oscillating nuclear deformations (Video 4, available at <http://www.jcb.org/cgi/content/full/jcb.200501042/DC1>). Peripherally dispersed telomere FISH signals have also been noted in fixed pachytene meiotic cells (Trelles-Sticken et al., 1999).

Rec8 deletion induces persistent clustering

To analyze the molecular requirements of meiotic telomere dynamics in more detail, we used FISH with pantelomeric and

Figure 2. Telomere clustering in *rec8Δ* meiosis is displaced from the SPB and requires actin polymerization. (A) Rap1-GFP-tagged telomeres (green) in *rec8Δ* meiocytes display one telomere cluster. (B) *rec8Δ* meiocyte nuclei before (–Lat B) and after (+Lat B) inhibition of actin polymerization by Lat B treatment, which leads to peripherally dispersed telomeres. (C) Anaphase I figures of wild-type (left) and *rec8Δ* meiocytes (right) show absence of a telomere cluster. Centromeres (red) lead the anaphase movements, whereas telomeres (green) are seen as numerous spots trailing behind. (D) Spatial relationships of the telomere cluster (green, Rap1-GFP) and SPB (red, Tub4) in *rec8Δ* meiocytes. Class a nuclei depict abundant patterns of telomere cluster–SPB association in wild-type meiosis. In *rec8Δ* meiosis, the telomere cluster is often separated from the SPB (class b; Table I). (E) Image field showing tubulin staining (FITC channel, gray scale) of cells from meiotic cultures without (left) and with (right) benomyl and nocodazole treatment. The treated cells display spots as a result of residual SPB staining that is resistant to MT drugs (Hasek et al., 1987; Lillie and Brown, 1998).



pancentromeric probes (telomere/centromere FISH; Trelles-Sticken et al., 2000) and screened for mutants with enduring telomere clustering. FISH analysis was first performed in mutants that lack proteins implied in perinuclear telomere location in vegetative budding yeast cells, such as Mlp1 and -2 nuclear pore extensions (Strambio-de-Castillia et al., 1999; Galy et al., 2000) and the Hdf1 (Ku70 in mammals) nonhomologous end-joining repair protein that is required for perinuclear tethering of vegetative telomeres (Laroche et al., 1998).

Telomere FISH revealed transient telomere clustering in *mlp1Δ*, *mlp2Δ*, and *hdf1Δ* meioses, which occurred with a delay in the latter mutant (unpublished data). In contrast, telomere clustering persisted in most prophase I cells lacking the meiotic cohesin Rec8 (Fig. 1 C and see Fig. 2 A), whereas the wild-type SK1 strain showed a maximum of 11% bouquet nuclei ($n = 100$) 270 min after the induction of meiosis (Fig. 1 C).

Rec8 is required for sister chromatid cohesion, recombination, AE/SC formation, and homologue segregation (Klein et al., 1999). Flow cytometry of our *rec8Δ* strain showed that it passed through premeiotic S-phase (Fig. 1 D). Centromere clustering was resolved with wild-type kinetics after induction of *rec8Δ* meiosis (see Fig. 3 A). In agreement with previous data (Klein et al., 1999), our *rec8Δ* strains underwent meiotic divisions, but these were greatly delayed (Fig. 1 E), and bi- and tetranucleate cells never exceeded 23% in prolonged time courses. After 24 h, only 22% of cells sporulated (vs. 94% in wild-type cells), as assayed by the formation of asci with one or more spores (>100 cells analyzed for each experiment). Altogether, these observations suggest that most *rec8Δ* meiocytes arrest in prophase I, most likely in an aberrant zygotene-like stage as DSBs are formed (Klein et al., 1999) and telomere clustering persists.

Most live *rec8Δ* Rap1-GFP meiocytes displayed persistent telomere clustering after 7 h in sporulation medium. In these bouquet-like meiocytes, telomeres formed a few mini-clusters that constantly underwent restricted movements within

a limited region of the nuclear periphery (Video 5, available at <http://www.jcb.org/cgi/content/full/jcb.200501042/DC1>). Recurrent reformation of a single, tight telomere cluster in bouquet-like *rec8Δ* meiocytes mirrored observations in the short-lived bouquet stage of wild-type SK1 meiocytes (Fig. 1 A and Video 3) and in meiosis of a W303 strain (not depicted). Most *rec8Δ* cells still displayed dynamic telomere clustering even 24 h after the induction of meiosis (unpublished data).

Trajectory analysis revealed that telomeres in premeiotic *rec8Δ* cells undergo locally restricted movements with a mean velocity of $9.2 \pm 2.6 \mu\text{m}/\text{min}$ ($n = 82$ trajectories), which is similar to the velocities observed in wild-type cells (Fig. 1 B), thus suggesting that the absence of Rec8 has no consequences for premeiotic telomere motility. In bouquet-like *rec8Δ* meiocytes, telomeres underwent short-range movements at the cluster site and had a mean velocity of $14.4 \mu\text{m}/\text{min}$ and a maximum velocity of $\leq 36.6 \mu\text{m}/\text{min}$ ($n = 123$ trajectories), which is almost as rapid as wild-type pachytene telomeres (mean velocity of $16.3 \mu\text{m}/\text{min}$ and maximum velocity of $35.1 \mu\text{m}/\text{min}$).

Telomere FISH time course analysis of *rec8Δ* meiosis revealed that 69% of nuclei ($n = 100$) had a single telomere cluster even 480 min after the induction of meiosis. After 10 h in sporulation medium, we observed a few *rec8Δ* meiocytes that had escaped prophase I arrest and were beginning to undergo divisions (Fig. 1 E). In such early anaphase I cells, telomere clustering had been dissolved (Fig. 2 C), which suggests that cells that escape the *rec8Δ* arrest resolve telomere clustering before entry into meiosis I. This makes it unlikely that the division itself disrupts the meiotic telomere cluster.

***rec8Δ*-dependent telomere clustering is maintained in the absence of DSBs**

Defects in recombination elicit an arrest in prophase I (Roeder and Bailis, 2000) and can lead to an extended duration of telo-

mere clustering (Scherthan, 2003; Storlazzi et al., 2003). To determine whether *rec8*Δ-dependent telomere cluster maintenance is altered when DSBs are absent, we investigated *rec8*Δ*spo11*Δ meiosis, which lacks DSBs as a result of the absence of the Spo11 trans-esterase (Bergerat et al., 1997; Keeney et al., 1997). In meiotic time courses, the frequency of *rec8*Δ *spo11*Δ bouquet-like cells was similar to *rec8*Δ meiosis but was reduced at late time points (Fig. 1 C). The frequency of telomere clustering in *rec8*Δ *spo11*Δ meiosis (41% at 330 min) was about twice as high as in the *spo11*Δ single mutant (Trelles-Sticken et al., 1999), suggesting that Rec8 is epistatic over Spo11 on telomere cluster maintenance. The *rec8*Δ *spo11*Δ strain underwent meiotic divisions with wild-type kinetics (Fig. 1 E). As DSBs are not repaired in *rec8*Δ meiosis (Klein et al., 1999), our data suggest that the prophase I arrest in *rec8*Δ meiosis is DSB dependent but that *rec8*Δ-promoted telomere cluster maintenance does not require DSBs.

Cohesin is required for telomere cluster-SPB colocalization

To assess whether the compromised assembly of AEs underlies persistent telomere clustering in *rec8*Δ meiosis, we investigated *red1*Δ meiosis, which lacks AEs (Smith and Roeder, 1997) and has defective recombination and significantly reduced homologue pairing (Rockmill and Roeder, 1990; Nag et al., 1995). *red1*Δ meiosis displayed delayed, but wild-type, telomere clustering frequencies with a maximum of 10% *red1*Δ bouquets at 330 min, whereas 10% wild-type bouquets were seen at 210 min ($n \geq 100$ in both strains; Fig. 1 C). Thus, defective AE formation in yeast does not lead to increased levels of telomere clustering.

Homology searching and pairing in yeast appears to be DSB dependent (Loidl et al., 1994; Weiner and Kleckner, 1994; Lichten, 2001). DSBs form but are hyperresected in *rec8*Δ meiosis (Klein et al., 1999). When we evaluated homologue pairing in *rec8*Δ meiosis by FISH with cosmids homologous to the chromosome III left telomere and an interstitial site on chromosome XI (Trelles-Sticken et al., 2000), we found that meiosis-specific pairing never installed in *rec8*Δ or in *rec8*Δ *spo11*Δ meioses (see Fig. 3 B and not depicted), which is consistent with synaptic defects in both *spo11*Δ and *rec8*Δ strains (Loidl et al., 1994; Klein et al., 1999). This indicates that even extensive telomere clustering cannot ameliorate the pairing defects that are caused by the absence of cohesin and axial cores.

Telomere cluster-SPB colocalization is considered a hallmark of the bouquet stage in budding yeast (Trelles-Sticken et al., 1999) and other organisms (Zickler and Kleckner, 1998; Bass, 2003). To determine whether this colocalization is affected by the absence of cohesin, the relative positioning of the telomere cluster and SPB was investigated by Rap1-GFP and tubulin/SPB immunostaining in *rec8*Δ meiosis. In the wild type, 68% of the cells displayed telomere cluster-SPB colocalization (Fig. 2 D, Table I, and see Fig. 3 C) 210 min after the induction of meiosis. This was significantly different in *rec8*Δ meiosis, in which only 23% of meiosis nuclei displayed this colocalization (Fig. 2 D, Table I, and see Fig. 3 C). As telomere cluster-SPB association was absent in 32% of wild-type meio-

Table I. **Telomere cluster-SPB colocalization**

	<i>n</i>	Overlap/ Touching (A)	Separated (B)
<i>rec8</i> Δ 210'	100	23	77
<i>rec8</i> Δ 330'	100	30	70
<i>rec8</i> Δ 480'	100	11	89
WT	60	68	32

Categories A and B as depicted in Fig. 2 D.

cytes, it could be that the telomere cluster first forms away from the SPB and becomes positioned at this location at a later time point. Alternatively, telomere cluster-SPB colocalization may be released before telomeres disperse from the cluster site. Because the single telomere cluster continuously splits into a few closely spaced miniclusters and reforms, it may be that Rec8, cohesin, and some yet unidentified cofactors may help to tether mobile telomeres to the SPB region.

Telomere cluster resolution depends on cohesin

To test whether cohesin or the Rec8 protein, by itself, is crucial for the maintenance of telomere clustering in the *rec8*Δ mutant, we studied telomere behavior in a strain that expressed the mitotic cohesin component Scc1 from the meiotic Rec8 promoter (hereafter designated P_{rec8} -*SCC1*; Toth et al., 2000). We found that the expression of Scc1 in P_{rec8} -*SCC1* meiosis (confirmed by Scc1-HA immunofluorescence; not depicted) restored both telomere cluster-SPB association (Fig. 3 C) and the timely resolution of the telomere cluster (Fig. 1 C). Moreover, the pairing defect in *rec8*Δ meiosis was restored by meiotic *SCC1* expression, albeit with a delay for the interstitial region of chromosome XI (Fig. 3 B). Restoration of pairing in P_{rec8} -*SCC1* meiosis suggests that the DSBs formed can now promote homologous strand interactions.

Meiotic telomere clustering is not controlled by Cdc5-dependent Rec8 phosphorylation

It has been shown that late aspects of Rec8 function are regulated by Cdc5 pololike kinase (Clyne et al., 2003). Thus, the influence of Rec8 phosphorylation on telomere clustering was analyzed by telomere FISH in a strain deficient for meiotic *CDC5* expression as a result of *SCC1* promoter control of *CDC5* (P_{scc1} -*CDC5*; Clyne et al., 2003). It was found that P_{scc1} -*CDC5* meiosis displayed the same dynamics of telomere cluster formation and resolution as the P_{rec8} -*SCC1* mutant (Fig. 1 C). The wild-type-like telomere dynamics that were observed suggest that the resolution of meiotic telomere clustering is not under the control of Cdc5-dependent Rec8 phosphorylation.

Meiotic telomere clustering in yeast occurs in the presence of MT-disrupting drugs

Next, we searched for other components that are involved in meiotic telomere clustering. Wild-type and *rec8*Δ meiosis

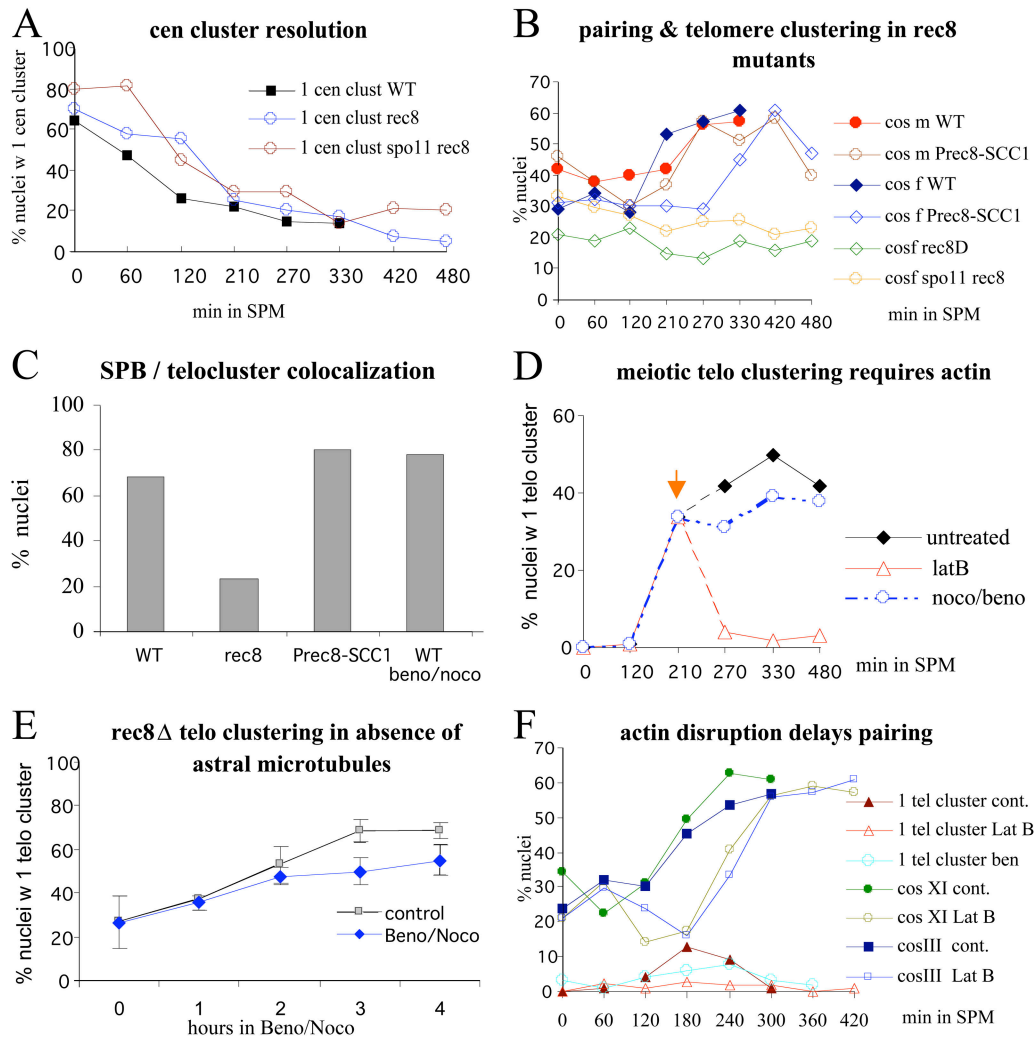


Figure 3. Cohesin and actin polymerization are required for normal telomere and chromosome dynamics in SK1 meiosis. (A) Centromere cluster resolution as measured by the frequency of nuclei with a single centromere FISH cluster (Trelles-Sticken et al. 1999). Centromere cluster resolution is similar in wild-type (WT), *rec8Δ* (*rec8*), and *rec8Δ spo11Δ* (*spo11 rec8*) time courses. (B) Cosmid FISH pairing (*cos f*, interstitial to XI; *cos m*, localizing to HML; Trelles-Sticken et al., 2000) as scored by the presence of one large FISH *cos*-signal cluster in *rec8Δ* strains. In *rec8Δ* and *rec8Δ spo11Δ* cells, this was only seen at a low level (*cos f*), and meiosis-specific pairing levels never installed with progression through sporulation. Similar values were noted for *cos m* (not depicted for clarity of display). It should be noted that up to four FISH signals were seen in *rec8Δ* meiosis, consistent with a lack of sister chromatid cohesion. A single FISH signal may relate to persistent premeiotic association and to a few *rec8Δ* cells not entering meiosis. Pairing is restored by meiotic expression of the mitotic cohesin SCC1 (P_{rec8} -SCC1). The interstitial chromosome XI region shows a delay in homologue pairing, whereas the telomere of III does not ($n > 100$ in all time points). (C) SPB colocalization with the telomere cluster in wild-type (WT; $n = 60$) cells as measured by FISH and Tub4 costaining. In *rec8Δ* meiocytes (*rec8*; $n = 100$), this colocalization is significantly reduced, whereas it is restored in cells expressing the mitotic cohesin Scc1 (*Prec8-SCC1*; $n = 100$). Telomere-SPB colocalization is not affected by the presence of benomyl/nocodazole MT drugs (*beno/noco*; $n = 60$; anti-Rap1-GFP and Tub4 costaining). (D) Time course experiments with addition of MT and actin poisons 210 min after meiosis induction (orange arrow) in *rec8Δ* SK1 meiosis. Combined nocodazole/benomyl treatment induced only a slight reduction in the frequency of bouquet-like meiocytes, consistent with results in live cells (C). However, Lat B treatment (*latB*) dissociated the telomere cluster ($n \geq 100$ nuclei were evaluated in all time points and strains). (E) Benomyl/nocodazole treatment of live *rec8Δ* Rap1-GFP meiocytes fails to eliminate telomere clustering. MT drugs were added 4 h after meiosis induction, and samples were taken before addition (0). After each hour, cells were evaluated for the presence of a single telomere cluster. Error bars represent SD of three experiments; $n = 100$. (F) Pairing of *cos f* and *m* (B) in MT-depleted (*beno*), and wild-type *REC8* (closed symbols, *cont.*) time courses as measured by FISH in actin-depleted (*Lat B*) meiosis. The frequency of cells with one telomere cluster is marked by red, open triangles. Inhibition of actin polymerization throughout sporulation prevents telomere clustering and delays homologous pairing for 1–2 h.

were treated with MT-disrupting drugs because, in plants, telomere clustering is sensitive to the MT drug colchicine (Cowan and Cande, 2002). The administration of a combination of the MT-disrupting drugs nocodazole and benomyl abolished astral MTs that were found in about one third of meiotic cells and left only residual fluorescence at the SPB (Fig. 2 E), which was noted previously (Hasek et al., 1987; Jacobs et al., 1988; Lillie and Brown, 1998). The absence of radial MTs was seen over

the course of 3 h after administration of the drugs, the time frame in which all live cell recordings were taken. However, the administration of MT drugs at different time points never eliminated telomere clustering (unpublished data). Although unlikely, the minimal MT fluorescence at the SPB could mean that some residual MTs drive telomere clustering.

Telomere FISH to MT drug-treated *rec8Δ* meiocytes revealed only a modest reduction of the high *rec8Δ*-induced bou-

quet frequency (Fig. 3 D). When MT drugs were added 4 h after meiosis induction to live *rec8Δ* meiocytes (before most *rec8Δ* cells engage in telomere clustering), telomere clustering increased with time but remained below untreated control levels during the next 4 h (Fig. 3 E). A recording of live benomyl/nocodazole-treated *rec8Δ* meiocytes revealed that nuclear deformations and clustering was present, and telomeres were motile during 7-min (Video 7, available at <http://www.jcb.org/cgi/content/full/jcb.200501042/DC1>) and 44-min (not depicted) recordings. Analysis of live MT drug-treated *rec8Δ* meiocytes ($n = 43$) showed that telomere miniclustering velocity was slightly reduced as compared with a DMSO-treated control ($n = 51$; i.e., $9.9 \pm 3.8 \mu\text{m}/\text{min}$ vs. $11.5 \pm 5.3 \mu\text{m}/\text{min}$, respectively, with the differences being statistically not significant; t test, $P = 0.078$). Because drug addition before bouquet formation did not inhibit telomere clustering (Fig. 3, E and F) or telomere cluster–SPB colocalization (Fig. 3 C), it is likely that MTs stabilize telomere clustering but are not absolutely necessary for telomere cluster formation and maintenance in budding yeast meiosis.

Meiotic telomere clustering in *S. cerevisiae* requires actin

Because recent studies suggest vital roles for actin in nuclear processes (Hofmann et al., 2004; Holaska et al., 2004; Forest et al., 2005), we tested the role of actin in telomere clustering by the administration of latrunculin B (Lat B), which binds to and reversibly prevents the polymerization of G-actin (Coue et al., 1987). Much to our surprise, Lat B addition to living *rec8Δ* meiocytes induced the rapid dispersion of clustered telomeres over the nuclear periphery (Fig. 3 D and Video 6, available at <http://www.jcb.org/cgi/content/full/jcb.200501042/DC1>). This dispersion was detected as a rimlike telomere FISH signal pattern in meiocytes that were fixed after Lat B treatment (Fig. 2 B). The continuous presence of Lat B in meiotic cultures inhibited telomere cluster formation in the wild type (Fig. 3 F) and prevented sporulation, which was accompanied by the presence of arrested cells with deformed nuclei in meiotic time courses (unpublished data). When we incubated *rec8Δ* meiocytes with cytochalasin D, which also inhibits actin polymerization (Peterson and Mitchison, 2002), there was a 1-h delay of telomere dispersion relative to the addition of the drug (the same effect as with Lat B), possibly owing to the reduced cell permeability of cytochalasin D (Greer and Schekman, 1982). Because vegetative telomere movements were not affected by the inhibition of actin polymerization (unpublished data), our observations suggest a novel role for actin in bringing about and maintaining meiotic telomere clustering, which is distinct from its known role in meiosis I division (Forer and Pickett-Heaps, 1998; LaFountain et al., 1999).

Nuclei of wild-type pachytene cells undergo deformations that are associated with rapid telomere movements (Video 4). When we investigated whether actin also plays a role in telomere movements at postbouquet stages, we found that inhibition of actin polymerization stopped rapid telomere movements and nuclear deformations and that Lat B-treated nuclei obtained a round shape (Video 8, available at <http://www.jcb.org/cgi/>

<http://www.jcb.org/cgi/content/full/jcb.200501042/DC1>). Telomere spots were still motile and had a mean velocity of $12.6 \pm 2.7 \mu\text{m}/\text{min}$ ($n = 23$ trajectories), which is similar to leptotene telomere velocities and is significantly reduced as compared with wild-type pachytene telomere velocities (t test, $P < 0.05$). These observations suggest that actin not only mediates telomere clustering but also drives nuclear deformations at pachytene.

Discussion

Prophase I telomeres are highly dynamic

Using a GFP-tagged version of the *S. cerevisiae* telomere-binding protein Rap1, we recorded, for the first time, dynamic telomere redistribution in synaptic meiosis. The strains that were used underwent meiotic divisions with wild-type kinetics. We recorded videos, which were, because of the bleaching of GFP signals, restricted to <500 frames. Live cell imaging showed that telomeres of vegetative cells formed a few peripheral clusters that underwent locally constrained movements, which coincides with earlier analysis (Heun et al., 2001). Leptotene telomeres rapidly moved around the periphery of nuclei (round-shaped nuclei discriminated leptotene from deformed pachytene nuclei). In bouquet nuclei, the motile telomeres accumulated in a limited region of the nuclear envelope. This conformation is indicative of the leptotene/zygotene transition and was only maintained for ~ 30 s in rapid SK1 wild-type meiosis. The short duration of this stage explains why telomere clustering could not be detected by time-lapse videos with large intervals between frames (Hayashi et al., 1998). In postbouquet pachytene cells, telomeres continuously underwent rapid perinuclear movements that were accompanied by nuclear deformation. Overall, the telomere dynamics observed in live cells confirm the sequence of peripheral telomere redistribution that was deduced from the FISH analysis of fixed yeast cells from meiotic time courses (Trelles-Sticken et al., 1999, 2000).

MTs are dispensable for telomere clustering

Previously, it was noted in the meiosis of plants and mammals that a colchicine-sensitive mechanism seems to control meiotic telomere clustering and/or nuclear movements (Salonen et al., 1982; Cowan and Cande, 2002). In the asynaptic meiosis of fission yeast, MT disruption inhibited nuclear movements, whereas telomeres remain clustered in *dhc⁻* cells that lack nuclear mobility (Yamamoto et al. 1999). The application of a combination of MT-disrupting drugs to *S. cerevisiae* meiocytes failed to inhibit or dissolve telomere clustering, which suggests that microtubules are not absolutely required either for telomere cluster formation or for its maintenance in this synaptic yeast. Nevertheless, the disruption of astral MTs led to a moderate reduction in telomere clustering in drug-treated wild-type and *rec8Δ* meiocytes, as measured by FISH or in live cells. Altogether, it may be that the polarized microtubule cytoskeleton provides a framework in which telomeres can undergo ordered motility to and from the SPB, which is a possibility that requires further experiments in live cells.

Actin is required for telomere clustering and nuclear motility

We were surprised to observe that the inhibition of actin polymerization by Lat B treatment prevented meiotic telomere clustering in the wild type and disintegrated the persistent telomere cluster in *rec8Δ* meiocytes. The latter was also seen, with a delay, in meiocytes treated with the actin drug cytochalasin D. However, actin disruption did not delocalize telomeres toward the nuclear interior, suggesting that actin somehow constrains telomere movements to a limited nuclear envelope sector but is dispensable for attachment. Bouquet disruption by actin depolymerization considerably delayed homologue pairing, which is reminiscent of delayed homologue pairing in yeast meiocytes lacking the meiotic telomere protein Ndj1 (Chua and Roeder, 1997; Conrad et al., 1997). However, unlike Lat B treatment, the *ndj1Δ* mutation delocalizes meiotic telomeres toward the nuclear interior (Trelles-Sticken et al., 2000).

Although our study could not determine whether cytoplasmic or nuclear actin is responsible for telomere clustering, we favor the hypothesis that nuclear actin (for reviews see Pederson and Aebi, 2002; Franke, 2004) might displace telomeres toward the SPB-bearing sector of the nuclear envelope. In this proposed mechanism, the actin-perpetuated gathering of motile telomeres would compress chromosome ends into a limited nuclear region. Interestingly, actin-related proteins localize to telomeric but not to centromeric heterochromatin in *Schizosaccharomyces pombe* (Ueno et al., 2004). The highly dynamic telomere movements and deformation of *S. cerevisiae* pachytene nuclei could involve cytoplasmic and nuclear actin. In other systems, nuclear actin has been localized to emerins and nuclear pore extensions at the nucleoplasmic face of the nuclear envelope (Hofmann et al., 2001; Holaska et al., 2004) and is implied in nuclear envelope assembly (Krauss et al., 2003) and in intranuclear transport (Forest et al., 2005). On the other hand, the actin cytoskeleton is important for control of the nuclear shape (Guilak, 1995; Abe et al., 2004).

Cohesin is required for exit from meiotic telomere clustering

Enforcement of telomere clustering seems to occur when neither the homologue nor the sister chromatid is available for repair or when defects in recombination are present (Trelles-Sticken et al., 1999, 2003; Scherthan, 2003; Storlazzi et al., 2003). Telomere clustering in the bouquet stage likely facilitates homology testing and pairing (Rockmill and Roeder, 1998; Niwa et al., 2000; Trelles-Sticken et al., 2000; Ding et al., 2004).

Telomere clustering was greatly increased in *rec8Δ* meiocytes that were arrested in prophase I. This arrest was likely the consequence of unrepaired DSBs, because a *spo11Δ* mutation removed DSBs and restored meiotic divisions and the resolution of telomere clustering before meiotic divisions. Nevertheless, extended telomere clustering was maintained in *rec8Δ spo11Δ* meiosis, suggesting that Rec8 imparts a DSB-independent role in telomere clustering. Given that bouquet formation plays a role in the instigation of homologue interactions, extended telomere clustering in *rec8Δ* meiosis may be a response

to the absence of sister chromatid cohesion. Initiation of the recombinational repair of DSBs would be impeded by spatially separate homologous chromosomes and chromatids. Alternatively, the processing of telomere repeats, which requires Ndj1, may occur during the bouquet stage (Joseph et al., 2005) and may also require cohesin function. Bouquet exit is regulated by cohesin because meiotic expression of the Scc1 cohesin in *rec8Δ* cells reestablished exit from telomere clustering. Moreover, meiotic Scc1 expression also restored defective telomere cluster–SPB colocalization in *rec8Δ* meiocytes.

Cohesin-dependent telomere cluster regulation does not require Cdc5 pololike kinase (Clyne et al., 2003) because shutting off Cdc5 activity in meiosis did not increase bouquet frequencies. It may be speculated that telomeric cohesin may recruit other factors contributing to the regulation of telomere cluster duration, which may involve the meiotic telomere protein Ndj1 and other factors influencing the proposed actin-dependent mechanism for the telomere clustering. Interestingly, Ndj1 is required for telomere clustering and contributes to sister chromatid cohesion at meiotic telomeres (Chua and Roeder, 1997; Conrad et al., 1997; Trelles-Sticken et al., 2000). Moreover, Ndj1 has been found to interact with the SPB component Msp3 (Uetz et al., 2000), which raises the possibility that Ndj1 could be involved in telomere/SPB tethering.

Recently, mice mutant for Rec8, unlike budding yeast mutants, assemble AEs (Bannister et al., 2004), and mice deficient for the meiotic cohesin Smc1 β are defective in recombination, telomere attachment, and clustering (Revenkova et al., 2004). Thus, further analysis is required to determine whether the cohesin- and actin-dependent telomere cluster maintenance in budding yeast meiosis applies to other species.

Materials and methods

Strains

Genotypes of SK1 strains that were used are listed in Table S1. Standard genetic procedures (Elble, 1992) were applied to generate the *rec8Δ rap1::RAP1-GFP-LEU2* strain. In brief, RAP1 gene replacement was performed in REC8-deleted haploid strains by transformation (Elble, 1992) with a PstI-digested plasmid coding for the GFP-tagged version of RAP1 (plasmid provided by A. Ehrenhofer-Murray, Max-Planck-Institute for Molecular Genetics, Berlin, Germany). Haploid clones of opposing mating types were mated, and zygotes were picked to obtain homozygous diploid *rec8Δ rap1::RAP1-GFP-LEU2* strains. Strains expressing Scc1 under the Rec8 promoter (Toth et al., 2000) and CDC5 under the Scc1 promoter (Clyne et al., 2003) were provided by K. Nasmyth (Institute of Molecular Pathology, Vienna, Austria).

Cell culture and drug treatment

Culture conditions, sporulation of strains, and meiotic cell preparation were performed as described previously (Scherthan and Trelles-Sticken, 2002). Nocodazole and benomyl (Calbiochem) were simultaneously used at 15 and 20 μ g/ml in culture medium, respectively. Lat B (Sigma-Aldrich) was used at 30 μ M, and cytochalasin D (Sigma-Aldrich) was used at 100 μ g/ml. Drugs were added 210 and 90 min after the induction of meiosis in *rec8Δ* and wild-type sporulation, respectively. Control cultures received a drug solvent (DMSO) at the same concentration.

For meiotic cell preparation, cultures were grown in presporulation medium to a density of 2×10^7 cells/ml and were transferred to sporulation medium (2% KAc) at a density of 4×10^7 cells/ml (Roth and Halvorson, 1969). Aliquots from the sporulating cultures were transferred at different time points during the meiotic time course to Eppendorf tubes at 0°C containing 0.1 vol of acid-free 37% formaldehyde (Merck). After 30 min, cells were removed from the fixative, washed with $1 \times$ SSC, and spheroplasted with 100 μ g/ml Zymolyase 100T (Seikagaku) in 0.8 M sorbitol, 2% KAc,

and 10 mM DTT. Spheroplasting was terminated by adding 10 vol of ice-cold 1 M sorbitol. Nuclear spreads were prepared as described previously (Trelles-Sticken et al., 2000). Sporulation was assayed by the formation of asci with one or more spores after 24 h in sporulation medium.

Flow cytometry

Cells were induced to sporulate, and 500 μ l of meiotic cells from each time point were harvested by centrifugation and fixed in 70% ethanol. After one wash in 50 mM citrate buffer, pH 7.4, cells were RNase A-digested for 2 h at 50°C (0.25 mg RNase A/ml citrate buffer) followed by the addition of 50 μ l proteinase K (from a 20 mg/ml-H₂O stock) and were further incubated at 50°C for 2.5 h. Finally, 1 ml of 16 μ g/ml propidium iodide and 50 mM sodium citrate solution were added to the tube, and DNA content was determined by flow cytometry using FACS (Becton Dickinson).

Fluorescent in situ hybridization, DNA probes, and immunofluorescence

FISH to yeast cells, DNA probes to all telomeres and centromeres, and their labeling were performed as described previously (Scherthan and Trelles-Sticken, 2002). Pairing of an internal locus on the left arm of chromosome XI and of HML near the left telomere of chromosome III was assessed as described previously (Trelles-Sticken et al., 2000). Antibodies to Tub4 (gift of L. Marschall, California State University, East Bay, Hayward, CA) and Zip1 (gift of G.S. Roeder, Yale University, New Haven, CT) were used in immunofluorescence as described previously (Trelles-Sticken et al., 2000).

Imaging

FISH preparations were evaluated by using the Isis fluorescence image analysis system (MetaSystems) fitted to an epifluorescence microscope (Axioskop; Carl Zeiss MicroImaging, Inc.) equipped with appropriate fluorescence filters (Chroma Technology Corp.).

Live cells were investigated by using a temperature-controlled microscope room equipped with an epifluorescence microscope (Axiovert; Carl Zeiss MicroImaging, Inc.). Cells were recorded with a 100 \times plan-neofluar oil-immersion lens (NA 1.35; Carl Zeiss MicroImaging, Inc.) attached to a PIFOC z-SCAN (Physik Instrumente) and a 12-bit black-and-white CCD digital camera (PCO; SensiCam) that were controlled by TILLVISION v4.0 software (TILL Photonics). GFP or fluorochromes were excited by using a monochromator (Polychrome IV; TILL Photonics) in combination with a GFP filter set or a quadruple band-pass beam splitter and barrier filter (Chroma Technology Corp.). The digital images displayed were processed by using Photoshop 6.0 (Adobe) and were adjusted in contrast and brightness to match the fluorescence image seen in the microscope.

Live cells were sporulated and attached to Con A-coated coverslips (Chikashige et al., 1994). Cells were immediately transferred to the microscope setting in a temperature-controlled room. Images of live cells were recorded every 0.5 s for 7 min or every 5 s for 44 min, with an exposure time of 300 ms for each frame, by using a preset focal plane at the nuclear equator. We chose to record two-dimensional images to limit bleaching and to be able to observe the cell for a longer time period. Telomere spot movements were analyzed with DiaTrack 2.3 software (Semasoph) in the time-lapse series by interactively tracking each visible spot in subsequent images until it left the focal plane. The same software was used to create histograms of velocity, which were created along trajectories and show the distribution of particle speed (the length of a trajectory, computed as the sum of the lengths of displacement vectors composing it divided by the lifetime of the particle). All trajectories that were created over the investigated time interval within one nucleus were superimposed to reveal a measure of the area of the focal plane over which telomere movements occurred. Generally, we were able to track spots over 13 consecutive images, whereas in a few cases, spots could be tracked for up to 69 frames.

Online supplemental material

The strains used in this study are listed in Table S1. Video 1 shows a vegetative wild-type nucleus with Rap1-GFP telomere clusters. Video 2 shows wild-type leptotene meiotic nuclei displaying small, whitish Rap1-GFP telomere dots that move rapidly over the nuclear periphery. Video 3 shows a wild-type meiotic nucleus displaying the transient formation of a telomere cluster after 1.4 min at the lower-right sector of the nuclear periphery. Video 4 shows pachytene wild-type nuclei displaying vigorous telomere movements and nuclear deformation. Video 5 shows three *rec8 Δ* bouquet nuclei, which display moving telomeres that are restricted to a limited region of the nuclear periphery. Video 6 shows the poisoning of telomere clustering in *rec8 Δ* meiotic nuclei by Lat B. Video 7 shows telomere cluster movements in the presence of nocodazole/benomyl. Video 8 shows telomere and nuclear movements before and at the addition of Lat

B in a wild-type pachytene cell. Online supplemental material is available at <http://www.jcb.org/cgi/content/full/jcb.200501042/DC1>.

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