Telomere binding of the Rap1 protein is required for meiosis in fission yeast

Yuji Chikashige and Yasushi Hiraoka

Telomeres are essential for chromosome integrity, protecting the ends of eukaryotic linear chromosomes during cell proliferation [1, 2]. Telomeres also function in meiosis; a characteristic clustering of telomeres beneath the nuclear membrane is observed during meiotic prophase in many organisms from yeasts to plants and humans [3–9], and the role of the telomeres in meiotic pairing and the recombination of homologous chromosomes has been demonstrated in the fission yeast *Schizosaccharomyces pombe* [9–14] and in the budding yeast *Saccharomyces cerevisiae* [15]. Here we report that *S. pombe* Rap1 is a telomeric protein essential for meiosis. While Rap1 is conserved in budding yeast and humans, schemes for telomere binding vary among species: human RAP1 binds to the telomere through interaction with the telomere binding protein TRF2 [16]; *S. cerevisiae* Rap1, however, binds telomeric DNA directly [17], and no orthologs of TRF proteins have been identified in this organism. In *S. pombe*, unlike in *S. cerevisiae*, an ortholog of human TRF has been identified. This ortholog, Taz1, binds directly to telomere repeats [18] and is necessary for telomere clustering in meiotic prophase [11, 12]. Our results demonstrate that *S. pombe* Rap1 binds to telomeres through interaction with Taz1, similar to human Rap1-TRF2, and that Taz1-mediated telomere localization of Rap1 is necessary for telomere clustering and for the successful completion of meiosis. Moreover, in *taz1*-disrupted cells, molecular fusion of Rap1 with the Taz1 DNA binding domain recovers telomere clustering and largely complements defects in meiosis, indicating that telomere localization of Rap1 is a key requirement for meiosis.

Address: CREST Research Project of the Japan Science and Technology Corporation, Kansai Advanced Research Center, Commmunications Research Laboratory, 588-2 Iwaoka, Iwaoka-cho, Nishi-ku, Kobe 651-2492, Japan.

Correspondence: Yasushi Hiraoka
E-mail: yasushi@crl.go.jp

Received: **25 June 2001**
Revised: **2 August 2001**
Accepted: **23 August 2001**

Published: **16 October 2001**

Current Biology 2001, 11:1618–1623

0960-9822/01/$ – see front matter

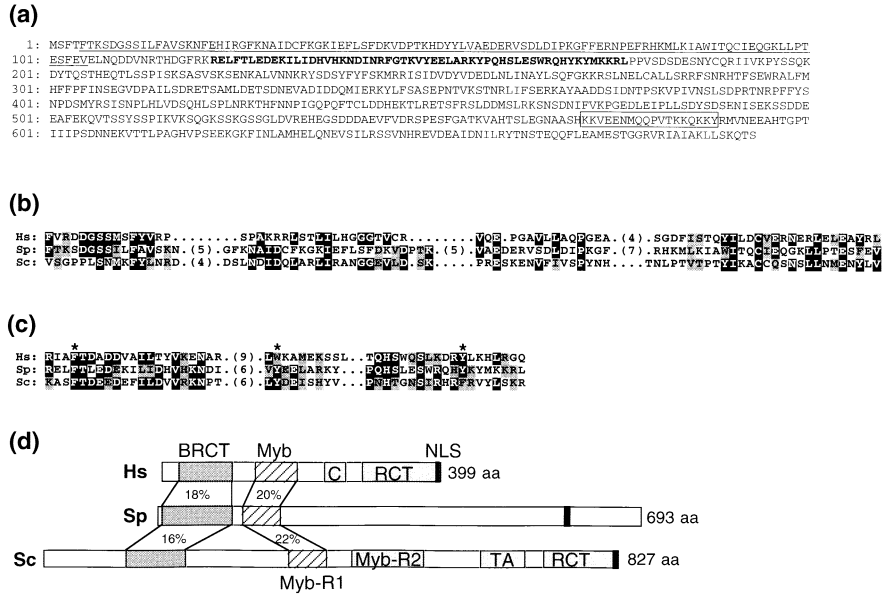
Results and discussion

Fission yeast Rap1 is a telomeric protein

Here, we identified the *rap1* gene in *Schizosaccharomyces pombe*. We first obtained a partial sequence of the *rap1* gene in our GFP-fusion genomic DNA library of *S. pombe* (clone TA14 in [19]). This fragment, containing a Myb DNA binding domain, shared significant homology with the DNA binding domain of *Saccharomyces cerevisiae* Rap1. We isolated the full-length cDNA of *S. pombe* Rap1 (GenBank accession number AB061738), and its predicted amino acid sequence shares significant homology with the *S. cerevisiae* and human Rap1 proteins, especially in the BRCT and Myb domains and in the putative bipartite nuclear localization signal (NLS) (see the legend to Figure 1).

To examine the intracellular localization of *S. pombe* Taz1 and Rap1 proteins, we constructed Taz1- and Rap1-green fluorescent protein (Taz1-GFP and Rap1-GFP) fusion proteins. During meiotic prophase of *S. pombe*, the nucleus oscillates back and forth between the cell poles with the telomeres clustered near the spindle-pole body (SPB), which is located at the leading edge of the moving nucleus [3]. Using fluorescence microscopy, we examined the localization of Taz1-GFP, Rap1-GFP, and the telomeres within meiotic prophase nuclei. As shown in Figure 2a–d, the telomeres together with Taz1 and Rap1 proteins were clustered at the SPB of the moving nucleus (an elongated shape shown in white). In 34 of 38 meiotic prophase nuclei examined, all the telomeres were clustered exclusively at the SPB (Figure 2a); in the other 4 nuclei, a cluster of telomere signals was associated with the SPB, and an extra telomere signal was observed away from the SPB. Similarly, Taz1-GFP signals were observed exclusively at the SPB in 49 out of 51 nuclei (Figure 2b), and in the two other nuclei, Taz1-GFP signals were observed clustered at the SPB with a single Taz1-GFP signal being observed away from the SPB. Observation of Rap1-GFP showed that Rap1 also localized to the SPB; a single signal of Rap1-GFP was observed at the SPB in all 48 meiotic prophase nuclei examined (Figure 2c). In addition, colocalization of Rap1-GFP with Taz1-HA was observed in all 27 meiotic prophase nuclei examined (Figure 2d). Taken together, Rap1 and Taz1 localize to the leading edge of the moving nucleus where the telomeres are clustered at the SPB. Also, Rap1 interacts with Taz1, as shown by the yeast two-hybrid assay (Figure 2e). These results indicate that Rap1 is a telomeric protein associated with Taz1.

Figure 1



Amino acid sequence of Rap1 proteins. **(a)** The predicted amino acid sequences of the *S. pombe* Rap1 protein. The BRCT domain is underlined. The Myb domain is indicated in bold. The putative bipartite NLS is boxed. **(b)** BRCT (BRCA1 C terminus) domain and **(c)** Myb domain aligned for human (Hs), *S. pombe* (Sp), and *S. cerevisiae* (Sc) Rap1. **(d)** Identity of Rap1 proteins in human (Hs), *S. pombe* (Sp), and *S. cerevisiae* (Sc). The Myb domain of *S. pombe* Rap1 shares characteristics found in that of *S. cerevisiae* and human Rap1 proteins, i.e., tryptophan residues conserved in typical Myb domains are replaced by phenylalanine or tyrosine in the Rap1 Myb domain (indicated by asterisks) [16]. No obvious similarities of amino acid sequences for Myb-R2 and transactivation (TA) domains are found in *S. pombe* or in human; coiled-coil region ("C") is found only in human RAP1. The RCT (Rap1 C terminus) domain defined in *S. cerevisiae* and human Rap1 [16] is not found in *S. pombe* Rap1 at its C terminus. The RCT domains contain the putative NLS that may arise sequence similarity in *S. cerevisiae* and human. *S. pombe* Rap1 has the putative NLS at an internal region, but not at the C terminus; no RCT domain is found near the internal NLS, either.

Fission yeast Rap1 is essential for meiosis

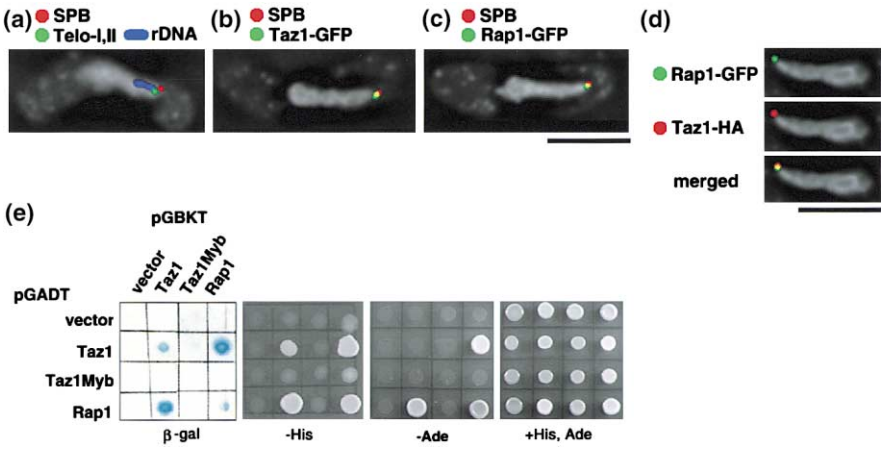
Disruption of the *taz1* gene does not affect mitotic growth but does lead to telomere elongation; it also leads to failure of telomere clustering in meiotic prophase, decreased meiotic recombination, and formation of inviable spores during meiosis [11, 12]. To examine the functions of *S. pombe* Rap1, we disrupted the *rap1* gene. Disruption of the *rap1* gene showed that Rap1 is nonessential for mitotic growth (data not shown) but leads to elongation of telomeres to an extent similar to *taz1* disruption (Figure 3a). Cells disrupted for both the *taz1* and *rap1* genes were also mitotically viable with elongated telomeres (Figure 3a); no synergistic effects were observed by double disruption of the *taz1* and *rap1* genes. During meiosis, *rap1*-disrupted cells (*rap1*Δ) fail to form normal spores (Figure 3b). Similar to *taz1*-disrupted cells (*taz1*Δ) [11, 12], the number of asci containing four normal spores decreased from 78% in *rap1*$^+$ cells (n = 115) to 8% in *rap1*Δ cells (n = 107), and random spore analysis showed that spore viability decreased from 81% to 7%. Thus, disruption of either the *taz1* or *rap1* gene has little effect on mitotic growth but causes severe defects in spore formation.

We then examined the localization of telomeres and Taz1 proteins during meiotic prophase of *rap1*Δ cells. In these cells, the telomeres failed to cluster efficiently at the SPB (Figure 3c; compare with Figure 2a); a single cluster of telomeres at the SPB was observed in only 6 of 65 nuclei examined (9%), and multiple telomere signals were observed in the remaining nuclei (91%). On the other hand, Taz1 was associated with each of the multiple telomeres in the absence of Rap1 (Figure 3d); 96% of 270 Taz1-GFP spots observed in 80 nuclei colocalized with telomeres. Next, we observed the localization of telomeres and the SPB in living *rap1*Δ cells by using Taz1-GFP and a fusion of the SPB protein Sad1 with red fluorescent protein (Sad1-DsRed). Time-lapse observation in living cells clearly showed that the SPB migrated back and forth and that the telomeres remained separate from the SPB (Figure 3e). Note that one of the telomere signals (green) appeares close to the SPB (red) in the top panel of Figure 3e but that the SPB moved away from the telomere spot at the later times (middle and bottom panels in Figure 3e). Such a situation may account for some of the telomere signals apparently colocalizing with the SPB in fixed cells (lower panel in Figure 3c). These results show that telomere binding of Taz1 does not require Rap1, but Taz1 bound telomeres do not cluster at the SPB in the absence of Rap1.

Telomere localization of Rap1 is mediated by Taz1

Next, to examine whether telomere binding of *S. pombe* Rap1 requires Taz1, we determined the localization of Rap1 during meiotic prophase of living *taz1*Δ cells. In these cells, Rap1-GFP showed diffuse nuclear staining in 39 of 61 meiotic prophase nuclei (64%), indicating no

Figure 2



Interaction of Rap1 with Taz1 at the telomere. **(a)** Localization of telomeres to the SPB in meiotic prophase cells. The telomeres of chromosomes 1 and 2 (green) were detected by fluorescence in situ hybridization by using the cosmid clone cos212 as a DNA probe, and the telomeres of chromosome 3 (blue) were detected by using rRNA genes as a DNA probe. SPB (red) was stained with anti-Sad1 antibodies. Sad1 is a well-characterized marker for the SPB in *S. pombe* [25]. The nucleus is displayed in white. **(b)** Localization of Taz1-GFP (green) at the SPB (red) stained with anti-Sad1 antibodies. **(c)** Localization of Rap1-GFP (green) at the SPB (red) stained with anti-Sad1 antibodies. **(d)** Colocalization of Rap1-GFP (green) with Taz1-HA (red). **(e)** Two-hybrid interaction of Rap1 and Taz1. Pairwise interaction of pGBKT and pGADT was assayed by three methods (see Materials and methods): β-galactosidase color assay (left), histidine auxotroph (second from left), and adenine auxotroph (third from left). The rightmost is the master plate of cells used for the assays. Interaction between Taz1 and Rap1 was detected in all three assays. Homodimeric interaction was detected between Rap1 and Rap1 (in all assays) and between Taz1 and Taz1 (in two of three assays). The Taz1Myb fragment did not interact with Taz1, Taz1Myb, or Rap1.

colocalization with telomeres (Figure 4a). In the remaining nuclei, Rap1-GFP visualized as a single spot in 20 nuclei (33%) and as two spots in two nuclei (3%) (Figure 4b). For cells having a single Rap1-GFP signal, we determined the position of the telomeres relative to the Rap1 spot in fixed specimens. As previously reported [11], telomeres did not cluster normally during meiotic prophase in *taz1*Δ cells, but instead, multiple telomere signals (3–8 spots; 4.6 on average) were observed in 54 of 57 meiotic prophase nuclei (Figure 4c, top); only in three nuclei was a single clustered telomere signal observed near the Rap1 signal (data not shown). In the nuclei with multiple telomere signals, most of the telomere signals did not colocalize with Rap1, although frequently one of them would do so (Figure 4c, top). Thus, we conclude that Rap1 does not localize properly to the telomeres in the absence of Taz1. Taz1-dependent telomere binding of Rap1 implies that the phenotype seen in *taz1*Δ cells is caused by the loss of telomeric Rap1 and explains why *taz1*Δ cells exhibit a phenotype similar to that of *rap1*Δ cells.

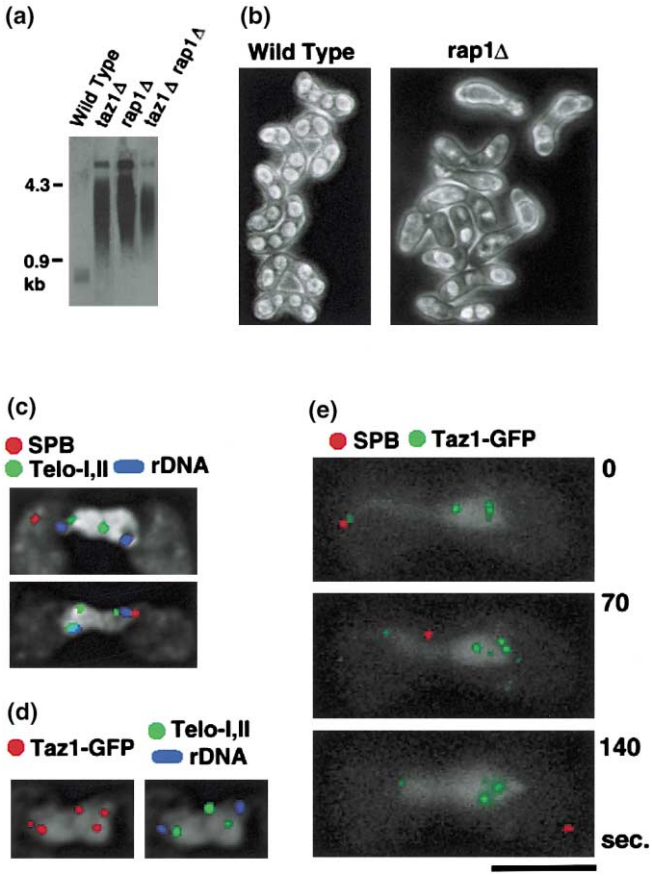
Telomere localization of Rap1 is a key requirement for meiosis

To see whether the Rap1-GFP signal localized to the SPB in *taz1*Δ cells that exhibited a single Rap1-GFP spot, we examined the position of the SPB in *taz1*Δ cells expressing Sad1-DsRed. In 20 of 21 living cells in which the Rap1-GFP signal visualized as a single spot, the spot colocalized with the SPB at the leading edge of the oscillating nucleus (Figure 4b). Thus, a certain fraction of Rap1 is able to localize to the SPB in meiotic prophase in the absence of Taz1. When Rap1 forms a single spot at the SPB in *taz1*Δ cells, the Rap1 spot frequently (50 out of 54 Rap1 spots) colocalizes with one of the telomere signals, as shown in the top panel of Figure 4c. On the other hand, in the majority of *taz1*Δ cells, the Rap1-GFP signal remained diffuse throughout meiotic prophase, and diffuse Rap1 does not seem to migrate to the SPB. These observations suggest a plausible model which is that Taz1 is necessary for localizing Rap1 efficiently to the telomere, but Rap1 is able to bind to a fraction of the telomeres even without Taz1; Rap1-bound telomeres, with or without Taz1, are tethered to the SPB.

To further examine this possibility, we constructed Rap1 fused with the C-terminal 167 amino acid residues of Taz1, which contains the Taz1 Myb DNA binding domain (designated Taz1Myb). The Taz1Myb fragment binds to the telomere but does not interact with Rap1 (Figure 2e), and expression of Taz1Myb in *taz1*Δ *rap1*$^+$ cells did not cluster telomeres at the SPB (Figure 4c, bottom; Figure 4d). In contrast, when the Rap1-Taz1Myb fusion was expressed in *taz1*Δ *rap1*Δ cells, telomeres frequently clustered at the SPB (Figure 4c, middle; compare with Rap1-GFP in the top panel); clustering of all the telomeres at the SPB was observed in 56% of *taz1*Δ *rap1*Δ cells expressing the Rap1-GFP-Taz1Myb fusion construct (Figure 4d). This frequency is high compared with 2% for *taz1*Δ *rap1*Δ cells expressing Rap1-GFP or 7% for *taz1*Δ *rap1*$^+$ cells expressing GFP-Taz1Myb (Figure 4d). Intriguingly, defects in spore formation and viability in *taz1*Δ *rap1*Δ cells were also largely rescued by expression of Rap1-Taz1Myb (Figure 4d). These results indicate that telomere binding of *S. pombe* Rap1 is a key requirement for telomere clustering and for progression through meiosis.

Our results highlight two major differences between *S.*

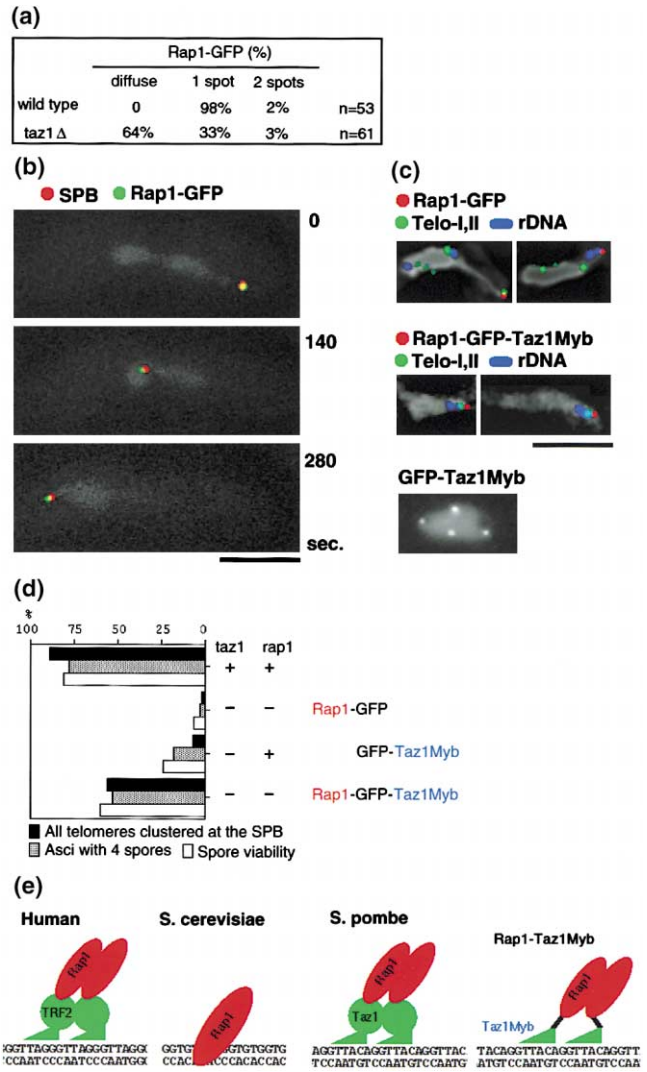
Figure 3



Phenotypes of Rap1 gene disruption. **(a)** Elongated telomeres in *rap1*Δ cells. Southern blots of ApaI-digested genomic DNA prepared from each strain were hybridized with telomere repeat sequences. **(b)** Aberrant spore formation. **(c)** Failure of telomere clustering at the SPB in *rap1*Δ cells. Two examples of fixed cells are shown. The SPB was stained with anti-Sad1 antibodies (red), and telomeres were stained by FISH (green for chromosomes 1 and 2; blue for chromosome 3). **(d)** Localization of Taz1-GFP (left) and telomeres (right) detected in the same *rap1*Δ cell. Taz1-GFP (red) was stained with anti-GFP antibodies, and telomeres were stained by FISH (green for chromosomes 1 and 2; blue for chromosome 3). **(e)** Failure of telomere clustering at the SPB demonstrated in a living *rap1*Δ cell. The SPB was stained with Sad1-DsRed (red), and telomeres were stained with Taz1-GFP (green). Optical section images of Taz1-GFP were projected onto a single section image of Sad1-DsRed at each time point.

pombe and *S. cerevisiae* Rap1 proteins (Figure 4e). First, *S. pombe* Rap1 is not essential for mitotic growth, whereas *S. cerevisiae* Rap1 is known to play a role in transcriptional regulation, and this function is essential for mitotic growth [20], and second, telomere binding of *S. pombe* Rap1 largely depends on Taz1, whereas *S. cerevisiae* Rap1 binds directly to telomeric DNA [17]. The *S. pombe* Rap1-Taz1 relationship, therefore, appears to more closely resemble the human case. Our results also demonstrate that the binding of *S. pombe* Rap1 to the telomere—naturally through interaction with Taz1 or by molecular fusion with

Figure 4



Localization of telomeres and Rap1 protein in *taz1*Δ cells. **(a)** The staining pattern of Rap1-GFP in wild-type and *taz1*Δ cells. **(b)** An example of *rap1* localization to the SPB in living *taz1*Δ cells. The SPB was stained with Sad1-DsRed (red), and Rap1 was stained with Rap1-GFP (green). **(c)** Top: the localization of telomeres and Rap1 in fixed *taz1*Δ cells. Rap1 was stained with Rap1-GFP (red), and telomeres were stained by FISH (green for chromosomes 1 and 2; blue for chromosome 3). Middle: clustering of telomeres in *taz1*Δ *rap1*Δ cells expressing Rap1-GFP-Taz1Myb (red). Telomeres were stained by FISH (green for chromosomes 1 and 2; blue for chromosome 3). Bottom: multiple signals of GFP-Taz1Myb in the nucleus of a *taz1*Δ *rap1*$^+$ cell. **(d)** Frequency of telomere clustering (complete clustering of all the telomeres at the SPB), spore formation, and spore viability. **(e)** Binding schemes of Rap1 to the telomere in human, *S. cerevisiae*, and *S. pombe*. Whereas *S. cerevisiae* Rap1 binds to the telomere as a monomer [17], human TRF2 binds to the telomere as a dimer [16]. *S. pombe* Taz1 also binds to the telomere as a dimer, but the Myb domain of Taz1 itself is capable of binding to the telomere as a monomer [26]. The Taz1Myb fragment does not form a dimer, but the Rap1-Taz1Myb fusion probably forms a dimer through dimerization of the Rap1 portion.

the Taz1 DNA binding domain—ensures the progression of meiosis in *S. pombe*. *S. cerevisiae* Rap1 associates with telomeres in meiotic cells [21], but its meiotic function has not been examined because appropriate mutant alleles that do not affect mitotic viability have not been made; human RAP1 also associates with meiotic telomeres [22]. Although schemes for telomere binding by Rap1 vary among species, the conservation of Rap1 in yeasts and human and its localization to meiotic telomeres suggest that the key role of Rap1 in *S. pombe* meiosis may be conserved among eukaryotes.

Materials and methods

Gene disruption

The *rap1* gene was disrupted by replacing a portion of the *rap1* gene (base pairs 290–2208) with the *S. pombe ura4*$^+$ gene by using the PCR-based gene targeting method [23]. Strains disrupted for the *taz1* gene are gifts of Dr. Julie P. Cooper [11].

Fusion constructs

Taz1-GFP and Rap1-GFP fusion constructs were made as follows: the entire coding sequence of the *taz1* gene with its own promoter region (1,989 base pairs of the coding sequence plus 744 base pairs immediately preceding) or the entire coding sequence of the *rap1* gene with its own promoter region (2,208 base pairs of the coding sequence plus 685 base pairs immediately preceding) was ligated in-frame to the 5′ end of the sequence encoding GFP-S65T followed by the *nmt1* terminator sequence and was cloned into the integration vector pYC36, a Bluescript-based plasmid vector with the *lys1* gene as a selection marker. The resulting plasmid was integrated into the chromosome at the *lys1* gene locus in cells disrupted for the corresponding gene. These *taz1*-GFP and *rap1*-GFP integrants form spores normally, indicating that these GFP-fusion genes are functional. Taz1-HA was constructed as follows: the *taz1* gene, which was fused at its 3′ end with triple tandem HA epitope tags, was integrated into the chromosome to replace the authentic *taz1* gene by using the *S. cerevisiae LEU2* gene as a selection marker. Taz1-HA was detected with anti-HA antibodies. Rap1-GFP-Taz1Myb was constructed as follows; the C-terminal 167 amino acid residues of Taz1, containing the Myb DNA binding domain (Taz1Myb), was ligated in-frame to the 3′ end of the Rap1-GFP fusion construct, which contains the *rap1* promoter as described above. The resulting plasmid was integrated into the chromosome at the *lys1* gene locus in cells disrupted for both *taz1* and *rap1* genes. To express the GFP-Taz1Myb protein, the GFP-Taz1Myb fusion construct was ligated to the 3′ end of the *nmt1* promoter in the integration vector, pYC36, containing the *lys1* gene, and the resulting plasmid was integrated into the chromosome at the *lys1* gene locus in cells disrupted for the *taz1* gene. Cells were cultured in medium containing thiamine to express GFP-Taz1Myb repressively under the *nmt1* promoter.

Yeast two-hybrid assay

The MATCHMAKER GAL4 Two-Hybrid System 3 (Clontech) was used for yeast two-hybrid assay. The entire coding sequence of the *taz1* gene was ligated in-frame to the pGBKT7 (pGBKT-*taz1*) or to the pGADT7 (pGADT-*taz1*); the entire coding sequence of the *rap1* gene was ligated in-frame to the pGBKT7 (pGBKT-*rap1*) or to the pGADT7 (pGADT-*rap1*). The diploid cells carrying these plasmids, indicated in Figure 2e, were tested for expression of the three reporter genes (*lacZ*, *HIS3*, and *ADE2*) according to the Clontech MATCHMAKER system manual.

Fluorescence microscopy

For fluorescence in situ hybridization and indirect immunofluorescence microscopy, fixed specimens of *S. pombe* meiotic cells were prepared as described previously [3, 4]. Plasmid YIp10.4 labeled with Texas red-dUTP and cosmid cos212 labeled with Cy5-dUTP were used as hybridization probes for telomeres; YIp10.4 hybridizes to ribosomal RNA gene repeats located at both ends of chromosome 3, and cos212 hybridizes to both ends of chromosomes 1 and 2. The SPB was detected with anti-Sad1 antibodies [24] and stained with Alexa green-conjugated second antibodies (in simultaneous staining with telomere probes) or Texas red-conjugated second antibodies (in simultaneous observation with GFP-tagged proteins). In in situ hybridizations, GFP-tagged proteins were stained with anti-GFP antibodies and Alexa green-conjugated second antibodies, since GFP fluorescence diminishes after hybridization procedures (otherwise GFP-tagged proteins were directly observed without further staining). Microscopic images were obtained using the computer-controlled fluorescence microscope system (DeltaVision; Applied Precision, Inc., Seattle, Washington, USA), as described previously [25]. To visualize the SPB in living cells, the *sad1* gene (1,542 base pairs of the coding sequence plus 614 base pairs immediately preceding; the entire *sad1* coding sequence including its promoter region) was ligated in-frame to the 5′ end of the sequence encoding the DsRed red fluorescent protein (Clontech) followed by the nmt1 terminator sequence. The Sad1-DsRed fusion construct was integrated into the chromosome at the *sad1* gene locus by using the *S. cerevisiae* LEU2 gene as a selection marker. Time-lapse observation of living *S. pombe* cells was carried out as described previously [25].

Supplementary material

Supplementary material including two movies of Taz1-GFP and Sad1-DsRed during meiotic prophase in living *rap1*$^+$ and *rap1*Δ cells is available online at http://images.cellpress.com/supmat/supmatin.htm.

Acknowledgements

We would like to thank Dr. Julia Cooper for providing *taz1*-disrupted strains; Kaori Tatsumi for technical assistance; and Drs. Elizabeth H. Blackburn, David Alexander, Hirohisa Masuda, and Tokuko Haraguchi for critical reading of the manuscript. This work was supported by grants from the Human Frontier Science Program and the Japan Science and Technology Corporation to Y.H.

References

1. Blackburn EH, Greider CW, eds.: *Telomeres*. New York: Cold Spring Harbor Laboratory Press; 1995.
2. Blackburn EH: **Telomere states and cell fates.** *Nature* 2000, **408:**53-56.
3. Chikashige Y, Ding DQ, Funabiki H, Haraguchi T, Mashiko S, Yanagida M, *et al.*: **Telomere-led premeiotic chromosome movement in fission yeast.** *Science* 1994, **264:**270-273.
4. Chikashige Y, Ding DQ, Imai Y, Yamamoto M, Haraguchi T, Hiraoka Y: **Meiotic nuclear reorganization: switching the position of centromeres and telomeres in the fission yeast Schizosaccharomyces pombe.** *EMBO J* 1997, **16:**193-202.
5. Scherthan H, Weich S, Schwegler H, Heyting C, Harle M, Cremer T: **Centromere and telomere movements during early meiotic prophase of mouse and man are associated with the onset of chromosome pairing.** *J Cell Biol* 1996, **134:**1109-1125.
6. Bass HW, Marshall WF, Sedat JW, Agard DA, Cande WZ: **Telomeres cluster de novo before the initiation of synapsis: a three-dimensional spatial analysis of telomere positions before and during meiotic prophase.** *J Cell Biol* 1997, **137:**5-18.
7. Trelles-Sticken E, Loidl J, Scherthan H: **Bouquet formation in budding yeast: initiation of recombination is not required for meiotic telomere clustering.** *J Cell Sci* 1999, **112:**651-658.
8. de Lange T: **Ending up with the right partner.** *Nature* 1998, **392:**753-754.
9. Hiraoka Y: **Meiotic telomeres: a matchmaker for homologous chromosomes.** *Genes Cells* 1998, **3:**405-413.
10. Shimanuki M, Miki F, Ding DQ, Chikashige Y, Hiraoka Y, Horio T, *et al.*: **A novel fission yeast gene, kms1+, is required for the formation of meiotic prophase-specific nuclear architecture.** *Mol Gen Genet* 1997, **254:**238-249.
11. Cooper JP, Watanabe Y, Nurse P: **Fission yeast Taz1 protein is required for meiotic telomere clustering and recombination.** *Nature* 1998, **392:**828-831.
12. Nimmo ER, Pidoux AL, Perry PE, Allshire RC: **Defective meiosis in telomere-silencing mutants of Schizosaccharomyces pombe.** *Nature* 1998, **392:**825-828.
13. Yamamoto A, West RR, McIntosh JR, Hiraoka Y: **A cytoplasmic dynein heavy chain is required for oscillatory nuclear**

movement of meiotic prophase and efficient meiotic recombination in fission yeast. *J Cell Biol* 1999, **145:**1233-1249.
14. Cooper JP: **Telomere transitions in yeast: the end of the chromosome as we know it.** *Curr Opin Genet Dev* 2000, **10:**169-177.
15. Trelles-Sticken E, Dresser ME, Scherthan H: **Meiotic telomere protein ndj1p is required for meiosis-specific telomere distribution, bouquet formation and efficient homologue pairing.** *J Cell Biol* 2000, **151:**95-106.
16. Li B, Oestreich S, de Lange T: **Identification of human Rap1: implications for telomere evolution.** *Cell* 2000, **101:**471-483.
17. König P, Rhodes D: **Recognition of telomeric DNA.** *Trends Biochem Sci* 1997, **22:**43-47.
18. Cooper JP, Nimmo ER, Allshire RC, Cech TR: **Regulation of telomere length and function by a Myb-domain protein in fission yeast.** *Nature* 1997, **385:**744-747.
19. Ding DQ, Tomita Y, Yamamoto A, Chikashige Y, Haraguchi T, Hiraoka Y: **Large-scale screening of intracellular protein localization in living fission yeast cells by the use of a GFP-fusion genomic DNA library.** *Genes Cells* 2000, **5:**169-190.
20. Shore D, Nasmyth K: **Purification and cloning of a DNA binding protein from yeast that binds to both silencer and activator elements.** *Cell* 1987, **51:**721-732.
21. Klein F, Laroche T, Cardenas ME, Hofmann JF, Schweizer D, Gasser SM: **Localization of RAP1 and topoisomerase II in nuclei and meiotic chromosomes of yeast.** *J Cell Biol* 1992, **117:**935-948.
22. Scherthan H, Jerratsch M, Li B, Smith S, Hulten M, Lock T, *et al.*: **Mammalian meiotic telomeres: protein composition and redistribution in relation to nuclear pores.** *Mol Biol Cell* 2000, **11:**4189-4203.
23. Bähler J, Wu JQ, Longtine MS, Shah NG, McKenzie A III, Steever AB, *et al.*: **Heterologous modules for efficient and versatile PCR-based gene targeting in Schizosaccharomyces pombe.** *Yeast* 1998, **14:**943-951.
24. Hagan I, Yanagida M: **The product of the spindle formation gene sad1+ associates with the fission yeast spindle pole body and is essential for viability.** *J Cell Biol* 1995, **129:**1033-1047.
25. Haraguchi T, Ding DQ, Yamamoto A, Kaneda T, Koujin T, Hiraoka Y: **Multiple-color fluorescence imaging of chromosomes and microtubules in living cells.** *Cell Struct Funct* 1999, **24:**291-298.
26. Spink KG, Evans RJ, Chambers A: **Sequence-specific binding of Taz1p dimers to fission yeast telomeric DNA.** *Nucleic Acids Res* 2000, **28:**527-533.