JCB Report

The fission yeast heterochromatin protein Rik1 is required for telomere clustering during meiosis

Creighton T. Tuzon,[1,2] Britta Borgstrom,[3] Dietmar Weilguny,[3] Richard Egel,[3] Julia Promisel Cooper,[1,2] and Olaf Nielsen[3]

[1]Telomere Biology Laboratory, Cancer Research UK, London WC2A 3PX, England, UK
[2]Department of Biochemistry and Molecular Genetics, University of Colorado Health Sciences Center, Denver, CO 80262
[3]Institute of Molecular Biology, University of Copenhagen, DK-1353 Copenhagen K, Denmark

Telomeres share the ability to silence nearby transcription with heterochromatin, but the requirement of heterochromatin proteins for most telomere functions is unknown. The fission yeast Rik1 protein is required for heterochromatin formation at centromeres and the mating-type locus, as it recruits the Clr4 histone methyltransferase, whose modification of histone H3 triggers binding by Swi6, a conserved protein involved in spreading of heterochromatin. Here, we demonstrate that Rik1 and Clr4, but not Swi6, are required along with the telomere protein Taz1 for crucial chromosome movements during meiosis. However, Rik1 is dispensable for the protective roles of telomeres in preventing chromosome end-fusion. Thus, a Swi6-independent heterochromatin function distinct from that at centromeres and the mating-type locus operates at telomeres during sexual differentiation.

Introduction

Telomeres are critical nucleoprotein structures that prevent degradation and fusion of chromosome ends and are required for faithful chromosome segregation. When cells progress from the mitotic cycle to sexual development and meiosis, telomeres acquire new functions, implying that their composition or structure must change. Telomere clustering at the nuclear periphery during early stages of meiosis is widespread among diverse eukaryotes (Dernburg et al., 1995) and has been shown to be crucial for successful meiotic chromosome segregation in fission yeast (Chikashige et al., 1994, 1997; Cooper et al., 1998; Nimmo et al., 1998).

Fission yeast cells lacking the telomere binding protein Taz1 exhibit multiple defects in the chromosome movements that accompany meiotic prophase (Cooper et al., 1998; Nimmo et al., 1998). In vegetatively growing wild-type cells, the centromeres localize to a single cluster adjacent to the spindle pole body (SPB) during interphase. Upon meiotic induction, this organization changes dramatically, with the telomeres gathering at the SPB in haploid cells responding to mating pheromone (Chikashige et al., 1997). Once haploids have mated, the centromeres separate from the SPB, leaving only the telomeres associated with it. This arrangement persists throughout the subsequent "horsetail" stage, during which the zygotic nucleus assumes an elongated shape and migrates back and forth across the zygote, with the SPB–telomere complex leading the nuclear movement, pulling the chromosomes in tow. Telomeres lacking Taz1 are unable to stably associate with the SPB, leading to a markedly disorganized zygotic nucleus, reduced homologue pairing, and defective meiotic chromosome segregation (Cooper et al., 1998; Nimmo et al., 1998). Taz1 must recruit a second telomere protein, Rap1, to organize these chromosomal rearrangements (Chikashige and Hiraoka, 2001; Kanoh and Ishikawa, 2001), but the mechanism underlying associations between the Taz1–Rap1 complex and the SPB remains elusive.

Fission yeast telomeres share structural features with centromeres and the mating-type locus, including the ability to form repressive chromatin domains (Nimmo et al., 1994).

Address correspondence to Olaf Nielsen, Department of Genetics, Institute of Molecular Biology, University of Copenhagen, Oster Farimagsgade 2A, DK-1353 Copenhagen K, Denmark. Tel.: 45 3532 2102. Fax: 45 3532 2113. email: onigen@biobase.dk; or Julia Promisel Cooper, Telomere Biology Laboratory, Cancer Research UK, 44 Lincoln's Inn Fields, London WC2A 3PX, UK. Tel.: 44 207 269 3415. Fax: 44 207 269 3258. email: julie.cooper@cancer.org.uk

D. Weilguny's present address is NatImmune A/S, Fruebjergvej 3, DK-2100 Copenhagen O, Denmark.

Key words: horsetail movement; histone methylation; chromosome behavior; silencing; *S. pombe*

Abbreviations used in this paper: NHEJ, nonhomologous end-joining; SPB, spindle pole body.

The Journal of Cell Biology, Volume 165, Number 6, June 21, 2004 759–765
http://www.jcb.org/cgi/doi/10.1083/jcb.200312061

The Journal of Cell Biology

The Rik1 protein functions in an early step of heterochromatin formation at those loci, as it is required for methylation of histone H3 on lysine 9. This modification is mediated by the chromo- and SET-domain protein Clr4 and allows recruitment of Swi6, a chromodomain-containing HP-1 orthologue (Ekwall et al., 1996; Nakayama et al., 2001). Consequently, mutants in *rik1*, *clr4*, and *swi6* are all compromised in centromere function. Each of these mutants also displays defects in telomere silencing but normal telomere length (Ekwall et al., 1996). Here, we characterize Rik1 function. Although it parallels Swi6 function in promoting mating, Rik1 acts together with Clr4 but independently of Swi6 to promote meiotic telomere clustering. These results establish that the heterochromatic nature of telomeres is specifically important for their meiotic function.

Results and discussion

Characterization of the *rik1*$^+$ gene

When depleted of a nitrogen source, fission yeast cells of opposite mating type (P and M) undergo morphological changes that allow them to mate to form diploid zygotes, which subsequently undergo meiosis and sporulation (Nielsen, 2004; Yamamoto, 2004). The *rik1* mutation causes both low sporulation efficiency and irregular asci (Egel et al., 1989), which is reflected by the unusually weak iodine staining reaction of sporulating colonies. We cloned the *rik1*$^+$ gene by rescue of this phenotype. A *rik1-304* strain was transformed with a *Schizosaccharomyces pombe* genomic library, and a clone that rescued the *rik1* sporulation defect was identified. Deletion analysis demonstrated that the complementation capacity resided within a DNA fragment encoding a 1040 amino acid uninterrupted open reading frame. We disrupted this gene in one chromosome of a diploid strain, and found by tetrad dissection that the disruption cosegregated with a sporulation defect identical to that of the original mutant. Furthermore, the disrupted allele was tightly linked to the original *rik1-304* mutation. Thus, we conclude that the cloned fragment represents the *rik1*$^+$ gene and not an exogenous suppressor and that *rik1*$^+$ is not essential for cell viability.

The predicted Rik1 protein shows homology over its entire length to the evolutionarily conserved Ddb1 group of proteins implicated in cullin-mediated protein ubiquitination during S-phase and nucleotide excision repair (Groisman et al., 2003; Liu et al., 2003). Thus, Rik1 displays 19% identity and 40% similarity to human DDB1, and 20% identity and 44% similarity to fission yeast Ddb1. As we have submitted the *rik1* sequence to GenBank (GenBank/EMBL/DDBJ accession no. AF136156), reference to it has already appeared in the literature, although the identity of the *rik1*$^+$ gene has not been previously reported.

Rik1 shares functions required for mating with Swi6

We investigated the basis for the severe sporulation defect seen in *rik1*$^-$ strains, which encompasses both reduced mating efficiency and aberrant sporulation of those zygotes that do form (Egel et al., 1989). Previously, it was reported that *rik1*$^-$ cells are defective in the generation of heterochromatin at the silenced *mat2-P* and *mat3-M* donor cassettes (Nakayama et al., 2001). Consequently, the normally nonrecombinational domain between *mat2-P* and *mat3-M* is rendered accessible to meiotic recombination (Egel et al., 1989), and the silent donor cassettes are partly expressed (Ekwall and Ruusala, 1994). This gives rise to a low level of sporulation from the haploid state and reduces mating efficiency, thus providing a partial explanation for the *rik1*$^-$ mating defect. Similar phenotypes have been observed in *swi6* and *clr1*, *2*, *3*, and *4* mutants (Klar and Bonaduce, 1991; Lorentz et al., 1992; Thon and Klar, 1992; Thon et al., 1994).

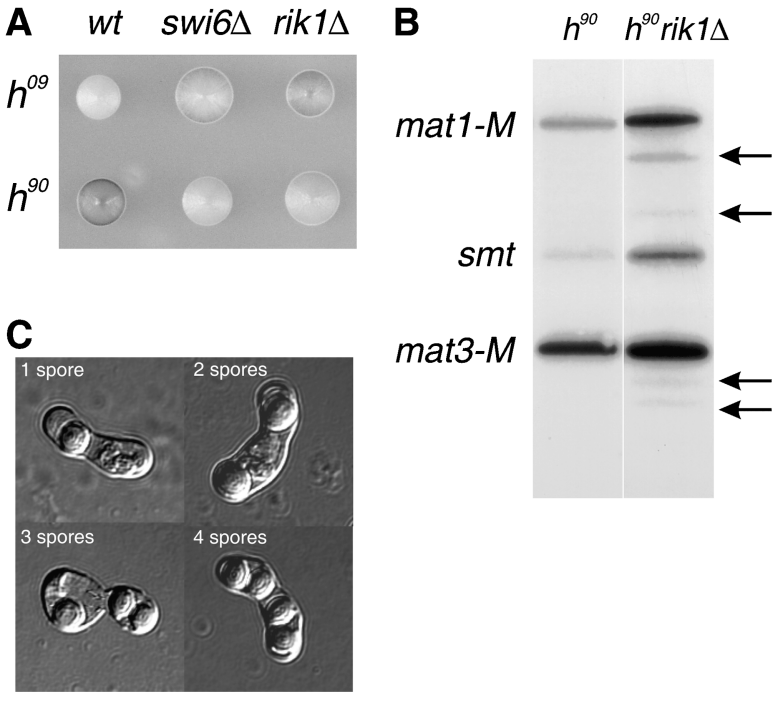


Figure 1. **Factors contributing to reduced sporulation of *rik1*Δ cells.** (A) *rik1*Δ cells have a defect in directionality of mating-type switching. Sporulation of colonies with the indicated genotypes was visualized by iodine staining, which causes spores to appear black. The directionality mechanism that causes a pronounced reduction in sporulation of h^{09} relative to h^{90} is lost in *rik1*Δ and *swi6*Δ cells. (B) The *rik1*Δ mutation gives rise to rearrangements in the mating-type region. Southern blot of HindIII-digested DNA hybridized to an M-specific probe. In addition to the *mat1-M*, *mat3-M*, and *smt* bands present in wild type, several rearrangement bands in the *rik1*Δ strain are indicated by arrows. White line indicates that intervening lanes have been spliced out. (C) Aberrant asci formed by sporulating *rik1*Δ cells.

In wild-type strains, a directionality mechanism ensures that cells preferentially switch to the opposite mating type. Thus, cells harboring *mat1-P* switch to *mat1-M* by copying the information stored at the silent *mat3-M* locus, and similarly, *mat1-M* cells preferentially choose the content of the silent *mat-2P* locus when switching. This switching pattern is subverted if the contents of the donor loci are swapped (Thon and Klar, 1993). Colonies of these so-called h^{09} strains sporulate at a much-reduced frequency due to extended sectors of cells with the same mating type, suggesting that donor choice is specified by genome position rather than mating-type information.

In *swi6* and *clr4* mutants, the directionality mechanism is lost and switching becomes random (Thon and Klar, 1993; Ivanova et al., 1998), suggesting that the heterochromatin structure around *mat2-P* and *mat3-M* is important for this mechanism. To determine if *rik1*$^+$ contributes to directionality, we compared the sporulation frequencies of h^{90}rik1 and h^{09}rik1 strains by iodine staining of sporulating colonies. As shown in Fig. 1 A, the h^{90} rik1 and h^{09} rik1 strains sporulate to a comparable extent, demonstrating that the directionality mechanism indeed requires *rik1*$^+$.

swi6 mutations lead to frequent rearrangements in the mating-type region (Egel et al., 1984), which give rise to subclones of unswitchable cells, providing another avenue to reduced mating efficiency. We found that *rik1* cells similarly accumulate aberrantly sized DNA fragments in the mating-type region (Fig. 1 B), demonstrating that *rik1* mutants acquire rearrangements therein. Thus, leakiness of the silent donor cassettes, impaired directionality of mating-type switching, and rearrangements in the *mat* region all confer reduced mating in *rik1* cells. Collectively, these phenotypes are shared with *swi6* mutants and likely reflect the failure of *rik1* cells to establish heterochromatin via Swi6 recruitment.

Rik1 has a function in meiotic telomere clustering not shared by Swi6

Although Rik1 appears to act in concert with Swi6 in regulating mating-type switching and centromere function, a pronounced departure from the behavior of *swi6* mutants is revealed by observing the products of *rik1*Δ meiosis. Although *swi6*Δ zygotes sporulate to produce asci of normal appearance containing four uniformly sized round spores apiece, *rik1*Δ asci often contain fewer than four spores, and the spores vary markedly in size and shape (Fig. 1 C and Table I).

The aberrant *rik1*Δ asci are reminiscent of those seen in strains lacking Taz1 (Table I). Observation of *rik1*Δ asci by FISH with telomere-adjacent probes and immunofluorescent localization of SPBs reveals that meiotic telomere clus-

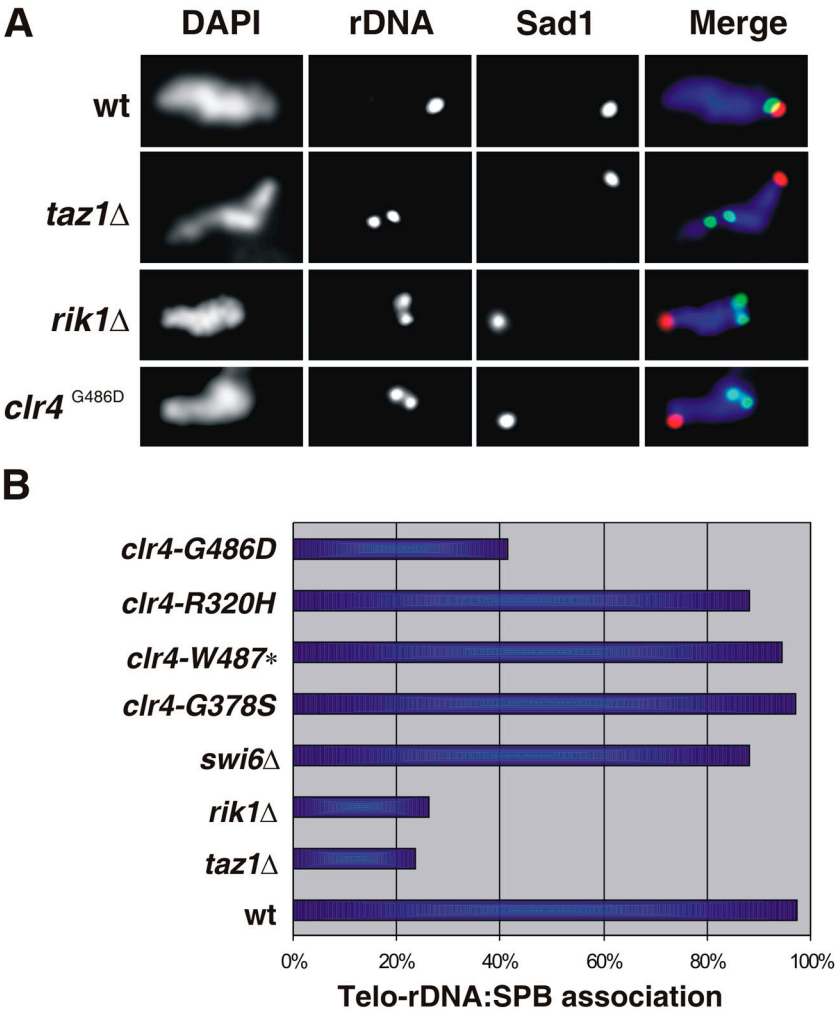


Figure 2. **Telomeres fail to associate with the SPB during meiotic prophase in *rik1* and *clr4* mutants.** (A) Indirect immunofluorescence against the Sad1 component of SPB and FISH to the telomere-adjacent rDNA were performed on zygotes in the horsetail stage. Examples are shown as merged images of anti-Sad1 (red), telomere-adjacent rDNA (green), and chromatin (blue). (B) The experiments in A were quantified for homozygous crosses of each strain indicated.

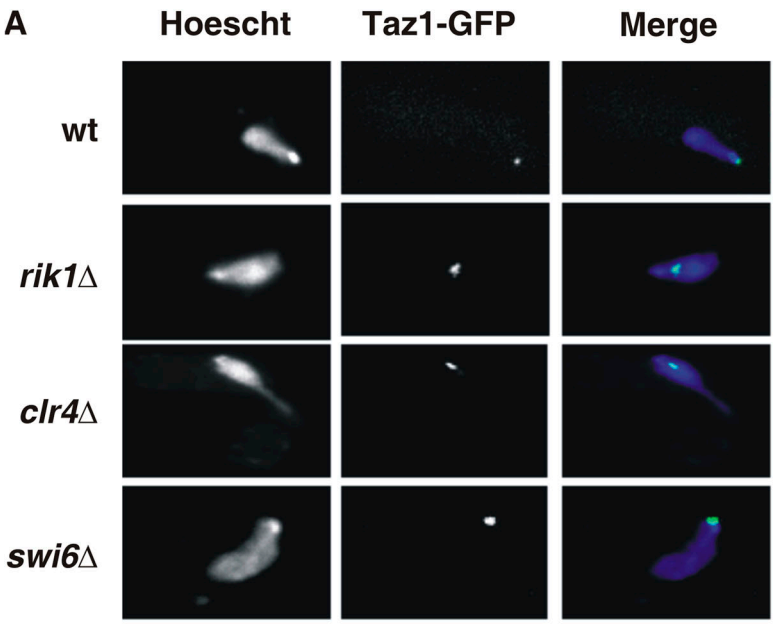
Table I. **Defects in asci morphology and number**

	Asci with aberrant size and number spores					
	1	2	3	4	Asci with four normal spores	*n*
	%	%	%	%	%	
wt	0	1	0	0	99	250
*taz1*Δ	12	25	23	20	20	215
*rik1*Δ	11	20	19	26	24	220
*clr4*Δ	2	12	15	33	37	228
*swi6*Δ	1	2	5	27	66	230
clr4-642^{G378S}	0	1	0	0	99	207
clr4-680^{W487*}	0	1	2	1	95	213
clr4-689^{R320H}	0	3	3	0	94	198
clr4-681^{G486D}	5	16	10	35	35	211

The frequency of zygotic asci having 1, 2, 3, and 4 spores was scored by light microscopy. Four-spored asci were further categorized on the basis of spore morphology and size to delineate normal and aberrant spores ("normal" means the four spores within an ascus are round and of approximately equal size). *n*, number of asci counted. *clr4-W487** contains a nonsense mutation.

tering is severely disrupted. Fig. 2 A shows examples of typical wild-type horsetail nuclei in which a single telomere signal colocalizes with the SPB, and *rik1*Δ horsetail zygotes in which the nuclear shape is lumpy rather than smoothly elongated and telomere signals are clearly separate from the SPB. Quantitation of telomere clustering in horsetail nuclei (Fig. 2 B) shows that telomere–SPB association is reduced in *rik1*Δ zygotes to the same extent as in *taz1*Δ zygotes (~22% of *taz1*Δ zygotes and ~25% of *rik1*Δ zygotes exhibit rDNA-SPB colocalization).

We also found that endogenously tagged Taz1-GFP, a marker for the telomere, fails to localize to the leading edge of meiotic horsetails in live *rik1*Δ cells (Fig. 3). Although the images in Fig. 3 show only one spot of Taz1-GFP, roughly one in three *rik1*Δ horsetail nuclei show more than one spot (not depicted). However, the weakness of the Taz1-GFP signal prevents us from using it to rigorously quantify telomere–telomere associations, as detection may require high local Taz1 concentrations found only at clustered telomeres. In *taz1*Δ horsetail nuclei, a residual level of telomere–telomere association is observed even when the telomeres are separated from the SPB (Cooper et al., 1998), and mitotic telomere clustering persists to a large extent in *taz1*Δ cells. Presumably, the tight clustering of telomeres seen during meiosis stems from the SPB association of telomeres rather than from meiosis-specific connections between telomeres, and the residual telomere clustering seen in mutants with disrupted telomere–SPB association is not stable enough to confer proper homologue pairing. Centromeric dissociation from the SPB upon meiotic induction occurs efficiently in



B

	Taz1-GFP at leading end of horsetail nuclei	n
taz1-GFP	96 %	50
*taz1-GFP rik1*Δ	30 %	50
*taz1-GFP clr4*Δ	28 %	50
*taz1-GFP swi6*Δ	94 %	50

Figure 3. **Telomeres fail to associate with the SPB in live *rik1*Δ or *clr4*Δ cells.** (A) Telomere clustering monitored via localization of endogenously tagged Taz1-GFP at the leading end of horsetail nuclei in live cells. (B) Quantitation of the experiments in A.

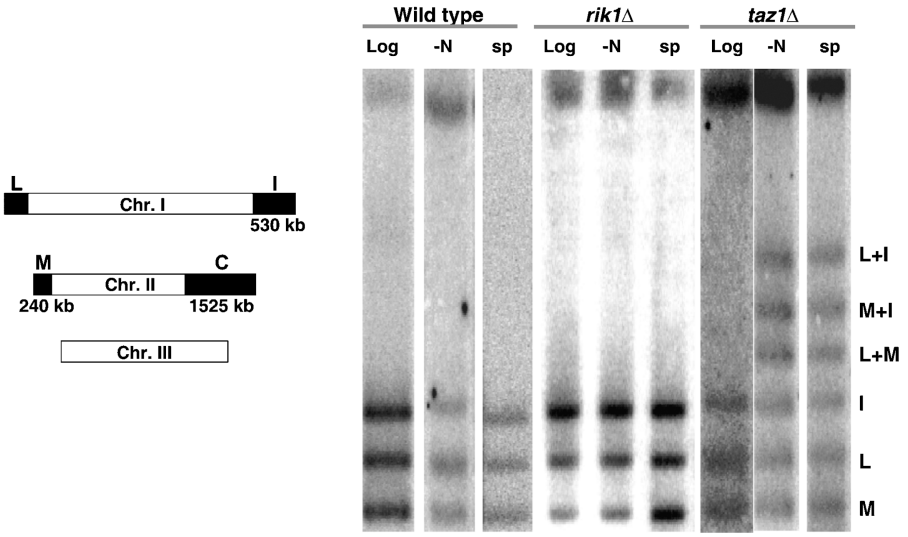


Figure 4. **Rik1p is dispensable for the prevention of telomere–telomere fusions.** PFGE of NotI-digested chromosomes. The diagram on the left shows the telomeric NotI restriction fragments in black. Unlike nitrogen-starved *taz1*Δ cells and spores, which display inter- and intrachromosomal telomere fusions, wild-type and *rik1*Δ strains lack telomere fusions. Log, log-phase cells; –N, nitrogen-starved G1-arrested cells; sp, spores. The telomeric restriction fragment C cannot be resolved from the fusion fragments C + I, C + L, and C + M under these conditions.

both strains, indicating that centromeres separate from the SPB independently of Rik1, Taz1, or telomere status (unpublished data).

Consistent with the normal ascus morphology seen in *swi6*Δ crosses, meiotic telomere clustering occurs at wild-type levels in *swi6*Δ zygotes (Figs. 2 B and 3 B). Hence, Rik1 promotes meiotic telomere–SPB association in a Swi6-independent manner.

Rik1 is not required for protection of telomeres against fusions

Not only do *taz1*Δ cells suffer from a lack of meiotic telomere clustering, but they also accumulate end-to-end chromosome fusions during the nitrogen starvation-induced G1 arrest period that precedes mating (Ferreira and Cooper, 2001). These fusions are formed by the nonhomologous end-joining (NHEJ) pathway of DNA double strand break repair, which acts predominantly in G1 cells and joins telomeres that have become unprotected via Taz1 loss. As Rik1 is required for the telomeric function of meiotic SPB association, we investigated whether or not it might also be required to protect chromosomes from NHEJ-induced end-fusions. To address this possibility, we used pulsed field gel electrophoresis to analyze chromosomes isolated from both nitrogen-starved *rik1*Δ haploids and germinated spores derived from *rik1*Δ/Δ meiosis. NotI restriction enzyme digestion of wild-type DNA yields four telomeric restriction fragments observed by probing Southern blots of pulsed field gels with telomeric oligonucleotides (Fig. 4). Whereas DNA from nitrogen-starved *taz1*Δ cells or from spores of *taz1*Δ/Δ meiosis yields additional bands corresponding to fusions between telomeres (Fig. 4), *rik1* deletion resulted in no such telomere fusion bands. Thus, Rik1 is dispensable for the telomere function of preventing chromosome end-to-end fusion.

Consistent with a role for Rik1 in meiotic telomere clustering, *rik1*Δ strains display reduced levels of spore viability intermediate between those of wild-type and *taz1*Δ strains (Fig. 5). The very low spore viability of *taz1*Δ cells is partially suppressed when NHEJ-mediated fusion is prevented by simultaneous deletion of the *pku70* gene (Fig. 5), yielding a spore viability value similar to that of *rik1*Δ cells, which is in line with our observation that meiotic *rik1*Δ cells do not sustain telomere fusions. The spore viability of *swi6* cells is also reduced relative to wild type due to centromeric cohesion defects (Kitajima et al., 2003), yet it is still higher than *rik1*Δ, which is consistent with meiotic *rik1*Δ cells suffering from both centromeric defects and telomere clustering defects.

Clr4 function is also required for meiotic telomere clustering

To address the possibility that Rik1 action is mediated by the Clr4 histone methyltransferase at meiotic telomeres as it is at centromeres and the mating-type loci, we examined meiosis in zygotes harboring various *clr4* mutations (Fig. 2

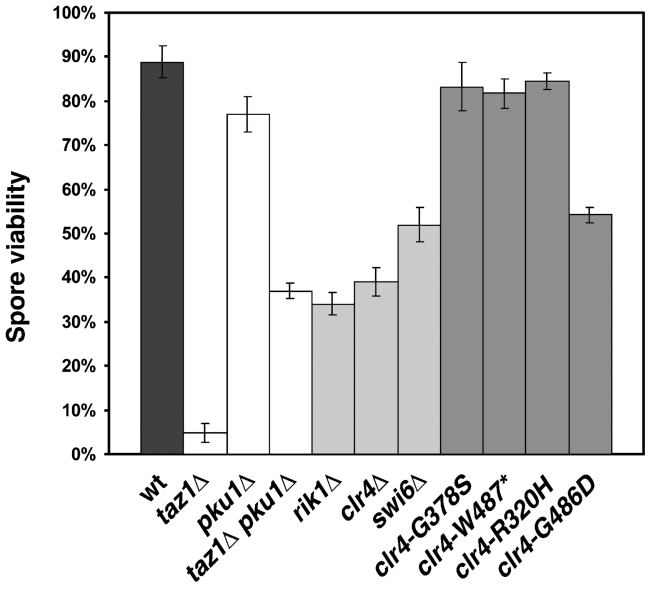


Figure 5. **Telomere and heterochromatin mutants display reduced spore viability.** Spore viability is represented as an average of three independent experiments, each performed in triplicate; error bars represent SD.

Table II. **The *clr4-G486D* allele is dominant**

	Asci with aberrant size and number spores					
	1	2	3	4	Asci with four normal spores	*n*
	%	%	%	%	%	
wt/wt	0	1	0	0	99	200
*taz1*Δ/*taz1*Δ	11	23	22	22	22	200
*rik1*Δ/*rik1*Δ	9	24	25	17	25	215
rik1$^+$/*rik1*Δ	0	4	3	1	94	279
clr4$^+$/*clr4*Δ	0	3	6	1	90	232
clr4-681^{G486D}/+	2	14	13	30	40	302

Stable diploid strains of the indicated genotype were sporulated, and the frequency of azygotic asci having 1, 2, 3, and 4 spores was scored by light microscopy as in Table I.

B, Fig. 3, Fig. 5, and Table I). Although ascus morphology, telomere clustering, and spore viability were only mildly affected in strains harboring the R320H, G378S, or W487* mutations, the G486D or null mutants show defects, yielding similar levels of aberrant asci, telomere clustering, and spore viability to *rik1*Δ mutants. Interestingly, the *clr4*$^+$/*clr4-G486D* diploid undergoes a defective azygotic meiosis (Table II), indicating that the G486D mutation is dominant. This finding suggests that Clr4 is partially redundant with some other protein for meiotic function, and that the Clr4-G486D protein interferes with the ability of this other protein to promote proper meiosis. Because the G486D mutant severely reduces histone H3 methylation activity (Nakayama et al., 2001), these observations suggest that telomere–SPB associations are mediated at least in part by Clr4-dependent histone methylation.

Conclusions

Previous studies have established an important role for Rik1 in recruiting the Clr4 histone methyltransferase to DNA regions targeted for Swi6 binding and subsequent heterochromatic silencing. Consequently, there is a substantial overlap between phenotypes displayed by *rik1* and *swi6* mutant cells, and our data on mating-type switching defects in *rik1*Δ cells reinforce this scheme. However, visual inspection of sporulating cells clearly reveals that Rik1 has a meiotic function not shared by Swi6, and we demonstrate that this function is required for telomere clustering during meiotic prophase.

We find that *clr4* mutant cells have a similar meiotic defect, suggesting that Rik1 regulates telomere behavior via Clr4 histone methyltransferase activity. Presumably, this methylation recruits some as yet unidentified factor distinct from Swi6. In addition to Swi6 and Clr4, the fission yeast genome encodes the two chromodomain proteins Chp1 and Chp2. However, our preliminary results suggest that neither Chp1 nor Chp2 are required for normal ascus formation (unpublished data). Recently, it was reported that Clr4 also has a Swi6-independent role in dicer-mediated degradation of centromeric transcripts into siRNAs (Schramke and Allshire, 2003). Cells lacking the RNAi machinery display markedly milder defects in meiotic telomere clustering than those observed in *rik1*Δ meiosis (Hall et al., 2003). Nevertheless, it will be interesting to see if Rik1 participates in the RNAi process, and if the Swi6-independent functions of Clr4 in centromeric RNAi processing and telomeric clustering involve a common target.

The telomeric protein Taz1 is required during sexual differentiation both to prevent NHEJ-mediated telomere fusions and to promote clustering of the telomeres at the SPB. The *rik1*Δ mutation allowed us to separate these two functions genetically, as Rik1 is required only for the latter. Hence, *rik1*Δ may provide a tool for studying the consequences of losing meiotic telomere clustering without the complication of simultaneous telomere fusion.

Materials and methods

Genetic procedures

Standard genetic procedures were performed as described previously (Gutz et al., 1974; Moreno et al., 1991). The *mat1-M*–specific RNA probe used for Southern blotting was transcribed from a 1016 bp BclI-TaqI fragment. Analysis of chromosome fusions was performed according to Ferreira and Cooper (2001). The *swi6* deletion strain was obtained from H. Schmidt (Technical University of Braunschweig, Braunschweig, Germany), whereas strains carrying point mutations in the *clr4* gene were obtained from G. Thon (University of Copenhagen, Copenhagen O, Denmark).

Cloning of the rik1$^+$ gene

A homothallic *h*^{90} *ura4-D18 rik1-304* strain was transformed with an *S. pombe* partial *Sau*3A genomic library (Weilguny et al., 1991). Approximately 10.000 ura$^+$ transformants were selected on sporulation medium and screened for rescue of the sporulation defect.

FISH, indirect immunofluorescence, and live analysis

Zygotes were prepared for indirect immunofluorescence using an anti-Sad1 antibody and FISH using a probe to the telomere-adjacent rDNA on chromosome III as described previously (Cooper et al., 1998). Images were captured on a microscope (model Axioplan 2; Carl Zeiss MicroImaging, Inc.) with an attached CCD camera (Hamamatsu). For live analysis, the endogenous *taz1*$^+$ gene was tagged at its COOH terminus using the Bahler gene-targeting method and plasmid pFA6a-GFP(S65T) (Bahler et al., 1998). Correct integration was confirmed by PCR and Western blot analysis. Taz1-GFP is fully functional, although strains harboring this allele show slight telomere elongation. Horsetail nuclei were visualized by Hoescht 33342 staining. Images were obtained using a Delta Vision microscope system (Applied Precision, Inc.).

We thank Henning Schmidt and Geneviève Thon for strains, Yoshinori Watanabe for comments on the manuscript, and Judith Jaehning, Paul Megee, and our lab members for discussions and support.

This work was supported by the Danish Cancer Society (O. Nielsen), the National Institutes of Health (C.T. Tuzon and J.P. Cooper), and Cancer Research UK (C.T. Tuzon and J.P. Cooper).

Submitted: 8 December 2003
Accepted: 13 May 2004

References

Bahler, J., J.Q. Wu, M.S. Longtine, N.G. Shah, A. McKenzie III, A.B. Steever, A. Wach, P. Philippsen, and J.R. Pringle. 1998. Heterologous modules for efficient and versatile PCR-based gene targeting in *Schizosaccharomyces pombe*. *Yeast.* 14:943–951.

Chikashige, Y., and Y. Hiraoka. 2001. Telomere binding of the Rap1 protein is required for meiosis in fission yeast. *Curr. Biol.* 11:1618–1623.

Chikashige, Y., D.Q. Ding, H. Funabiki, T. Haraguchi, S. Mashiko, M. Yanagida, and Y. Hiraoka. 1994. Telomere-led premeiotic chromosome movement in fission yeast. *Science.* 264:270–273.

Chikashige, Y., D.Q. Ding, Y. Imai, M. Yamamoto, T. Haraguchi, and Y. Hiraoka. 1997. Meiotic nuclear reorganization: switching the position of

centromeres and telomeres in the fission yeast *Schizosaccharomyces pombe*. *EMBO J.* 16:193–202.

Cooper, J.P., Y. Watanabe, and P. Nurse. 1998. Fission yeast Taz1 protein is required for meiotic telomere clustering and recombination. *Nature.* 392:828–831.

Dernburg, A.F., J.W. Sedat, W.Z. Cande, and H.W. Bass. 1995. Cytology of telomeres. *In* Telomeres. E.H. Blackburn and C.W. Greider, editors. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. 295–338.

Egel, R., D.H. Beach, and A.J. Klar. 1984. Genes required for initiation and resolution steps of mating-type switching in fission yeast. *Proc. Natl. Acad. Sci. USA.* 81:3481–3485.

Egel, R., M. Willer, and O. Nielsen. 1989. Unblocking of meiotic crossing-over between the silent mating-type cassettes of fission yeast, conditioned by the recessive, pleiotropic mutant rik1. *Curr. Genet.* 14:407–410.

Ekwall, K., and T. Ruusala. 1994. Mutations in rik1, clr2, clr3 and clr4 genes asymmetrically derepress the silent mating-type loci in fission yeast. *Genetics.* 136:53–64.

Ekwall, K., E.R. Nimmo, J.P. Javerzat, B. Borgstrom, R. Egel, G. Cranston, and R. Allshire. 1996. Mutations in the fission yeast silencing factors clr4+ and rik1+ disrupt the localisation of the chromo domain protein Swi6p and impair centromere function. *J. Cell Sci.* 109:2637–2648.

Ferreira, M.G., and J.P. Cooper. 2001. The fission yeast Taz1 protein protects chromosomes from Ku-dependent end-to-end fusions. *Mol. Cell.* 7:55–63.

Groisman, R., J. Polanowska, I. Kuraoka, J. Sawada, M. Saijo, R. Drapkin, A.F. Kisselev, K. Tanaka, and Y. Nakatani. 2003. The ubiquitin ligase activity in the DDB2 and CSA complexes is differentially regulated by the COP9 signalosome in response to DNA damage. *Cell.* 113:357–367.

Gutz, H., H. Heslot, U. Leupold, and N. Loprieno. 1974. *Schizosaccharomyces pombe*. *In* Handbook of Genetics. Vol. 1. R.C. King, editor. Plenum Press, New York. 395–446.

Hall, I.M., K. Noma, and S.I. Grewal. 2003. RNA interference machinery regulates chromosome dynamics during mitosis and meiosis in fission yeast. *Proc. Natl. Acad. Sci. USA.* 100:193–198.

Ivanova, A.V., M.J. Bonaduce, S.V. Ivanov, and A.J. Klar. 1998. The chromo and SET domains of the Clr4 protein are essential for silencing in fission yeast. *Nat. Genet.* 19:192–195.

Kanoh, J., and F. Ishikawa. 2001. spRap1 and spRif1, recruited to telomeres by Taz1, are essential for telomere function in fission yeast. *Curr. Biol.* 11: 1624–1630.

Kitajima, T.S., S. Yokobayashi, M. Yamamoto, and Y. Watanabe. 2003. Distinct cohesin complexes organize meiotic chromosome domains. *Science.* 300: 1152–1155.

Klar, A.J., and M.J. Bonaduce. 1991. swi6, a gene required for mating-type switching, prohibits meiotic recombination in the mat2-mat3 "cold spot" of fission yeast. *Genetics.* 129:1033–1042.

Liu, C., K.A. Powell, K. Mundt, L. Wu, A.M. Carr, and T. Caspari. 2003. Cop9/signalosome subunits and Pcu4 regulate ribonucleotide reductase by both checkpoint-dependent and -independent mechanisms. *Genes Dev.* 17:1130–1140.

Lorentz, A., L. Heim, and H. Schmidt. 1992. The switching gene swi6 affects recombination and gene expression in the mating-type region of *Schizosaccharomyces pombe*. *Mol. Gen. Genet.* 233:436–442.

Moreno, S., A. Klar, and P. Nurse. 1991. Molecular genetic analysis of fission yeast *Schizosaccharomyces pombe*. *Methods Enzymol.* 194:795–823.

Nakayama, J., J.C. Rice, B.D. Strahl, C.D. Allis, and S.I. Grewal. 2001. Role of histone H3 lysine 9 methylation in epigenetic control of heterochromatin assembly. *Science.* 292:110–113.

Nielsen, O. 2004. Mating-type control and differentiation. *In* The Molecular Biology of *Schizosaccharomyces pombe*. R. Egel, editor. Springer, Heidelberg, Germany. 281–296.

Nimmo, E.R., G. Cranston, and R.C. Allshire. 1994. Telomere-associated chromosome breakage in fission yeast results in variegated expression of adjacent genes. *EMBO J.* 13:3801–3811.

Nimmo, E.R., A.L. Pidoux, P.E. Perry, and R.C. Allshire. 1998. Defective meiosis in telomere-silencing mutants of *Schizosaccharomyces pombe*. *Nature.* 392: 825–828.

Schramke, V., and R. Allshire. 2003. Hairpin RNAs and retrotransposon LTRs effect RNAi and chromatin-based gene silencing. *Science.* 301:1069–1074.

Thon, G., and A.J. Klar. 1992. The clr1 locus regulates the expression of the cryptic mating-type loci of fission yeast. *Genetics.* 131:287–296.

Thon, G., and A.J. Klar. 1993. Directionality of fission yeast mating-type interconversion is controlled by the location of the donor loci. *Genetics.* 134:1045–1054.

Thon, G., A. Cohen, and A.J. Klar. 1994. Three additional linkage groups that repress transcription and meiotic recombination in the mating-type region of *Schizosaccharomyces pombe*. *Genetics.* 138:29–38.

Weilguny, D., M. Praetorius, A. Carr, R. Egel, and O. Nielsen. 1991. New vectors in fission yeast: application for cloning the his2 gene. *Gene.* 99:47–54.

Yamamoto, M. 2004. Initiation of meiosis. *In* The Molecular Biology of *Schizosaccharomyces pombe*. R. Egel, editor. Springer, Heidelberg, Germany. 297–309.