Meiotic nuclear reorganization: switching the position of centromeres and telomeres in the fission yeast *Schizosaccharomyces pombe*

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In fission yeast meiotic prophase, telomeres are clustered near the spindle pole body (SPB; a centrosome-equivalent structure in fungi) and take the leading position in chromosome movement, while centromeres are separated from the SPB. This telomere position contrasts with mitotic nuclear organization, in which centromeres remain clustered near the SPB and lead chromosome movement. Thus, nuclear reorganization switching the position of centromeres and telomeres must take place upon entering meiosis. In this report, we analyze the nuclear location of centromeres and telomeres in genetically well-characterized meiotic mutant strains. An intermediate structure for telomere-centromere switching was observed in haploid cells induced to undergo meiosis by synthetic mating pheromone; fluorescence *in situ* hybridization revealed that in these cells, both telomeres and centromeres were clustered near the SPB. Further analyses in a series of mutants showed that telomere-centromere switching takes place in two steps; first, association of telomeres with the SPB and, second, dissociation of centromeres from the SPB. The first step can take place in the haploid state in response to mating pheromone, but the second step does not take place in haploid cells and probably depends on conjugation-related events. In addition, a linear minichromosome was also co-localized with authentic telomeres instead of centromeres, suggesting that telomere clustering plays a role in organizing chromosomes within a meiotic prophase nucleus.

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Introduction

It is generally believed that a major function of telomeres is to stabilize the ends of chromosomes with their special structures and special schemes of synthesis. In addition to this basic function, recent studies of telomere behavior demonstrate that telomeres play multiple roles in mitosis as well as in meiosis (reviewed in Blackburn, 1994). Special functions of meiotic telomeres have been inferred from cytological observations of the characteristic chromosome configuration in meiotic prophase. In meiotic

prophase, chromosomes are kept bundled at the telomeres to form a bouquet-like arrangement. This chromosome arrangement has been observed in a wide range of animals and plants and is generally called a bouquet structure because of its appearance (reviewed in Fussel, 1987; John, 1990). In addition, it is reported in some organisms that the centrosome is located near the cluster of meiotic telomeres (Schreiner and Schreiner, 1905; Hughes-Schrader, 1943; reviewed in Therman and Susman, 1992; Dernburg *et al.*, 1995). Universality of the close apposition of meiotic telomeres to the centrosome was confirmed in studies of nuclear organization in fission yeast meiosis; it was demonstrated that telomeres form a single cluster near the spindle pole body (SPB; a centrosome-equivalent structure in fungi) specifically during the limited periods of karyogamy and meiotic prophase in fission yeast (Chikashige *et al.*, 1994).

A large body of cytological observations of meiotic chromosomal events has been accumulated in a wide range of animals and plants (reviewed in John, 1990) but their underlying molecular mechanisms are poorly understood. In an attempt to combine cytological observations with the powerful tools of molecular biology in a single organism, cytological approaches have recently been developed in budding yeast (Hollingsworth *et al.*, 1990; Klein *et al.*, 1992; Scherthan *et al.*, 1992; Weiner and Kleckner, 1994; Sym and Roeder, 1995) as well as in fission yeast (Uzawa and Yanagida, 1992; Bähler *et al.*, 1993; Funabiki *et al.*, 1993; Chikashige *et al.*, 1994; Scherthan *et al.*, 1994).

There are several advantages in studying meiotic chromosomal events in the fission yeast *Schizosaccharomyces pombe*. First, fission yeast, a unicellular organism, provides a simple experimental system for examining meiotic events directly under a microscope, with its ease of induction of and rapid progress of meiosis. A number of mutants affecting the progression of meiosis have been isolated and characterized in fission yeast (reviewed in Egel, 1989; Nielsen, 1993; Yamamoto, 1996). In addition, the fission yeast has only three chromosomes which can be distinguished cytologically (Umesono *et al.*, 1983), which simplifies analysis of spatial arrangement and dynamics of chromosomes. It is also possible to examine chromosome dynamics in individual living cells in fission yeast (Chikashige *et al.*, 1994).

Taking advantage of the small number of chromosomes, the mitotic nuclear organization of chromosomes has been well studied in fission yeast (summarized in Figure 1B). The nucleus in mitotic interphase comprises two hemispherical compartments, one hemisphere rich in RNA (a nucleolus) and the other hemisphere of chromatin with two protrusions of chromatin extending into the RNA-rich hemisphere (Yanagida *et al.*, 1986). Three chromosomes are organized within the hemispherical region with

Fig. 1. Genome and nuclear organization in fission yeast. **(A)** Genome organization. Telomere repeats are indicated by red-filled boxes; the centromere is indicated by a green-filled circle labeled 'Cen'; the telomere-adjacent sequence is indicated by a yellow-filled circle labeled 'Telo'; the repeats of rRNA genes are indicated by a light blue-filled circle labeled 'rRNA'; the rest of the chromosomes are indicated by blue bars. **(B)** Nuclear organization in mitotic and meiotic nuclear divisions (left) and in meiotic prophase (right). Polarized orientation of centromeres and telomeres was observed both in mitosis and meiosis. The telomere-centromere orientation switches the polarity relative to the SPB during the process of meiosis. Centromeres are close to the SPB in mitosis (left), telomeres take a position near the SPB during karyogamy and meiotic prophase (right) and centromeres resume their position near the SPB in the first meiotic division (left). **(C)** Nuclear location of telomeres of chromosomes I–III. Red signals represent telomeres and green signals represent centromeres; nuclear DNA is displayed in blue. Telomeres of chromosomes I and II were probed with the telomere-adjacent sequence (left panel) and telomeres of chromosome III were probed with the rRNA genes (right panel). Scale bar 5 μ m.

a polarized configuration of centromeres and telomeres. Fluorescence *in situ* hybridization showed that the protrusions of chromatin embedded in the nucleolus are repeats of rRNA genes located at both ends of chromosome III (Uzawa and Yanagida, 1992). Centromeres are clustered at a single site near the SPB located at the opposite side to the nucleolus, whereas telomeres are located at several locations at the nuclear periphery (Funabiki *et al.*, 1993).

On the other hand, the nucleus in meiotic prophase shows an elongated morphology which is generally called 'horse tail' because of its shape (Robinow, 1977; Robinow and Hyams, 1989). Our previous studies in living cells showed that horse tail nuclei migrate within the cell at meiotic prophase; further analysis by *in situ* hybridization revealed that during this movement, the telomeres remain clustered at the SPB located at the leading end of movement, whereas centromeres are separated from the SPB (Chikashige *et al.*, 1994). This nuclear organization in meiotic prophase contrasts with the mitotic chromosome arrangement, in which centromeres are clustered near the SPB and lead chromosome movement (Funabiki *et al.*, 1993). Thus, it follows that nuclear reorganization

switching the position of centromeres and telomeres takes place upon entering meiosis.

In this report, we first describe an intermediate structure for telomere-centromere switching observed in haploid cells induced to undergo meiosis by synthetic mating pheromone. Fluorescence *in situ* hybridization revealed that in these cells, telomeres formed a cluster near the SPB. In such haploid cells, however, centromeres also remained clustered at the SPB. Further analyses of nuclear organization were made in genetically well-characterized meiotic mutant strains. Detachment of centromeres from the SPB was observed in mutant strains which arrested at later stages of meiosis. These results showed that telomere-centromere switching takes place in two steps; first, telomeres become associated with the SPB in response to mating pheromone and, second, centromeres detach from the SPB in conjugation-related events.

Furthermore, we found that an artificial linear minichromosome derived from chromosome III also co-localized with authentic telomeres and separated from authentic centromeres in the meiotic prophase nucleus. Minichromosome III contains a 390 kbp portion of chromosome III directly flanked by telomeric repeats (Matsumoto *et al.*, 1987; Niwa *et al.*, 1989; see Figure 5A). It has been shown that meiotic recombination between authentic chromosome III and minichromosome III is rare in spite of their sharing 390 kbp of DNA sequence spanning the centromere (Niwa *et al.*, 1986, 1989). The spatial separation of minichromosome III from authentic centromeres provides an explanation for the reduced recombination between chromosome III and minichromosome III. The nuclear location of minichromosome III also suggests that telomeres, rather than the DNA sequence homology, play a role in the initial step of aligning homologous chromosomes in meiotic prophase.

Results

Nuclear organization in fission yeast

We examined the locations of telomeres and centromeres within the so-called horse tail nucleus in fission yeast meiotic prophase by DNA *in situ* hybridization to chromosomal DNA. DNA probes used in this paper are summarized in Figure 1A. The genome of the fission yeast *S.pombe* is composed of three chromosomes. All three chromosomes have telomeric repeats of 5'-TTACAGG-3' with some variations in the length of G tracts (Sugawara and Szostak, 1986; reviewed in Henderson, 1995). Chromosomes I and II share a telomere-adjacent sequence which we used to probe the telomeres in a previous work (Chikashige *et al.*, 1994) and in this paper. Chromosome III does not have this telomere-adjacent sequence but instead the telomeric repeats are immediately flanked by repeats of rRNA genes (Yanagida *et al.*, 1991). We used the rRNA genes to probe the ends of chromosome III in this paper. Centromeres were probed with the centromeric repetitive sequences shared by all three chromosomes (Chikashige *et al.*, 1989). The nuclear organization of the chromosomes in mitotic interphase is summarized in the left panel of Figure 1B (see also Introduction).

Previous studies showed that telomeres are clustered near the SPB located at the leading end of the horse tail nucleus, whereas centromeres are located on the opposite

Fig. 2. Chromosome dynamics in living meiotic cells. Schematic diagrams of the cellular response to the mating pheromone in the wild-type (A), a *mam2*⁻ mutant (B) and a *sxa2*⁻ *cyr1*⁻ mutant (C). Dynamics of chromosomes observed in living cells of a *mam2*⁻ mutant (D) and a *sxa2*⁻ *cyr1*⁻ mutant (E) in response to the mating pheromone. During this movement, a specific portion of the nucleus, which can be seen as a bright spot, is kept at the leading end of movement, as documented in Chikashige *et al.* (1994); this bright spot co-localizes with a cluster of telomeres as shown by fluorescence *in situ* hybridization with the subtelomeric DNA probe (data not shown). Numbers on the right side of the figure indicate time in minutes for both (D) and (E). The observation was started 16 h after nitrogen starvation in (D) and 7 h after the P factor treatment in (E). Scale bar 5 μ m.

side to the SPB (Chikashige *et al.*, 1994). Double *in situ* hybridization for telomeres (red spot) and centromeres (green spot) revealed a polarized orientation of chromosomes within the horse tail nucleus (Figure 1C). *In situ* hybridization using the chromosome I and II telomere-adjacent sequence probe revealed that telomeres of these chromosomes form a single cluster at the leading end of the horse tail nucleus (Figure 1C, left). When probed with rRNA genes, the ends of chromosome III were also localized at the leading end of the horse tail nucleus (Figure 1C, right). Thus, telomeres of all three chromosomes are clustered near the SPB without regard to the presence of the telomere-adjacent sequences. These results are summarized in the right panel of Figure 1B.

Nuclear movement and morphology in meiosis

In the fission yeast *S.pombe*, haploid cells of the opposite mating type, *h*⁺ and *h*⁻, conjugate upon nitrogen starvation and enter the process of meiosis. Nitrogen starvation leads to the expression of mating type genes. Under the regulation of these genes, *h*⁺ and *h*⁻ cells secrete the mating pheromones, P factor and M factor respectively, while producing receptors for the mating pheromone from the opposite mating type cell (Egel, 1973b; Willer *et al.*, 1995; see Figure 2A). Response to the mating pheromone leads to conjugation and zygotic gene expression commits the cells to meiosis.

We observed changes in cell and nuclear morphology

during the process of meiosis in fixed and living cells of fission yeast. Upon nitrogen starvation, the nucleolar region disappears but overall nuclear morphology remains spherical in the absence of mating partners (data not shown). In the presence of cells of the opposite mating type, cells form a conjugation tube toward the mating partner; the nucleus turns to a tear drop shape and moves toward the site of the conjugation tube. Immediately after nuclear fusion, the fused nucleus elongates and starts to move within the cell, as demonstrated in living cells (Chikashige *et al.*, 1994).

To test the effect of mating pheromone on nuclear organization, we first examined nuclear movement in haploid cells of a *mam2* mutant, *mam2*-A84. The *mam2* mutant lacks a functional receptor to P factor and thus shows an *h*⁻ specific sterile phenotype (Kitamura and Shimoda, 1991; Leupold *et al.*, 1991). When *h*⁻ *mam2*⁻ cells and *h*⁺ *mam2*⁻ cells were mixed in nitrogen-depleted sporulating medium EMM2-N (see Materials and methods), *h*⁺ *mam2*⁻ cells formed a conjugation tube toward *h*⁻ *mam2*⁻ cells in response to M factor from the *h*⁻ cell but *h*⁻ cells did not respond to P factor from the *h*⁺ cells (Figure 2B and D). Formation of a conjugation tube is an indication that that cell has responded to the mating pheromone. We observed nuclear movement in living cells of the *mam2* mutant stained with a DNA-specific fluorescent dye, Hoechst 33342. Nuclear movement in a pair of *h*⁺ and *h*⁻ *mam2*⁻ cells is shown in Figure 2D. The nucleus in the *h*⁺ *mam2*⁻ cell took on the tear drop shape and started nuclear movement, whereas the nucleus in the *h*⁻ *mam2*⁻ cell retained the interphase nuclear morphology with no movement. The movement observed in *h*⁺ *mam2*⁻ cells was similar to that in karyogamy in normal meiosis, limited to a relatively small region of the cell, unlike horse tail nuclear movement spanning the entire cell.

To confirm involvement of the mating pheromone in nuclear movement, we examined *h*⁻ haploid cells treated with synthetic P factor (Imai and Yamamoto, 1994). For this experiment, we used a *h*⁻ *sxa2*⁻ *cyr1*⁻ strain which is sensitive to P factor (Imai and Yamamoto, 1992). As shown in Figure 2C and E, *h*⁻ haploid cells treated with synthetic P factor show a morphological change, forming a conjugation tube as in the normal process of conjugation in which a pair of cells form conjugation tubes towards each other. Nuclei in cells treated with P factor showed karyogamy-like movement similar to that in *h*⁺ *mam2*⁻ cells (Figure 2E). On the other hand, nitrogen starvation alone does not induce formation of a conjugation tube or nuclear movement. These results show that nuclear movement during karyogamy as well as formation of a conjugation tube start in response to mating pheromone.

Telomeres form a cluster near the SPB in response to mating pheromone

To examine the effect of mating pheromone on telomere-centromere switching, we determined the positions of telomeres and centromeres in haploid cells that had responded to mating pheromone, using fluorescence *in situ* hybridization. We first confirmed that nitrogen starvation alone did not induce association of telomeres with the SPB in haploid cells (data not shown). Then we examined nuclear location of telomeres and centromeres in haploid

Fig. 3. Position of telomeres and centromeres after P factor treatment. (A and B) Before P factor treatment. (C and D) 3 h after P factor treatment. (E and F) 7 h after P factor treatment. Red signal represents the SPB in all examples. Green signal represents either the telomere (A, C and E) or the centromere (B, D and F). Nuclear DNA was counterstained with DAPI and is displayed in blue. In (E), a telomere signal is slightly out of focus in one of the examples indicated by an arrowhead; an image of the same cell at 0.3 μm focus separation is shown in the inset. Scale bar 5 μm .

cells of a $h^- \text{ sxa2}^- \text{ cyl1}^-$ strain treated with synthetic P factor. Before P factor treatment, telomeres were separated from the SPB (Figure 3A) and centromeres were clustered near the SPB (Figure 3B and Table I). As shown in Figure 3C–F, h^- haploid cells treated with synthetic P factor for 3–7 h show a morphological change, forming a conjugation tube from one end of the cell. In such haploid cells having a conjugation tube, a cluster of telomeres was located

near the SPB in most of the cells (Figure 3C and E and Table I). Strikingly, however, centromeres were also found to remain clustered near the SPB (Figure 3D and F and Table I). Thus, it follows that both centromeres and telomeres are confined within a small region near the SPB in haploid cells that have responded to mating pheromone.

To eliminate the possibility that the results obtained from P factor treatment are due to the $\text{sxa2}^- \text{ cyl1}^-$ mutations, we analyzed nuclear organization in $h^+ \text{ mam2}^-$ cells that had responded to mating pheromone. When $h^- \text{ mam2}^-$ cells and $h^+ \text{ mam2}^-$ cells are mixed in sporulating medium, only $h^+ \text{ mam2}^-$ cells form a conjugation tube in response to mating pheromone. We analyzed mam2^- cells that had formed a conjugation tube; in these cells, both centromeres and telomeres were clustered near the SPB (Figure 4A and Table I). In these examples, co-localization of centromeres and telomeres with the SPB was demonstrated by simultaneous staining. This result is consistent with the observations in haploid cells treated with P factor. These results indicate that the association of telomeres with the SPB takes place in response to mating pheromone and does not require conjugation with a cell of the opposite mating type. Telomere clustering at the SPB demonstrates an example in which an extracellular signal, mating pheromone, leads to drastic reorganization of nuclear structures.

Centromeres detach from the SPB in a pair of conjugating cells

Mating pheromone alone was not sufficient for detachment of centromeres from the SPB as described above. On the other hand, it has been demonstrated that centromeres are separated from the SPB in conjugated diploid cells (Chikashige *et al.*, 1994; see also Table I). To examine when in meiosis centromeres detach from the SPB, we analyzed the nuclear location of telomeres and centromeres in genetically well-characterized mutant strains, fus1-D20 and mei1-B102 , which are arrested at later stages of meiosis. Cells of the fus1 mutant form a conjugation tube towards a mating partner but do not complete conjugation (Bresch *et al.*, 1968; Petersen *et al.*, 1995; see also Figure 6H). Pairs of fus1^- cells are not stably held together and separate during the hybridization procedure; thus, analysis was in the haploid nucleus in separate cells. In the mei1 mutant, cell conjugation and nuclear fusion are completed, but these cells are not committed to meiosis (Egel, 1973a; see also Figure 6I); analysis was in a diploid nucleus in a conjugated zygote. In both the fus1 and mei1 mutants, centromeres were separated from the SPB and telomeres were associated with the SPB in most of the cells, as summarized in Table I. Typical examples of fus1^- cells are shown in Figure 4B(a) for complete association of telomeres with the SPB and in Figure 4B(d) for complete separation of centromeres from the SPB. Figure 4B(b) shows an example of partial separation of telomeres from the SPB; in these cases, minor signals were observed in addition to a major signal close to the SPB. Figure 4B(c) shows an example of partial association of centromeres with the SPB; in these cells, a faint signal was observed near the SPB although the majority of centromere signals were separated from the SPB. A typical example of mei1^- cells is shown in Figure 4C(a); in these cells, telomeres are clustered near the SPB. Figure 4C(b) shows an example

Table I. Telomere/centromere association with the SPB

	Telomere–SPB				Centromere–SPB			
	Completely associated	Partially separated	Completely separated	Total No.	Completely associated	Partially separated	Completely separated	Total No.
<i>h⁻ sxa2⁻ cyr1⁻</i>								
P factor 0 h	0	0	51	51	8	0	0	8
3 h	44	7	14	65	48	5	6	59
7 h	8	0	0	8	30	6	7	43
<i>h⁺ mam2⁻</i>	42	4	1	47	65	7	1	73
<i>fus1⁻</i>	22	14	3	39	3	16	80	99
<i>mei1⁻</i>	26	12	0	38	0	2	42	44
<i>h⁺/h⁻</i> wild-type diploid	200	0	0	200	0	1	121	122

Fig. 4. Position of telomeres and centromeres in mutant cells. (A) *h⁺ mam2-A84* cells. (B) *h⁹⁰ fus1-D20* cells. (C) *h⁹⁰ mei1-B102* cells. Red signal represents the SPB in all examples. In (A), green signal represents the telomere and blue signal represents the centromere while DAPI counterstaining is displayed in orange. In (B) and (C), green signal represents either the telomere or centromere while DAPI counterstaining is displayed in blue. Scale bar 5 μ m.

of partial separation of telomeres from the SPB; in these cases, minor signals were observed in addition to a major signal close to the SPB. Figure 4C(c) shows a typical

example of centromeres in *mei1⁻* cells; centromeres were completely separated from the SPB in most *mei1⁻* cells (Table I). In this mutant, centromeres tended to disperse,

Fig. 5. Nuclear location of minichromosome III. (A) Construction of minichromosome III. The 450 kbp minichromosome III is made up of a 390 kbp portion of chromosome III spanning the centromere and 60 kbp vector sequences inserted into the centromeric region (designated Chr33-Tr29 in Niwa *et al.*, 1989). (B) Three possible locations of minichromosome III within a horse tail nucleus. In (A) and (B), red-filled squares represent telomeric repeats and green-filled circles represent centromeric repetitive sequences; hatched regions near the centromere represent the sequence shared by chromosome III and minichromosome III while blue squares represent the vector sequences specific to minichromosome III. The purple cross-hatched circle in (B) represents the SPB. (C) Cells bearing minichromosome III probed with a minichromosome III-specific sequence; the inset shows cells bearing minichromosome III probed with the centromere probe. The red spot represents the SPB and the green spot represents the hybridization signal. Nuclear DNA was counterstained with DAPI and is displayed in blue. Scale bar 5 μ m.

as shown in Figure 4C(c); out of 42 cases of complete separation, centromeres were diffused in 29 cases and were clustered in 13 cases.

Nuclear location of minichromosomes in meiotic prophase

To test whether the rare meiotic recombination between chromosome III and minichromosome III, which share DNA sequences spanning the centromere, reflects the spatial organization of chromosomes bundled at the telomere, we examined the nuclear location of minichromosome III in horse tail nuclei. As shown in Figure 5A, minichromosome III shares with chromosome III a 390 kbp sequence spanning the centromere; in addition, minichromosome III contains a 60 kbp extra sequence derived from a vector which can be used as a specific probe for minichromosome III (Niwa *et al.*, 1989). Both ends of minichromosome III are directly flanked by the telomeric

repeats without the telomere-adjacent sequence or the rRNA genes. It is known that meiotic recombination between chromosome III and minichromosome III is rare in spite of their 390 kbp DNA sequence homology (Niwa *et al.*, 1986, 1989).

If chromosome III and minichromosome III are aligned by DNA sequence homology, minichromosome III is expected to be found at the site of the centromere cluster (Figure 5B, left); if aligned by telomeres, on the other hand, minichromosome III should be found at the site of the telomere cluster (Figure 5B, right). Minichromosome III is not long enough for its centromere and its telomeres all to colocalize with their authentic counterparts on chromosome III, unless the centromere and telomeres of authentic chromosome III form a cluster at the site of minichromosome III (Figure 5B, middle).

When cells bearing minichromosome III were probed with the minichromosome III-specific sequence, only one hybridization signal (green spot) was observed near the SPB (red spot), as shown in Figure 5C. The same result of a unique signal associated with the SPB was obtained in 27 out of 31 cases examined; the remainder had faint signals in addition to one major signal associated with the SPB. On the other hand, when probed with the centromere sequence common in authentic chromosomes and minichromosome III (green spot), extra centromeres signals separated from the SPB were observed in addition to one signal associated with the red spot of the SPB (Figure 5C, inset). These results of *in situ* hybridization showed that minichromosome III is located at the SPB and is separated from the centromere of authentic chromosomes. This chromosome arrangement accounts for the rare recombination between authentic chromosome III and minichromosome III (Niwa *et al.*, 1986, 1989).

Discussion

Two-step nuclear reorganization

Previous studies demonstrated that telomeres take the position of centromeres upon entering meiosis (Chikashige *et al.*, 1994). Upon nitrogen starvation, fission yeast cells are induced to undergo meiosis in response to mating pheromone (Fukui *et al.*, 1986; Leupold, 1987). The mating pheromone response is followed by cell conjugation in the presence of a mating partner and telomere-centromere switching is completed in conjugated zygotes. In an attempt to find intermediate structures for telomere-centromere switching, we designed haploid meiosis experiments to separate cell conjugation from mating pheromone response. An intermediate structure of telomere-centromere switching was observed in haploid cells that had responded to mating pheromone; both centromeres and telomeres were co-localized near the SPB in these cells (Figure 6F and G).

Thus, telomeres first become associated with the SPB in response to mating pheromone and this association does not require cell conjugation with an opposite mating type cell (Figure 6A and B). On the other hand, the mating pheromone response is not sufficient for centromere-SPB detachment; centromeres detach from the SPB at a later stage of meiosis, as discussed later (Figure 6B and C). Further analyses of the nuclear location of telomeres and centromeres in a series of meiotic mutants confirmed that

Fig. 6. Nuclear reorganization in fission yeast meiosis. Centromeres, telomeres and the SPB are represented by open circles, filled circles and an open square respectively. At the time of induction of meiosis, centromeres form a single cluster near the SPB while telomeres are separated from the SPB (A). In response to the mating pheromone, telomeres form a cluster close to the SPB and centromeres remain clustered near the SPB (B). After cell conjugation, centromeres detach from the SPB (C). Association of homologous chromosomes begins at the telomeres led by the SPB (D). Chromosomes have a polarized centromere–telomere configuration with an orientation opposite to each other in parental nuclei (D). After several rounds of oscillation, parental sets of chromosomes are suddenly pulled by the SPB moving in one direction and adopt the horse tail shape (D and E). By this motion, parental sets of chromosomes now have the same orientation as the polarized centromere–telomere configuration in a horse tail nucleus (E). Nuclear reorganization in mutant strains; *ah- sxa2- cyr1-* haploid cell treated with P factor (F), *mam2-A84* (G), *fus1-D20* (H) and *mei1-B102* (I).

telomere–centromere switching takes place in two steps, as summarized in Table I (also see Figure 6F–I). This scheme of two-step switching ensures that the SPB is kept associated with either or both centromeres and telomeres at all times during switching.

We infer that centromeres detach from the SPB in a conjugation-related manner, probably by the combined actions of opposite mating type cells or by zygotic gene expression in diploid cells. Centromeres are separated from the SPB in *fus1* mutant cells, whereas both telomeres and centromeres remain associated with the SPB in *h+* *mam2-* cells. In *mam2* mutant cells, the sterile phenotype is specific to *h-* cells and *h+* *mam2-* cells respond to mating pheromone normally. Thus, the nuclear organization in *h+* *mam2-* cells represents the terminal state that can be reached in the haploid state in response to mating pheromone. Unlike the case of *mam2-* cells, in which *h-* cells do not form a conjugation tube (Figure 6G), cells with both mating types form a conjugation tube in *fus1* mutant cells, although cell conjugation is abortive (Figure 6H). This suggests that centromere–SPB detachment requires a mating partner with a conjugation tube but does not require the completion of conjugation.

Alternatively, centromere–SPB detachment may require the diploid state. In spite of the fact that *fus1-* cells do not achieve the diploid state, mutation in the *fus1+* gene may cause centromere–SPB detachment prematurely in the haploid state. One of the candidates for a signal monitoring the haploid/diploid state is a mating type-specific gene product such as *mat1-Pm* in *h+* cells and *mat1-Mm* in *h-* cells, cooperation between which commits cells to meiosis. It is unlikely that centromeres detach from the SPB under the regulation of the *mat1-Pm* and

mat1-Mm genes because centromeres separate from the SPB in a *mei1-B102* mutant bearing a point mutation in the *mat1-Pm* gene. However, we cannot eliminate the possibility that *mei1-B102* is deficient in commitment to meiosis but is functional for centromere–SPB detachment.

Telomere first in meiotic prophase

An interesting question in meiotic chromosomal events is how homologous chromosomes find each other. The analysis of minichromosome locations reported here indicates that homologous chromosomes are aligned first at telomeres, instead of by DNA sequence homology, during meiotic prophase. The nuclear location of minichromosome III spatially separated from authentic centromeres also accounts for the rare recombination between authentic chromosome III and minichromosome III. This is not because minichromosome III lacks the capability for meiotic recombination; in fact, the meiotic recombination rate per chromosome length between minichromosomes III was similar to that between authentic chromosomes III (O.Niwa, personal communication). Intriguingly, a *kms1* mutant strain which is deficient in telomere clustering showed a reduced rate of meiotic recombination (O.Niwa, personal communication). Moreover, in spite of the reduced recombination rate in authentic chromosomes, meiotic recombination rate between authentic chromosome III and minichromosome III was elevated in *kms1* mutant cells (O.Niwa, personal communication). These observations suggest that recombination between homologous chromosomes is affected by telomere positioning within a nucleus.

It should be emphasized that in fission yeast, the horse tail period of a few hours is the only chance to align, pair

and recombine homologous chromosomes because fusion of haploid nuclei is immediately followed by the horse tail period and then by meiotic segregation. It is obvious from observations of karyogamy that the telomere is the first contact site in nuclear fusion (Chikashige *et al.*, 1994; see also Figure 6C). Immediately after nuclear fusion, telomere-led chromosome movement alters the polarized telomere-centromere configuration of homologous chromosomes from antiparallel to parallel (Figure 6D and E). This parallel orientation of homologous chromosomes aligned at the telomere is a prerequisite for pairing.

Possible roles of meiotic telomeres

The characteristic chromosome arrangement in meiotic prophase, the bouquet structure, implies functions of meiotic telomeres required specifically for meiotic chromosomal events such as association of homologous chromosomes. During this period of meiotic prophase, heterologous chromosomes caught between homologous chromosomes need to be moved out of the way to allow a pair of homologous chromosomes to be aligned. It may be reasonable to bundle chromosomes, homologous or heterologous, into a small confined volume at the telomeres and then to shuffle the clustered telomeres around each other to search for a homologous partner.

In a wide range of animals, a special structure called an attachment plaque is observed at the ends of chromosomes in meiotic prophase, although its biochemical properties have not been characterized (reviewed in Fussell, 1987). In budding yeast, some protein species have been characterized as telomere proteins; protein Rap1 binds to non-nucleosomal DNA of the telomeric repeats and proteins Sir3 and Sir4 are involved in proper positioning of telomeres on the nuclear membrane (Klein *et al.*, 1992; Palladino, 1993; Gotta *et al.*, 1996). It has also been reported that the telomere region has no nucleosome structure in the fission yeast *S.pombe* (Chikashige *et al.*, 1989) and that protein swi6 co-localizes with telomeres as well as centromeres and the mating-type loci (Ekwall *et al.*, 1995). However, meiosis-specific components of the telomere have not been reported so far.

Telomeres of all three chromosomes as well as an artificial minichromosome co-localized near the SPB. Minichromosome III does not share a telomere-adjacent sequence with chromosomes I and II nor rRNA genes with chromosome III (Matsumoto *et al.*, 1987). Since the telomeric repeats are the only sequences shared by the ends of the three authentic chromosomes and minichromosome III, it is inferred that the telomeric repeat 5'-TTACAGG-3' at the very ends of the chromosomes are sufficient DNA sequences for telomere clustering in meiotic prophase.

Because telomeric repeats are well conserved among species, we expect that molecular mechanisms for telomere clustering are conserved in eukaryotes. For a better understanding of the roles of the meiotic telomere in nuclear organization, a combination of molecular biological approaches and structural approaches in a single organism will be necessary. Our microscopic studies of nuclear organization in fission yeast will provide a new insight into meiosis-specific functions of telomeres.

Materials and methods

Microscope system set-up

Fluorescence microscopic images were obtained with a cooled, charge-coupled device (CCD) as image detector. A Peltier-cooled CCD camera (Photometrics Ltd, Tucson, AZ) with a 1317×1035 pixel CCD chip (KAF1400) was attached to an Olympus inverted microscope IMT-2; the microscope lamp shutter, focus movement, CCD data collection and filter combinations were controlled by a Silicon Graphics Iris 4D35/TG. Details of the microscope system set-up are described in Hiraoka *et al.* (1991), except that a MicroVAX was replaced by the Silicon Graphics workstation.

Strains and media

Strains used in this study were as follows: HM143 (*h⁹⁰ ade6-210*), JZ916 (*h⁻ leu1-32 ura4-D18 ade6-216 sxa2::ura4⁺ cyl1::ura4⁺*), CRL109 (*h⁹⁰ ade6-210 Chr33-Tr29*), C114-2D (*h⁹⁰ leu1-32 ade6-216 mam2-A84*), C112-2C (*h⁹⁰ leu1-32 ade6-216 fus1-D20*) and CRL101 (*mei1-B102 leu1-32 ade6-210*). Complete medium YEade (YE medium containing 75 mg/ml adenine sulfate) and minimal medium EMM2 were used for routine culture of *S.pombe* strains (Moreno *et al.*, 1991). For observation of mating and meiosis, *h⁹⁰* strains were cultured in EMM2 supplemented with appropriate nutrients, washed in EMM2 -N (EMM2 deprived of nitrogen) and incubated in EMM2 -N at 25°C either for 6–10 h (HM143 and CRL109) or 16–24 h (C114-2D, C112-2C and CRL101). P factor treatment was carried out as described in Imai and Yamamoto (1994) with some modifications. JZ916 cells (*h⁻ sxa2⁻ cyl1⁻*) were grown in EMM2 supplemented with appropriate nutrients to 2–4×10⁶ cells/ml and synthetic P factor was added to a final concentration of 0.5 µg/ml. After incubation at 30°C for 3–7 h, cells were examined either live or after fixation.

Live analysis of chromosome movement

Cells were stained with Hoechst 33342 (0.5 µg/ml) in distilled water for 10 min at a room temperature and then resuspended in EMM2 -N as previously described (Chikashige *et al.*, 1994). Stained cells were mounted either on a coverslip or in a 35 mm glass-bottomed culture dish (MatTek Corp., Ashland, MA) coated with concanavalin A (1 mg/ml). Fluorescence microscopic images of Hoechst 33342-stained living cells were obtained on a cooled CCD with an exposure of 0.1–0.2 s under mercury arc lamp illumination using an excitation filter with a narrow peak at 380 nm (Chroma Technology, Brattleboro, VT). For live imaging, an Olympus oil immersion objective lens (SPlan Apo 60/NA=1.4) was used. Each pixel represents 0.11 µm in the specimen plane.

Immunofluorescence microscopy and fluorescence in situ hybridization

Immunofluorescence microscopy and fluorescent *in situ* hybridization were carried out as described previously (Chikashige *et al.*, 1994) with some modifications. Cells were fixed with 3% formaldehyde (freshly prepared from paraformaldehyde) and 0.2% glutaraldehyde in PEM buffer (100 mM PIPES, pH 6.9, 1 mM EGTA, 1 mM MgCl₂) for 10 min at room temperature. After fixation, cells were permeabilized and treated with RNase A as described in Funabiki *et al.* (1993). Position of the SPB was determined by indirect immunofluorescence microscopy with an antibody against the fission yeast *sad1⁺* gene product, which is well characterized as a marker of the SPB in fission yeast mitotic cells (Hagan and Yanagida, 1995), and a Texas Red-conjugated second antibody. Immunofluorescence labeling was carried out in PEM buffer as described in Hagan and Hyams (1988). After immunofluorescence labeling, cells were re-fixed with 3% formaldehyde for 5 min at room temperature and subjected to *in situ* hybridization.

Plasmid pRS140, which hybridizes to centromeric repeats in all three chromosomes (Chikashige *et al.*, 1989), was used to probe centromeres. Cosmid cos212 was used to probe the ends of chromosomes I and II (Funabiki *et al.*, 1993), while the end of chromosome III was probed by rRNA genes (Uzawa and Yanagida, 1992). For detection of minichromosome Chr33-Tr29 (Niwa *et al.*, 1989), plasmid YIp32 was used. DNA fragments were labeled with digoxigenin-dUTP by a terminal transferase reaction using an oligonucleotide tailing kit (Boehringer Mannheim). Heat-denatured DNA probe was hybridized to chromosomal DNA that had been denatured in 0.1 M NaOH. Hybridization was carried out in 50% formamide and 2× SSC at 37°C for 1 h. Hybridization signals were detected with the use of fluorescein-conjugated anti-digoxigenin antibody (Boehringer Mannheim).

For double *in situ* hybridization (Figure 1C), one of the DNA probes

was labeled with fluorescein-dUTP; the other DNA probe was labeled with digoxigenin-dUTP and detected with rhodamine-conjugated anti-digoxigenin antibody (Boehringer Mannheim). Nuclear DNA was counterstained with 1 µg/ml DAPI. In the case of quadruple staining (Figure 4A), telomeres and centromeres were stained with cos212 DNA fragments labeled with fluorescein-dUTP and pRS140 DNA fragments labeled with Cy3-dUTP respectively; the SPB was detected with an antibody against *S.pombe* γ -tubulin (Masuda and Shibata, 1996) and Cy5-conjugated second antibody; nuclear DNA was counterstained with DAPI.

Three or four color fluorescent images were obtained on a computerized CCD microscope using an Olympus oil immersion objective lens (DPlan Apo 100/NA=1.3). Each pixel represents 0.07 µm in the specimen plane. High selectivity excitation and barrier filter combinations (Chroma Technology, Brattleboro, VT) for DAPI, fluorescein, Texas Red and Cy5 were used. For wavelength switching during data collection, excitation and barrier filters were mounted on revolving wheels controlled by the Silicon Graphics workstation. A single dichroic mirror with quadruple bandpass properties designed for the wavelengths of DAPI, fluorescein, Texas Red and Cy5 (Chroma Technology, Brattleboro, VT) was used to eliminate significant displacement of images during wavelength switching and thus no further alignment was necessary (Hiraoka *et al.*, 1991).

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