The Fission Yeast Meiotic Regulator Mei2p Forms a Dot Structure in the Horse-Tail Nucleus in Association with the *sme2* Locus on Chromosome II

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Fission yeast Mei2p is an RNA-binding protein essential for induction of both premeiotic DNA synthesis and first meiotic division. Mei2p forms a dot structure at an apparently fixed position in the horse-tail nucleus during meiotic prophase. This dot formation requires a meiosis-specific RNA species, meiRNA, which is indispensable for meiosis I, and the emergence of the dot is an indicator of the ability of the cell to perform meiosis I. Herein, we have sought the identity of this dot. Analyses using chromosome segregation in haploid meiosis, reciprocal translocation of chromosomes, and gene translocation have led us to conclude that the Mei2p dot is in association with the *sme2* gene on the short arm of chromosome II, which encodes meiRNA. Transcripts of *sme2*, rather than the DNA sequence of the gene, seem to be the determinant of the localization of the Mei2p dot. However, evidence suggests that the dot may not be a simple reflection of the attachment of Mei2p to meiRNA undergoing transcription. We speculate that the Mei2p dot is a specialized structure, either to foster the assembly of Mei2p and meiRNA or to perform some unidentified function indispensable for meiosis I.

INTRODUCTION

The RNA-binding protein Mei2p is a key regulator of meiosis in fission yeast *Schizosaccharomyces pombe*, which is required for the induction of both premeiotic S-phase and first meiotic division (meiosis I) (Watanabe and Yamamoto, 1994; Yamamoto *et al.*, 1997). The *mei2* gene is expressed very poorly, if at all, in cells growing mitotically (Shimoda *et al.*, 1987; Watanabe *et al.*, 1988). In addition, Pat1(Ran1) kinase, which is active during the mitotic cell cycle, represses the function of Mei2p by phosphorylating it on two amino-acid residues (Watanabe *et al.*, 1997). To date, two mechanisms have been elucidated concerning how the phosphorylation by Pat1 kinase down-regulates Mei2p activity. 1) Phosphorylated Mei2p is more susceptible to ubiquitin-mediated proteolysis (Kitamura *et al.*, 2001). 2) 14-3-3 protein binds preferentially to phosphorylated Mei2p and thereby decreases the affinity of Mei2p for RNA (Sato *et al.*, 2002).

In natural meiosis, the activity of Pat1 kinase is turned off by the binding of a pseudosubstrate Mei3p, which is ex-

pressed only in diploid cells heterozygous for the mating type genes under starved conditions (McLeod and Beach, 1988; Li and McLeod, 1996). Nutritional starvation also results in enhancement of *mei2* transcription (Shimoda *et al.*, 1987). Thus, diploid cells exposed to starvation accumulate unphosphorylated Mei2p, which is a critical step to commit the cells to meiosis (Watanabe *et al.*, 1997). Mei2p shuttles between the cytoplasm and the nucleus (Sato *et al.*, 2001), and the ability of Mei2p to bind to RNA is essential for its activity to stimulate meiosis (Watanabe and Yamamoto, 1994).

One peculiar property of Mei2p is that it forms a distinct “dot” in the nucleus at meiotic prophase (Watanabe *et al.*, 1997; Yamashita *et al.*, 1998). The nucleus assumes an elongated shape like a horse tail and moves back and forth during meiotic prophase, led by the spindle pole body (SPB) (Chikashige *et al.*, 1994). The relative position of the Mei2p dot is apparently fixed in the horse-tail nucleus (Yamashita *et al.*, 1998). Formation of the Mei2p dot requires an RNA species called meiRNA, which specifically binds to Mei2p and colocalizes with it in the dot (Yamashita *et al.*, 1998). meiRNA is indispensable for the Mei2p function to stimulate meiosis I (Watanabe and Yamamoto, 1994). Because the emergence of this dot seems to correlate with the ability of the cell to perform meiosis I (Yamashita *et al.*, 1998; Sato *et al.*, 2001), we have set out to clarify the identity of the dot,

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Table 1. *S. pombe* strains used

Strain	Genotype
JW215	<i>h⁹⁰ ade6-M216 leu1 gar2⁺-GFP<<kan^r</i>
JW329	<i>h⁹⁰ ade6-M216 leu1 ura4-D18 taz1::ura4⁺ gar2⁺-GFP<<kan^r</i>
JW916	<i>h⁺ ade6-M216 leu1 pat1-114 mei2⁺-GFP<<kan^r</i>
JW917	<i>h⁺ ade6-M216 leu1 lys1::(leu1⁺<<mat1-M) mei2⁺-GFP<<kan^r</i>
JW935	<i>h⁹⁰ ade6-M26&469 leu1 ura4-D18 sad1⁺-GFP<<kan^r mei2⁺-CFP<<kan^r site G-(ade6⁺<<ura4⁺) tII;III</i>
JW936	<i>h⁹⁰ ade6-M26&469 leu1 ura4-D18 sad1⁺-GFP<<kan^r mei2⁺-CFP<<kan^r site H-(ade6⁺<<ura4⁺) tII;III</i>
JW937	<i>h⁹⁰ ade6-M216 leu1 ura4-D18 lys1::(ura4⁺<<sme2⁺) mei2⁺-GFP<<kan^r</i>
JW938	<i>h⁹⁰ ade6 leu1 ura4-D18 lys1::(ura4⁺<<sme2⁺) sme2::ura4⁺ mei2⁺-GFP<<kan^r</i>
JW940	<i>h⁹⁰ ade6 leu1 ura4-D18 lys1::(ura4⁺<<sme2⁺) sme2::ura4⁺ mei2⁺-CFP<<kan^r lys1<<LacO his7⁺<<GFP-LacI-NLS</i>
JW941	<i>h⁹⁰ ade6 leu1 ura4-D18 lys1::(ura4⁺<<sme2-m) mei2⁺-GFP<<kan^r</i>
JW942	<i>h⁹⁰ ade6 leu1 ura4-D18 lys1::(ura4⁺<<sme2-m) sme2::ura4⁺ mei2⁺-GFP<<kan^r</i>
JW456	<i>h⁹⁰ ade6-M210 leu1 ura4-D18 sme2::ura4⁺ mei2⁺-GFP<<kan^r</i>
JW231	<i>h⁹⁰ ade6-M216 leu1 mei2⁺-GFP<<kan^r</i>
JW859	<i>h⁻ ade6-D19 leu1 ura4-D18</i>
JW943	<i>h⁹⁰ ade6-M26 leu1 ura4-D18 sad1⁺-GFP<<kan^r mei2⁺-CFP<<kan^r</i>
JW968	<i>h⁹⁰ leu1 ura4-D18 mei2⁺-GFP<<kan^r site G-(ade6-469<<ura4⁺)</i>

An unconventional expression *gar2⁺-GFP<<kan^r* indicates that the *kan^r* allele is inserted in the close vicinity of *gar2⁺-GFP*. *tII;III* indicates that the strain bears reciprocal translocation between chromosomes II and III.

hoping that the results may provide insight into the molecular function of Mei2p. In this report, we describe detailed analysis of the location of the dot. The obtained results lead us to conclude that the dot is closely associated with the gene for meiRNA, namely, *sme2*, positioned on the short arm of chromosome II. Implications of this finding are discussed.

MATERIALS AND METHODS

Fission Yeast Strains, Genetic Methods, and Media

S. pombe strains used in this study are listed in Table 1. General genetic procedures for *S. pombe* were carried out as described previously (Gutz *et al.*, 1974). Complete medium YE and minimal medium SD (Sherman *et al.*, 1986) were used for the routine culture of *S. pombe*. Minimal medium MM (Moreno *et al.*, 1990) was used to induce transcription from the *nmt1* promoter. Transformation of *S. pombe* cells was done by the lithium acetate method (Okazaki *et al.*, 1990).

Microscopy

Fluorescence images were detected by a cooled, charge-coupled device (Photometrics Peltier-cooled charge-coupled device camera Quantix), which was attached to a fluorescence microscope Axio-plan 2 (Carl Zeiss, Jena, Germany) and controlled by the MetaMorph program (Universal Imaging, Downingtown, PA). Immunofluorescence analysis was done essentially as described previously (Yamashita *et al.*, 1998). Briefly, *S. pombe* cells were fixed with 3% formaldehyde freshly prepared from paraformaldehyde and with 0.2% glutaraldehyde in PEM buffer (100 mM PIPES, pH 6.9, 5 mM EGTA, 5 mM MgCl₂) for 45 min at room temperature. After fixation, cells were treated with Zymolyase 100T (0.1 mg/ml; Seikagaku America, Rockville, MD) for 70 min at 37°C. They were suspended in 1% Triton X-100 and washed three times with 1 mg/ml sodium borohydride. The hemagglutinin (HA)-tagged Mei2 protein was stained with mouse monoclonal anti-HA antibody 16B12 (Babco, Richmond, CA), followed by Texas Red-conjugated sheep antibodies to mouse IgG (Amersham Biosciences, Piscataway, NJ). The SPB was visualized with anti-Sad1p, which recognizes its component

(Hagan and Yanagida, 1995). DNA was counterstained with Hoechst 33342.

Integration of Epitope-tagged mei2 Alleles into the Chromosomal mei2 Locus

We prepared an *EcoRI* fragment carrying the *mei2* open reading frame (ORF), which was truncated at the N terminus, tagged with an epitope at the C terminus, and followed by the *nmt1* terminator. The epitope used was either green fluorescent protein (GFP), or enhanced cyan fluorescent protein (CFP) (BD Biosciences Clontech, Palo Alto, CA), or three copies of HA. The *EcoRI* fragment was cloned into the integration vector pFA6a-kanMX6 (Bahler *et al.*, 1998). The resulting plasmid was linearized by cutting at the *KpnI* site within the *mei2* ORF and transformed into *S. pombe* cells. Stable Kan^r transformants were isolated, and they were confirmed by genomic polymerase chain reaction (PCR) to carry the epitope-tagged *mei2* gene generated by integration of the plasmid at the chromosomal *mei2* locus.

Induction of Haploid Meiosis

Two distinctive ways to induce haploid meiosis were used. 1) Cells carrying both *mat1-M* and *mat1-P* were cultured in MM+N medium at 30°C. At the concentration of $\sim 1 \times 10^7$ cells/ml, they were transferred to MM-N medium lacking a nitrogen source to induce meiosis. 2) The *pat1*-driven meiosis (Iino and Yamamoto, 1985; Nurse, 1985) was induced in haploid cells, adopting the protocol described for diploid cells homozygous for the mating-type genes (Murakami and Nurse, 1999). Haploid *pat1-114* cells were cultured in MM+N medium at 25°C. At the concentration of $\sim 1 \times 10^7$ cells/ml, they were transferred to MM-N medium containing 50 μ g/ml leucine, and incubated for 14–16 h at 25°C. NH₄Cl and leucine were added to the cultures at 500 and 50 μ g/ml, respectively, immediately before the shift to 34°C. The cells were kept at 34°C to induce meiosis.

Fluorescence Marking of Specific Chromosomal Loci

Marking of chromosomes by the LacI-LacO system was done as described originally for budding yeast (Straight *et al.*, 1996). We constructed various derivatives from the parental *S. pombe* strains

Table 2. LacO/*ade6-469* integration sites in noncoding regions

Site	Contig	Chromosome structure
A	SPAC521	991 AGGATGTCAG 1000 -LacO- <i>ura4</i> ⁺ - <i>kan</i> ^r - 1101 GGAAGCAATT
B	SPBC31F10	5991 TATCAGCATA 6000 -LacO- <i>ura4</i> ⁺ - <i>kan</i> ^r - 6101 AATCTGAATT
C	SPCC663	3991 TAATGAGATC 4000 -LacO- <i>ura4</i> ⁺ - <i>kan</i> ^r - 4101 TTTTGGACTG
D	SPBC428	32891 AAATAAAGAG 32900 -LacO- <i>ura4</i> ⁺ - <i>kan</i> ^r - 33001 TTAATTCAGT
E	SPBC56F2	22991 AAATTATAGA 23000 -LacO- <i>ura4</i> ⁺ - <i>kan</i> ^r - 23101 TTACCTTTTT
F	SPBC1271	8991 CTATTTAATC 9000 -LacO- <i>ura4</i> ⁺ - <i>kan</i> ^r - 9101 TTCGCGCAGA
G	SPBC106	<i>ade6-469-ura4</i> ⁺ was integrated into the <i>NdeI</i> site at 2319
H	SPBC1271	<i>ade6-469-ura4</i> ⁺ was integrated into the <i>SphI</i> site at 3372

either described previously (Nabeshima *et al.*, 1998) or further developed by A. Yamamoto (Kansai Advanced Research Center, Kobe, Japan). These strains expressed a GFP-LacI-nuclear localization signal (NLS) fusion protein to recognize the LacO sequence. The fusion gene for GFP-LacI-NLS was integrated at the *his7* locus and driven by the *dis1* promoter. Insertion of the 8-kb-long LacO sequence to each specific chromosomal locus was carried out using plasmids pCT32-6 and pCT31-13, provided by A. Yamamoto. pCT32-6 was a variant of pFA6a-kanMX6, and carried the *ura4* gene truncated at its N terminus (*ura4*ΔN) and the *kan*^r gene. The *ura4*ΔN and *kan*^r genes were integrated into a specific chromosomal locus, according to the PCR protocol described for pFA6a-kanMX6 by Bahler *et al.* (1998). Stable Kan^r transformants were selected and then transformed with pCT31-13, which carried the *ura4* gene truncated at its C terminus (*ura4*ΔC) and 256 copies of the *lacZ* operator sequence. pCT31-13 was linearized by cutting at the *StuI* site within the *ura4* ORF before transformation. Thus, the resulting Ura⁺ strain bore the LacO sequence at each specific locus on the chromosome. Genomic PCR and Southern blot hybridization were carried out to confirm the integration of the LacO sequence at the targeted chromosomal locus. The LacO integration sites A through F, which are located in noncoding regions, are defined precisely in Table 2. Expression of GFP-LacI-NLS visualized the LacO sequence on the chromosome by fluorescence.

Reciprocal Chromosome Translocation

We followed the method developed by Virgin *et al.* (1995). A 1.8-kb-long *ura4*⁺ fragment was inserted into the *HindIII* site on the vector pBluescript (Stratagene, La Jolla, CA). A 2.9-kb-long *PvuII*-*SpeI* fragment carrying the *ade6-469* allele was then inserted into the same plasmid by using the *SmaI* and *SpeI* sites. The resulting plasmid was digested with *ClaI* and *SpeI*, which generated a 4.7-kb-long fragment containing *ura4*⁺ and *ade6-469*. Plasmids harboring either site G or site H (Figure 3B) were constructed by PCR amplification of genomic DNA, and the *ClaI*-*SpeI* fragment was cloned into either the *NdeI* site (site G) or the *SphI* site (site H). The resulting plasmid carrying site G was digested with *SacI* and *KpnI* and that carrying site H was digested with *NotI* and *KpnI*. The fragments thus obtained were transformed into JW859 (*h*⁻ *ade6-D19 leu1 ura4-D18*), a derivative of the strain provided by G. Smith (Fred Hutchinson Cancer Research Center, Seattle, WA), and stable Ura⁺ transformants were isolated. Genomic PCR was carried out to confirm that the *ura4*⁺ and *ade6-469* markers were properly integrated at either site G or site H. The resultant strains were crossed with an *h*⁹⁰ *ade6-M26* strain JW943. Sporulation was induced and Ade⁺ spores were selected to isolate strains with arms of chromosomes II and III translocated reciprocally. Genomic PCR was carried out to confirm that the chromosomes were crossed over at the *ade6* sequence on chromosome II (site G or H) and the authentic *ade6* locus on chromosome III.

RESULTS

The Mei2p Dot Is Outside of the Nucleolus

We first examined whether the Mei2p dot was inside the nucleolus, because Mei2p accumulated in the nucleolus of mammalian COS-7 cells when it was artificially expressed together with meiRNA (Yamashita *et al.*, 1998). To visualize nucleoli, we expressed a nucleolar protein Gar2p (Gulli *et al.*, 1995) conjugated with GFP, from a fusion gene that carried the authentic *gar2* promoter and was integrated at the original chromosomal locus. Electron microscopic observation has shown previously that Gar2p spreads throughout the nucleolus in interphase cells (Sicard *et al.*, 1998). Consistently, the Gar2p-GFP fusion protein occupied half of the spherical interphase nucleus, where no staining of DNA was observed (Figure 1A). This region has been assigned previously as the nucleolus (Toda *et al.*, 1981).

Using the Gar2p-GFP fusion construct, we visualized the nucleolar region in the meiotic prophase nucleus. The nucleolus was found to reside in the front region of the horse-tail nucleus, adjoining the SPB. It was rather elongated and flattened (Figure 1B). Similar images were observed when we performed indirect immunostaining by using the anti-NOP1 (Aris and Blobel, 1988), which recognizes another nucleolar protein fibrillarin (Girard *et al.*, 1993) (our unpublished data). Electron microscopic observation has also assigned an electron-dense region near the SPB as the putative nucleolus (Bahler *et al.*, 1993). To determine the position of the Mei2p dot relative to the nucleolus, we fixed meiotic cells expressing Gar2p-GFP and 3HA-tagged Mei2p. We then immunostained the cells with anti-HA to detect Mei2p and anti-Sad1p to detect SPB (Hagan and Yanagida, 1995). Gar2p-GFP emitted enough fluorescence for detection even after fixation. A typical result is shown in Figure 1B. In most cells the Mei2p-HA dot was close to the rear edge of the nucleolus, but it was always outside the nucleolus, indicating that Mei2p was not associated with part of the nucleolus.

The Mei2p Dot Segregates Like a Chromosome in Haploid Meiosis

We next examined the possibility that the Mei2p dot might be associated with chromosomes. As a characteristic of the meiotic nuclei in fission yeast, telomeres cluster near the SPB during prophase (Chikashige *et al.*, 1994, 1997). Centromeres are away from the SPB and each part of the chromosomes apparently occupies a specific position relative to the SPB (Niwa *et al.*, 2000). However, the telomere clustering is dis-

Figure 1. Relative localization of the Mei2p dot, the SPB, and the nucleolus in the meiotic prophase nucleus. The Mei2p dot was visualized with anti-HA, the SPB, with anti-Sad1p, and the nucleolus, with GFP-tagged Gar2p. DNA was counterstained with Hoechst 33342. (A) Cells of JW215 (*h90 gar2-GFP*) growing mitotically were live observed. (B) JW215 cells carrying pREP41-Mei2p-3HA were fixed under the meiotic conditions and stained for DNA, Mei2p-3HA, and SPB. (C) Cells of the *taz1Δ* strain JW329 (*h90 taz1Δ gar2-GFP*) were analyzed as in B. The merged images display DNA (blue), Gar2-GFP (green), Mei2p-3HA (red), and SPB (white). Bar, 10 μ m.

rupted and the prophase-specific chromosome arrangement is distorted in the *taz1Δ* mutant, which lacks a telomere-binding protein (Cooper *et al.*, 1998). We visualized the Mei2p dot by immunostaining in *taz1Δ* cells, and stained the cells also with anti-Sad1p, which marked the SPB at the leading edge of the horse-tail nucleus. Compared with wild-type cells (Figure 1B), the Mei2p dot was far distant from the SPB in *taz1Δ* cells (Figure 1C). These results suggested that the location of the Mei2p dot was unlikely to be directed by the SPB. Rather, the Mei2p dot seemed to have been displaced together with the bulk of chromosomes disconnected from the SPB in *taz1Δ* cells.

To investigate the possibility that the Mei2p dot is attached to one of the three chromosomes, we used a combination of two haploid-meiosis systems in fission yeast. The first system was a strain that carried the *mat1-M* gene in addition to its endogenous *mat1-P* gene, which could perform haploid meiosis under nutrient starvation (Kelly *et al.*, 1988). In this system, meiosis is activated through mating pheromone signaling, and the chromosome segregation in meiosis I is reductional (Yamamoto and Hiraoka, 2003). In other words, the two sister chromatids generated by replication do not separate in the first division but move together into one daughter nucleus (Figure 2A). In the second system, haploid meiosis was induced by inactivation of thermolabile Pat1p kinase (Iino and Yamamoto, 1985; Nurse, 1985). The first division in *pat1*-driven haploid meiosis, which proceeds in the absence of mating pheromone signaling, is mostly equational, i.e., the sister chromatids tend to segregate into two daughter nuclei at meiosis I (Yamamoto and Hiraoka, 2003) (Figure 2A).

In natural meiosis, the Mei2p dot persists until telophase of meiosis I and segregates into two daughter nuclei (Yamashita *et al.*, 1998). Thus, we expected that, if the Mei2p dot was linked to a chromosome, the dot would be seen in only one of the two split daughter nuclei in the first haploid meiosis system, whereas in both of them in the second system. The experimental results indicated that the expectation was correct. In the first system, the Mei2p dot was observed in only one daughter nucleus at telophase of meiosis I (Figure 2B). In contrast, we could observe the dot in each daughter nucleus in the second system (Figure 2C). Thus, the Mei2p dot was suspected to be associated with one of the three chromosomes.

Association of the Mei2p Dot with Chromosome II

We used the first haploid-meiosis system further to identify the chromosome with which the Mei2p dot cosegregated. We marked each of the three chromosomes by using the *lac* repressor/operator system (Nabeshima *et al.*, 1998). The *lac* operator sequence LacO, which could be visualized by the binding of the GFP-conjugated *lac* repressor protein LacI, was integrated into specific sites on chromosomes. Two sites were chosen for each chromosome: chromosome I, *lys1* and "site A" (between SPAC521.01 and SPAC521.02); chromosome II, *cut3* and "site B" (between SPBC31F10.05 and SPBC31F10.06); and chromosome III, *ade6* and "site C" (between SPCC663.02 and SPCC663.03). These sites are schematically shown in Figure 3A. A haploid strain carrying both the *mat1-P* and *mat1-M* mating-type genes, which was marked with LacO at one of these sites and was expressing CFP-conjugated Mei2p, was subjected to nutrient starvation to induce meiosis. Because the first division was reductional in this system, the Mei2p dot segregated into one nucleus at

Figure 2. Haploid meiosis in which the first division is either reductional or equational. (A) Schematic illustration of haploid meiosis that is enabled either by the expression of the two mating-type genes (*mat1-M* and *mat1-P*) (top) or by the inactivation of Pat1 kinase (bottom). Chromosome segregation is reductional in the former, whereas it is equational in the latter (Yamamoto and Hiraoka, 2003). Bars indicate sister chromatids and open circles indicate centromeres. Surrounding large circles indicate nuclei. (B) A typical haploid cell that carried both mating-type genes (JW917). The arrowhead indicates the Mei2p-GFP dot at telophase of meiosis I. DNA counterstained with Hoechst 33342 is shown in blue. (C) A typical haploid cell that underwent *pat1*-driven meiosis (JW916). The arrowheads indicate the separated Mei2p-GFP dots. Horizontal edges of B and C, 10 μ m.

Figure 3. A map of chromosomes indicating the sites where the LacO sequence was integrated. (A) LacO integration sites on the three chromosomes, which were used in the early stage of analysis to determine the Mei2p dot position. Bars indicate chromosomes and open circles indicate centromeres. This panel was drawn according to the following references (Hoheisel *et al.*, 1993; Mizukami *et al.*, 1993; Yamamoto *et al.*, 1999) and the genome sequence information presented by Sanger Centre (Cambridge, United Kingdom). (B) A detailed chart of the vicinity of *sme2* on the short arm of chromosome II. The centromere is rightward. Site D shown in A corresponds to the right end of this chart. New integration sites F, G, and H are shown. The genes shown in this chart are presented for references and are not LacO integration sites. Cosmids covering this area are also presented for references.

telophase of meiosis I, and so did each chromosome marker. We then calculated the frequency of cosegregation of the Mei2p dot with each chromosome marker. As summarized in Table 3, the LacO marker integrated in chromosome II frequently cosegregated with the Mei2p dot, whereas that in either chromosome I or chromosome III showed no positive correlation. These results suggested that the Mei2p dot was coupled with chromosome II. Also, the data in Table 3 seem to suggest that chromosomes I and II might preferentially segregate from each other in this haploid meiosis system, whereas chromosomes II and III behaved randomly with respect to each other.

Localization of the Mei2p Dot at the *sme2* Locus on Chromosome II

To pinpoint the location of the Mei2p dot, we marked a number of sites on chromosome II by integrating the LacO sequence, as indicated in Figure 3A, in homothallic *h⁹⁰* strains carrying *mei2⁺-CFP*. Meiosis after self-conjugation was induced in each strain, and the distance between the Mei2p dot and the respective marked site in a horse-tail nucleus was measured. In an initial attempt, we examined four loci, namely, *cut3*, *his2*, *ade1*, and *ade8*. Each of these loci (Figure 4, green dot) occupied a position in the horse-tail nucleus that was colinear with its map distance from the nearest telomere. The Mei2p dot (Figure 4, red dot) seemed to localize very close to the *ade8* marker, which is ~300 kb away from the long-arm telomere, and moderately close to the *cut3* marker, which is ~1 Mb away from the short-arm telomere (Figure 3A).

Based on these observations, we examined two additional loci, namely, site D between SPBC428.16 and SPBC428.17, and site E between SPBC 56F2.05 and SPBC56F2.06 (Figure 3A). These two loci are ~400 kb away from the short-arm and long-arm telomeres, respectively. The average distance observed between the Mei2p dot and each of these sites was <1 μ m in either case (Figure 4), but the SD of the distance was significantly smaller between the Mei2p dot and site D than between the Mei2p dot and site E (our unpublished data). We suspected from these results that the dot might be associated with the short arm. This was subsequently confirmed by using the *taz1* mutant, in which the prophase chromosomal arrangement is distorted (Cooper *et al.*, 1998). The distance between the Mei2p dot and either site D or site E was measured in this mutant at meiotic prophase (Figure 4), and the average distance $\pm$ SD was calculated. Site D was always close to the Mei2p dot ($0.58 \pm 0.23 \mu$ m, $n = 22$), whereas the distance of site E from the dot was more variable ($1.2 \pm 0.75 \mu$ m, $n = 25$). These observations indicated that the location of the Mei2p dot was near to site D on the short arm of chromosome II. It is reported that pairing of homologous chromosomes is affected in the *taz1* mutant (Cooper *et al.*, 1998). We observed two Mei2p dots sitting near the split LacO/LacI dots at site D in *taz1* mutant cells, although not frequently, reinforcing that the Mei2p dot was linked to site D (our unpublished data). Given these results, an idea occurred to us that the Mei2p dot might be associated with *sme2*, the gene for meiRNA, which is located ~250 kb away from the end of the short arm of chromosome II. Therefore, we integrated the LacO sequence at site F be-

Table 3. Cosegregation frequency of the Mei2p dot with each LacO marker at a specific site on chromosomes

Chromosome	LacO locus	Location of the Mei2p dot and the LacO marker		
		Same nucleus	Separated nuclei	Total examined
Ch I	<i>lys1</i>	8	23	31
	site A	5	12	17
Ch II	<i>cut3</i>	30	1	31
	site B	20	2	22
Ch III	<i>ade6</i>	15	16	31
	site C	11	16	27

Figure 4. Location of the Mei2p dot (red) relative to a specific LacO marker (green) in a horse-tail nucleus undergoing zygotic meiosis. LacO was inserted at each genetic locus on chromosome II as indicated in the panel. DNA (blue) was counterstained with Hoechst33342. The LacO marker at *cut3* seems to be split probably due to temporary separation of the homologous chromosomes. The LacO marker at either site D or site E was visualized also in the *taz1Δ* mutant. Bar, 10 μ m.

tween SPBC1271.13 and SPBC1271.14, which is only 2 kb away from the *sme2* gene (Figure 3B). The Mei2p dot and the LacO/LacI dot overlapped closely in this strain (Figure 4).

We then set out to examine strains in which the telomeric regions were translocated reciprocally between chromosomes II and III. Strain construction was carried out essentially as described previously (Virgin *et al.*, 1995). Briefly, a DNA fragment containing the *ade6-469* allele and the *ura4⁺* marker was integrated at either site G (between SPBC106.02 and SPBC 106.03) or site H (between SPBC1271.14 and SPBC 1271.15, ~3 kb apart from *sme2*) on chromosome II (Figure 3B). Site G was on the centromere-proximal side of *sme2*, whereas site H was on the telomere-proximal side. An *ade6*-deletion (*ade6-D19*) strain with the *ade6-469* allele integrated at either site G or site H was crossed with an *ade6*-M26 strain. Recombination between the *ade6-469* allele on chromosome II and the *ade6*-M26 allele on chromosome III resulted in the generation of an *ade6⁺* strain with chromosome arms translocated (Figure 5). When arms were exchanged at site G (Figure 5A), the resultant strain exhibited the Mei2p dot at nearly the same position as the wild-type cells (Figure 6A). In contrast, when chromosome translocation was induced at site H and hence the *sme2* locus was expected to be 1.8 Mb away from the telomere (Figure 5B), the position of the Mei2p dot shifted toward the rear of the nucleus and became much distant from the leading edge (Figure 6A). These results indicate that the Mei2p dot resides between site G and site H, where *sme2* is located.

Finally, we removed a 3-kb-long DNA segment, which carried the entire *sme2* but no other probable gene, from the original *sme2* locus on chromosome II, and inserted it into the *lys1* locus near the centromere of chromosome I. In the resulting strain, the Mei2p dot was seen to be far away from the leading edge and overlapped with the LacO marker integrated at the *lys1* locus (Figure 6B). Together, we conclude that the *sme2* gene directs assembly of Mei2p into a dot structure around it.

Transcription of the *sme2* Gene Is Essential for the Dot Assembly around It

The question we addressed next was whether the DNA sequence of *sme2* directs the position of the dot or its tran-

scripts do so. To answer this question, we newly constructed a mutant allele of *sme2* (*sme2-m*), which carried a number of substitutions in the TATA sequence (Figure 7A) and was much reduced in the transcription activity (Figure 7C). We inserted this mutant allele into the *lys1* locus on chromosome I. The resultant strain (JW941), which carried the authentic *sme2⁺* allele at its original locus, was compared with a control strain (JW937), which was isogenic to JW941 except that it had the *sme2⁺* allele integrated at the *lys1* locus instead of *sme2-m*. When the Mei2p dot was visualized in

Figure 5. A schematic illustration of chromosome translocation. Red and green bars represent chromosome II and chromosome III, respectively. Open circles indicate centromeres. (A) Possible crossover when the inserted *ade6-469* allele is closer to the centromere than the *sme2* gene is. (B) Possible crossover when the *sme2* gene is closer to the centromere than the inserted *ade6-469* allele is.

Figure 6. Position of the Mei2p dot in a strain with rearranged chromosomes. (A) A homothallic strain with arms of chromosomes II and III translocated at site G (JW935; left), and a homothallic strain with arms of chromosomes II and III translocated at site H (JW936; right). A zygote of each strain is shown. The red dot, indicated by an arrowhead, represents the Mei2p-CFP dot, and the green dot, indicated by an arrow, represents the SPB marked by Sad1p-GFP. (B) The Mei2p dot (red) was visualized in a zygote of JW940, in which the *sme2* gene was translocated to the *lys1* locus on chromosome I. The *lys1* locus was marked with LacO and can be seen as a green dot. Bar, 10 μ m.

these two strains, the former showed only one nuclear dot at the original *sme2* locus (Figure 7B, left). In contrast, the latter strain harbored two nuclear dots, which corresponded to the original *sme2* locus and the *lys1* locus (Figure 7B, right). These observations suggest that the *sme2* allele must be transcriptionally active to generate the Mei2p dot around it and that meiRNA may play a major role in positioning of the dot. It seems that the DNA sequence of the gene is not a key determinant of the dot position, although we cannot deny the possibility that the DNA sequence may also participate in the positioning.

The Dot Is Unlikely to Be a Simple Reflection of *sme2* Transcription

The above-mentioned results suggested that the dot was likely to be assembled on nascent meiRNA, which might not have detached from the *sme2* gene. At the same time, however, we suspected that the dot was unlikely to be a simple visualization of *sme2* transcription by binding of Mei2p, because our previous observations had indicated that nascent meiRNA transcripts alone would not be detectable as a dot-like structure. The rationale was as follows. In situ hybridization analysis has shown that meiRNA can be observed as a dot overlapping the Mei2p dot in meiotic prophase nuclei, but no meiRNA dot is detectable in a mutant strain that either lacks Mei2p (*mei2Δ*) or carries Mei2p defective in binding to RNA (*mei2-644A*) (Yamashita *et al.*, 1998). It has been also shown that transcription of meiRNA is inducible by nutrient starvation irrespective of the presence or absence of Mei2p (Watanabe and Yamamoto, unpublished data; see below). These observations together lead to a conclusion that nascent meiRNA transcripts are not detectable as a dot unless they are bound by Mei2p.

To confirm the above-mentioned inference, we reexamined the previous observations more quantitatively. Northern blotting indicated that the amount of meiRNA in *mei2Δ* cells subjected to starvation was equal to, or slightly more abundant than that in wild-type cells (our unpublished data). This suggested that transcription of *sme2* must be ongoing in *mei2Δ* cells under meiotic conditions. We then examined the *mei2Δ* strain carefully for generation of a dot-like structure by meiRNA. In situ hybridization performed as described previously (Yamashita *et al.*, 1998) revealed no

Figure 7. Mei2p dot formation in a strain with a *sme2* allele that is transcriptionally inactive. (A) Nucleotide alterations introduced into the *sme2-m* allele. The putative TATA sequence, doubly underlined, was modified into the sequence shown below. The rightmost three nucleotides, underlined, correspond to the 5' terminus of meiRNA. (B) The Mei2p dot was visualized in a zygote of either JW941 (left), which carried the *sme2-m* allele inserted at the *lys1* locus in addition to the authentic *sme2* gene, or JW937 (right), which carried the *sme2+* allele inserted at the *lys1* locus together with the authentic *sme2* gene. The arrows indicate the Mei2p dot (green). DNA (blue) was counterstained with Hoechst33342. Bar, 10 μ m. (C) Comparison of the level of meiRNA in JW231 (*h90* wild type), JW456 (*h90 sme2Δ*), JW938 (*h90 sme2Δ lys1::ura4+-sme2+*), and in JW942 (*h90 sme2Δ lys1::ura4+-sme2-m*). Cells of these four strains were cultured in MM+N medium to the density of 8×10^6 cells/ml and then transferred to MM-N medium. Samples were taken 0 and 4 h after the transfer. The amount of meiRNA was measured by Northern blotting. rRNA stained with ethidium bromide is shown as loading controls.

nuclear meiRNA dot in any of 10 *mei2Δ* cells that evidently bore meiRNA in the cytoplasm. In contrast, similar analysis visualized a nuclear meiRNA dot in 17 of 24 *mei2+* cells. Therefore, we conclude that the Mei2p dot is unlikely to reflect simple association of Mei2p to nascent meiRNA being transcribed from the *sme2* gene, but that it is probably a more sophisticated and organized structure.

Effects of the Translocation of the *sme2* Gene on Meiosis

We finally investigated whether translocation of the *sme2* gene might affect the proficiency of the host cell to perform meiosis. JW938, in which *sme2* was moved to the *lys1* locus, formed spores as efficiently as the wild type. JW936, in which the 1.8-Mb telomeric region of chromosome III was exchanged with the telomeric region of chromosome II distal to *sme2*, could also sporulate as efficiently as the wild type.

In the case of JW935, in which *sme2* was moved to chromosome III together with the telomeric region of chromosome II distal to it, we noticed a sporulation deficiency. However, this deficiency was observed also in the strain JW968, which carried an insertion of *ade6-469* at site G on untranslocated chromosome II. Thus, the insertion at site G apparently affected function of a certain gene required for sporulation. 4,6-Diamidino-2-phenylindole staining of JW935 cells shifted to the sporulation medium revealed that many of them carried four nuclei, indicating that the meiotic divisions could proceed normally. Together, we conclude that relocation of the *sme2* gene from its original chromosomal locus does not significantly hamper the progression of meiosis.

DISCUSSION

The analyses performed in this study, including chromosome segregation, reciprocal translocation of chromosomes, and gene translocation, have led us to conclude that the Mei2p dot is in association with the chromosomal locus where the *sme2* gene, encoding meiRNA, resides. This explains well our previous observation that the Mei2p dot occupies a fixed position in the horse-tail nucleus (Yamashita *et al.*, 1998), because chromosomes are thought to align linearly in the prophase nucleus, with their telomeres held at the SPB (Chikashige *et al.*, 1994, 1997; Niwa *et al.*, 2000).

Our results have indicated unambiguously where the Mei2p dot is located. However, the question what function this dot performs is still left open. Roughly speaking, we can imagine two types of possibilities about its function. One possibility is that the dot represents an assembly farm for Mei2p and meiRNA and does not have an intrinsic function to stimulate meiosis. The emergence of the dot will be an indication of active transcription of meiRNA and vigorous assembly of it with Mei2p in this case. Mei2p coupled with meiRNA may then fulfill a function critical for meiosis I, either within the nucleus or after migrating to the cytoplasm. Even if this is the case, we like to emphasize that the dot is unlikely to be a simple reflection of the attachment of Mei2p to meiRNA undergoing transcription, as substantiated in the RESULTS. The dot may be regarded as a specialized structure for the assembly of Mei2p and meiRNA, just like the nucleolus for the assembly of ribosomal proteins and RNAs.

The other possibility is that the dot structure itself performs some function essential for meiosis I. So far, however, we have no concrete evidence to support this idea. Rather, the results we have obtained seem to deteriorate this possibility. Only one of the three chromosomes bears the Mei2p dot, and translocation of the dot to another chromosome does not significantly affect the ability of the cell to undergo meiosis. These suggest that the dot is unlikely to be involved in the meiosis-specific modification of the chromosome structure. Nevertheless, we cannot abandon this possibility completely. We have previously observed that Mei2p-NLS, which can suppress *sme2Δ*, forms a nuclear dot in the absence of meiRNA, probably attached by another RNA species (Yamashita *et al.*, 1998). The *rad24Δ* mutant, missing the major 14-3-3 isoform (Ford *et al.*, 1994), allows formation of a Mei2p dot under the conditions where *sme2* is unlikely to

be actively transcribed (Sato *et al.*, 2002). These observations suggest that Mei2p, bound with RNA, has an intrinsic tendency to coagulate and form a complex, which may be vital for its function.

Our preliminary analysis of the Mei2p dot by electron microscopy has shown that the dot is likely to be electron dense (Yoneda, Kamasawa, Osumi, Yamashita, and Yamamoto, unpublished data), suggesting that it may be identical to the "black bodies," an entity found electron microscopically in the meiotic prophase nucleoplasm (Bahler *et al.*, 1993). Together, whatever its function is, the Mei2p dot seems to possess a specialized structure relevant to the progression of meiosis, significance of which deserves further investigation.

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