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Note

Ectopic Overproduction of a Sporulation-Specific Transcription Factor Induces Assembly of Prespore-Like Membranous Compartments in Vegetative Cells of Fission Yeast

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ABSTRACT

Mei4 is a key sporulation-specific transcription factor in fission yeast. Ectopic expression of Mei4 in vegetative cells caused formation of nucleated membranous compartments, which shared common features with normal forespore membranes, thereby perturbing nuclear division. These results suggest why expression of development-specific transcription factors must be strictly controlled.

SPORULATION is a major developmental phase accompanying meiosis in the fission yeast *Schizosaccharomyces pombe*. Spore formation initiates with assembly of double unit membranes, termed forespore membranes (FSMs) (Yoo *et al.* 1973), which develop into the spore plasma membrane (Hirata and Tanaka 1982). During meiosis II, membrane vesicles are recruited to the vicinity of spindle pole bodies (SPBs) and fuse there to generate FSMs. The FSM expands and eventually encapsulates each of the four nuclei generated by meiosis. Most sporulation-related genes are transcriptionally induced during sporulation (Mata *et al.* 2002), indicating that alteration of the transcriptional program underlies this dynamic cellular event. Mei4 is a sporulation-specific transcription factor possessing a forkhead domain (Horie *et al.* 1998). A *mei4* mutant arrests at prophase I (Shimoda *et al.* 1985). More than 400 meiosis-upregulated genes are governed by Mei4 (Mata *et al.* 2007). Previous studies have shown that overproduction of Mei4 in vegetative cells induces its target genes (Horie *et al.* 1998; Abe and Shimoda 2000; Mata *et al.* 2007). Thus, these observations indicate that Mei4 is a key regulator of sporulation. To better understand the significance of this development-specific transcription factor, we report here the morphological consequences of ectopic overproduction of Mei4 in vegetative cells.

Mei4 was overproduced under the *nmt1* promoter in a strain carrying GFP-tagged Psy1 to observe FSM-like membranous structures within the cytoplasm (Table 1). Psy1 is an *S. pombe* homolog of syntaxin, localizes to the plasma membrane during vegetative growth, and translocates to the FSM after meiosis I (Nakamura *et al.* 2001), and thus GFP-Psy1 was used here as an FSM marker. Overproduction of Mei4 was confirmed by Western analysis, and the level was much higher than that during meiosis (supporting information, Figure S1). In vegetative cells, the GFP-Psy1 signal was observed mainly on the plasma membrane and the septum (Figure 1A, control). About 70% of Mei4-overproducing cells contained cytoplasmic membranous structures that were visualized by GFP-Psy1 (Figure 1A). A significant proportion of these structures appeared to be either anucleate or nucleate membrane compartments (Figure 1A, wild-type Mei4op). Because most of these structures were in close contact with SPBs (Figure 1A), we then determined whether these membrane-like structures were formed from spindle poles. As shown in Figure 1B, four cup-shaped GFP-Psy1 signals were observed at both ends of two spindle microtubules in Mei4-overproducing cells. In contrast, a typical mitotic spindle formed in cells harboring the control plasmid (Figure 1B). Prior to FSM formation, a meiotic SPB component Spo13 is expressed in a Mei4-dependent manner and recruited to the SPB, which is essential for initiation of FSM formation (Nakase *et al.* 2008). Spo13-GFP fluores-

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TABLE 1

Strains used in this study

Strain	Genotype	Source
MKW5 (FY7456)[a]	*h*^{90}	Nakamura-Kubo *et al.* (2003)
MM72-4C (FY6845)[a]	*h*$^{-}$ *leu1-32 ura4-D18*	YGRC
YM24 (FY12104)[a]	*h*^{90} *ade6≪GFP-psy1 leu1-32*	This study
TN427	*h*^{90} *ade6≪GFP-psy1 leu1≪nmt1-mei4*	This study
YN310	*h*$^{-}$ *leu1≪GFP-psy1 ade6-M210*	Nakase *et al.* (2008)
YN311	*h*$^{-}$ *leu1≪GFP-psy1 mes1-B44 ura4-D18*	This study
YN312 (FY12490)[a]	*h*^{90} *spo13::ura4*$^{+}$ *leu1≪GFP-psy1 ade6-M210 ura4-D18*	Nakase *et al.* (2008)
HM4832	*h*$^{+}$*/h*$^{+}$ *pat1-114/pat1-114 mei4-HA::Kan*^{r}*/mei4*$^{+}$ *ade6-M210/ade6-M216 leu1-32/leu1*$^{+}$	Murakami-Tonami *et al.* (2007)

[a] These strains were obtained from the Yeast Genetic Resource Center of Japan, supported by the National BioResource Project (YGRC/NBRP) (http:/yeast.lab.nig.ac.jp/nig/). *S. pombe* strains constructed in this study will be deposited at the YGRC/NBRP.

cence was not detected in vegetative cells (data not shown). In Mei4-overproducing vegetative cells, Spo13-GFP was observed as dots in the periphery of nuclei. Immunofluorescence microscopy with a Sad1 antibody showed that Spo13 colocalized with Sad1, an SPB-resident protein. These data suggest that ectopic expression of Mei4, in turn, results in production of Spo13 and activates the SPB to form the membranous structure.

Thin-section electron microscopy confirmed membranous compartments in the cytoplasm of Mei4-over-

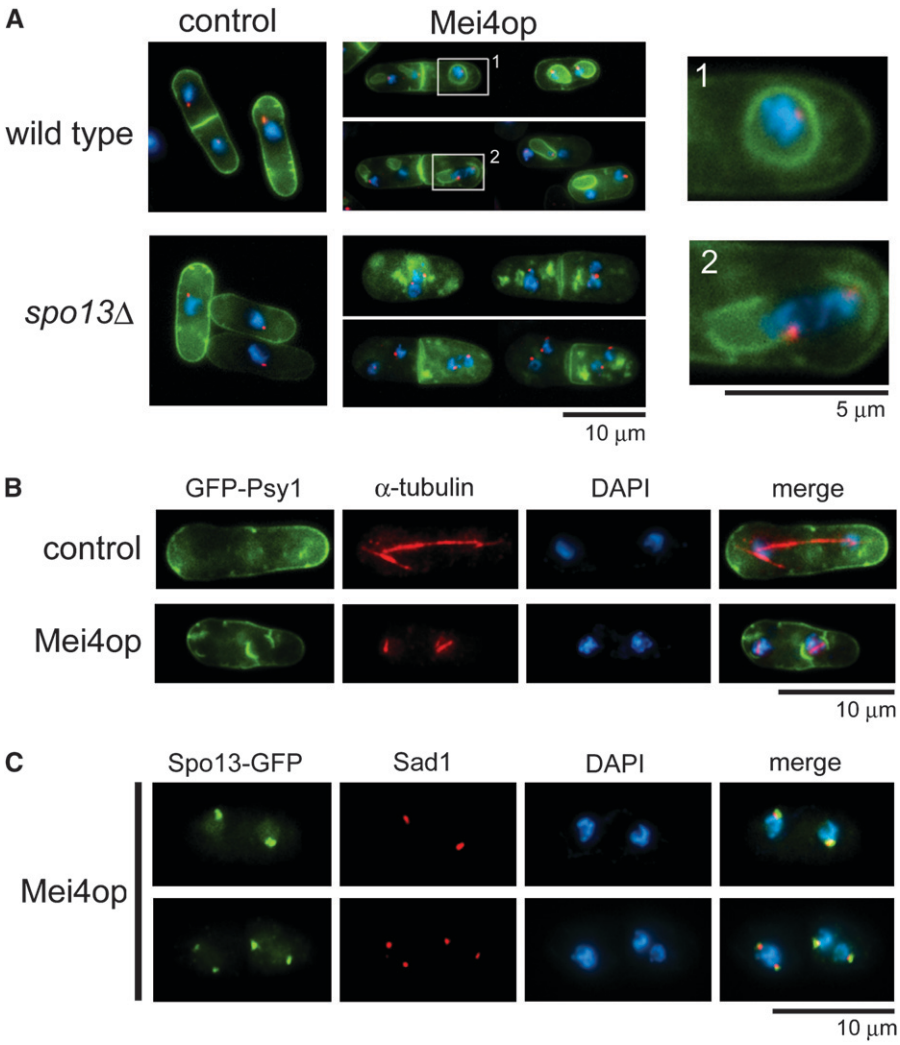

Figure 1.—Ectopic overproduction of Mei4 causes formation of membrane-like structures resembling FSMs. (A) Wild-type (YN310) and *spo13*Δ mutant cells (YN312) carrying either the control plasmid pREP1A or pREP1A(mei4) were incubated in MM + N (thiamine-free medium) at 30° for 16 hr. Cells were fixed with glutaraldehyde and paraformaldehyde as described (Hagan and Hyams 1988). The SPB was visualized by indirect immunofluorescence microscopy, using rabbit anti-Sad1 antibody (a generous gift from O. Niwa) and Alexa 546-conjugated goat anti-rabbit IgG (Molecular Probes, Eugene, OR). To visualize the nuclear chromatin region, cells were stained with 4′,6-diamidino-2-phenylindole (DAPI) at 1 μg/ml. Stained cells were observed by fluorescence microscopy, using a microscope (model BX50; Olympus, Tokyo, Japan) equipped with a charge-coupled device (CCD) camera (Cool-SNAP; Roper Scientific, San Diego). Blue, chromatin; red, SPB; green, GFP-Psy1. Magnified images (wild-type cells expressing Mei4) are also shown in the right panels. (B) Wild-type cells (YN310) carrying either pREP1A or pREP1A(mei4) were fixed and microtubules were visualized by mouse anti-α-tubulin antibody TAT-1 (Woods *et al.* 1989) and Cy3-conjugated secondary antibody (Sigma, St. Louis). Blue, chromatin; red, microtubules; green, GFP-Psy1. (C) Wild-type cells (MM72-4C) carrying pAL (spo13-GFP) and pREP2 (mei4) were incubated in MM + N at 30° for 16 hr. Fixed cells were examined by DAPI and GFP, as well as with an anti-Sad1 antibody.

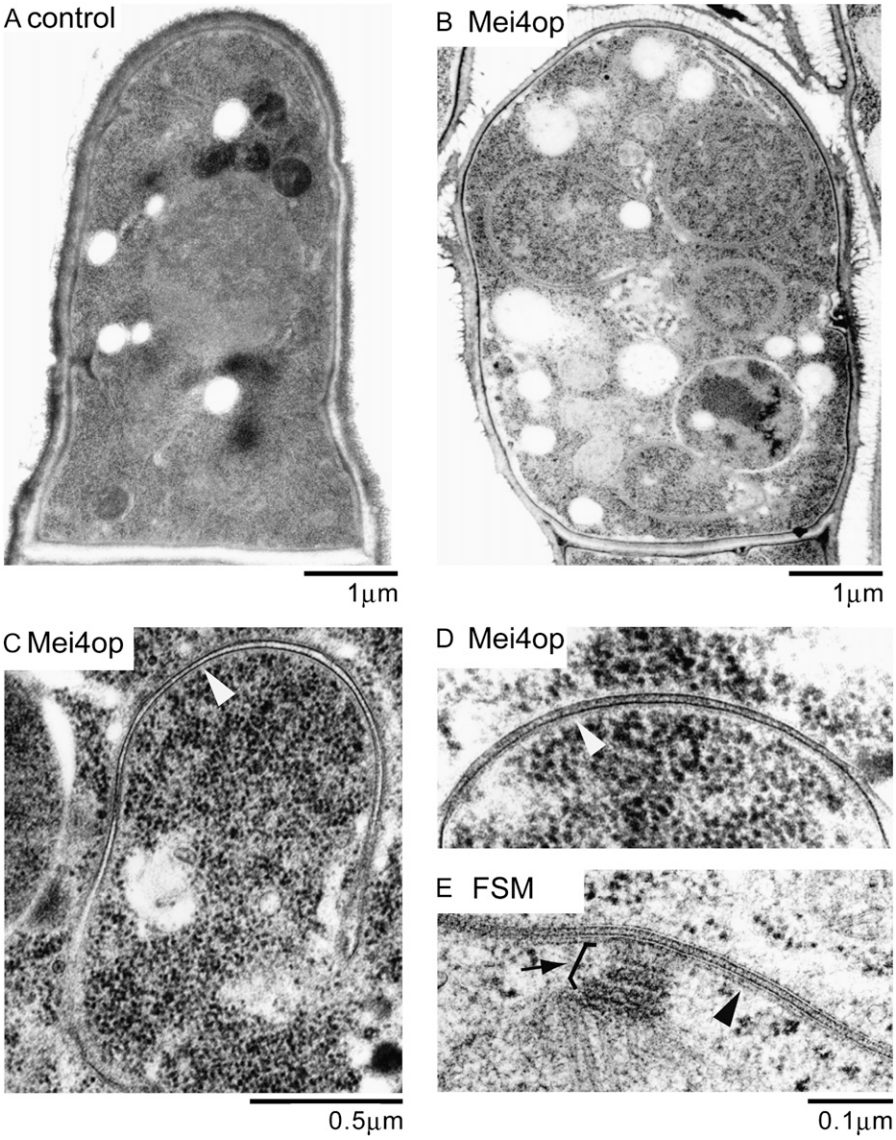

Figure 2.—Fine structure of Mei4-overproducing cells. (A–D) Wild-type cells (YN310) carrying pREP1A or pREP1A(mei4) were cultured in MM + N at 30° for 16 hr. Samples for electron microscopy were prepared as described (Ye *et al.* 2007) and sections were viewed using an electron microscope (H-7600; Hitachi, Tokyo) at 100 kV. White arrowheads indicate the FSM-like membranous structure. (E) Fine structure of sporulating cell (MKW5). Arrowhead and arrow indicate the FSM and the SPB, respectively.

producing cells (Figure 2B). The membranes were composed of double-unit membranes like the FSM. Furthermore, the interval between unit membranes was very similar to the FSM (Figure 2, C–E). In contrast, no such compartment was observed in control cells (Figure 2A). It is known that many ribosomes attach to the endoplasmic reticulum (ER) membrane (Nakamura-Kubo *et al.* 2003). However, no ribosomes were found on the membranous structure, indicating that the structure induced by Mei4 overproduction is not related to the ER. We conclude that the intracellular membranous compartments induced by Mei4 overproduction are an FSM-equivalent structure.

During vegetative growth, most cells are mononucleate because the cell cycle of *S. pombe* has a long G2 phase, and cell separation occurs by the end of S phase. However, multinucleate cells were often observed by Mei4 overproduction (Figure 1, A and C). Approximately 60% of the cells were binucleate, and ~25% were tri- or tetranucleate, while only 17% of control cells were binucleate (Table 2). We examined nuclear division in the *mes1* mutant, which is defective in the second meiotic division (Bresch *et al.* 1968). In Mei4-overproducing cells, the *mes1* mutation significantly increased the number of mononucleate cells and, remarkably, diminished the number of tri- or tetranucleate cells (Table 2). A unique nuclear division pattern caused by Mei4 overproduction may require Mes1-mediated meiotic function. Interestingly, these abnormal nuclear divisions occurred with concomitant formation of abnormal FSM-like membranous structures. These membranous structures were observed in >80% of the binucleate and multinucleate cells. In contrast, only 8.6% of the mononucleate cells formed such structures. In *mes1* cells overproducing *mei4*$^{+}$, no membranous structures were observed (data not shown). Although the cytoplasmic membranous compartments were not formed in *spo13*Δ cells, abnormal nuclear division occurred to the same extent in wild-type cells (data not shown). These observations suggest that ectopic formation of FSM-like

TABLE 2

Cell types observed during ectopic overproduction of Mei4

	No. of nuclei per cell			
	1	2	3	4
	Cell type (%)			
Wild type				
Control	83.4	16.6	0	0
Mei4op	19.0	56.0	15.5	9.8
mes1				
Control	83.7	16.3	0	0
Mei4op	55.5	41.1	2.4	1.3

Wild-type cells (YN310) carrying pREP1A (control) or pREP1A(*mei4*) (Mei4op) and *mes1* cells (YN311) carrying pREP1A (control) or pREP1A(*mei4*) (Mei4op) were incubated in MM + N medium for 18 hr at 30°. Cells were fixed and stained with DAPI. For each sample, >200 cells were counted. The experiment was performed three times with reproducible results. The results presented are from a representative single experiment.

membranes *per se* does not cause the unusual nuclear division pattern.

In this study, we provide evidence that untimely expression of a key sporulation-specific transcription factor Mei4 causes a striking morphological consequence, assembly of prespore-like membrane compartments. A genomewide DNA microarray analysis has shown that expression of few meiosis-upregulated genes is not reduced in *mei4*Δ but increased by Mei4 overproduction (Mata *et al.* 2007). *S. pombe* has four forkhead transcription factors other than Mei4. If the recognition sequences of these forkhead proteins resemble each other, overproduced Mei4 might enhance expression of genes that are normally governed by other forkhead transcription factors. As Fkh2 has been reported to be implicated in sporulation (Szilagyi *et al.* 2005), there is a possibility that some target genes of Fkh2 are induced by overproduced Mei4. Such artifactual expression might contribute partly to the observed morphological consequences.

Several spore wall biosynthetic enzymes such as β-glucan synthase Bgs2 are known to localize to the FSM (Liu *et al.* 2000; Martin *et al.* 2000). However, Bgs2-GFP did not localize to the membranous structure but dispersed in the cytoplasm in Mei4-overproducing cells (data not shown). Because no viable spores could be produced, Mei4 expression is insufficient to mediate differentiation of vegetative cells to asci.

In addition, Mei4 overproduction perturbed nuclear division, depending partly on meiotic function, including Mes1 activity. In addition to transcriptional control of *mei4*$^+$, the transcript is selectively removed by the determinant of selective removal (DSR)-Mmi1 system, in which mRNAs containing a *cis*-acting region called the DSR are recognized by a YTH-family RNA-binding protein Mmi1 and are degraded in the exosome (Harigaya *et al.* 2006). In the present study, overproduction of Mei4 from the multicopy plasmid pREP1 or pREP1A may have compensated for normal elimination of *mei4* mRNAs by the DSR-Mmi1 system. Indeed, a substantial amount of Mei4 was detected in these cells (Figure S1). In summary, our observations indicate that expression of development-specific transcription factors must be under strict control. We presume that fission yeast has the DSR-Mmi1 system to ensure this control.

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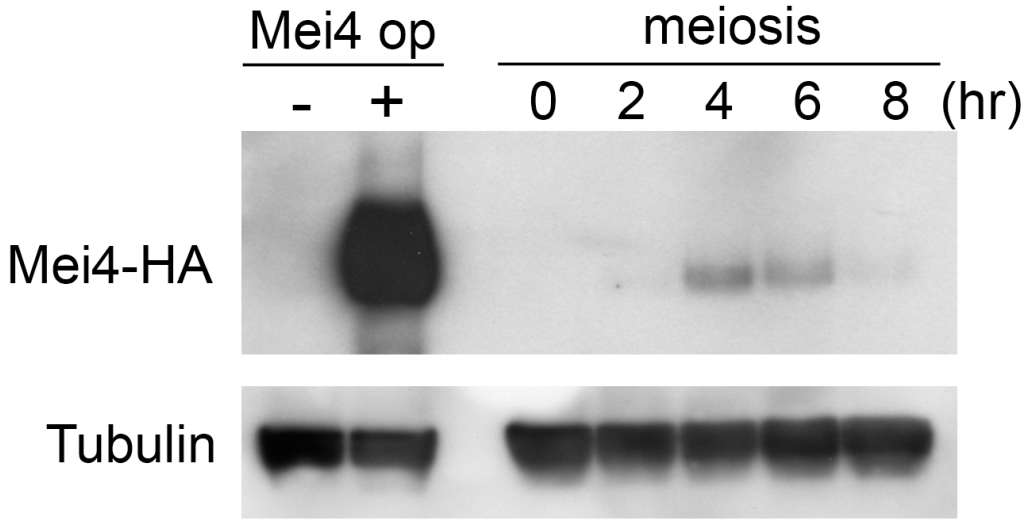

FIGURE S1.—Expression of Mei4. (Mei4 OP) YM24 cells harboring plasmid pREP1 or pREP1(mei4-HA) were grown in MM medium to derepress the *nmt1* promoter. After 16 hr's incubation, samples were prepared. (meiosis) Cells homozygous harboring the mei4-HA allele (HM4832) were induced to undergo synchronous meiosis (NAKAMURA *et al.* 2001). Samples were prepared at the indicated times. Abundance of Mei4-HA was analyzed by Western blot with the rat anti-HA antibody 3F10, as well as with anti-a-tubulin antibody as the loading control (NAKAMURA *et al.* 2001).