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S. pombe sporulation-specific coiled-coil protein Spo15p is localized to the spindle pole body and essential for its modification

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SUMMARY

Spindle pole bodies in the fission yeast *Schizosaccharomyces pombe* are required during meiosis, not only for spindle formation but also for the assembly of forespore membranes. The *spo15* mutant is defective in the formation of forespore membranes, which develop into spore envelopes. The *spo15*$^+$ gene encodes a protein with a predicted molecular mass of 223 kDa, containing potential coiled-coil regions. The *spo15* gene disruptant was not lethal, but was defective in spore formation. Northern and western analyses indicated that *spo15*$^+$ was expressed not only in meiotic cells but also in vegetative cells. When the *spo15-GFP* fusion gene was expressed by the authentic *spo15* promoter during vegetative growth and sporulation, the fusion protein colocalized with Sad1p, which is a component of spindle pole bodies. Meiotic divisions proceeded in *spo15Δ* cells with kinetics similar to those in wild-type cells. In addition, the morphology of the mitotic and meiotic spindles and the nuclear segregation were normal in *spo15Δ*. Intriguingly, transformation of spindle pole bodies from a punctate to a crescent form prior to forespore membrane formation was not observed in *spo15Δ* cells. We conclude that Spo15p is associated with spindle pole bodies throughout the life cycle and plays an indispensable role in the initiation of spore membrane formation.

Key words: Fission yeast, Meiosis, Spindle pole body, Sporulation

INTRODUCTION

Gametogenesis of multicellular organisms accompanies haploidization by meiotic nuclear divisions and the cellular specialization necessary for fertilization. The fundamental processes of meiosis are shared by different organisms. In contrast, the morphogenetic processes of gamete differentiation differ markedly among organisms. Spatial and temporal coordination between meiotic events and cellular specialization processes appear to be important for normal gametogenesis. Many species of lower eukaryotes such as yeasts have a sexual reproduction phase in their life cycle. Yeast meiosis is followed by the formation of ascospores. The budding yeast *Saccharomyces cerevisiae* and the fission yeast *Schizosaccharomyces pombe*, both amenable to genetic analysis, have been used as models for the study of meiosis and sporulation (Esposito and Klapholz, 1981; Kupiec et al., 1997; Yamamoto et al., 1997). Genetic analysis of sporulation-deficient mutants has defined many specific genes, some of which have been cloned and their products analyzed at the molecular level (Esposito and Klapholz, 1981; Kupiec et al., 1997; Yamamoto et al., 1997).

The fission yeast *S. pombe* proliferates in a haploid state, and arrests at G_1 phase in the cell cycle when starved of nutrients, especially sources of nitrogen (Egel, 1971, 1989; Yamamoto et al., 1997). Haploid cells of different mating types, designated h^+ and h^-, conjugate to form diploid zygotes. Diploid zygotes or vegetative cells undergo one round of premeiotic DNA replication followed by two consecutive meiotic divisions, meiosis-I and meiosis-II, and finally differentiate into asci, each of which contains four haploid ascospores (Egel, 1989; Yamamoto et al., 1997). During meiosis-II, precursors of the spore envelope, called forespore membranes, are assembled by the fusion of vesicles (Hirata and Tanaka, 1982; Tanaka and Hirata, 1982). In the course of meiotic second division, the spindle pole body (SPB), which plays a central role in spindle microtubule formation, undergoes a morphological alteration by differentiating into multi-layered structures (Hirata and Tanaka, 1982; Tanaka and Hirata, 1982). In the vicinity of the cytoplasmic side of the modified SPBs, membrane vesicles, probably derived from endoplasmic reticulum (ER) and/or Golgi apparatus, are gathered and fused for assembly into forespore membranes (Hirata and Tanaka, 1982; Moens, 1971; Tanaka and Hirata, 1982). These membranes extend to encapsulate each of four haploid nuclei, which have been produced by meiosis-II. Finally spore walls are constructed by accumulating wall materials in the lumen of forespore membranes (Tanaka and Hirata, 1982). Thick walls confer resistance to various environmental stresses on fission yeast spores.

Numerous sporulation-deficient mutants of *S. pombe* have been isolated and genetically characterized (Bresch et al., 1968; Kishida and Shimoda, 1986). To date many genes specifically involved in meiosis-I (*mei1*$^+$-*mei4*$^+$, *pat1*$^+$, *sme2*$^+$), meiosis-II (*mes1*$^+$) and sporulation (*spo1*$^+$-*spo20*$^+$, *cam1*$^+$) have been defined (Bresch et al., 1968; Iino and Yamamoto, 1985; Shimoda et al., 1985; McLeod and Beach, 1986; Kishida and Shimoda, 1986; Takeda et al., 1989; Watanabe and Yamamoto, 1994). The *spo* gene products are thought to be required for structural modification of SPBs, supply of precursor vesicles for forespore membrane assembly, controlled extension of forespore membranes, encapsulation of sister nuclei by membranes, synthesis of spore wall materials and so forth.

In the present study, we have analyzed the *spo15*$^+$ gene product, because our previous electron microscopic study (Hirata and Shimoda, 1994) indicated that *spo15* mutants are defective in the assembly of forespore membranes. We cloned the *spo15*$^+$ gene, partially sequenced it, and found that putative Spo15p was a 223-kDa protein containing a potential coiled-coil region and associated with spindle pole bodies. The role of Spo15p in sporulation is discussed.

MATERIALS AND METHODS

Yeast strains and culture conditions

The *S. pombe* strains used are as follows: B225 (h^{90} *ade6-M210 spo15-B225*), MK15 (h^{90} *leu1 spo15-B225*), TN29 (h^{90} *leu1 ura4-D18*), SI51 (h^{90} *leu1 ura4-D18 spo15::ura4*$^+$), SI52 (h^{90} *ura4-D18 spo15::ura4*$^+$) and SI53 (h^{90} *spo15*$^+$). The media used have been described previously (Egel and Egel-Mitani, 1974; Gutz et al., 1974; Moreno et al., 1990; Sherman and Hicks, 1986). *S. pombe* cells were grown at 30°C and sporulation cultures were done at 28°C. Yeast transformation was carried out by a highly efficient lithium acetate method (Okazaki et al., 1990).

Sensitivity of *spo15Δ* cells to the microtubule-destabilising drug thiabendazole (TBZ) was tested by streaking cells on YEA medium containing 0-50 μg/ml TBZ.

Cloning of *spo15*$^+$

A homothallic *spo15* mutant, MK15, was transformed with the *S. pombe* genomic libraries, ATW2 (a gift from Y. Watanabe, University of Tokyo) and pTN-L1 (this study) containing partial *Sau*3AI fragments constructed in a multicopy plasmid, pDB248′ (Beach and Nurse, 1981) and pAL-KS (a gift from K. Tanaka, University of Tokyo), respectively. The Leu$^+$ transformants were sporulated on SSA plates, and then treated with 30% ethanol for 30 minutes to kill non-sporulated vegetative cells (Gutz et al., 1974). Cells were then spread on SSA sporulation medium. The plates were exposed to iodine vapor (Gutz et al., 1974), and those colonies that turned brown were removed as candidates for sporulation-proficient transformants. Plasmids were transferred from such Spo$^+$ and Leu$^+$ transformants to *E. coli* cells (DH5). Two different plasmids, named pDB(spo15) and pTN(spo15), were independently isolated. A restriction map of the insert is presented in Fig. 1A.

Gene disruption of *spo15*

The *spo15::ura4*$^+$ alleles, *spo15-Δ1* and *spo15-Δ2*, were produced by one-step gene replacement (Rothstein, 1983) using a 1.7-kb *ura4*$^+$ cassette (Grimm et al., 1988). A 1.9-kb *Nru*I/*Nsi*I fragment (*spo15-Δ1*) or a 4.1-kb *Nru*I/*Bgl*II fragment (*spo15-Δ2*) was replaced by the *ura4*$^+$ cassette (Fig. 1A). Each of the *Xba*I/*Pst*I fragments bearing *spo15::ura4*$^+$ were transformed into a strain, TN29. Disruption was confirmed by genomic Southern hybridization (data not shown).

Plasmid construction

The plasmid pAL(spo15-GFP) was constructed as follows. A 1.8-kb *Not*I-*Sac*I fragment, which contains a mutant version of the *Aequorea* green fluorescent protein gene (GFP^{S65T}) and the *nmt1* terminator (Maundrell, 1990) of plasmid pAH(GFP)1 (a gift from H. Asakawa, Osaka City University) was fused to the same sites of pAL-KS to create pTN143. The 8.5-kb *Sal*I-*Not*I fragment of pTN(spo15) was inserted at the same site in pTN143, yielding pAL(spo15IM)GFP. Two oligonucleotides were used to amplify the C terminus of the *spo15*$^+$ gene by PCR: 5′-TGCGCTGCAG(*Pst*I)A-AGAAAAGGAAAAACAAGCTAC-3′ and 5′-CCCGCGGCCGC-(*Not*I)AAACACAAGAGAGGTTCTCGATAAC-3′. The PCR product was digested with *Pst*I and *Not*I, and then ligated into the same site of pAL(spo15IM)GFP, yielding pAL(spo15-GFP).

Southern and northern analysis

Genomic DNA was restricted, fractionated on a 1% agarose gel and then transferred onto nylon membranes (Biodyne A, Nihon Pall Co., Tokyo). Total RNA was prepared from *S. pombe* cultures (Jensen et al., 1983) and fractionated on a 1.5% gel containing 3.7% formaldehyde as previously reported (Thomas, 1980).

Western blotting

A *spo15* disruptant strain (SI51) carrying pAL(spo15-GFP) was cultured in liquid sporulation medium (MM–N), and at intervals samples were removed. Cell extracts were prepared as described by Masai et al. (1995). Polypeptides were separated by SDS-PAGE in a 7.5% gel and then blotted to nitrocellulose filters. Filters were probed with the rabbit anti-GFP antibody (Clontech) at a 1:1000 dilution. Blots were also probed with anti-α-tubulin antibody, TAT-1, to ensure that the approximately equal amounts of protein were loaded. Immunoactive bands were revealed with horse radish-conjugated secondary antibody at a 1:1000 dilution and chemiluminescence (NEN Life Sciences).

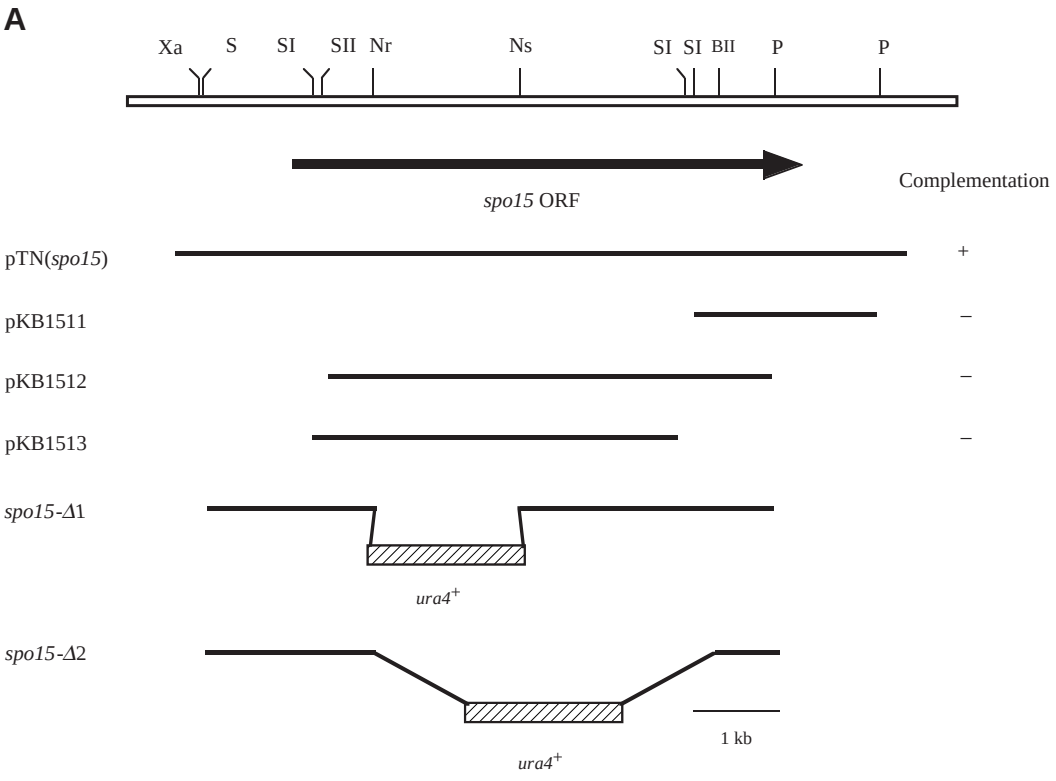
Immunofluorescence microscopy

For cell fixation, we followed the procedure of Hagan and Hyams (1988) using glutaraldehyde and paraformaldehyde. The SPB was visualized by indirect immunofluorescence microscopy using anti-Sad1 antibody (Hagan and Yanagida, 1995) and a Cy3-conjugated secondary antibody at a 1:1000 dilution (Pharmacia). For microtubule staining, TAT-1 anti-α-tubulin antibody (Wood et al., 1989) and Cy3- or Alexa488-conjugated secondary antibody (Molecular Probe Co.) were used. To visualize the nuclear chromatin region, samples were stained with 4′,6-diamidino-2-phenylindole (DAPI) at 1 μg/ml. Stained cells were observed under a fluorescence microscope (model BX50; Olympus, Tokyo).

RESULTS

Cloning of *spo15*$^+$

To elucidate the molecular function of the *spo15*$^+$ gene product, we attempted to isolate *spo15*$^+$ from *S. pombe* genomic libraries by functional complementation. Two clones that could complement the *spo15-B225* mutation were independently isolated as pDB(spo15) and pTN(spo15). As these plasmids had overlapping inserts, only pTN(spo15) was analyzed further. Since the subclones tested could not complement *spo15-B225* (Fig. 1), a partial nucleotide sequence (approximately 0.5 kb) of the insert of pTN(spo15) was determined. We searched for this sequence in the *S. pombe* genome sequence database (The Sanger Centre, UK) and found a match in the cosmid C1F3 (Accession number, Z70690) of chromosome-I. The 5.9-kb fragment contained one complete



B

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SPILDINSVTQKSNLLNELKQVRSKLAALEHENGILSLQLSSSNKKDKNTSSVTTLTSEE   180
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EQNFSSLDTSFKKLNESHQELENNHQTITKQLKDTSSKLQQLQLERANFEQKESTLSDEN   660
NDLRTKLLKLEESNKSLIKKQEDVDSLEKNIQTLKEDLRKSEEALRFSKLEAKNLREVID   720
NLKGKHETLEAQRNDLHSSLSDAKNTNAILSSELTKSSEDVKRLTANVETLTQDSKAMKQ   780
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SAQLNSGLKSLEAEKQLLHTENEELHIRLDKLTGKLKIEESKSSDLGKKLTARQEEISNL   960
KEENMSQSQAITSVKSKLDETLSKSSKLEADIEHLKNKVSEVEVERNALLASNERLMDDL  1020
KNNGENIASLQTEIEKKRAENDDLQSKLSVVSSEYENLLLISSQTNKSLEDKTNQLKYIE  1080
KNVQKLLDEKDQRNVELEELTSKYGKLGEENAQIKDELLALRKKSKKQHDLCANFVDDLK  1140
EKSDALEQLTNEKNELIVSLEQSNSNNEALVEERSDLANRLSDMKKSLSDSDNVISVIRS  1200
DLVRVNDELDTLKKDKDSLSTQYSEVCQDRDDLLDSLKGCEESFNKYAVSLRELCTKSEI  1260
DVPVSEILDDNFVFNAGNFSELSRLTVLSLENYLDAFNQVNFKKMELDNRLTTTDAEFTK  1320
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FGYFFKTNRVEMEVLDSFKQQVAKLQFLAGAEFIVKFKEDLEKCAAEEKEKQATFDNYSE  1860
KVENLGKSIEALYFALNREISFRKSLALSKSAYHNLLVRDSPKFNPDSQITYSIPVTNTK  1920
QSLLRSAILCVISLQRLRLLGQRHSFCEEVIENLSCV*                         1957
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Fig. 1. Structure of the *spo15*$^+$ gene and its disruption. (A) Restriction map, subcloning and disruption construct. The arrow indicates the region and direction of the *spo15* ORF, which encodes a protein composed of 1,957 amino acids. All the subclones were derived from pTN(spo15). Complementation by each subclone: +, complements; –, does not complement. Restriction enzyme sites: BII, *Bgl*II; P, *Pst*I; Ns, *Nsi*I; Nr, *Nru*I; SI, *Sac*I; SII, *Sac*II; S, *Sal*I; Xa, *Xba*I. (B) The deduced amino acid sequence of the *spo15*$^+$ gene. The coiled-coil region was predicted using the COILS program with the 21-residue window setting (Lupas et al., 1991). The putative coiled-coil regions (P>0.9) are underlined.

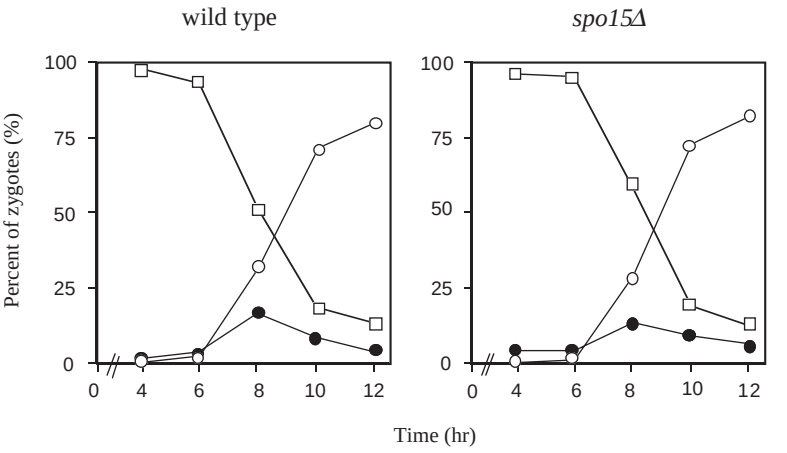

Fig. 2. Kinetics of meiosis in a wild-type and a *spo15* disruptant strain. SI52 (h^{90} *spo15::ura4*$^+$) and SI53 (h^{90} *spo15*$^+$) precultured overnight in liquid growth medium (MM+N) were incubated with shaking at 28°C in liquid sporulation medium (MM–N). A portion of the culture was taken every 2 hours and stained with DAPI. Zygotes were classified based on the number of nuclei per cell. For each sample, about 600 zygotes were counted. Values depict one representative result of four independent experiments. Open squares, mononucleate zygotes; closed circles, binucleate zygotes; open circles, tri- or tetranucleate zygotes.

open reading frame (ORF) encoding 1,957 amino acids (SPAC1F3.06c) (Fig. 1), which did not appear to have introns.

Our previous genetic analysis mapped *spo15* to the left arm of chromosome-I, where it is closely linked to *spo20* (Kishida and Shimoda, 1989). Recently we have cloned a DNA fragment containing *spo20*$^+$ (Y. Nakase, unpublished). This genomic fragment contained the overlapping region of two contiguous cosmids C1F3 and C3H8, indicating that the two ORFs are located close together. These results strongly suggest that the cloned DNA fragment complementing the *spo15* mutation carries the genetically defined *spo15*$^+$ itself. This was directly verified by genetic complementation testing, as described below.

The predicted amino acid sequence of the *spo15*$^+$ product was run through a protein sequence database. Although we could find no known proteins with a high degree of sequence similarity with Spo15p, it has partial similarity with the putative coiled-coil region of *S. pombe* Myo2p (Kitayama et al., 1997). Spo15p was predicted (Lupas et al., 1991) to be rich in potential coiled-coil structures.

Phenotype of the *spo15* disrupted strain

Disruption of *spo15*$^+$ was conducted by one-step gene replacement to analyze the role of *spo15*$^+$. Firstly, to genetically identify the cloned DNA fragment, SI51 (h^{90} *spo15::ura4*$^+$) was crossed to B225 (h^{90} *spo15-B225*). The resulting diploid strain could not sporulate on nitrogen-free (–N) medium (data not shown), indicating that the cloned gene is *spo15*$^+$ itself, not a multicopy suppressor gene.

The *spo15Δ* cells were viable and their growth rate in complete medium (+N), cell size and morphology, and sensitivity to the microtubule-destabilising drug TBZ were not different from *spo15*$^+$ cells (data not shown), indicating that *spo15*$^+$ was not essential for normal growth. Although homothallic *spo15Δ* cells conjugate at a frequency comparable with that of wild-type cells in nitrogen-free medium, diploid zygotes could not form any asci. This sporulation-deficient phenotype was the same as the original *spo15-B225* mutant.

We examined the kinetics of meiosis by DAPI staining. A homothallic strain, SI52, harboring the *spo15::ura4*$^+$ allele was incubated in sporulation medium (MM–N). Cells were stained with DAPI every 2 hours of incubation and mono-, bi- and tetra-nucleate zygotes were differentially counted. Meiotic first and second divisions proceeded with similar kinetics to the isogenic wild-type strain (Fig. 2). Furthermore, the final yields of tetranucleate zygotes reached about 85% in both *spo15Δ* and *spo15*$^+$ strains.

Expression of *spo15*$^+$ and identification of Spo15p

Generally, the *S. pombe* genes responsible for mating, meiosis and sporulation are transcribed under conditions of nutritional starvation (Yamamoto et al., 1997). Northern analysis revealed that the transcription of *spo15*$^+$ occurred during vegetative growth and was not further enhanced after the shift to a nitrogen-free medium (data not shown). Using the GFP-fused *spo15* gene on a plasmid, the level of Spo15 protein expressed under control of the authentic spo15 promoter was assayed. Western analysis using an anti-GFP antibody revealed the Spo15-GFP fusion protein as an approximately 250 kDa polypeptide on SDS-PAGE gels (Fig. 3). This apparent molecular mass corresponds well with that deduced from sequence data. This band was not detected in the extract from cells bearing an empty vector (data not shown). Fig. 3 also indicates that the Spo15-GFP fusion protein was expressed in vegetative cells (0 hours) and its abundance was almost

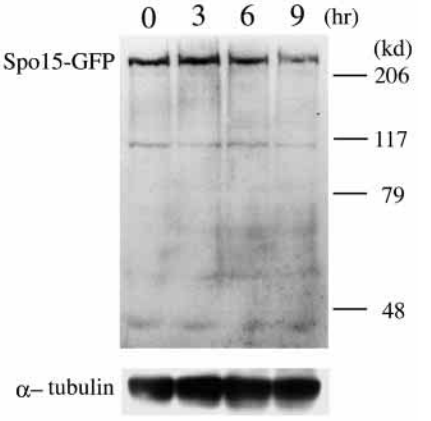

Fig. 3. Detection of Spo15p by western blotting. SI51 cells transformed with pAL(spo15-GFP) was precultured overnight in MM+N medium and then transferred to MM–N sporulation medium. Protein extracts were prepared at intervals and subjected to western analysis with anti-GFP antibody, as well as with anti-α-tubulin antibody as the loading control. Spo15-GFP was detected as a band of approximately 250 kDa.

constant during the first 6 hours, but a little decreased between 6 and 9 hours in nitrogen-limited sporulation medium. Similar results were obtained with Spo15-HA (data not shown). In conclusion, *spo15*$^+$ is constitutively expressed in contrast to most genes responsible for sexual reproduction.

Localization of Spo15p

To obtain a clue as to the function of Spo15p, we examined the intracellular localization of Spo15-GFP. A multicopy plasmid, pAL(spo15-GFP), was introduced into a *spo15* disruptant (SI51). The sporulation defect of the host was fully complemented. The GFP signal in vegetative cells was detected as one or two dots in the periphery of nuclei (Fig. 4A). Two GFP dots were localized at both ends of mitotic spindles, as revealed by indirect immunofluorescence microscopy using the anti-tubulin antibody, TAT-1 (Wood et al., 1989) (Fig. 4A). These observations suggest that Spo15p is localized to the SPB. The *S. pombe sad1*$^+$ gene encodes a constitutive component of SPB that is essential for normal bipolar spindle formation (Hagan and Yanagida, 1995). Immunofluorescence microscopy with the Sad1 antibody showed that Spo15p was colocalized with Sad1p (Fig. 4B). Spo15-GFP was not detected in post-mitotic MTOC (microtubule-organizing centers). These facts strongly suggest that Spo15-GFP is closely associated with SPB. Similarly, we observed the localization of Spo15-GFP during meiosis. The GFP signal was colocalized with signals for anti-Sad1 antibody during meiosis (Fig. 5A,B). The GFP signals were intense during meiosis-I and meiosis-II, and weak in prophase-I. These facts indicate that Spo15p was localized to SPBs not only in mitotic but also in meiotic cells.

Structures of SPBs and spindles in *spo15Δ*

The *sad1-1* temperature-sensitive mutant exhibits aberrant X-shaped spindles at restrictive temperatures (Hagan and Yanagida, 1990, 1995). Although, in contrast to *sad1*$^+$, *spo15*$^+$ was not essential for growth, it is still possible that *spo15* mutants would show an aberrant morphology of spindle microtubules and SPBs. As shown in Fig. 6A, *spo15Δ* cells have normal appearance of spindles and SPBs during mitosis. In addition, being consistent with the normal kinetics of meiosis in *spo15Δ*, spindle formation, duplication of SPBs and separation of sister nuclei during meiosis-I and -II appeared normal (Fig. 6B).

Hagan and Yanagida (1995) reported that SPBs visualized by Sad1 staining underwent structural changes from a compact dot to a crescent during meiosis-II, and suggested that the latter morphology corresponds to the enlarged SPBs having multilayered outer plaques prior to forespore membrane assembly, as revealed by electron microscopy (Hirata and Tanaka, 1982; Tanaka and Hirata, 1982). This SPB modification was presumed to be indispensable to the spore formation (Hirata and Shimoda, 1994). Our previous electron microscopic observations revealed that *spo15* mutants are defective in normal spore membrane formation. We next tested whether such alterations in SPB structure occur even in *spo15Δ* cells. Intriguingly, virtually no crescent-shaped SPBs were found in *spo15Δ* cells at the binucleate and tetranucleate stages, though approximately half of the SPBs in wild-type cells during meiosis-II underwent morphological alterations (Fig. 7). A crescent morphology of SPBs in wild-type cells was also observable by Spo15-GFP (Fig. 5B). These observations imply that Spo15p localizes to SPBs and when cells enter meiosis-II, it is implicated in the modification of SPBs.

DISCUSSION

SPBs in fission yeast function as an organizing center of spindle microtubules in both mitosis and meiosis. In addition, SPBs have been presumed indispensable to the initiation of forespore membrane organization. In the present study, we show that the sporulation-specific *spo15*$^+$ gene of *S. pombe* encodes a novel 223-kDa protein with potential coiled-coil regions and that Spo15p is associated with SPBs and is implicated in the latter function of SPBs.

spo15$^+$ was identified as a sporulation-specific gene by Bresch et al. (1968). Most *S. pombe* genes specific to sexual processes are transcriptionally regulated. The *spo15*$^+$ gene was unique in that it is expressed even in vegetative cells, as revealed by northern and western analyses (Fig. 3). Fluorescence microscopy also showed that Spo15-GFP localized in SPBs throughout mitotic cell cycles and meiotic nuclear divisions, verifying the constitutive expression of *spo15*$^+$. The structure and behavior of SPBs and spindles appear normal in *spo15Δ*, consistent with the fact that *spo15* null mutants had no apparent defects in either mitosis or meiosis. The cytological examination techniques used in this study do not exclude the possibility that *spo15* mutants are defective in meiotic recombination and accurate segregation of homologous chromosomes during meiosis-I and of sister chromatids during meiosis-II. The possibility that Spo15p plays some dispensable role in mitotically growing cells also remains to be tested.

The components of SPBs and their architecture have been less well documented in *S. pombe*. Of the SPB components, only a few proteins, calmodulin (Moser et al., 1997), Sad1p (Hagan and Yanagida, 1995), Cut12p (Bridge et al., 1998) and Kms1p (Tange et al., 1998), have been reported. Mutational analysis suggested that these proteins have indispensable roles in mitosis and meiosis. For example, in temperature-sensitive *sad1-1* mutants, aberrant X-shaped spindles are frequently produced and sister chromatids segregate insufficiently in mitotic anaphase (Hagan and Yanagida, 1995). In the *kms1-1* mutant, the nuclear structure in meiotic prophase-I is partially disrupted, most notably, the clustering of telomeres is abolished, and the integrity of the SPBs is impaired (Shimanuki et al., 1997). In contrast to these mutants, *spo15* null mutants are viable and do not exhibit apparent defects in SPBs or spindle microtubules. The only defect found was a sporulation deficiency. In this connection, it is interesting that a missense mutation, *cam1-F116*, in the calmodulin-encoding gene causes a sporulation-specific defect (Takeda et al., 1989). The relationship between Spo15p and calmodulin remains to be explored.

An interesting question is how Spo15p is implicated in sporulation. As far as we could ascertain, there was no abnormality in meiotic nuclear division. Phase-contrast and electron microscopy showed that forespore membranes and spore walls are not formed in the mutant. The assembly of internal spore membranes is initiated by fusion of small membranous vesicles near SPBs. Prior to membrane assembly, SPB undergoes a structural transformation to form multiple

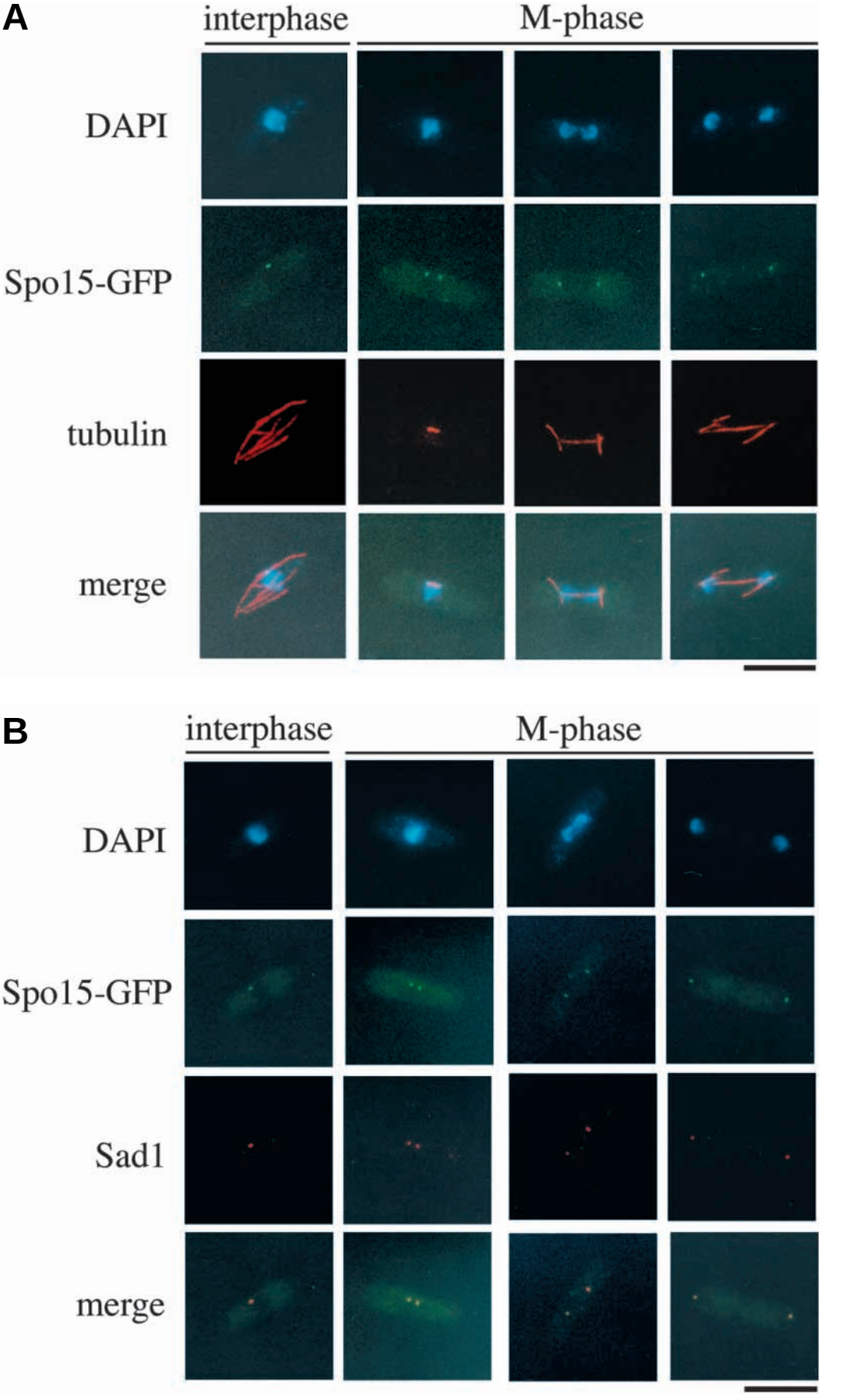

Fig. 4. Spo15-GFP is localized to SPBs during mitotic cell division. SI51 (h^{90} *spo15::ura4*$^{+}$) cells transformed with pAL(spo15-GFP) were cultured in MM+N. Fixed cells were examined at different stages of mitotic division using DAPI and GFP, as well as anti-α-tubulin (A) or anti-Sad1p (B) antibodies. Bars, 10 μm.

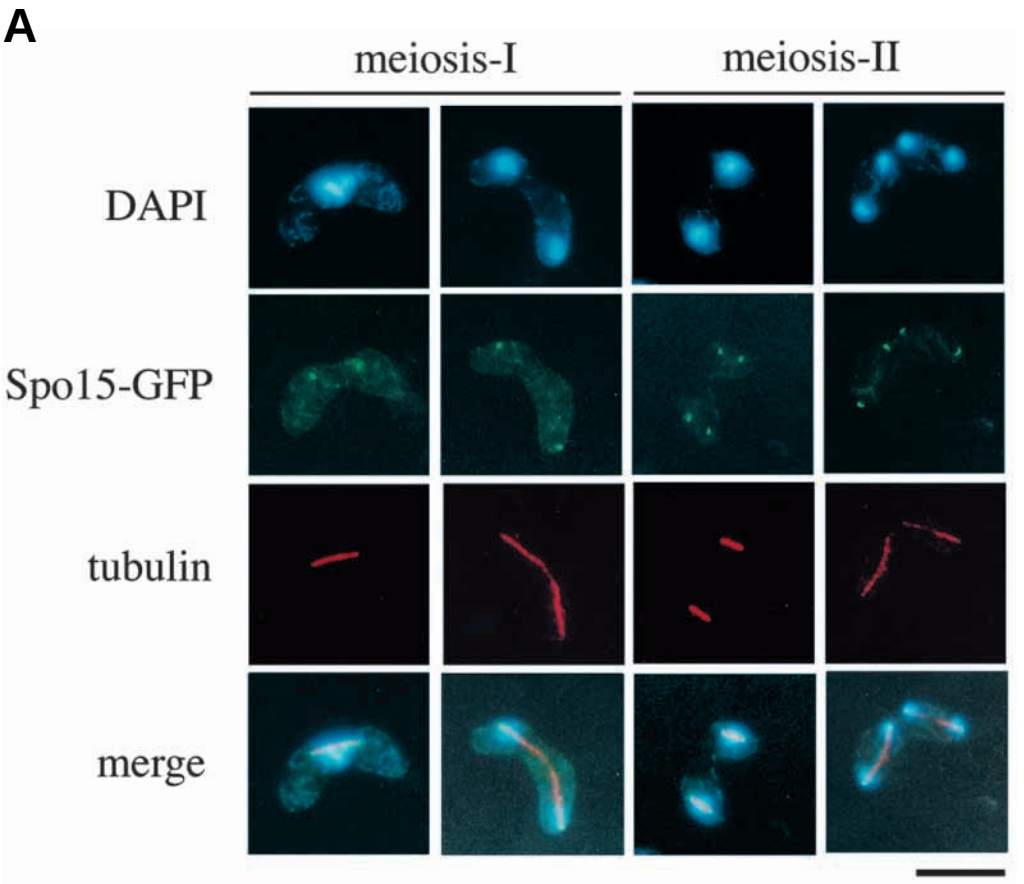

Fig. 5. Spo15-GFP is localized to SPBs during meiotic division. SI51 (h^{90} *spo15::ura4*$^{+}$) cells transformed with pAL(spo15-GFP) were cultured in MM–N to induce meiosis. Fixed cells were examined at different stages of mitosis using DAPI and GFP, as well as anti-α-tubulin (A) or anti-Sad1p (B) antibodies. Bar, 10 μm.

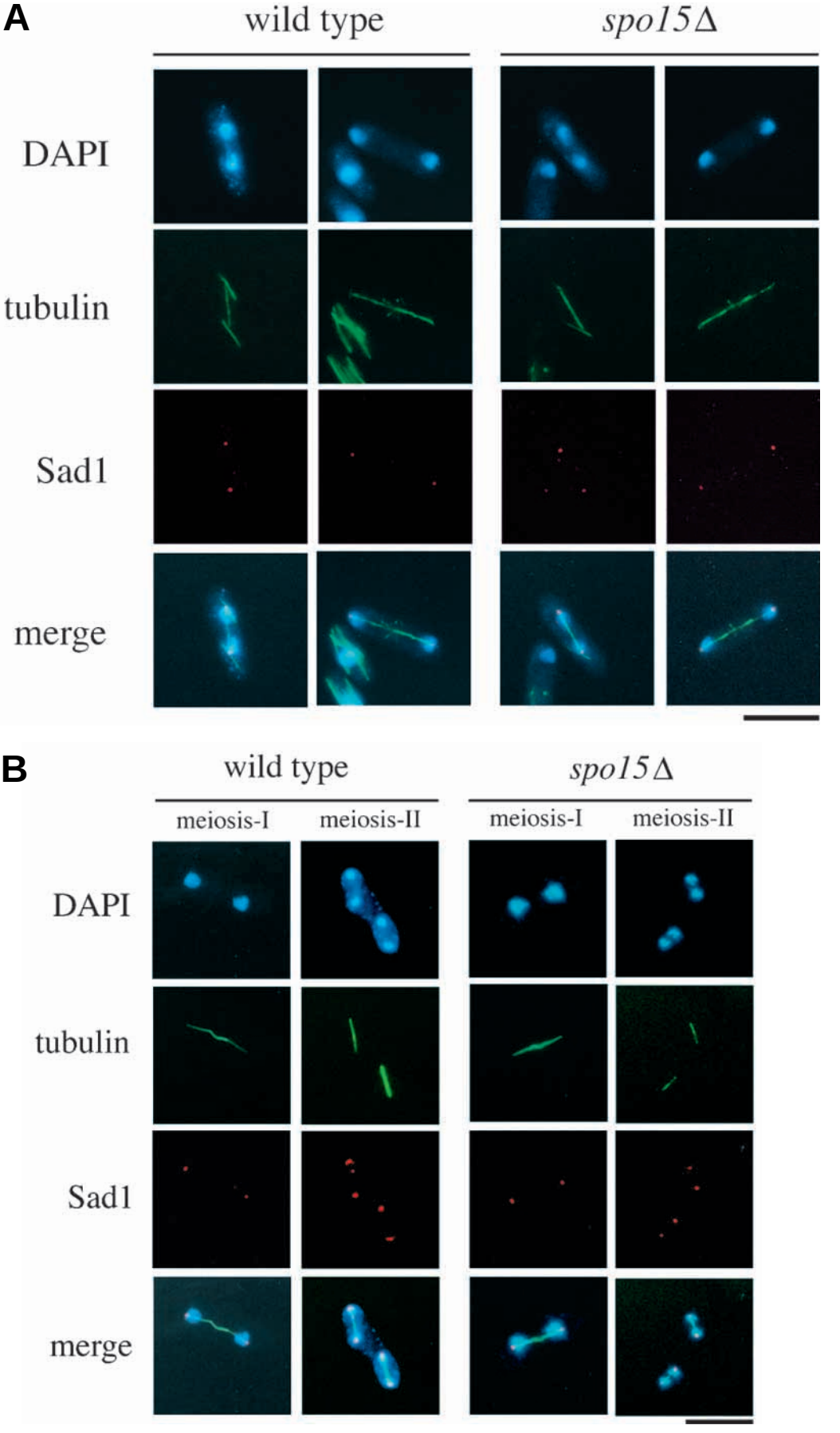

Fig. 6. Normal appearance of SPBs and spindles in *spo15Δ* cells. SI53 (h^{90} *spo15*$^{+}$) and SI52 (h^{90} *spo15*::*ura4*$^{+}$) were incubated in MM+N for mitotic cells (A) or in MM–N for meiotic cells (B). Fixed cells were examined using DAPI, anti-α-tubulin and anti-Sad1p antibodies. Bars, 10 μm.

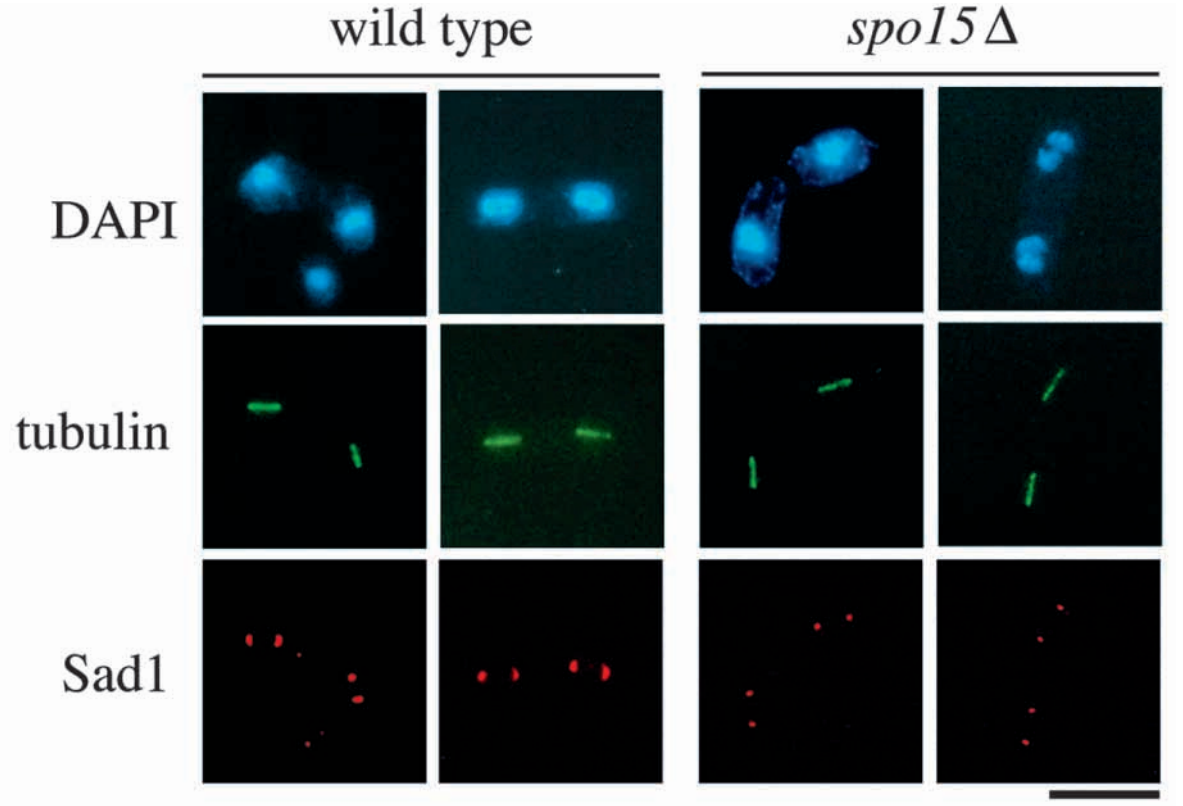

Fig. 7. The morphological change of SPBs from dot to crescent forms during meiosis-II is lacking in *spo15Δ*. SI53 (h^{90} *spo15*$^{+}$) and SI52 (h^{90} *spo15::ura4*$^{+}$) were cultured in MM–N sporulation medium. Fixed cells were examined by DAPI, anti-α-tubulin and anti-Sad1p antibodies. Note that typical crescent-shaped SPBs are seen in wild-type cells but no such modified SPBs were found in *spo15Δ* cells during meiosis-II. Bar, 10 μm.

outer plaques. This modification might correspond to the change in the SPB structure from a dot to a crescent form during meiosis-II, as observed by Hagan and Yanagida (1995). No such modification was observed in *spo15Δ* cells. This indicates that the SPB function directing forespore membrane assembly is specifically impaired by *spo15Δ*, while its meiotic and mitotic functions are apparently normal. The sporulation-specific defect of a mutant SPB-associating protein, Spo15p, supports the view that the structural modification of SPBs is essential for spore membrane formation. Modified SPBs might produce certain signals to induce the formation of ER/Golgi-derived vesicles and/or fusion of those accumulated vesicles. The *S. cerevisiae* sporulation gene, *SPO14*, encodes phopholipase D, which is required for vesicle budding from the ER/Golgi apparatus. Spo14p relocalizes to SPB during meiosis-I and then distributes spore membranes. Membrane transport is governed by lots of protein secretion (*SEC*) genes in *S. cerevisiae*. Neiman (1996) discovered a sporulation-specific SNAP-25 homologue called Spo20p in *S. cerevisiae*, suggesting that a vesicular transport system is needed for spore formation and some of the components are differentially used in vegetative growth and sporulation. We also found that at least two *S. pombe Sec* homologs play essential roles in sporulation (M. Kubo, unpublished; Y. Nakase, unpublished). From these observations, we propose that Spo15p localized in SPBs stimulates the budding and/or fusion of membrane vesicles by recruiting certain proteins necessary for membrane metabolism such as phopholipase D in the vicinity of the cytoplasmic side of SPBs. To test this model, a two-hybrid screening of Spo15p-interacting proteins is now in progress.

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