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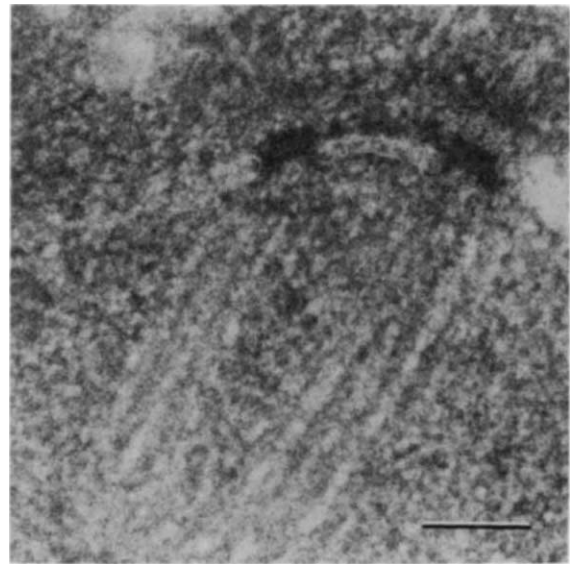


FIG. 1 An electron micrograph of a thin section of a *spo15* cell after 14 h in sporulation medium. Note the duplication of the spindle pole body. Cells were grown to mid-logarithmic phase in YPA medium (1% potassium acetate, 2% bacto-peptone, 1% yeast extract) and transferred to sporulation medium (1% potassium acetate). Cells were prepared for electron microscopy as described by Byers and Goetsch¹¹. Bar, 0.1 μ m.

A dynamin-like protein encoded by the yeast sporulation gene *SPO15*

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THE tightly centromere-linked gene *SPO15* is essential for meiotic cell division in the yeast *Saccharomyces cerevisiae*. Diploid cells without the intact *SPO15* gene product are able to complete premeiotic DNA synthesis and genetic recombination, but are unable to traverse the division cycles. Electron microscopy of blocked cells reveals a duplicated but unseparated spindle-pole body. Thus cells are unable to form a bipolar spindle. Sequence analysis of *SPO15* DNA reveals an open reading frame that predicts a protein of 704 amino acids. This protein is identical to *VPS1*, a gene involved in vacuolar protein sorting in yeast which has significant sequence homology (45% overall, 66% over 300 amino acids) to the microtubule bundling-protein, dynamin. The *SPO15* gene product expressed in *Escherichia coli* can be affinity-purified with microtubules. *SPO15* encodes a protein that is likely to be involved in a microtubule-dependent process required for the timely separation of spindle-pole bodies in meiosis.

The *SPO15* gene is located 200 base pairs from the centromere on the left arm of chromosome XI (ref. 1). Homozygous disruption mutations of the *SPO15* coding sequence result in an early block in meiosis, the terminal phenotype being a mononucleated cell¹. Mutant cells respond to the appropriate nutritional cues, complete DNA replication and recombination with wild-type efficiency, and upon return to a rich carbon source (glucose) re-enter the mitotic cycle. Thus *spo15::HIS3* mutants execute the transition to recombination, but arrest before the meiotic divisions.

The sequence of the *SPO15* gene was determined by the dideoxy method. Sequence analysis predicts a 704-amino-acid open reading frame with a relative molecular mass of 78,687 (EMBL accession number X54316). The DNA sequence is homologous (99.6% at the amino-acid level) to a recently sequenced gene, *VPS1* (ref. 2), identified in a protein-sorting

screen for secretion of carboxypeptidase Y (ref. 3). The sequence of *SPO15* differs by 3 out of 704 amino acids from *VPS1*. Two amino-acid differences result from single-base changes in the nucleotide sequence (*VPS1*: Asn 33 (AAT), Lys 141 (AAG); *SPO15*: Thr 33 (ACT), Gln 141 (CAG)). There is a discrepancy between the nucleotide sequence encoding Asn 111 which is GAA in *VPS1* and AAC in *SPO15*. The *VPS1* gene has been genetically mapped, and is identical to *SPO15*. Complete deletion of *SPO15/VPS1* uncovers a temperature-sensitive process, making cells unable to grow mitotically at elevated temperature². The mutant we describe carries a disruption (*spo15::HIS3*), which leaves the first two-thirds of the gene intact¹; the mutant shows mitotic growth at 37 °C. Differences in scoring temperature-sensitive mitotic lethality may arise from the different nature of the mutations being examined. As described by Rothman *et al.*² the predicted protein product shows homology to interferon-induced Mx proteins and has a consensus sequence for GTP binding. More recently, the sequence of the microtubule-bundling protein dynamin has been determined. This exhibits 45% overall identity to *VPS1* and is 66% identical to *VPS1* over approximately 300 amino acids spanning the GTP-binding consensus sites⁴.

To define precisely the *spo15::HIS3* defect in meiosis, the terminal phenotype has been examined by immunofluorescence and electron microscopy. In *spo15::HIS3* mutants, a meiotic spindle never develops, and the tubulin immunofluorescence remains a diffusely staining spot in thousands of cells examined. Electron microscopy at 14 h following induction of sporulation reveals mostly single or duplicated but unseparated spindle-pole bodies (Fig. 1). Thus the terminal phenotype of *spo15::HIS3* is arrest in the pachytene stage of meiosis. This defect could reflect the perturbation of a regulatory step governing spindle development through the misrouting of vacuolar proteins, or the loss of a direct interaction between the *SPO15* gene product and spindle components.

Microtubules are the major spindle components and are required for separation of spindle-pole bodies in mitosis⁵. The homology of *SPO15* to dynamin is certainly indicative of the potential for microtubule binding. To test the ability of the *SPO15* protein to interact with microtubules, *SPO15* was

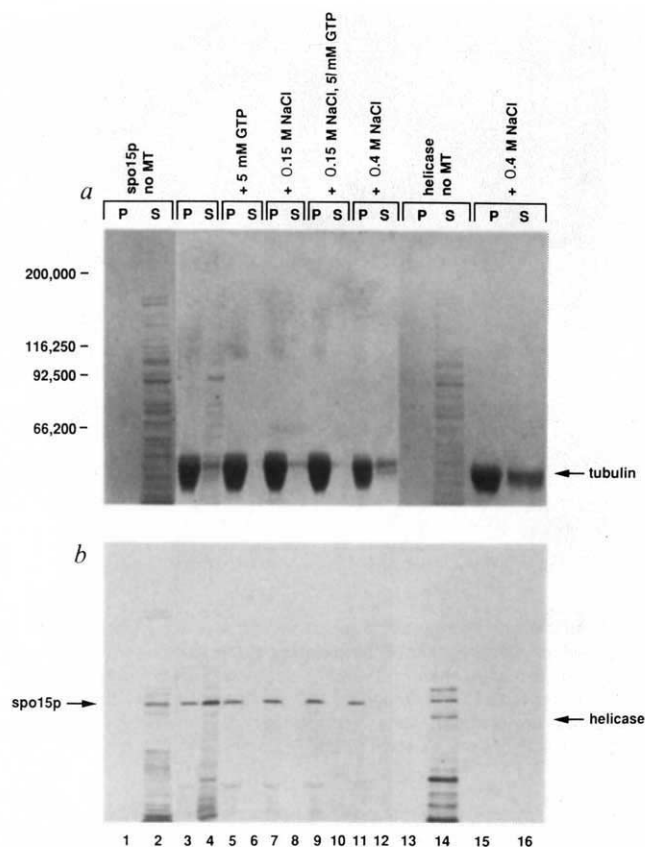


FIG. 2 Expression and microtubule-binding of *spo15*. *a*, Coomassie blue-stained 7.5% SDS-polyacrylamide gel showing the microtubule-dependent sedimentation of *spo15* and helicase II produced from *E. coli* cells carrying the appropriate T7-7 vector. *b*, Corresponding autoradiograph of ^{35}S -labelled proteins. A fraction of the labelled *spo15* binds to microtubules and remains bound in the presence of nucleotide triphosphates. Molecular weight markers are indicated to the left. Lanes 1 and 2, pellet (p) and supernatant (s) of the *E. coli* extract (pT7-7 SPO15) in buffer containing taxol in the absence of microtubules (no MT); lanes 3 and 4, *E. coli* extract (pT7-7 SPO15) plus microtubules; lanes 5 and 6, pellet in lane 3 incubated with 5 mM GTP; lanes 7 and 8, *E. coli* extract (pT7-7 SPO15) plus microtubules, bound in the presence of 150 mM NaCl; lanes 9 and 10, pellet in lane 7 incubated with 5 mM GTP and 150 mM NaCl; lanes 11 and 12, *E. coli* extract (pT7-7 SPO15) from a microtubule-pellet fraction incubated with 0.4 M NaCl; lanes 13 and 14, *E. coli* extracts containing pH12-2 (helicase II) in buffer containing taxol in the absence of microtubules; lanes 15 and 16, pH12-2 extracts sedimented with microtubules as in lanes 11 and 12.

METHODS. A 2.4-kilobase (kb) DNA fragment, starting 3 base pairs (bp) upstream of the initiation codon AUG, was inserted 8 bp downstream of the ribosome binding site in the T7 polymerase expression vector pT7-7 (ref. 6), and the junction verified by DNA sequencing. The resulting plasmid (pT7-7 SPO15) was introduced into the *E. coli* strain K38 (Hfr λ) harbouring a plasmid pGp1-2 and labelled *E. coli* extracts were prepared as described⁶. The microtubule-sedimentation assay of proteins expressed from pT7-7 SPO15 was performed with taxol-stabilized microtubules. Porcine-brain tubulin purified on a phosphocellulose column (2 mg ml⁻¹) was assembled into microtubules by incubation at 37 °C for 5 min followed by 20 min at 30 °C in 1 \times PME (0.1 M PIPES, pH 6.9, 5 mM magnesium acetate, 5 mM EGTA, 1 mM DTT) with 1 mM GTP, 20 μ M taxol and protease inhibitors. The microtubules were sedimented at 69,000 \times g at 30 °C for 10 min. The pellet was washed twice in 1 \times PME with 20 μ M taxol plus protease inhibitors. ^{35}S -labelled proteins treated with apyrase (2.5 U per ml) in 1 \times PME with 20 μ M taxol were mixed with microtubules and incubated at room temperature for 20 min, then centrifuged through a 10% sucrose cushion (1 \times PME, 20 μ M taxol, with protease inhibitors) at 50,000 \times g in a swinging-bucket rotor at 30 °C for 30 min. The supernatants were removed and the pellets washed once in 1 \times PME with 20 μ M taxol plus protease inhibitors. The nucleotide-sensitivity and salt-extraction properties of *spo15* binding were tested by addition of the appropriate concentration of nucleotide (ATP or GTP) or NaCl to the buffers after the sucrose-cushion sedimentation and incubating the pellet at room temperature for 15 min before the washes.

expressed in *E. coli*. SPO15 coding sequences were placed downstream from a promoter for T7 RNA polymerase and introduced into *E. coli* cells containing a regulated bacteriophage T7 RNA polymerase⁶. SPO15 protein (*spo15*) was labelled selectively following induction of T7 RNA polymerase in the presence of ^{35}S -containing amino acids. Taxol-stabilized porcine brain microtubules were incubated with clarified ^{35}S -labelled *E. coli* extracts in the presence or absence of nucleotides, and sedimented through a sucrose cushion. The soluble proteins and microtubule-containing pellets were examined by PAGE and autoradiography (Fig. 2). The *E. coli* extract contains a predominant labelled protein of apparent relative molecular mass 85,000. The presence of this protein is completely dependent on SPO15 coding information in the vector. The size estimated from the migration relative to molecular mass standards is in close agreement to that of the predicted open reading frame. Inspection of the autoradiograph in Fig. 2 reveals a significant fraction (>40%) of this protein to cosediment with the microtubule pellet, in the presence or absence of 150 mM NaCl. Incubation of extracts containing the *E. coli* protein, helicase II labelled and prepared in the same fashion, does not reveal significant microtubule-dependent sedimentation. No *spo15* was observed to sediment in the absence of polymerized microtubules. Other proteins in the preparation, as visualized by Coomassie- or silver-staining methods, do not sediment with microtubules. In addition, microtubule-bound *spo15* is not released by incubation with 5 mM GTP plus 150 mM NaCl, 10 mM ATP (data not shown) or 0.4 M NaCl. Thus *spo15*, in the absence of other eukaryotic proteins, binds but does not release microtubules in the presence of adenosine or guanosine nucleotide triphosphates, and is resistant to 1 M NaCl extraction (data not shown). This result is of particular interest when considering the bundling function of the related protein dynamin⁷. The ability of dynamin to cross-link microtubules has led to the notion of two microtubule-binding domains⁷. The conserved GTP-binding site may be one, with the other elsewhere in the protein. These may reflect nucleotide-sensitive and -insensitive binding sites, respectively. Alternatively, in the absence of any cellular factors these proteins may not be able to convert from a GTP- to GDP-bound form. Many G proteins are associated with activating proteins required for regulation of GTP hydrolysis⁸, and the mechanochemical activity of dynamin does require the presence of a cofactor⁷. The identification of activating molecules for *spo15* will be an important step in determining the role of this new class of microtubule binding-protein in meiosis.

The role of these related proteins (*spo15/vps1*, Mx, dynamin) in seemingly diverse processes may reflect the multifunctional roles of cellular microtubules. In the case of *spo15/vps1*, the meiotic role may involve microtubule sliding mechanisms required for spindle-pole body separation, or post-translational regulation of proteins involved in spindle morphogenesis. The requirement for microtubule-binding proteins in spindle function has been recently demonstrated by analysis of *KAR3* in bakers' yeast⁹, and *bimC4* in *Aspergillus nidulans*¹⁰. These proteins have homology to the microtubule-motor protein, kinesin, and *bimC4* mutants arrest with duplicated, but unseparated spindle-pole bodies. The role of dynamin-like proteins in similar processes emphasizes the pivotal role of microtubules and associated molecules in intracellular sorting and spindle morphogenesis. □

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A suppressor of a yeast splicing mutation (*prp8-1*) encodes a putative ATP-dependent RNA helicase

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FIVE small nuclear RNAs (snRNAs) are required for nuclear pre-messenger RNA splicing: U1, U2, U4, U5 and U6^{1,2}. The yeast U1 and U2 snRNAs base-pair to the 5' splice site and branch-point sequences of introns respectively¹. The role of the U5 and U4/U6 small nuclear ribonucleoprotein particles (snRNPs) in splicing is not clear, though a catalytic role for the U6 snRNA has been proposed³. Less is known about yeast splicing factors, but the availability of genetic techniques in *Saccharomyces cerevisiae* has led to the identification of mutants deficient in nuclear pre-mRNA splicing (*prp2-prp27*)^{4,5}. Several *PRP* genes have now been cloned and their protein products characterized. The *PRP8* protein is a component of the U5 snRNP and associates with the U4/U6 snRNAs/snRNP to form a multi-snRNP particle believed to be important for spliceosome assembly⁶. We have isolated extragenic suppressors of the *prp8-1* mutation of *S. cerevisiae* and present here the preliminary characterization of one of these suppressors, *spp81*. The predicted amino-acid sequence of the *SPP81* protein shows extensive similarity to a recently identified family of proteins thought to possess ATP-dependent RNA helicase activity. The possible role of this putative helicase in nuclear pre-mRNA splicing is discussed.

To identify genes encoding splicing factors that interact with the *PRP8* protein, we isolated extragenic suppressors of a *prp8-1* mutation. We sought suppressors that can not only suppress a *prp8-1* mutation but also render the growth of the cells cold-sensitive, as this would greatly aid the analysis of the mutants. Eleven such mutants were isolated which define three complementation groups termed *spp81*, *spp82* and *spp83* (Table 1). Tetrad analysis showed that the cold-sensitive phenotype and the ability to suppress a *prp8-1* mutation were genetically linked in each case and that the cold-sensitive mutations were unlinked to the *prp8-1* mutation and segregated as single recessive nuclear mutations (data not shown). Nine (at least seven of which were independent isolates) of the eleven mutations defined the *SPP81* gene. The ability of the *spp81-3* mutation to suppress the splicing defect of a *prp8-1* temperature-sensitive mutant was confirmed at the molecular level by examining the amount of precursor RNA accumulation at various temperatures. Compared with that observed for the *prp8-1* mutant SPJ8.31, the strain DJY65 (*prp8-1 spp81-3*) failed to accumulate significant amounts of precursor RNA at 34 °C (Fig. 1).

In the process of determining whether the suppressor mu-

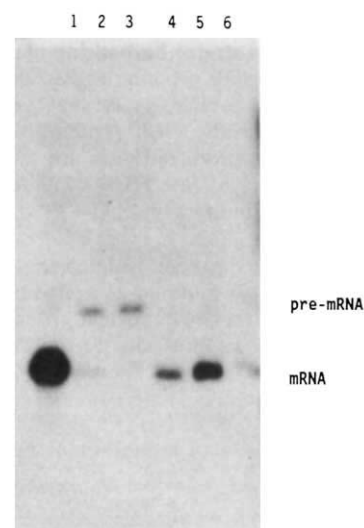


FIG. 1 Northern blot analysis of the *prp8-1* suppressor mutant DJY65 (*MATa his3 leu2 ura3-52 prp8-1 spp81-3*). The yeast actin gene, which contains one intron²¹, was used as a probe to demonstrate suppression of the *prp8-1* temperature-sensitive mutation by the *spp81-3* mutation. Lanes 1-3, SPJ8.31 (*prp8-1*) 23 °C, 34 °C and 36 °C; lanes 4-6, DJY65 (*prp8-1 spp81-3*) 23 °C, 34 °C and 36 °C.

METHODS. The actin-encoding plasmid pYA301 (ref. 21) was labelled by the random-priming method²². RNA was prepared from cultures which had been grown at 30 °C before shifting them to either 23 °C, 34 °C, or 36 °C for 2 h as described by Jackson *et al.*⁷. Total RNA was extracted as described by Hopper *et al.*²³ and equivalent amounts of total RNA were run on a 1.2% agarose gel. Northern-blotting prehybridization, hybridization and washing conditions were as described by Jackson *et al.*⁷.

tations were linked to the *PRP8* gene, plasmids carrying the wild-type *PRP8* gene and flanking DNA were introduced into *spp81* mutants. The *spp81-3* mutants grew very slowly when transformed with plasmid p8000 (2 μ *URA3 PRP8*) compared with those transformed with the control plasmid, *YEP24* (2 μ *URA3*). With *spp81-2* mutants the growth defect was more pronounced (no transformants could be obtained), whereas wild-type yeast, *spp82* mutants and *spp83* mutants transformed with plasmid p8000 did not give this phenotype (Table 1). The effect was specific to the *PRP8* gene and was not observed with 2 μ plasmids carrying the wild-type *PRP2*, *PRP4* or *PRP11* genes. Transformation of both *spp81-2* and *spp81-3* mutants with either high (p8000) or low (p8500, *CEN URA3 PRP8*) copy-number plasmids carrying the wild-type *PRP8* gene

TABLE 1 Effect of extra copies of the *PRP8* gene on *spp81* mutants

Relevant genotype	No. of isolates	<i>CEN PRP8</i> p8500	2 μ <i>PRP8</i> p8000
<i>PRP8 spp81-2</i>	9	+/-	-
<i>PRP8 spp82-1</i>	1	+	+
<i>PRP8 spp83-1</i>	1	+	+

Cultures of strain SPJ8.31 (*MATa ura3-52 his3 leu2 prp8-1*) were mutagenized with ultraviolet light to a level at which roughly 90% of cells were killed. Mutagenized cells were divided into aliquots and grown in the dark at 30 °C for 2 days. Aliquots of these pools were spread on YPD plates²⁷ and incubated at 34 °C. Putative cold-sensitive suppressors were identified by screening for weak or no growth at 18 °C and 15 °C on YPD plates. Putative suppressor mutations were then crossed to the wild-type strain KY118 (*MATa ura3-52 trp1-289 lys2-801^{am} adel-101^{oc} his3 Δ 200*)²⁸ and then crossed into the parental strain SPJ8.31 as described by Sherman *et al.*²⁷. Complementation analysis was performed as described by Sherman *et al.*²⁷. To test for interactions with the wild-type *PRP8* gene yeast strains were transformed with *PRP8*-containing plasmids as described by Ito *et al.*²⁹ and plated out at 30 °C. +, growth; +/-, poor growth; -, no growth.

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