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Regulation of Spindle Pole Function by an Intermediary Metabolite

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Spore formation in the yeast *Saccharomyces cerevisiae* depends on a modification of spindle pole bodies (SPBs) at the onset of meiosis II that allows them to promote de novo membrane formation. Depletion of the environmental carbon source during sporulation results in modification of only one SPB from each meiosis II spindle and formation of a two-spored ascus, called a nonsister dyad (NSD). We have found that mutants impaired in the glyoxylate pathway, which is required for the conversion of acetate to glucose, make NSDs when acetate is the primary carbon source. Wild-type cells make NSDs when the carbon source is glycerol, which is converted to glucose independently of the glyoxylate pathway. During NSD formation in glycerol, only the two SPBs created at the meiosis I/II transition ("daughters") are modified. In these conditions, the SPB components Mpc70p and Spo74p are not recruited to mother SPBs. Moreover, cooverexpression of Mpc70p and Spo74p suppresses NSD formation in glycerol. Our findings indicate that flux through the glyoxylate pathway during sporulation regulates modification of mother SPBs via recruitment of Mpc70p and Spo74p. These results define a cellular response in which the accumulation of an intermediary metabolite serves as a measure of biosynthetic capacity to regulate the number of daughter cells formed.

INTRODUCTION

Sporulation in *Saccharomyces cerevisiae* is a specialized form of cell division in which a single diploid cell produces four haploid spores within the cytoplasm of the original mother cell (Esposito, 1981). Sporulation is triggered as a starvation response to nitrogen deficiency and requires the presence of a nonfermentable carbon source. During spore formation, the carbon source is metabolized to produce energy via the Krebs cycle and to provide precursors for synthesis of macromolecules, such as nucleic acids, lipids, and polysaccharides. The optimal carbon source to promote sporulation is acetate, but other nonfermentable carbon sources can also be used (Miller, 1957).

The limiting membranes of the spores are synthesized de novo during meiotic segregation of nuclear DNA via the redirection of the secretory pathway (Neiman, 1998). In yeast, both meiotic divisions occur within a single, continuous nuclear envelope, in which the spindle pole bodies (SPBs), the functional equivalents of centrosomes in higher eukaryotes, are embedded. At the onset of the second meiotic division, the cytoplasmic face of each SPB, termed the outer plaque, expands to serve as a site for docking and fusion of Golgi-derived vesicles (Moens and Rapport, 1971; Neiman, 1998). Fusion of these vesicles creates four discrete membrane compartments, the prospore membranes, which extend to engulf the adjacent lobes of the nucleus. As nuclear division occurs at the end of meiosis II, the leading edge of each prospore membrane pinches together to enclose a haploid nucleus within two continuous membranes. Spore wall material is deposited into the lumen between the two new membranes to produce a mature spore (Lynn and Magee, 1970).

Modification of the outer plaque of the SPB during meiosis is essential for spore formation (Davidow *et al*., 1980; Knop and Strasser, 2000; Bajgier *et al*., 2001). Morphological expansion of the outer plaque during sporulation is due to a change in its protein composition that shifts the function of the plaque from the anchoring of cytoplasmic microtubules to the initiation of prospore membrane synthesis. During meiosis, Spc72p, a mitotic component of the outer plaque that binds to the γ-tubulin complex, disappears from SPBs, and the meiosis-specific components Ady4p, Mpc54p, Mpc70p/Spo21p, and Spo74p are recruited via interactions with the constitutive components Cnm67p and Nud1p/Spc94p (Knop and Strasser, 2000; Bajgier *et al*., 2001; Nickas *et al*., 2003). Cnm67p, Mpc54p, Mpc70p, and Spo74p are essential for outer plaque modification and prospore membrane formation, and these processes occur aberrantly in the absence of Ady4p (Knop and Strasser, 2000; Bajgier *et al*., 2001; Nickas *et al*., 2003). It has been proposed that Mpc54p, Mpc70p, and Spo74p make up the ordered protein matrix of the expanded outer plaque, that Ady4p promotes the stability of this structure, and that Cnm67p and Nud1p bring the meiosis-specific components into proximity to each other and anchor them to the SPB (Knop and Strasser, 2000; Bajgier *et al*., 2001; Nickas *et al*., 2003).

Regulation of SPB modification in response to environmental conditions is critical for determining how many spores a cell will form. When acetate is abundant throughout sporulation, meiotic outer plaques (MOPs) are assembled on all four SPBs, and a four-spored ascus (tetrad) is formed. When acetate is depleted after meiosis has been initiated, however, MOPs are assembled on only one SPB

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Abbreviations used: GFP, green fluorescent protein; MOP, meiotic outer plaque; NSD, nonsister dyad; RFP, red fluorescent protein; SPB, spindle pole body.

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from each meiosis II spindle (Davidow *et al*., 1980). The result of this selective MOP assembly is the packaging of a single haploid nucleus from each meiosis II spindle into a spore and the formation of a two-spored ascus called a nonsister dyad (NSD). NSDs can be distinguished from dyads that result from random packaging of haploid nuclei by analysis of segregation of centromere-linked markers (Davidow *et al*., 1980).

The formation of NSDs in response to depletion of acetate from the environment can be mimicked by mutations that affect MOP assembly. Heterozygous deletion of *MPC54*, *MPC70*, or *SPO74*, the three known essential, meiosis-specific components of the MOP, results in elevated formation of NSDs (Bajgier *et al*., 2001; Wesp *et al*., 2001; Nickas *et al*., 2003). The addition of a green fluorescent protein (GFP) tag onto the 3′ end of *MPC70* also results in NSD formation, presumably due to effects on the folding or stability of the expressed polypeptide (Bajgier *et al*., 2001).

Yeast cells that sporulate in the presence of acetate as the sole carbon source use acetate for both catabolic and anabolic functions (Esposito *et al*., 1969). In both instances, acetate is converted to acetyl CoA. Acetyl CoA that is used for the production of energy is oxidized to CO_2 via the Krebs cycle and drives ATP synthesis through oxidative phosphorylation. Oxidation to CO_2 is the primary fate of acetate in yeast cells sporulating in the absence of other carbon sources (Esposito *et al*., 1969), and respiration is essential for sporulation even in media that contain glucose.

One of the main anabolic uses of acetate during sporulation is in hexose biosynthesis. Acetyl CoA to be used for the synthesis of glucose enters the glyoxylate pathway, a metabolic pathway present in microorganisms, plants, and some metazoans. The glyoxylate pathway shares several enzymatic steps with the Krebs cycle, but the two pathways serve different metabolic purposes (Lorenz and Fink, 2002). Whereas the Krebs cycle results in the oxidation of acetyl CoA to CO_2 to yield energy, the glyoxylate pathway results in the net conversion of acetyl CoA to oxaloacetate that can be used for gluconeogenesis (Figure 1). Glucose can be converted into pentoses and other hexoses that are required for the synthesis of essential macromolecules, such as nucleic acids and cell wall polymers. Thus, the ability to synthesize glucose from acetyl CoA via the glyoxylate pathway enables yeast to grow and differentiate when acetate is the only available carbon source.

In this report, we provide evidence that assembly of MOPs on mother SPBs during sporulation is dependent on accumulation of an intermediate of the glyoxylate pathway. Mutants of the glyoxylate pathway make NSDs on solid medium when acetate is the primary carbon source, but mutants defective in gluconeogenesis do not. Both sets of mutants form tetrads when the primary carbon source is pyruvate, which can be converted to oxaloacetate independently of the glyoxylate pathway. Wild-type cells form NSDs in liquid medium when the sole carbon source is glycerol, which bypasses the glyoxylate pathway entirely when it enters gluconeogenesis. When wild-type cells form NSDs in glycerol, MOPs assemble only on daughter SPBs due the failure to recruit Mpc70p and Spo74p to mother SPBs, and wild-type cells that overexpress both Mpc70p and Spo74p form tetrads in glycerol. Our results suggest that accumulation of an intermediary metabolite regulates assembly of MOPs on mother SPBs via the recruitment of Mpc70p and Spo74p. This study provides a molecular outline for the mechanism by which flux through a metabolic pathway regulates a key developmental decision in response to environmental conditions.

MATERIALS AND METHODS

Yeast Strains and Methods

Standard *S. cerevisiae* genetic methods and media were used (Rose *et al*., 1990). Sporulation assays in liquid medium were performed by preculturing cells for 12–18 h in either YP glycerol (1% yeast extract, 2% bacto-peptone, 3% glycerol) (for experiments shown in Figures 3 and 9) or YP acetate (1% yeast extract, 2% bacto-peptone, 2% potassium acetate) (for all other experiments)

Table 1. *S. cerevisiae* strains used in this study

Strain	Genotype	Source
AN117-4B	*MATα arg4-NspI his3ΔSK hoΔ::LYS2 leu2 lys2 rme1Δ::LEU2 trp1::hisG ura3*	Neiman *et al.*, 2000
AN117-16D	*MAT***a** *his3Δ/SK hoΔ::LYS2 leu2 lys2 trp1::hisG ura3*	Neiman *et al.*, 2000
AN120	*MAT***a***/MATα ARG4/arg4-NspI his3ΔSK/his3ΔSK hoΔ::LYS2/hoΔ::LYS2 leu2/leu2 lys2/lys2 RME1/rme1Δ::LEU2 trp1::hisG/trp1::hisG ura3/ura3*	Neiman *et al.*, 2000
AN241	*MAT***a***/MATα ARG4/arg4-NspI his3ΔSK/his3ΔSK hoΔ::LYS2/hoΔ::LYS2 leu2/leu2 lys2/lys2 RME1/rme1Δ::LEU2 trp1::hisG/trp1::pRS304-TRP1 ura3/ura3*	This study
ADY131	*MAT***a***/MATα ARG4/arg4-NspI his3/his3 fbp1Δ::HIS3MX6/fbp1Δ::HIS3MX6 hoΔ::LYS2/hoΔ::LYS2 leu2/leu2 lys2/lys2 RME1/rme1Δ::LEU2 trp1::hisG/trp1::hisG ura3/ura3*	This study
ADY132	*MAT***a***/MATα ARG4/arg4-NspI his3/his3 hoΔ::LYS2/hoΔ::LYS2 leu2/leu2 lys2/lys2 pck1Δ::HIS3MX6/pck1Δ::HIS3MX6 RME1/rme1Δ::LEU2 trp1::hisG/trp1::hisG ura3/ura3*	This study
MJY7	*MAT***a***/MATα ARG4/arg4-NspI his3/his3 hoΔ::LYS2/hoΔ::LYS2 icl1Δ::HIS3MX6/icl1Δ::HIS3MX6 leu2/leu2 lys2/lys2 RME1/rme1Δ::LEU2 trp1::hisG/trp1::hisG ura3/ura3*	This study
MJYI1	*MAT***a***/MATα ARG4/arg4-NspI his3/his3 hoΔ::LYS2/hoΔ::LYS2 leu2/leu2 lys2/lys2 RME1/rme1Δ::LEU2 sfc1Δ::HIS3MX6/sfc1Δ::HIS3MX6 trp1::hisG/trp1::hisG ura3/ura3*	This study
MND66	*MAT***a***/MATα ARG4/arg4-NspI his3/his3 hoΔ::LYS2/hoΔ::LYS2 leu2/leu2 lys2/lys2 RME1/rme1Δ::LEU2 SPC72::YFP-HIS3MX6/SPC42::RFP-HIS3MX6 trp1/trp1 ura3/ura3*	This study
MND70	*MAT***a***/MATα ARG4/arg4-NspI his3/his3 hoΔ::LYS2/hoΔ::LYS2 leu2/leu2 lys2/lys2 RME1/rme1Δ::LEU2 SPC42::RFP-KanMX6/SPC42::RFP-KanMX6 trp1/trp1 ura3/ura3*	This study
MND79	*MAT***a***/MATα ARG4/arg4-NspI his3ΔSK/his3ΔSK hoΔ::LYS2/hoΔ::LYS2 leu2/leu2 lys2/lys2 NUD1::RFP-KanMX6/NUD1::RFP-KanMX6 RME1/rme1Δ::LEU2 trp1::hisG/trp1::hisG ura3/ura3*	This study
MND80	*MAT***a***/MATα ARG4/arg4-NspI CNM67::RFP-KanMX6/CNM67::RFP-KanMX6 his3ΔSK/his3ΔSK hoΔ::LYS2/hoΔ::LYS2 leu2/leu2 lys2/lys2 RME1/rme1Δ::LEU2 trp1::hisG/trp1::hisG ura3/ura3*	This study
MND92	*MAT***a***/MATα ARG4/arg4-NspI his3/his3 hoΔ::LYS2/hoΔ::LYS2 leu2/leu2 lys2/lys2 mls1Δ::HIS3MX6/mls1Δ::HIS3MX6 RME1/rme1Δ::LEU2 trp1::hisG/trp1::hisG ura3/ura3*	This study

Table 2. Plasmids used in this study

Plasmid	Source
pFA6a-His3MX6	Longtine *et al*., 1998
pFA6a-YFP-His3MX6	This study
pRS314-MPC54-GFP	Nickas *et al*., 2003
pRS314-SPO74-GFP	Nickas *et al*., 2003
pRS424	Christianson *et al*., 1992
pRS424-TEF	Mumberg *et al*., 1995
pRS424-MPC54-GFP	This study
pRS424-SPO74	This study
pRS424-MPC54/MPC70	This study
pRS424-MPC54/SPO74	This study
pRS424-MPC70/SPO74	This study
pRS424-MPC54/MPC70/SPO74	This study
pSB18 (pRS424-MPC70)	This study
pSB33 (pRS314-MPC70-GFP)	This study
pSM822	Pereira *et al*., 2001

to an OD_{600} of 1.0–1.3 and then transferring cells to either 2% potassium acetate, 2% glycerol, or other indicated medium at a culture density of $\sim 3 \times 10^7$ cells/ml. Sporulation assays on solid medium were performed by preculturing cells on YPD (1% yeast extract, 2% bacto-peptone, 2% dextrose, 2% agar) for 18–24 h and then culturing cells on either spo acetate or spo pyruvate (0.05% yeast extract, 0.05% dextrose, 0.05% no arginine amino acid dropout mix [Rose *et al*., 1990], 2% agar, and either 1% potassium acetate or 1% sodium pyruvate).

All strains used in this study were derived from SK-1 and are listed in Table 1. Strains AN117-4B, AN117-16D, and AN120 have been described previously (Neiman, *et al*., 2000). All diploid strains have the same combination of markers as AN120 except as indicated. The following diploid strains were made by independently disrupting a gene in both AN117-4B and AN117-16D and mating the resulting haploids: ADY131, ADY132, MJY7, MJY11, and MND92. AN241 was made by targeted integration of pRS304 into the *trp1* locus of AN120. MND66 and MND70 were made by tagging *SPC72* and *SPC42* with yellow fluorescent protein (YFP) and red fluorescent protein (RFP), respectively, in AN117-4B, crossing these strains to a strain related to AN117-16D, and mating two of the haploid segregants. MND79 and MND80 were made by tagging *NUD1* and *CNM67*, respectively, with RFP in AN117-4B, crossing the resulting strains to either AN117-16D or a related strain, and mating two of the haploid segregants.

Gene insertions and replacements were performed by polymerase chain reaction (PCR)-generated DNA cassettes and verified by PCR. The cassettes used to replace *FBP1*, *ICL1*, *MLS1*, *PCK1*, and *SFC1* in ADY131, MJY7, MND92, ADY132, and MJY11, respectively, were amplified from pFA6a-His3MX6 (Longtine *et al*., 1998). The cassettes used to add RFP tags to *CNM67* and *NUD1* in MND80 and MND79, respectively, and to *SPC42* in MND70 were amplified from pSM822 (Pereira *et al*., 2001). The cassette used to add a YFP tag to *SPC72* was amplified from pFA6a-YFP-His3MX6. Oligonucleotide names and sequences used for construction of strains and plasmids are available upon request.

Plasmids

Plasmids used in this study are listed in Table 2 Plasmids created for this study were made as follows: pFA6a-YFP-His3MX6 was made by digesting pDH5 (from K. Tatchell, Louisiana State University, Baton Rouge, LA) with *Msc*I and *Asc*I, isolating the 0.5-kb fragment, and using it to replace the *Msc*I-*Asc*I fragment of similar size in pFA6a-yEGFP-His3MX6 (Nickas and Neiman, 2002). pSB18 was made by amplifying a fragment containing *MPC70* from genomic DNA, digesting the PCR product with *Not*I and *Xho*I, subcloning it into pRS426, excising the same *Not*I-*Xho*I fragment from this plasmid, and subcloning it into pRS424. pSB33 was made by amplifying a fragment containing *MPC70-GFP* from genomic DNA of hapoid strain NY74 (Tachikawa, unpublished data), digesting the PCR product with *Xho*I, and subcloning it into the *Sma*I and *Xho*I sites of pRS314. pRS424-MPC54-GFP was made by amplifying a fragment containing *MPC54-GFP* from genomic DNA of NY556 (Nickas *et al*., 2003), digesting the PCR product with *Xho*I, and subcloning it into the *Xho*I site of pRS424. pRS424-MPC70/SPO74 was made in two steps. First, a fragment containing *SPO74-GFP* was amplified from genomic DNA of AN282 (Nickas *et al*., 2003), and the PCR product was digested with *Eco*RI and *Bam*HI and subcloned into pRS424 to create pRS424-SPO74-GFP. Second, a 1.8-kb fragment containing *SPO74* was amplified from pRS424-SPO74-GFP, and the PCR product was cut with *Not*I and *Sac*II and subcloned into pSB18. pRS424-SPO74 was made by excising the 1.8-kb *Not*I-*Sac*II fragment from pRS424-MPC70/SPO74 and subcloning it into the *Not*I and *Sac*II sites of pRS424. pRS424-MPC54/MPC70/SPO74 was made by amplifying a 2.1-kb fragment containing *MPC54* from pRS424-MPC54-GFP and subcloning it into the *Xho*I and *Kpn*I sites of pRS424-MPC70/SPO74. pRS424-MPC54/MPC70 was made by digesting pRS424-MPC54/MPC70/SPO74 with *Not*I and *Sac*II to remove the1.8-kb fragment, blunting the DNA ends, and religating the large fragment. pRS424-MPC54/SPO74 was made by digesting pRS424-MPC54/MPC70/SPO74 with *Not*I and *Xho*I to remove the 2.4-kb fragment containing *MPC70*, blunting the DNA ends, and religating the large fragment.

Fluorescence Microscopy

Preparation and analysis of cells by fluorescence microscopy were performed as described previously (Nickas *et al*., 2003) except that cells that expressed RFP fusions were not fixed with formaldehyde but were incubated for 5 min in 0.5% NaN_3.

Analysis of Segregation of Centromere-linked Markers

Frequencies of dyad types expected from NSDs were calculated based on centromere linkage values of 12 and 7 cM for *ARG4* and *RME1*, respectively, obtained from analysis of an isogenic strain that makes NSDs due to a mutation (Bajgier *et al*., 2001) and a centromere linkage value of 1 cM for *TRP1* from *Saccharomyces* genome database. All segregants from dyads were competent to mate with either *MAT***a** or *MAT*α strains and thus inferred to be haploid. P values were calculated based on the null hypothesis that dyads resulted from random packaging of spores.

RESULTS

Mutants of the Glyoxylate Pathway form NSDs on Acetate Solid Medium

To understand how the availability of an environmental carbon source determines how many spores a yeast cell will make, we sought to identify genes involved in this process by looking for mutants that form NSDs under conditions that normally promote formation of tetrads. The ability to synthesize glucose from acetate is essential for sporulation when other carbon sources are unavailable, so genes involved in the glyoxylate pathway and gluconeogenesis were tested as candidates (Figure 1). *ICL1* and *MLS1* encode isocitrate lyase and malate synthase, respectively, the two enzymes of the glyoxylate pathway that are not shared with the Krebs cycle (Hartig *et al*., 1992). *SFC1* is coregulated with *ICL1* and *MLS1* and encodes a putative mitochondrial succinate-fumarate antiporter that may be involved in shuttling intermediates of the glyoxylate pathway into the appropriate subcellular locations (Fernandez *et al*., 1994; Lorenz and Fink, 2001). *PCK1* and *FBP1* encode phosphoenolpyruvate carboxykinase and fructose 1,6-bisphosphatase, respectively, which catalyze the two irreversible reactions of gluconeogenesis (Entian *et al*., 1988; Valdes-Hevia *et al*., 1989). Mutants lacking any of these five genes grew poorly using acetate as the sole carbon source, and all of these mutants but *fbp1*Δ were able to grow well using glycerol as the sole carbon source (our unpublished data), demonstrating that these strains are competent for respiration but defective in the conversion of acetate to glucose.

Mutants of the glyoxylate and gluconeogenic pathways were assayed for sporulation on standard solid medium that contains 1% potassium acetate as the primary carbon source and 0.05% glucose. Under these conditions, the glyoxylate pathway mutants *icl1*Δ and *mls1*Δ displayed an elevated frequency of dyads, as did *sfc1*Δ (Figure 2A). In contrast, the gluconeogenesis mutants *fbp1*Δ and *pck1*Δ formed predominantly tetrads (Figure 2A). Analysis of centromere-linked markers in dyads formed by *ic1*Δ, *mls1*Δ, and *sfc1*Δ mutants revealed that they were nonsisters (Table 3). These results indicate that impairment of the glyoxylate pathway triggers NSD formation even in the presence of acetate and suggest that accumulation of an intermediary metabolite produced by this pathway but upstream of Pck1p function is required to promote tetrad formation.

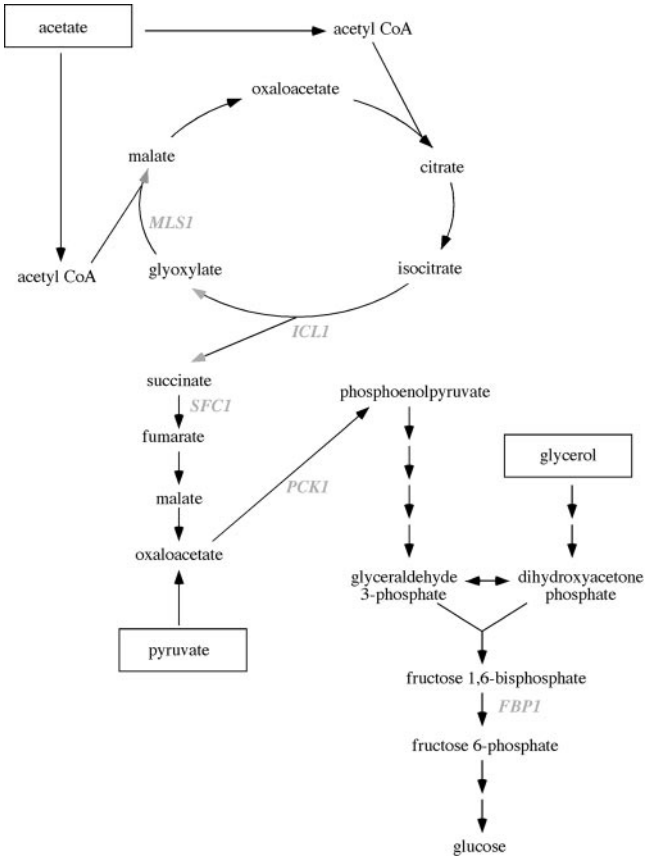

Figure 1. Pathways for conversion of selected nonfermentable carbon sources to glucose. Glyoxylate cycle is shown at top, shunting of succinate to feed gluconeogenesis is shown by downward arrows leading to oxaloacetate on left, and gluconeogenesis is shown by downward arrows leading from phosphoenolpyruvate to glucose on right. Key intermediates are shown. Carbon sources used in this study are in boxes, enzymatic steps unique to the glyoxylate pathway have gray arrowheads, and genes that block specific steps when mutated are italicized in gray.

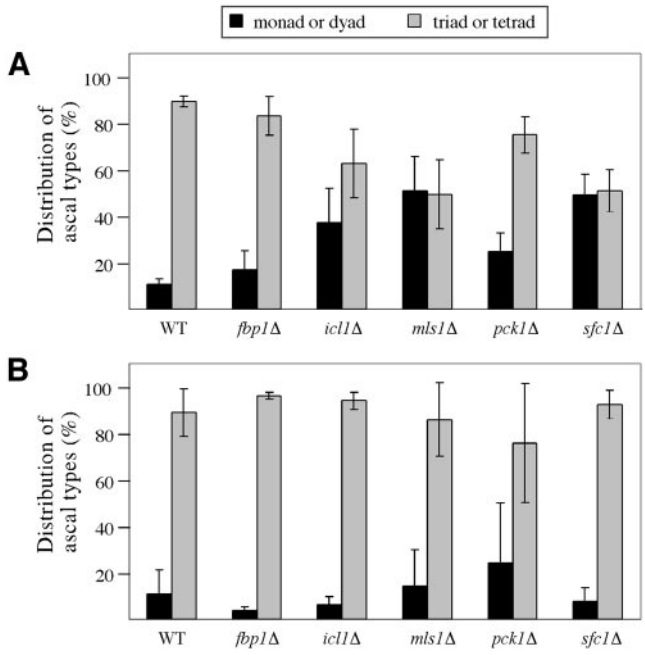

Figure 2. Ascal type distribution of glyoxylate pathway and gluconeogenesis mutants after sporulation on acetate and on pyruvate. AN120 (WT), ADY131 (*fbp1*Δ), MJY7 (*icl1*Δ), MND92 (*mls1*Δ), ADY132 (*pck1*Δ), and MJY11 (*sfc1*Δ) cells were induced to sporulate on solid medium containing either acetate (A) or pyruvate (B) as the primary carbon source, and the distribution of ascal types among 200 cells for each clone was analyzed after 24–48 h. Data shown are averages of at least six assays of individual clones performed in at least two independent experiments.

Mutants of the Glyoxylate Pathway Form Tetrads on Pyruvate Solid Medium

To test the idea that NSD formation is regulated by the accumulation of an intermediary metabolite, mutants of the glyoxylate pathway were assayed for sporulation on solid medium in which the primary carbon source is pyruvate. Pyruvate can be converted to oxaloacetate directly by pyruvate carboxylase and thus bypass the requirement for the glyoxylate pathway to make glucose (Figure 1). *icl1*Δ, *mls1*Δ, and *sfc1*Δ mutants all formed predominantly tetrads when induced to sporulate on solid medium that contains pyruvate, as did the gluconeogenesis mutants *fbp1*Δ and *pck1*Δ (Figure 2B). These data demonstrate that the glyoxylate pathway mutants are capable of forming tetrads. Furthermore, these findings support the idea that NSD formation by these strains on acetate is triggered by the lack of some product of the glyoxylate pathway that is produced during the metabolism of pyruvate.

Wild-Type Cells Form NSDs in Glycerol Liquid Medium

We next wanted to establish a liquid sporulation protocol to facilitate microscopic analysis of cells in the process of forming NSDs. Mutants of the glyoxylate pathway could not be used for this purpose because they fail to sporulate in liquid when acetate is the primary carbon source. Wild-type cells that sporulate after brief exposure to acetate predominantly form NSDs (Sriviastava *et al.*, 1981), but we found the ratio of dyads to tetrads produced by this method to be variable, making it difficult to ascertain during microscopic analysis whether an individual cell in meiosis was in the process of making an NSD.

Wild-type cells were tested for the ability to sporulate in liquid by using glycerol, which can be converted to glucose without the use of the glyoxylate pathway (Figure 1), as a carbon source. When glycerol was the sole carbon source, wild-type cells sporulated efficiently and formed almost exclusively monads and dyads (Figure 3). Analysis of segregation of centromere-linked markers and mating competency of progeny from glycerol-induced dyads indicated that they were haploids of nonsister origin (Table 4). In sporulation media that contained only glycerol, the sporulation efficiency and distribution of ascal types were comparable when the glycerol concentration ranged from 0.09 to 0.3 M (Figure 3; our unpublished data), suggesting that the accumulation of NSDs in glycerol was not due to an insufficiency of carbon source. When induced to sporulate in medium containing 0.2 M glycerol and 0.1 M acetate, however, a majority of wild-type cells formed triads and tetrads (Figure 3). These results indicate that metabolism of glycerol promotes NSD formation in the absence of other carbon sources but does not prevent formation of tetrads when acetate is simultaneously consumed. These observations suggest that the metabolic basis for NSD formation is the same in wild-type cells in glycerol liquid medium as in

Table 3. Segregation of centromere-linked markers in dyads formed by *icl1*Δ, *mls1*Δ, and *sfc1*Δ mutants on acetate-containing solid medium

	Expected frequency (%)			Observed frequency[a] (%)					
		Nonsister		*icl1*Δ		*mls1*Δ		*sfc1*Δ	
Dyad type[b]	Random	*ARG4*	*RME1*	*ARG4*	*RME1*	*ARG4*	*RME1*	*ARG4*	*RME1*
+/+	16.7	6.0	3.5	2.7	0.0	3.1	3.1	5.1	10.0
+/−	66.7	88.0	93.0	97.3	100	96.9	96.9	94.9	87.5
−/−	16.7	6.0	3.5	0.0	0.0	0.0	0.0	0.0	2.5

[a] Forty dyads of MJY7 (*icl1*Δ), 64 dyads of MND92 (*mls1*Δ), and 37 dyads of MJY11 (*sfc1*Δ) were analyzed. Observed distributions of dyad types for both markers in all three mutants were significantly different ($P < 0.025$ for RME1 in *sfc1*Δ, $P < 0.001$ for all others) from expected frequency for random dyads.

[b] +/+ represents a dyad in which both spores received the wild-type allele of the indicated locus; +/−, a dyad in which one spore received the wild-type allele and the other received the mutant allele; −/−, a dyad in which both spores received the mutant allele.

glyoxylate pathway mutants on acetate solid medium: the failure to accumulate an intermediate in acetate metabolism.

Recruitment of Mpc70p and Spo74p to SPBs Is Regulated during Formation of NSDs

We next wanted to elucidate how SPB function is regulated by the metabolic state of the cell during NSD formation. To determine whether recruitment of individual MOP components to SPBs is affected during NSD formation, localization of meiosis-specific proteins of the outer plaque was analyzed in cells that were sporulating in either acetate or glycerol. GFP fusions of Mpc54p, Mpc70p, and Spo74p were expressed in wild-type cells, the number of fluorescent SPBs in meiosis II was examined, and the final distribution of ascal types was analyzed. Results are shown in Figure 4. In cells sporulating in acetate, all three MOP components were localized to three or four SPBs in the majority of cells, suggesting that MOPs were being assembled on most SPBs. Consistent with this idea, most cells sporulating in acetate went on to make triads or tetrads (Figure 4). In cells sporulating in glycerol, however, only Mpc54p-GFP was localized to three or four SPBs in the majority of cells. In contrast, Mpc70p-GFP and Spo74p-GFP gave strong signals at only one or two SPBs in the majority of cells sporulating in glycerol, suggesting that MOPs were only being assembled at one or two SPBs per cell. Consistent with this idea, very few cells sporulating in glycerol ultimately formed triads or tetrads. Similar results were obtained in cells induced to form NSDs by brief exposure to KOAc (our unpublished data). The observation that Mpc70p and Spo74p display similar profiles of SPB localization during formation of NSDs is consistent with previous findings that these two proteins are mutually dependent for localization to SPBs when cells are making tetrads (Nickas *et al*., 2003). These results indicate that recruitment of Mpc70p and Spo74p to SPBs during meiosis is regulated in response to environmental conditions that determine how many spores a cell will make.

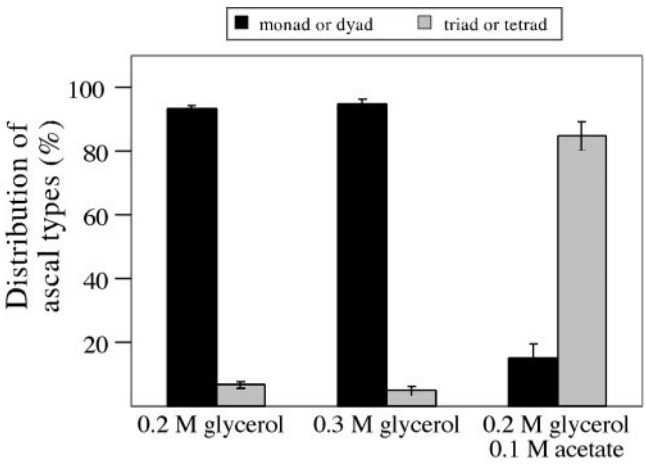

Figure 3. Ascal type distribution of wild-type cells after sporulation in glycerol. AN120 cells were induced to sporulate in liquid media containing 0.2 M glycerol, 0.3 M glycerol, or 0.2 M glycerol and 0.1 M sodium acetate, and the distribution of ascal types among 200 asci was analyzed after 24–30 h. Data shown are averages of three independent experiments. Sporulation efficiency was 86–90% in all three media.

To determine whether the constitutive layers of the outer plaque are present at all meiosis II SPBs during formation of NSDs in glycerol, a strain that expresses functional Nud1p-YFP homozygously from the *NUD1* locus was analyzed. When this strain was induced to sporulate in glycerol, the majority of cells had Nud1p-YFP localized to at least three SPBs in meiosis II (our unpublished data). These results indicate that the constitutive components of the outer plaque are present at most SPBs during NSD formation.

MOPs are Preferentially Assembled on Daughter SPBs during Formation of NSDs

Analysis was then performed to determine which SPBs become modified during formation of NSDs. During meiosis, the SPB duplicates at meiosis I and again at meiosis II. NSDs result from modification of the outer plaque on a single SPB from each meiosis II spindle, so three possibilities exist for selection of SPBs to become modified during the NSD response: 1) the two SPBs that exist before the second round of duplication (mothers), 2) the two SPBs that are created during the second round of duplication (daughters), or 3) random use of one SPB on each spindle.

To identify the pattern of SPB modification during the NSD formation, two fluorescent markers were analyzed in cells making NSDs. A fusion of the central plaque component Spc42p to a version of RFP that takes several hours to fold into a conformation that fluoresces in the red spectrum was used as a marker for mother SPBs (Pereira *et al*., 2001). Most cells in meiosis II that express this protein display red fluorescence at either one or two spindle poles, representing

Table 4. Segregation of centromere-linked markers in dyads formed by wild-type cells in 2% glycerol liquid medium

	Expected frequency (%)						
		Nonsister			Observed frequency[a] (%)		
Dyad type[b]	Random	*ARG4*	*RME1*	*TRP1*	*ARG4*	*RME1*	*TRP1*
+/+	16.7	*6.0*	3.5	0.5	3.8	1.3	1.3
+/−	66.7	*88.0*	93.0	99.0	87.5	93.8	97.5
−/−	16.7	*6.0*	3.5	0.5	8.8	5.0	1.3

[a] Eighty dyads of AN241 were analyzed. Observed distributions of dyad types for all three markers were significantly different ($P < 0.001$) from expected frequency for random dyads.

[b] +/+ represents a dyad in which both spores received the wild-type allele of the indicated locus; +/−, a dyad in which one spore received the wild-type allele and the other received the mutant allele; −/−, a dyad in which both spores received the mutant allele.

one or both of the mother SPBs (Figures 5 and 6B), demonstrating that duplication of the SPB central plaque in meiosis, as in mitosis, is conservative (Pereira *et al*., 2001). Mpc70p-GFP was used as a marker for outer plaque modification because it is essential for MOP assembly and its recruitment to SPBs is regulated. The number and position of red fluorescent SPBs were analyzed in meiosis II cells that had two Mpc70p-GFP$^+$ SPBs and were therefore presumed to be in the process of making NSDs. In cells with two Mpc70p-GFP$^+$ SPBs, the signal from Spc42p-RFP almost never overlapped with the Mpc70p-GFP signal, indicating that modification of the outer plaque rarely occurred at mother SPBs (Figure 5). These results demonstrate that daughter SPBs are preferentially modified during formation of NSDs in glycerol.

Nud1p and Cnm67p from Mother SPBs Remain Stably Localized during Formation of Tetrads

The role of the constitutive outer plaque components in MOP assembly was analyzed next. Assembly of MOPs at SPBs requires Cnm67p and may also depend on Nud1p (Bajgier *et al*., 2001), so one potential mechanism for preventing modification of mother SPBs is the removal of the Cnm67p- and/or Nud1p-containing layers of the outer plaque. It is unknown, however, whether these layers remain assembled on mother SPBs during tetrad formation, so we first examined what happens to the constitutive components of the outer plaque on mother SPBs during formation of tetrads in acetate.

To determine whether Cnm67p and Nud1p from mother SPBs are incorporated into daughter SPBs at the onset of meiosis II, functional fusions of these two proteins to slow-folding RFP were analyzed. During sporulation in acetate, red fluorescence from Cnm67p-RFP and Nud1p-RFP was apparent at only one or two SPBs per meiosis II cell, similar to the distribution observed for the central plaque component Spc42p-RFP (Figure 6). These results demonstrate that the Cnm67p- and Nud1p-containing layers of the outer plaque of mother SPBs remain largely intact during tetrad formation.

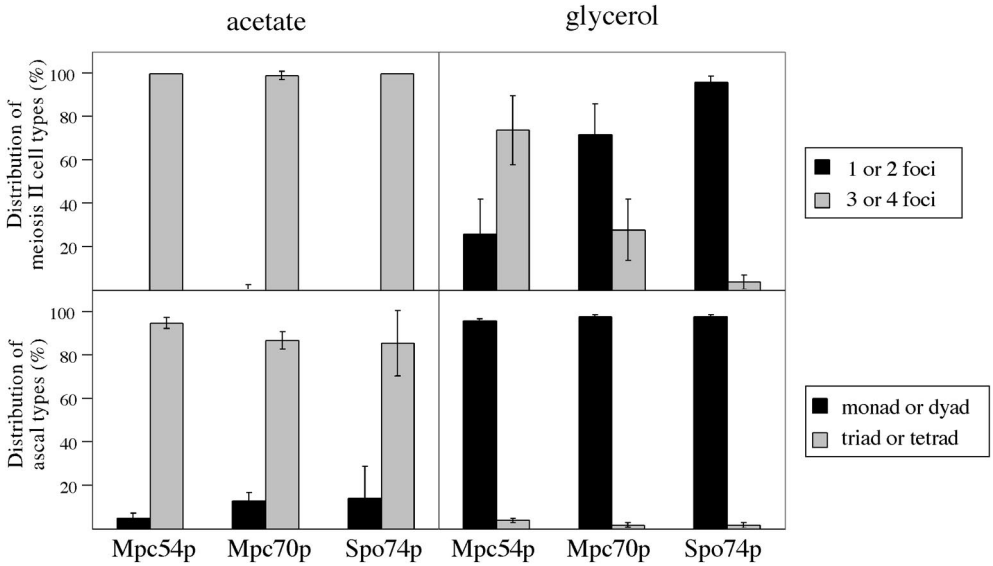

Figure 4. Localization of MOP components to meiosis II spindle poles during sporulation in acetate and in glycerol. AN120 cells that expressed fusions of GFP to the indicated proteins from centromeric plasmids were induced to sporulate in 2% potassium acetate or 2% glycerol, the number of GFP foci at spindle poles in at least 50 meiosis II cells was analyzed, and the final distribution of ascal types among 200 cells was analyzed. GFP foci were analyzed at 6–8 h in acetate and at 12–14 h in glycerol, and asci were analyzed at 24–30 h. Data shown are averages of at least three independent experiments for each fusion.

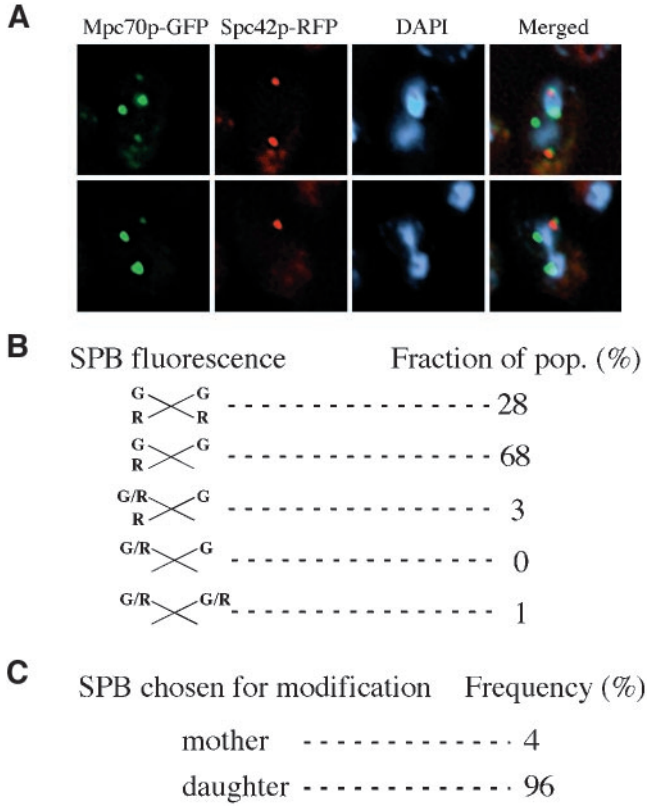

Figure 5. Localization of Mpc70p-GFP and mature Spc42p-RFP to meiosis II spindle poles during formation of NSDs in glycerol. MND70 cells that expressed Mpc70p-GFP from a centromeric plasmid were induced to sporulate in 2% glycerol, stained with 4′,6 diamidino-2-phenylindole (DAPI), and visualized by fluorescence microscopy. (A) Images of representative cells. (B) Population analysis of cells that had Mpc70p-GFP bound to two spindle poles. Seventy-four cells were analyzed. G represents green fluorescence from Mpc70p-GFP, R represents red fluorescence from mature Spc42p-RFP, and lines represent meiosis II spindles. (C) Frequency at which mother and daughter SPBs were chosen for modification. SPBs that displayed red fluorescence were designated mothers, and SPBs on the opposite pole of the same spindle from a red fluorescent signal were designated daughters; 196 SPBs were designated either mothers or daughters. SPBs that displayed green fluorescence were considered to be modified.

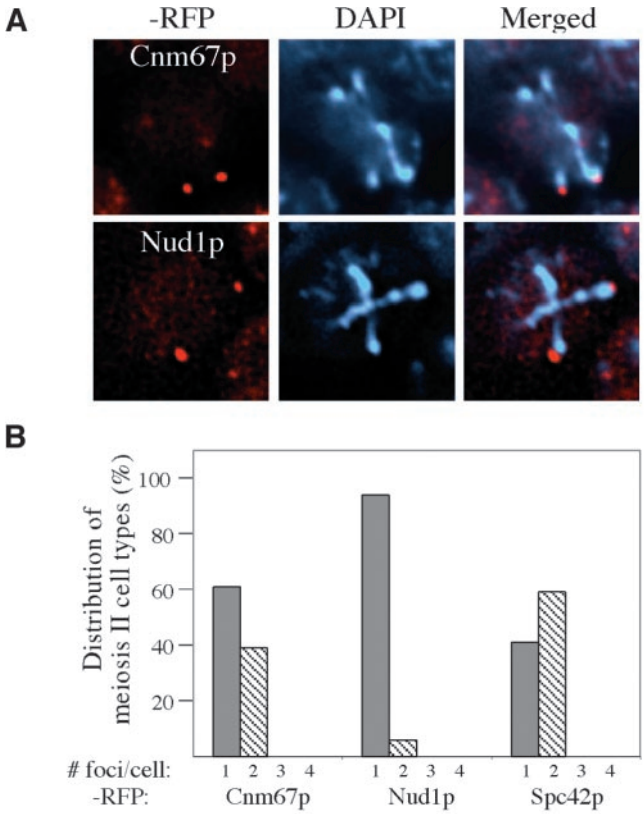

Figure 6. Localization of preexisting SPB components to meiosis II spindle poles during formation of tetrads in acetate. MND70 (Spc42p-RFP), MND79 (Nud1p-RFP), and MND80 (Cnm67p-RFP) cells were induced to sporulate in 2% potassium acetate, stained with 4′,6 diamidino-2-phenylindole (DAPI), and visualized by fluorescence microscopy. (A) Images of representative cells with mature Cnm67p-RFP and Nud1p-RFP. Top, images of an MND80 cell. Bottom, images of an MND79 cell. (B) Population analysis of localization of mature Cnm67p-RFP, Nud1p-RFP, and Spc42p-RFP. For each strain, 100 cells in meiosis II in which a red fluorescent signal from the indicated RFP fusion was detected at one or more spindle poles were analyzed. Dark gray bars represent cells with one red fluorescent spindle pole, and cross-hatched bars represent cells with two red fluorescent spindle poles. No cells with three or more red fluorescent spindle poles were observed in any of the strains.

Nud1p and Cnm67p from Mother SPBs Remain Stably Localized during Formation of NSDs

The behavior of the constitutive components of the outer plaque was then examined in cells forming NSDs. To determine whether Cnm67p and Nud1p remain bound to mother SPBs, Mpc70p-GFP was coexpressed with either Cnm67p-RFP or Nud1p-RFP in cells sporulating in glycerol, and the position of red fluorescent SPBs was analyzed in meiosis II cells that had two Mpc70p-GFP$^+$ SPBs. In cells with two Mpc70p-GFP$^+$ SPBs, the signals from Cnm67p-RFP and Nud1p-RFP almost never overlapped with the Mpc70p-GFP signal (Figure 7). These results demonstrate that mother SPBs retain Cnm67p and Nud1p during formation of NSDs and exclude the possibility that the failure to assemble MOPs on mother SPBs is due to the removal of the constitutive layers of the outer plaque.

Spc72p Is Removed from Mother SPBs during Formation of NSDs

To determine whether Spc72p, a component of the outer plaque during vegetative growth, plays a role in modification of mother SPBs in meiosis, localization of this protein was analyzed in cells during sporulation. A strain that expresses functional Spc72p-YFP homozygously from the *SPC72* locus was induced to sporulate in either acetate or glycerol. During both tetrad formation in acetate and NSD formation in glycerol, Spc72p-YFP could be detected at SPBs in meiosis I but disappeared from SPBs in meiosis II (Figure 8). These results show that Spc72p is removed from mother SPBs during NSD formation and rule out the possibility that the failure to assemble MOPs on mother SPBs is due to retention of this protein.

Overexpression of MOP Components Promotes Formation of Tetrads in Glycerol Liquid Medium

We next tested whether overexpression of one or more MOP components could promote formation of tetrads during sporulation in glycerol. Our results support the idea that the absence of Mpc70p and Spo74p from mother SPBs during NSD formation in glycerol is due to the failure to accumulate

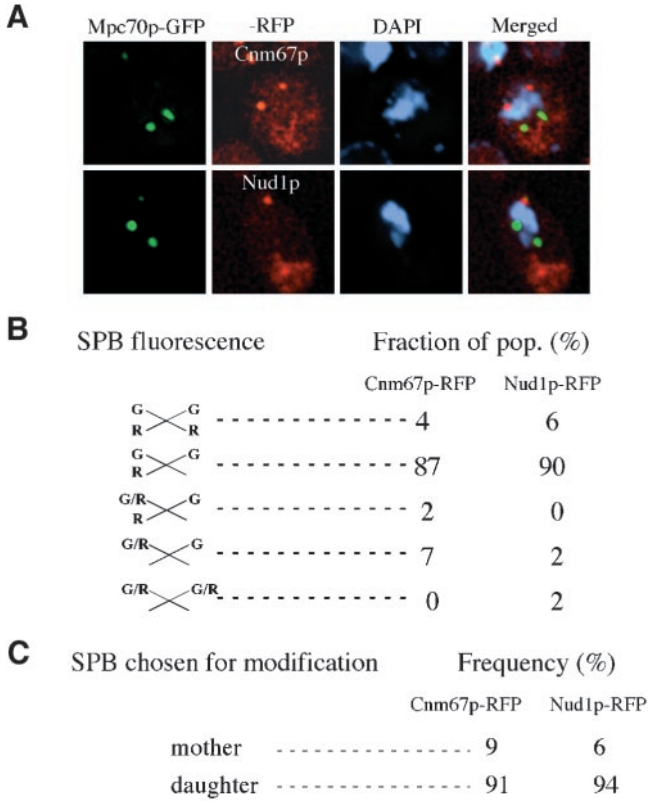

Figure 7. Localization of preexisting SPB components to meiosis II spindle poles during formation of NSDs in glycerol. MND79 (Nud1p-RFP) and MND80 (Cnm67p-RFP) cells that expressed Mpc70p-GFP from a centromeric plasmid were induced to sporulate in 2% glycerol, stained with 4′,6 diamidino-2-phenylindole (DAPI), and visualized by fluorescence microscopy. (A) Images of representative cells with mature Cnm67p-RFP and Nud1p-RFP. Top, images of an MND80 cell. Bottom, images of an MND79 cell. (B) Population analysis of cells that had Mpc70p-GFP bound to two spindle poles. At least 50 cells were analyzed for each strain. G represents green fluorescence from Mpc70p-GFP, R represents red fluorescence from mature RFP fusion, and lines represent meiosis II spindles. (C) Frequency at which mother and daughter SPBs were chosen for modification. SPBs that displayed red fluorescence were designated mothers, and SPBs on the opposite pole of the same spindle from a red fluorescent signal were designated daughters; 58 SPBs from Cnm67p-RFP cells and 54 SPBs from Nud1p-RFP cells were designated either mothers or daughters. SPBs that displayed a green fluorescence were considered to be modified.

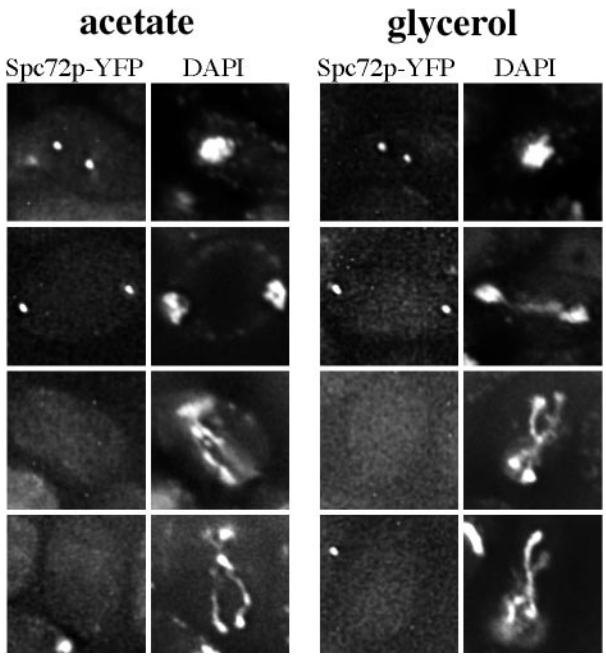

Figure 8. Localization of Spc72p-YFP during sporulation in acetate and in glycerol. MND66 cells were induced to sporulate in either 2% potassium acetate or 2% glycerol, stained with 4′,6 diamidino-2-phenylindole (DAPI), and visualized by fluorescence microscopy. Images of representative cells at various stages of meiotic division are shown.

an intermediary metabolite that triggers modification of mother SPBs. If glycerol provides sufficient biosynthetic carbon to support tetrad formation but triggers NSD formation due to the absence of a tetrad-inducing signal, supplying additional MOP components might bypass the requirement for this signal and allow formation of tetrads when glycerol is the sole carbon source.

To test whether providing an excess of MOP components could obviate the requirement for a metabolic signal to promote tetrads, wild-type cells that overexpressed Mpc54p, Mpc70p, and Spo74p individually, in pairwise combinations, or all together were assayed for sporulation in glycerol. Results are shown in Figure 9. When overexpressed individually, only Mpc54p (expressed as a fully functional Mpc54p-GFP fusion) caused a substantial increase in tetrad formation, whereas Spo74p and Mpc70p had little or no effect, respectively, on distribution of ascal types. When multiple MOP components were simultaneously overexpressed from the same plasmid, however, all combinations produced an increase in tetrad formation to various degrees. Significantly, cooverexpression of Mpc70p and Spo74p resulted in a higher percentage of tetrads than did overexpression of either protein alone, supporting the idea that these two proteins are interdependent for recruitment to SPBs. A similar effect on distribution of ascal types was observed when these MOP components were overexpressed in *mls1*Δ cells sporulating on solid medium containing acetate as the primary carbon source (our unpublished data), suggesting that the mechanistic basis for NSD formation is the same in this mutant as in wild-type cells in glycerol. These results demonstrate that an increase in the amount and/or function of the meiosis-specific MOP components promotes modification of mother SPBs and tetrad formation under conditions that normally lead to formation of NSDs.

DISCUSSION

Microorganisms adapt their life cycles according to the availability of nutrients in the environment. In *S. cerevisiae*, *MAT***a**/*MAT*α diploid cells respond to the absence of a nitrogen source and the presence of a nonfermentable carbon source by undergoing meiosis and packaging the haploid meiotic products into spores. After the commitment to enter sporulation has been made, the number of haploid nuclei that become packaged into spores is influenced by the type and abundance of the carbon source. In this report, we have found that the decision of whether to make tetrads or nonsister dyads in response to an environmental carbon source is dependent on flux through the gloxylate pathway and is effected by the regulated recruitment of the MOP

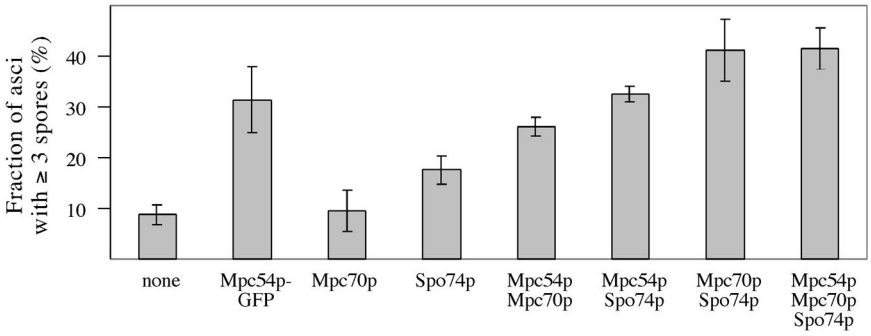

Figure 9. Ascal type distribution of wild-type cells overexpressing MOP components after sporulation in glycerol. AN120 cells that overexpress indicated MOP components from a single multicopy plasmid were induced to sporulate in 2% glycerol, and the distribution of ascal types among 500 asci for each transformant was analyzed after 24–30 h. Fraction of asci that had either three or four spores is shown for each transformant. Data shown are averages of three independent experiments.

components Mpc70p and Spo74p to mother SPBs at the onset of the second meiotic division.

A Metabolic Product of the Glyoxylate Pathway Promotes Formation of Tetrads

Our analysis of sporulation of various mutants unable to convert acetate to glucose suggests that the number of spores a cell will make is determined by the accumulation of a metabolic product of the glyoxylate pathway. The glyoxylate pathway results in the net conversion of acetyl CoA to oxaloacetate, the precursor for gluconeogenesis, and both pathways are essential for synthesis of glucose from acetate (Figure 1). Using acetate as the primary carbon source on solid sporulation medium, the glyoxylate pathway mutants *icl1*Δ, *mls1*Δ, and *sfc1*Δ accumulate NSDs (Figure 2 and Table 3). Under these sporulation conditions, the gluconeogenesis mutants *pck1*Δ and *fbp1*Δ make predominantly tetrads, demonstrating that the low level of glucose in the medium is sufficient for the biosynthetic requirements to support tetrad formation (Figure 2). Thus, formation of NSDs in glyoxylate pathway mutants is not due to a shortage of biosynthetic glucose but rather to the absence of a signal to promote formation of tetrads.

The idea that accumulation of a glyoxylate pathway intermediate promotes tetrad formation is further supported by analysis of wild-type cells sporulating in glycerol liquid medium. The conversion of glycerol to glucose requires only the last five steps of gluconeogenesis and does not involve synthesis of intermediates of the glyoxyate pathway, and wild-type cells form NSDs when glycerol is the sole carbon source (Figure 3). The observation that overexpression of MOP components results in increased tetrad formation demonstrates that wild-type cells have the biosynthetic capacity to make tetrads in glycerol but lack the signal to do so (Figure 9). Consistent with this idea, wild-type cells sporulating in glycerol make predominantly tetrads when the medium is supplemented with acetate (Figure 3). In sum, the accumulation of a product of acetate metabolism during sporulation acts as a measure of biosynthetic capacity to promote formation of tetrads.

Our data suggest that the critical metabolite that determines whether a sporulating cell will form a tetrad or NSD is one of the intermediates of the glyoxylate pathway that lies downstream of succinate dehydrogenase but upstream of phosphoenolpyruvate carboxykinase (Figure 1). The finding that *sfc1*Δ cells, which are likely defective in the conversion of succinate to fumarate, accumulate NSDs on acetate implicates fumarate, malate, oxaloacetate, or a derivative of one of these compounds as the key metabolite. The observation that glyoxylate pathway mutants make tetrads by using pyruvate as a carbon source indicates that the tetrad-inducing metabolite accumulates under these conditions. Cytoplasmic oxaloacetate is the only common intermediate that lies upstream of the *PCK1*-dependent step in the conversion of both acetate and pyruvate to glucose and is therefore a strong candidate for the compound that promotes tetrad formation. However, because the reactions that convert fumarate to malate and malate to oxaloacetate are readily reversible, it remains possible that malate or fumarate accumulate during sporulation on pyruvate and that one of these intermediates triggers formation of tetrads.

Modification of Mother SPBs during Meiosis Is Regulated

Our results indicate that assembly of MOPs on mother SPBs during sporulation is regulated in response to carbon source availability. During formation of NSDs in glycerol, the majority of cells in meiosis II have Mpc70p and Spo74p bound to only one or two SPBs, and the SPBs to which Mpc70p is preferentially recruited are the daughters (Figures 4 and 5). Similar results were obtained when NSD formation was triggered by brief exposure to acetate (our unpublished data). Thus, assembly of MOPs on daughter SPBs occurs by default once the commitment to enter meiosis has been made, whereas modification of mother SPBs remains sensitive to the metabolic state of the cell during sporulation.

The finding that MOP assembly occurs preferentially on daughter SPBs during formation of NSDs refutes earlier models proposing that MOP assembly is restricted to mother SPBs when NSDs are made. Previous reports have suggested that only mother SPBs acquire MOPs and initiate synthesis of prospore membranes when NSDs are formed due to limitation of a MOP component or carbon source availability (Bajgier *et al*., 2001; Wesp *et al*., 2001). Our results demonstrate that daughter SPBs preferentially acquire MOPs during sporulation when glycerol is the sole carbon source (Figure 5). We have also found that MOP assembly occurs predominantly on daughter SPBs when NSDs are formed in a strain homozygous for a partially functional *mpc70-GFP* allele (our unpublished data). It is likely that the NSDs formed by *mpc70-GFP/mpc70-GFP* cells are due to a reduction in the amount of functional Mpc70p because the sporulation efficiency of this strain is low and a *mpc70-GFP/mpc70*Δ strain fails to sporulate (Bajgier *et al*., 2001). Thus, assembly of MOPs occurs only on daughter SPBs during formation of NSDs due to either environmental conditions or to mutations that affect MOP structure.

How is regulation of MOP assembly restricted to mother SPBs? One potential model comprises two tenets: that assembly of MOPs on daughter SPBs precedes assembly of MOPs on mother SPBs and that the abundance or function of one or more MOP components is regulated in response to the metabolic state of the cell. According to this model,

sporulation conditions that do not lead to the accumulation of the intermediary metabolite would limit the amount of components available for MOP assembly, and assembly of MOPs on daughter SPBs would deplete the pool of components available for modification of mother SPBs. Two pieces of evidence are consistent with this temporal model. First, the observation that heterozygous deletion of *MPC54*, *MPC70*, or *SPO74* results in formation of NSDs suggests that each of these MOP components is normally expressed at quantities just sufficient to support formation of four MOPs per cell and that a 50% reduction in the abundance of any one of them leads to NSD formation (Bajgier *et al*., 2001; Wesp *et al*., 2001; Nickas *et al*., 2003). Second, the finding that overexpression of MOP components promotes formation of tetrads in glycerol indicates that an increase in the abundance of these proteins can promote assembly of MOPs at mother SPBs in the absence of a metabolic signal to do so (Figure 9).

An alternative model for selectively regulating MOP assembly on mother SPBs is that mother and daughter SPBs are structurally distinct. Mother SPBs may be marked in a way that inhibits MOP assembly, e.g., by covalent modification of a constitutive SPB component, and accumulation of the intermediary metabolite could provide a signal that relieves this inhibition. The observation that overexpression of MOP components promotes tetrad formation in glycerol demonstrates that assembly of MOPs on mother SPBs is not completely blocked in the absence of a putative metabolic signal, but mother SPBs may bear a molecular mark that renders MOP assembly inefficient when the biosynthetic capacity of the cell is low.

Our results suggest that the mutually dependent recruitment of Mpc70p and Spo74p is the critical step in the regulation of mother SPB modification. Both proteins are absent from mother SPBs during formation of NSDs in glycerol or after brief exposure to acetate (Figures 4 and 5; our unpublished data). Cooverexpression of Mpc70p and Spo74p dramatically increases tetrad formation during sporulation in glycerol, indicating that modification of mother SPBs is unregulated when the both of these proteins are overabundant (Figure 9). Overexpression of Mpc54p alone also increases tetrad formation in glycerol, indicating that modification of mother SPBs is unregulated when this protein is overabundant as well (Figure 9). Mpc54p is localized to mother SPBs during NSD formation and is capable of binding to both Mpc70p and Spo74p (Figure 4; Knop and Strasser, 2000). Thus, overexpression of Mpc54p may act by mass action to recruit Mpc70p and Spo74p to mother SPBs in the absence of the appropriate metabolic signal and thereby promote MOP assembly.

Model for Control of the Tetrad versus NSD Decision

Taken as a whole, our results suggest a model by which the availability of an environmental carbon source during sporulation determines how many spores a yeast cell will make. As cells enter meiosis, accumulation of an intermediary metabolite (oxaloacetate, malate, or fumarate) serves as an indicator of biosynthetic capacity. The meiosis-specific components of the outer plaque are expressed and recruited by default to daughter SPBs at the onset of meiosis II. In conditions under which the metabolite accumulates, signaling that biosynthetic carbon is abundant, Mpc70p and Spo74p are recruited to mother SPBs. Consequently, MOPs are assembled on all four SPBs under these conditions, and tetrads are formed. In conditions under which levels of metabolite remain low; however, Mpc70p and Spo74p are not recruited to mother SPBs. As a result, MOPs assemble only on daughter SPBs under these conditions, and NSDs are formed.

Similarities between Regulation of MOP Assembly and Other Signaling Systems

Our results elucidate a connection between two regulatory mechanisms that have parallels in other biological systems. Asymmetric distribution of proteins between mother and daughter spindle poles has been described in a variety of systems (Sohrmann *et al*., 1998; Chang and Stearns, 2000; Chang *et al*., 2003; Liakopoulos *et al*., 2003; Maekawa *et al*., 2003). Differential marking of mother and daughter spindle poles may thus be a common strategy to allow a cell to generate daughter cells with distinct fates. Monitoring the intracellular level of an intermediary metabolite has also been proposed to regulate cell function. For example, pancreatic β-cells release insulin from secretory granules in response to glucose, and it has been suggested that this response is partially due to elevated levels of citrate in the cytosol produced by glucose catabolism (Farfari *et al*., 2000; Flamez *et al*., 2002). Analysis of regulation of MOP assembly in yeast may reveal insight into the mechanisms by which asymmetric spindle pole function and metabolic flux regulate cellular processes and developmental decisions in other systems.

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