The creation of haploid gametes in yeast, termed spores, requires the de novo formation of membranes within the cytoplasm. These membranes, called prospore membranes, enclose the daughter nuclei generated by meiosis. Proper growth and closure of prospore membranes require the highly conserved Vps13 protein. Mutation of \textit{SPO71}, a meiosis-specific gene first identified as defective in spore formation, was found to display defects in membrane morphogenesis very similar to those seen in \textit{vps13Δ} cells. Specifically, prospore membranes are smaller than in the wild type, they fail to close, and membrane vesicles are present within the prospore membrane lumen. As in \textit{vps13Δ} cells, the levels of phosphatidylinositol-4-phosphate are reduced in the prospore membranes of \textit{spo71Δ} cells. \textit{SPO71} is required for the translocation of Vps13 from the endosome to the prospore membrane, and ectopic expression of \textit{SPO71} in vegetative cells results in mislocalization of Vps13. Finally, the two proteins can be coprecipitated from sporulating cells. We propose that Spo71 is a sporulation-specific partner for Vps13 and that they act in concert to regulate prospore membrane morphogenesis.

\textbf{SPO71 Encodes a Developmental Stage-Specific Partner for Vps13 in \textit{Saccharomyces cerevisiae}}

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\textbf{S}tarvation of diploid cells of the yeast \textit{Saccharomyces cerevisiae} induces their differentiation into haploid spores, the equivalent of gametes in metazoans (1, 2). During meiosis, two sequential nuclear divisions produce four haploid nuclei, and each nucleus is then packaged within newly formed membranes to generate spores. As is frequently seen during differentiation in higher cells, sporulation requires rearrangement of the secretory pathway (3–5). In meiosis II, secretory vesicles are redirected from the plasma membrane to the poles of the meiotic spindle (6). At each of the four spindle pole bodies, the vesicles dock and fuse with each other to form small membrane compartments termed prospore membranes (5). As meiosis II proceeds, these membranes expand, and at the end of meiosis each prospore membrane encloses a nucleus to give rise to four immature spores. A spore wall then forms in the lumen of the prospore membrane, resulting in a mature spore (7).

Membrane closure is a type of cytokinesis, resulting in the physical separation of daughter cell and mother cell cytoplasm. Membrane closure requires removal of a protein complex that localizes to the lip of the prospore membrane termed the leading edge protein complex (LEP) (8). The LEP is composed of at least three proteins, SspI, Ady3, and Don1 (9, 10). SspI is targeted for degradation at the end of meiosis II through the action of the anaphase-promoting complex and its meiosis-specific activator, Ama1 (11). Degradation of Ssp1 leads to release of Ady3 and Don1 from the lip of the prospore membrane and is a prerequisite for membrane closure (8, 11). An \textit{ama1} mutant, therefore, is unable to complete meiotic cytokinesis and shows persistent localization of the leading edge complex proteins at the lip of the prospore membrane.

Recently, we described a role for the \textit{VPS13} gene in promoting prospore membrane closure (12). \textit{VPS13} was originally identified by its role in vesicle traffic between the Golgi body and the vacuole (13–15). In vegetative cells, the protein is localized to the endosome, but during sporulation \textit{VPS13} relocates to the prospore membrane (12). \textit{vps13Δ} mutants display a variety of prospore membrane defects, including small prospore membranes, the appearance of intraluminal vesicles within prospore membranes, and a failure to complete membrane closure. The small prospore membrane phenotype of the \textit{vps13Δ} mutant results from reduced levels of phosphatidylinositol-4,5-bisphosphate [PtdIns(4,5)P\(_2\)] and its precursor, phosphatidylinositol-4-phosphate [PtdIns (4)P], in the prospore membrane (12). These results suggest that \textit{VPS13} is repurposed during sporulation to regulate PtdIns phosphate levels at the prospore membrane. \textit{VPS13} is highly conserved, and mutations of the human orthologs \textit{VPS13A} and \textit{VPS13B} give rise to the inherited disorders chorea acanthocytosis and Cohen syndrome, respectively (16, 17). Understanding the mechanism by which \textit{Vps13} influences PtdIns phosphate levels in yeast may therefore provide insight into the basis for these human diseases.

During sporulation, a highly regulated program of gene expression results in the induction of sporulation-specific proteins that can alter the function of constitutive gene products (1). For example, the sporulation-specific SNARE protein Spo20 works in concert with the constitutive SNARE molecules Sso1 and Snc1/2 to promote vesicle fusion at the prospore membrane (5, 18, 19). This work shows that Vps13 function is similarly changed during sporulation by the presence of a sporulation-specific protein encoded by \textit{SPO71}. \textit{SPO71} is required for sporulation and for proper prospore membrane growth (20). Here, we report that the \textit{spo71Δ} sporulation defect results from a defect in membrane closure. \textit{spo71Δ} cells share several other phenotypes with \textit{vps13Δ} cells as well, including small prospore membranes and intraluminal vesicles. The similarity of phenotypes in \textit{vps13Δ} and \textit{spo71Δ} cells suggests that they work together to promote membrane morpho-
Table 1. Strains used in this study.

| Strain | Genotype | Source |
|--------|----------|--------|
| AN117-4B | MATα ura3 leu2 his3ΔSK trp1::hisG arg4-NpsI lys2 ho::LYS2 rme1Δ::LEU2 | 23 |
| AN117-16D | MATα ura3 leu2 trp1::hisG his3ΔSK lys2 ho::LYS2 | 23 |
| AN120 | MATα/MATα ura3/ura3 his3ΔSK/his3ΔSK trp1::hisG/trp1::hisG ARG4/arg4-NpsI lys2/lys2 | 23 |
| AN361-6A | MATα ura3 leu2 trp1::hisG his3ΔSK lys2 ho::LYS2 spo71Δ::his5+ RME1/rme1Δ::LEU2 leu2/leu2 | This study |
| AN363 | MATα/MATα ura3/ura3 his3ΔSK/his3ΔSK trp1::hisG/trp1::hisG ARG4/arg4-NpsI lys2 ho::LYS2 spo71Δ::his5+ RME1/rme1Δ::LEU2 leu2/leu2 vps13Δ::his5+ /vps13Δ::his5+ | This study |
| HI29 | MATα ura3/ura3 his3ΔSK/his3ΔSK spo71Δ::his5+ spo71Δ::his5+ | 32 |
| JSP163 | MATα/MATα ura3/ura3 trp1::trp1 ARG4/arg4 LEU2/leu2 spo71Δ::his5+/spo71Δ::his5+ TEF2::GFP::his5+/TEF2::GFP::his5+ | This study |
| JSP247 | MATα ura3 trp1::hisG arg4-NpsI lys2 ho::LYS2 rme1Δ::LEU2 leu2 VPS13::GFP::his5+ | This study |
| JSP248 | MATα ura3 trp1::hisG ARG lys2 ho::LYS2 leu2/leu2 VPS13::GFP::his5+ | This study |
| JSP257 | MATα/MATα ARG4/arg4-NpsI LEU2/leu2 ura3/ura3 trp1::trp1 trp1 VPS13::GFP::his5+/VPS13::GFP::his5+ | 12 |
| JSP290 | MATα ura3 leu2 trp1::hisG ho::LYS2 arg4-NpsI spo71Δ::his5+ VPS13::GFP::his5+ | This study |
| JSP291 | MATα ura3 leu2 trp1::hisG ho::LYS2 spo71Δ::his5+ VPS13::GFP::his5+ | This study |
| JSP294 | MATα/MATα ura3/ura3 leu2/leu2 trp1::trp1 ARG4/arg4 leu2/leu2 his3ΔSK VPS13::HA::his5+ | This study |
| JSP391 | MATα ura3 trp1::hisG arg4-NpsI lys2 ho::LYS2 rme1Δ::LEU2 leu2 his3Δ SK VPS13::HA::his5+ | This study |
| JSP392 | MATα ura3 trp1::hisG lys2 ho::LYS2 leu2 his3ΔSK VPS13::HA::his5+ | This study |
| JSP394 | MATα ura3 trp1::hisG arg4-NpsI lys2 ho::LYS2 leu2 leu2 VPS13::HA::his5+ | This study |
| JSP401 | MATα/MATα ura3/ura3 trp1::trp1::trp1 hisG ho::LYS2 ho::LYS2 leu2/leu2 ARG4/arg4-NpsI VPS13::HA::his5+ /VPS13::HA::his5+ | This study |

ogenesis. We propose that meiotic induction of SPO71 triggers re-localization of Vps13 to the prospore membrane, where the complex of the two proteins functions to regulate PtdIns phosphate levels.

**Materials and Methods**

Yeast strains and media. Yeast strains used for this study are listed in Table 1. Unless otherwise mentioned, standard yeast media and genetic techniques were used (21). JSP247 and JSP248 were segregants from a cross of AN117-4B to a strain carrying a green fluorescent protein (GFP)-tagged VPS13 allele (15). JSP290 and JSP291 were segregants from a cross of JSP247 with AN361-6A. AN361-6A is a segregant from a cross of AN117-4B and a SPO71 gene deletion strain (22). AN363 was generated by mating of AN361-6A with a second segregant from the same cross. Mating of JSP290 to JSP291 generated strain JSP294.

To construct JSP163 for the fluorescence loss in photobleaching (FLP) assay, a spo71Δ segregant was mated with a strain containing a GFP-tagged TEF2 allele (15). After tetrad dissection, segregants containing both spo71Δ::his5+ and TEF2::GFP::his5+ were mated to generate strain JSP163. This strain was transformed with pRS426-RFP-SP202/21R prior to the FLP assay.

To generate JSP401, VPS13 was first tagged with 3× hemagglutinin (HA) in haploid strains AN117-4B and AN117-16D (23) by PCR-based gene tagging using primers JSP08 and JSP02, creating JSP391 and JSP392, respectively. All oligonucleotide sequences are listed in Table 2. JSP394 is a segregant from the cross of JSP391 and AN117-16D. A cross of JSP392 and JSP394 generated the diploid strain JSP401.

Plasmids. Plasmids used in this study are listed in Table 3. To construct pRS426-VPS13, 500 bp of upstream and downstream sequence were first amplified by PCR using primer pair JSP08 and JSP06 and primer pair JSP01 and JSP02, respectively. The oligonucleotides used to amplify the upstream region (P_{VPS13}) introduce the restriction enzyme sites SacII and NotI. After SacII and NotI double digestion, this fragment was ligated into SacII/NotI-digested pRS426 to generate pRS426-P_{VPS13}. The downstream fragment (VPS13-Ter) was then similarly cloned into pRS426-P_{VPS13} using Xhol and KpnI to create pRS426-P_{VPS13}VPS13-Ter. Because of the large size of the VPS13 coding region (~10 kb), this sequence was introduced into the plasmid by homologous recombination of overlapping segments in S. cerevisiae (24). Overlapping fragments of the VPS13 coding region were amplified by PCR using the primers JSO70 and JSO64, JSO64 and JSO65, JSO65 and JSO66, and JSO66 and JSO67. The 5′ ends of the primers JSO70 and JSO67 carry 27 and 36 nucleotides, respectively, which allow recombination with the plasmid pRS426-P_{VPS13}TER. vps13Δ mutant diploid cells (H29) were cotransformed with the four PCR products and NotI/Xhol-linearized pRS426-P_{VPS13}TER. Transformants were screened for rescue of the vps13Δ sporulation defect, and the assembled pRS426-VPS13 was recovered from a rescued strain.

pRS424-SPO71 was similarly constructed by homologous recombination in S. cerevisiae (24). Overlapping fragments of the SPO71 coding region including 500 bp upstream and downstream were amplified by PCR using the primers JSO159 and JSO160 and correctly assembled plasmids were identified by complementation of the sporulation defect. pRS426-P_{TEF2-SP071-RFP} was constructed as pRS424-SPO71, except that pRS426-P_{TEF2-RFP} was used as the linearized plasmid and the oligonucleotides and JSO191 and JSO196 were used in place of JSO159 and JSO160 and JSO161. The 5′ ends of the primers JSO159 and JSO161 carry 40 nucleotides that allow recombination with the plasmid pRS424 linearized with SacI. The PCR products and linearized plasmid were cotransformed into spo71Δ mutant diploid cells (AN363), and correctly assembled plasmids were identified by complementation of the sporulation defect. pRS426-P_{TEF2-SP071-RFP} was constructed as pRS424-SPO71, except that pRS426-P_{TEF2-RFP} was used as the linearized plasmid and the oligonucleotides and JSO191 and JSO196 were used in place of JSO159 and JSO161, respectively, to provide homology to the ends of the linearized plasmid. EcoRI/HindIII-digested pRS426-P_{TEF2-RFP} and the two PCR products were introduced into spo71Δ diploid cells. pRS426-P_{TEF2-SP071-RFP} was recovered from a transformant whose sporulation defect was complemented. To generate pRS424-SPO71-GFP, the chromosomal copy of SPO71 was first fused to GFP by PCR-mediated transformation using HT290 and HT291 and pFA6a-yEGFP-HISMX6 as a template (10). SPO71-GFP with the SPO71 promoter was then amplified from genomic DNA of the transformant using HT293 and HT66 and cloned into pRS424.

### Fluorescence microscopy.

For examination of localization of fluorescent markers to the prospore membrane, cells were replica plated from selective medium to SPO (sporulation) plates and incubated overnight at room temperature. Cells were transferred to microscopy slides, and...
Table 2 Oligonucleotides used in this study

| Name  | Sequence (5′–3′) |
|-------|-----------------|
| JSO61 | CGG CTC GAG TCA CAT ATG AAA GTA TAT AC |
| JSO62 | GGG GTA CCT TTG ATC TTT AAG AGC GCA T |
| JSO64 | TGA GTC TCA GTA GAT AAA ATT TGG A |
| JSO64-r | TCC AAA TTT TAT TCT AGC GTA CTC A |
| JSO65 | CGT CAC CAA TTA AAA TAA ATC TCA G |
| JSO65-r | CTG AGA TTT ATT TTA ATT GGT GAC G |
| JSO66 | GTGTATTAACCTTTTATTCAC |
| JSO66-r | GAT GAG GAT GAG AAG GGT ATT AAT ACA C |
| JSO67 | TAG TGT ACA AAA GCG GGT ATC TAC TTT CAT |
| JSO68 | TCC CCG GGG GAA GAT GGT AGA ACG CTC TG |
| JSO69 | ATA AGA ATG CCG CCG CTT AAT TGG TCT TAA |
| JSO70 | GAA AAA AGG AAA ATT AAG AGT TAA GCG |
| JSO71 | GCC GCA TTA TTA TAT ATT AGA TGG AGC TGC TAA |
| JSO81 | ACA TCG CCA TTG CTG TTA GAT AAT ACA ATG AGT ATG GTG AAT GTA CTA TCC TAC GAG TCC GGT TAA TTA A |
| JSO82 | GAA TTA TAG CTA CAT AGT GTA CAA AAG CCG GTG TAT ACT TGT ATG TAT AAG TAC TGC TGG CTT TAA A |
| JSO159 | CGG GGG ATC CAC TAG TCC TTC TAG AGC GGC GGC CAC CGG GGT GGA GCT CCT TTT AGT GGA CAG TGG |
| JSO160 | AAC TCG TAT AAT GAT TTA CCA AA |
| JSO160-r | TTT TGG AAT ATT AGG GTA TTA AAG TT |
| JSO161 | CAA GGG GGC AAT TAA CCC TCA GTA AAG GGA ACA AAA GGT GCA GGT CGC TAG AGT TCC GGT GTA AGT TT |
| JSO195 | TGG CCG CCG CTC TAG AAG TGG ATG ACC CCC GGT GCA GCA ATT CAT GGA TTA TGC TAT GAT TTA AAA CAG |
| JSO196 | AGC GTA TCA ACT CCT TGA CGT CCT CCG AGG AGG CCA TAA GCT TCA TAG TTT GAT TCC GGT AAT TTG |
| JSO197 | AAA GAC TAA GAA GAA CTG GGT CCA TTA CAA ATT CAC GAC GTA AAT CTA CGG TCA CGG CCT CCG GGT TAA TTA A |
| HT290 | AGT ATA CAC TAA ATT TTA TGC AAT AAT AAA AAG AGAAGA CAG CTG CCG CAG CAA AAT AGG AG TAC CTC TTT AAA C |
| HT291 | AAA TGA CAT GAA GAT GAG AAT CTA AGA AAA GAT TTT GGT |
| HT293 | GAA GAA GGT ACC AAA TGA TGA TGA AAG GAC TGA |
| HT66 | GAA GAA TTC AGA TCT TCA CTA TAA CCC TGT TAT CC |

Table 3 Plasmids used in this study

| Name     | Gene(s) expressed | Source |
|----------|-----------------|--------|
| pRS426-RFP-SPO20 | Ppex2-RFP-SPO20 | 33     |
| pRS424-DTR1-RFP | DTR1-RFP | 12     |
| pRS426-RFP-SPO20 | Ppex2-RFP-SPO20 | 12     |
| DON1-GFP | DON1-GFP | -      |
| pRS426-Ppex2-GFP-PH | GFP-PH | 27     |
| pRS426-GFP-2×PHPLC | GFP-2×PHPLC | 26     |
| pRS426-VPS13 | VPS13 | -      |
| pRS424-SPO71 | SPO71 | -      |
| pRS424-SPO71-GFP | SPO71-GFP | -      |
| pRS426-Ppex2-SPO71-RFP | Ppex2-SPO71-RFP | -      |

The results of the experiment show...

**RESULTS**

*SPO71* is required for membrane closure during spore formation. We have previously described a fluorescence loss in photobleaching assay to identify mutants defective in prosop membrane closure (11). The FLIP assay differentiates whether prosop membrane are open or closed by measuring the fluorescence intensity inside the prosop membrane of a diffusible, cytoplasmic green fluorescent protein in response to repetitive photobleaching of the cytoplasm outside the prosop membrane. If the membrane is closed, then fluorescence persists within the prosop membrane but is lost in the cytoplasm of the surrounding mother cell. If closure has not occurred, GFP bleaches evenly both inside and outside the prosop membrane. Both *ama1* and *vps13* cells exhibit closure defects in this assay (11, 12). In addition, a significant closure defect was observed for *spo71A* cells. In postmeiotic spo71A cells, 72% of the prosop membranes were still open to the asc cytoplasm, as opposed to 0% in wild-type cells at a similar stage (Table 4).

Prospor membrane closure requires removal of the LEF from the mouth of the prosop membrane (8, 11). Don1-GFP is a...
convenient marker for LEP disassembly, as it is localized at the leading edge during prospore membrane growth and relocalizes throughout the spore cytoplasm coincidentally with membrane closure \((8, 11)\). The previously identified closure \(ama1\Delta/H9004\) and \(vps13\Delta/H9004\) mutants differ with respect to removal of the LEP. In \(ama1\Delta/H9004\) mutants, Don1-GFP persists at the leading edge in the majority of cells, whereas in \(vps13\Delta/H9004\) mutants a majority of the cells display Don1-GFP redistributed to the cytoplasm (Table 4).

To determine if the closure defect of the \(spo71\Delta/H9004\) mutant is upstream or downstream of LEP removal, the distribution of Don1-GFP was monitored in postmeiotic \(spo71\Delta/H9004\) cells. Don1-GFP relocalizes to the spore cytoplasm in 67% of such cells, though the FLIP results suggest that the majority of prospore membranes are not yet closed (Table 4). Therefore, similar to what is seen in the \(vps13\Delta\) mutant, the \(spo71\Delta\) closure defect occurs after LEP removal \((12)\).

**Intraluminal vesicles accumulate in \(spo71\Delta\) prospore membranes.** \(spo71\Delta\) cells were examined for other \(vps13\Delta\) sporulation phenotypes. In addition to the closure defects, \(vps13\Delta\) mutants display a unique phenotype, the accumulation of membrane vesicles within the lumen of the prospore membrane \((12)\). Transmission electron microscopy was performed to examine the prospore in \(spo71\Delta\) cells. In wild-type cells, the luminal width between inner and outer prospore membrane is constant (Fig. 1A). In \(spo71\Delta\) cells, this uniform distance is disrupted by the presence of intraluminal vesicles, just as in \(vps13\Delta\) cells (Fig. 1B and C). These vesicles were visible in 37% of prospore membrane profiles in \(spo71\Delta\) cells \((n = 57)\), a percentage lower than that reported for \(vps13\Delta\) cells \((75% \ [12])\) but still significant given that these vesicles were never seen in wild-type cells \((n > 100)\). Expression of VPS13 from a high-copy-number plasmid did not suppress the sporulation defect of, or the formation of these vesicles in, the \(spo71\Delta\) mutant, suggesting that VPS13 does not function downstream of \(SPO71\) (Fig. 1D).

**Prospore membrane PtdIns-phosphate pools are reduced in \(spo71\Delta\) cells.** Many of the \(vps13\Delta\) prospore membrane defects are caused by reduced levels of PtdIns(4)P and PtdIns(4,5)P\(_2\) within

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### TABLE 4 Comparison of prospore membrane closure in wild-type, \(ama1\Delta\), \(vps13\Delta\), and \(spo71\Delta\) cells

| Expt                      | Wild-type cells\(^a\) | \(ama1\Delta\) cells\(^a\) | \(vps13\Delta\) cells\(^b\) | \(spo71\Delta\) cells\(^c\) |
|---------------------------|------------------------|-----------------------------|-----------------------------|-----------------------------|
| Membrane closure          | Closed                 | 100                         | 31                          | 0                           | 3                           |
| by FLIP assay (%)         | Open                   | 0                           | 53                          | 94                          | 72                          |
|                           | Indeterminate\(^d\)    | 0                           | 16                          | 6                           | 25                          |
| Don1-GFP distribution (%) | Cytoplasm              | 100                         | 34                          | 66                          | 67                          |
|                           | Leading edge           | 0                           | 66                          | 34                          | 33                          |

\(^a\) Data are from references 11 and 12.

\(^b\) Fluorescence loss was intermediate between open and closed patterns.

\(^c\) More than 50 prospore membranes were scored in the FLIP assay. More than 110 prospore membranes were examined in the Don1-GFP distribution experiment.

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**FIG 1** Intraluminal vesicle accumulation within the prospore membrane. Transmission electron microscope images of prospore membranes in wild-type (AN120) cells (A), \(vps13\Delta\) (HI29) cells (B), \(spo71\Delta\) (AN363) cells (C), and \(spo71\Delta\) cells overexpressing VPS13 (D). In all images, yellow arrows indicate prospore membranes and red arrows indicate the accumulation of vesicles within the lumen of a prospore membrane. Bars, 500 nm.
the prospore membrane (12). To investigate if these pools are reduced in *spo71/H9004* cells, the localizations of two lipid-binding sensors—GFP-PH OSH2, a marker for PtdIns(4)P, and GFP-2xPHPLC/H9254, a marker for PtdIns(4,5)P2—were monitored during sporulation (25–27). In wild-type cells, both sensors colocalized with the prospore membrane marker Dtr1-RFP in 70% of the cells (Fig. 2). In *spo71* cells, however, only 36% or 38% of the prospore membranes detectable by Dtr1-RFP displayed colocalization with GFP-PH OSH2 or GFP-2xPHPLC/H9254, respectively (Fig. 2). These results suggest that the levels of both of these lipids are reduced in the *spo71/H9004* mutant, demonstrating another parallel between the *spo71/H9004* and *vps13/H9004* sporulation phenotypes.

**Spo71-GFP localizes to the prospore membrane during sporulation.** Vps13 localizes to prospore membranes during meiosis (12). If Spo71 and Vps13 work together directly, then Spo71 should also be found on these membranes. When the chromosomal copy of *SPO71* was C-terminally tagged with GFP, no detectable fluorescence signal was seen in sporulating cells. Therefore, a *SPO71::GFP* fusion under the control of the constitutive *TEF2* promoter in mitotic cells might alter Vps13 localization. To test this hypothesis, *SPO71::RFP* was ectopically expressed under the control of the *TEF2* promoter in mitotic cells that also expressed Vps13-GFP. The *SPO71::RFP* fusion is functional as shown by its ability to rescue the sporulation defect of a *spo71/H9004* mutant (J.-S. Park, unpublished observation). In the absence of *SPO71::RFP* the prospore membrane (12). To investigate if these pools are reduced in *spo71Δ* cells, the localizations of two lipid-binding sensors—GFP-PH OSH2, a marker for PtdIns(4)P, and GFP-2xPHPLC/H9254, a marker for PtdIns(4,5)P2—were monitored during sporulation (25–27). In wild-type cells, both sensors colocalized with the prospore membrane marker Dtr1-RFP in >70% of the cells (Fig. 2). In *spo71* cells, however, only 36% or 38% of the prospore membranes detectable by Dtr1-RFP displayed colocalization with GFP-PH OSH2 or GFP-2xPHPLC/H9254, respectively (Fig. 2). These results suggest that the levels of both of these lipids are reduced in the *spo71Δ* mutant, demonstrating another parallel between the *spo71Δ* and *vps13Δ* sporulation phenotypes.

**Spo71-GFP localizes to the prospore membrane during sporulation.** Vps13 localizes to prospore membranes during meiosis (12). If Spo71 and Vps13 work together directly, then Spo71 should also be found on these membranes. When the chromosomal copy of *SPO71* was C-terminally tagged with GFP, no detectable fluorescence signal was seen in sporulating cells. Therefore, a *SPO71::GFP* fusion under the control of the constitutive *TEF2* promoter in mitotic cells might alter Vps13 localization. To test this hypothesis, *SPO71::RFP* was ectopically expressed under the control of the constitutive *TEF2* promoter in mitotic cells that also expressed Vps13-GFP. The *SPO71::RFP* fusion is functional as shown by its ability to rescue the sporulation defect of a *spo71Δ* mutant (J.-S. Park, unpublished observation). In the absence of *SPO71::RFP*

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**FIG 2** Effect of the *spo71Δ* mutation on prospore membrane PtdIns(4)P and PtdIns(4,5)P2 pools. (A) Sporulating wild-type (AN120) and *spo71Δ* (AN363) cells expressing both a PtdIns(4)P sensor (GFP-PH OSH2) and a prospore membrane marker (Dtr1-RFP). The red arrows indicate a prospore membrane without the GFP marker in the *spo71Δ* cell. Bar, 1 μm. (B) Quantitation of GFP-PH OSH2/Dtr1-RFP colocalization. Bars indicate standard errors of the means. More than 100 prospore membranes were scored in each of four separate experiments. (C) Sporulating wild-type (AN120) and *spo71Δ* (AN363) cells expressing both a PtdIns(4,5)P2 sensor (GFP-2xPHPLC/H9254) and a prospore membrane marker (Dtr1-RFP). The red arrows indicate a prospore membrane without the GFP marker in the *spo71Δ* cell. Bar, 1 μm. (D) Quantitation of GFP-PHPLC/H9254/Dtr1-RFP colocalization. Bars indicate standard errors of the means. More than 100 prospore membranes were scored in each of two separate experiments. The chi-square test indicates that the differences between wild-type and *spo71Δ* cells are significant ($P < 0.001$).

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**FIG 3** Localization of Spo71-GFP in sporulating cells. Wild-type (AN120) cells carrying pRS424-*SPO71*-GFP and the prospore membrane marker pRS426-RFP-Spo2051-91 were sporulated, and fluorescence was examined during prospore membrane growth. Colocalization to the prospore membrane was seen in 100% of the cells examined ($n = 25$). Bar, 1 μm.
expression, Vps13-GFP foci are observed in the cytoplasm, likely at endosomes as previously reported (Fig. 5). Expression of SPO71::RFP caused a redistribution of the Vps13-GFP signal. Rather than cytoplasmic foci, GFP fluorescence was diffuse in the cytosol, with some concentration at the plasma membrane and bud neck (Fig. 5). Vps13-GFP signal at the plasma membrane was not seen in wild-type cells (Fig. 5).

Ectopically expressed SPO71 has been reported to localize to the plasma membrane in mitotic cells (20). This suggests that induction of Spo71-RFP might lead to colocalization with Vps13 at the plasma membrane and bud neck. However, we were unable to test this prediction, as the RFP fluorescence from our Spo71-RFP was seen uniformly throughout the cell, suggesting that the RFP moiety may be cleaved from Spo71 in vivo. Nonetheless, these results demonstrate that expression of SPO71::RFP is sufficient to alter Vps13 localization.

**Spo71 physically interacts with Vps13 during sporulation.**

To test for a physical interaction between Vps13 and Spo71, co-immunoprecipitation experiments were performed. Extracts made from sporulating cells expressing SPO71::GFP, VPS13::3xHA, or both fusions were used for immunoprecipitations with both anti-HA and anti-GFP antibodies. A Western blot of the anti-HA immunoprecipitates with anti-GFP antibodies revealed that Spo71-GFP precipitated only when coexpressed with Vps13-3xHA (Fig. 6). Reciprocally, pulldown of Spo71-GFP was able to specifically coprecipitate Vps13-3xHA. Thus, consistent with the similar phenotypes of the mutants, Spo71 forms a sporulation-specific complex with Vps13. The anti-HA pulldown reveals extensive degradation of Vps13-3xHA in the extracts. In contrast, only the full-length Vps13-3xHA protein is seen in the anti-GFP precipitates. As the HA epitopes are fused to the C-terminal end of Vps13, this observation suggests that the N terminus of Vps13 is essential for interaction with Spo71.

**DISCUSSION**

The data presented provide a model for how constitutive secretory pathway functions can be repurposed during differentiation. The Spo71 and Vps13 proteins form a sporulation-specific complex that regulates PtdIns phosphate levels in the prospore membrane. While VPS13 is constitutively expressed, SPO71 is sporulation specific in its expression. Our data suggest that induction of SPO71 when cells progress out of meiotic prophase leads to Spo71 protein binding to Vps13. In turn, interaction with Spo71 leads to...
release of Vps13 from the endosome, and both proteins then localize to the prospore membrane and influence PtdIns phosphate levels.

The spo71Δ prospore membrane phenotypes described are similar to those of vps13ΔΔ cells; however, in all cases—e.g., membrane size, the percentage of membranes that fail to close, the fraction of membranes displaying intraluminal vesicles—the phenotype of the spo71Δ mutant is quantitatively less severe than that of the vps13ΔΔ mutant. This suggests the possibility that the critical role of Spo71 is simply to recruit VPS13 to the prospore membrane, where Vps13 would be responsible for stimulating PtdIns phosphate levels. If so, overexpression of VPS13 might be expected to rescue some of the spo71Δ phenotypes. As overexpression of VPS13 does not bypass spo71, we prefer a model in which the two proteins act in concert to regulate PtdIns phosphates.

Another important issue that remains to be resolved is whether Vps13 performs the same molecular function in two different locations, the endosome or the prospore membrane, at different stages of the life cycle, or if the function of Vps13 at the prospore membrane is different from its vegetative function in endosomal-Golgi body retrograde transport. While the answer to this question will require identifying the precise molecular function of Vps13 in both processes, the fact that SPO71 is required at the prospore membrane but not at the endosome may indicate that Vps13 has two independent functions.

Another critical question is how the Vps13/Spo71 complex affects PtdIns phosphate levels. As the levels of these lipids are based, ultimately, on the rate of their synthesis and degradation, Vps13/Spo71 likely acts either by inhibition of PtdIns-4-phosphate phosphatases or by activation of PtdIns-4 kinases. For the kinases, the likely targets would be either Pik1, responsible for the Golgi body pool of PtdIns(4)P in vegetative cells, or Stt4, responsible for the plasma membrane pool (28, 29). A previous study has implicated PIK1 in contributing to PtdIns phosphates in the prospore membrane (30). However, these effects could be indirect, as Pik1-generated PtdIns(4)P in the Golgi body could be delivered to the prospore membrane via vesicular transport. For example, initial pools of PtdIns(4)P might be from a Pik1-generated Golgi body pool delivered by vesicular transport, while maintenance of PtdIns(4)P levels might require Stt4 activity and the latter activity could be influenced by Vps13/Spo71. Consistent with this possibility, Stt4 localizes to the prospore membrane (J.-S. Park and H. Tachikawa, unpublished observations). To date, however, we have been unable to observe a physical interaction of Vps13 with either Pik1 or Stt4; however, it is possible that any effect on enzymatic activity of the kinases may not be mediated through direct binding.

VPS13 is highly conserved in eukaryotic organisms, including four paralogs in human cells (31). Mutations in two of these paralogs result in different genetic syndromes (16, 17). It is of interest, therefore, whether the Spo71/Vps13 complex or its function is conserved in higher organisms. While SPO71 is not as highly conserved as VPS13—putative SPO71 orthologs can be found in many fungi, but are absent from metazoans—the protein contains domains present in higher cells. The Spo71 protein contains 2 pleckstrin homology (PH) domains and a third conserved domain, which sequence alignments suggest may be a third, degenerate PH domain (20, 27; A. M. Neiman, unpublished observation). While no direct orthologs of SPO71 are present in metazoans, there are many PH domain-containing proteins. It is possible that one or more of these mammalian PH domain proteins interact with a Vps13 ortholog similar to Spo71.

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