YEASTBOOK

CELL SIGNALING & DEVELOPMENT

Sporulation in the Budding Yeast *Saccharomyces cerevisiae*

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ABSTRACT In response to nitrogen starvation in the presence of a poor carbon source, diploid cells of the yeast *Saccharomyces cerevisiae* undergo meiosis and package the haploid nuclei produced in meiosis into spores. The formation of spores requires an unusual cell division event in which daughter cells are formed within the cytoplasm of the mother cell. This process involves the *de novo* generation of two different cellular structures: novel membrane compartments within the cell cytoplasm that give rise to the spore plasma membrane and an extensive spore wall that protects the spore from environmental insults. This article summarizes what is known about the molecular mechanisms controlling spore assembly with particular attention to how constitutive cellular functions are modified to create novel behaviors during this developmental process. Key regulatory points on the sporulation pathway are also discussed as well as the possible role of sporulation in the natural ecology of *S. cerevisiae*.

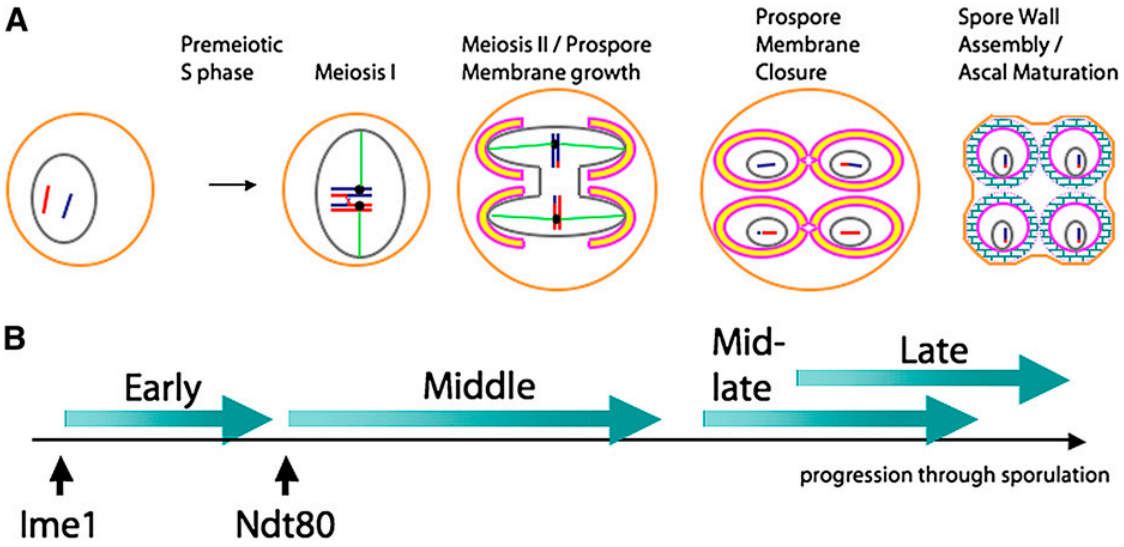

Figure 1 The morphogenetic events of spore formation are driven by an underlying transcriptional cascade. (A) The landmark events of meiosis and sporulation are shown in temporal order. Orange lines indicate the mother cell plasma membrane (which becomes the ascal membrane). Gray lines indicate the nuclear envelope. Blue and red lines represent homologous chromosomes. Green lines represent spindle microtubules. Prospore membranes are indicated by pink lines and the lumen of the prospore membrane is highlighted in yellow. After membrane closure, the prospore membrane is separated into two distinct membranes. The one closest to the nucleus serves as the plasma membrane of the spore, while the outer membrane, indicated by thin, dashed pink line, breaks down during spore wall assembly. Blue hatching represents the spore wall. (B) The shaded arrows indicate the relative timing of the different transcriptional classes with respect to the events in A. The black arrows indicate the points at which the transcription factors Ime1 and Ndt80 become active.

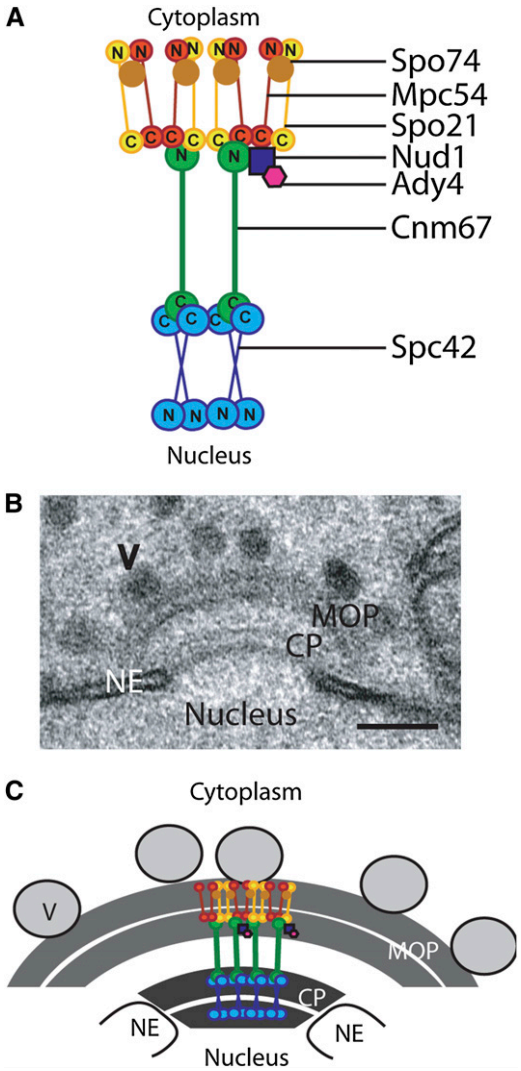

Figure 2 Organization of meiosis II outer plaque. (A) Diagram of the arrangement of meiosis II outer plaque subunits within the complex. The coiled-coil proteins Mpc54, Spo21, Cnm67, and Spc42 are depicted as dumbbells with their N- and C termini indicated. The likely positions of Spo74, Nud1, and Ady4 are also shown. (B) Electron micrograph of a meiosis II SPB prior to prospore membrane formation. V, prospore membrane precursor vesicle; CP, central plaque; MOP, meiosis II outer plaque; NE, nuclear envelope. Bar, 100 nm. (C) Cartoon of image in B overlaid with the schematic from A to show the positions of proteins within the structure. This figure is adapted from Mathieson *et al.* (2010b).

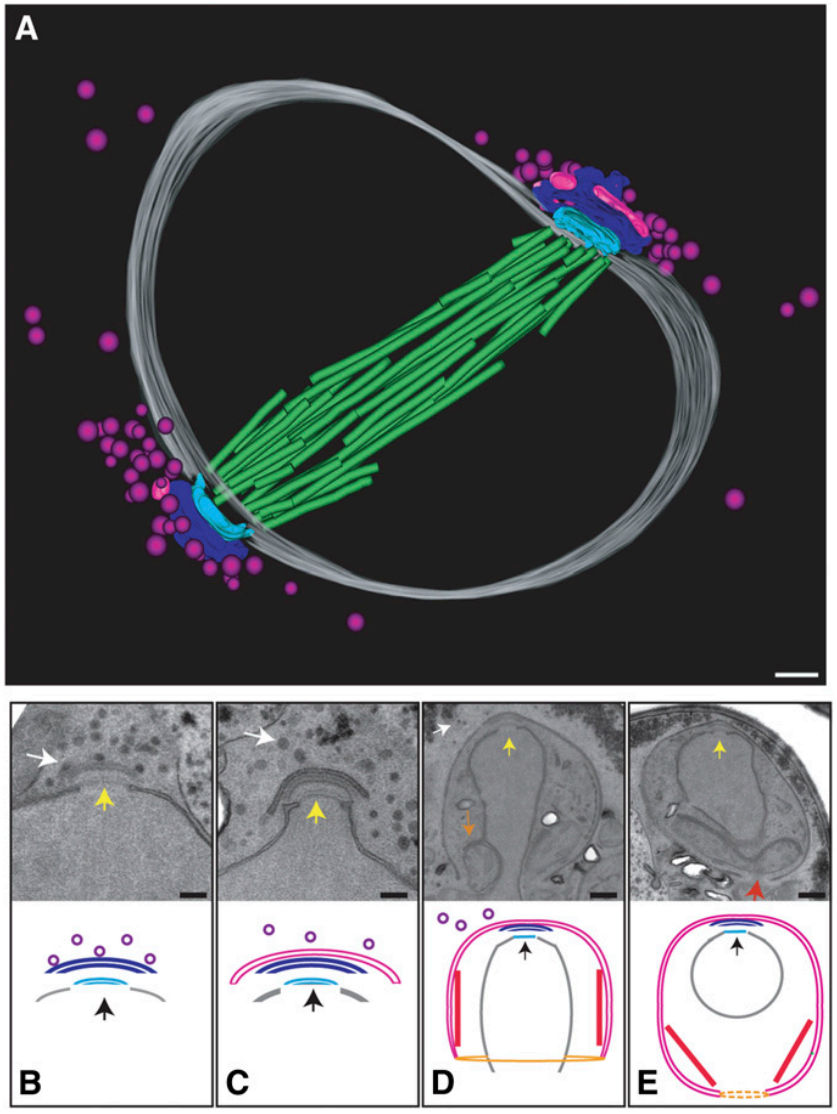

Figure 3 Stages of prospore membrane growth. (A) Model of a meiosis II spindle at the time prospore membrane formation initiates on the basis of a 3D EM tomographic reconstruction. Green cylinders indicate the position of spindle microtubules and the gray lines the location of the nuclear envelope. Dark blue structures are the MOP, while light blue indicates the central plaque of the SPB. Purple spheres are vesicles while bright pink shows prospore membranes beginning to form on the MOP surface. Bar, 100 nm. (B–E) (Upper) Electron micrographs of prospore membranes at different stages of growth. (Lower) Cartoons corresponding to the EM images. (B) Docking of vesicles to the MOP prior to fusion. Yellow arrows are within the nucleus and point to the position of the SPB. White arrow indicates precursor vesicles. Bar, 100 nm. (C) Initial fusion of vesicles creates a prospore membrane "cap" on the MOP. Labels are as in B. (D) Expansion of the prospore membrane, the lobe of the nucleus. White and yellow arrows are as in B. Orange arrow indicates an extension of nuclear envelope wrapping around a mitochondrion. Bar, 200 nm. (E) Just prior to closure, the prospore membrane has engulfed a divided nucleus. Yellow arrow is as in B. Red arrow indicates the site where the prospore membrane is closing. Bar, 400 nm. In the cartoons, structures are colored as in A. In addition, the red bars and orange rings in D and E indicate the positions of the septins and the leading edge complex, respectively, though these structures are not visible in the EM images. Stippling of the orange ring in E indicates that the leading edge complex is removed from the membrane prior to closure (see text).

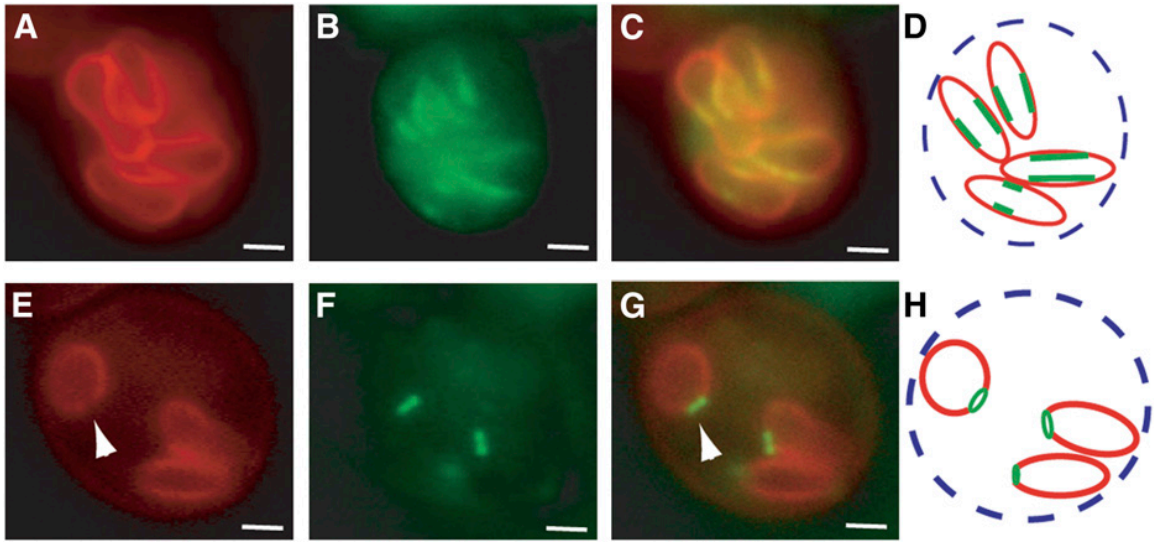

Figure 4 Prospore membrane associated cytoskeletal elements. (A) Prospore membranes are indicated by Spo20$^{51\text{-}91}$–RFP. (B) Septins are shown by Spr28–GFP. (C) Merge of the images in A and B. (D) Representation of the fluorescence image in C. Dashed line indicates the outline of the cell, red lines the prospore membranes, and green the position of the septins. (E) Prospore membranes are indicated by Spo20$^{51\text{-}91}$–RFP. (F) Leading edge complex is visualized by Don1–GFP. (G) Merge of images in D and E. (H) Representation of the fluorescence image in G. Dashed line indicates the outline of the cell, red lines the prospore membrane, and green the position of the leading edge complex. The arrowheads in E and G indicate the mouth of one prospore membrane. Bars, 1 μm.

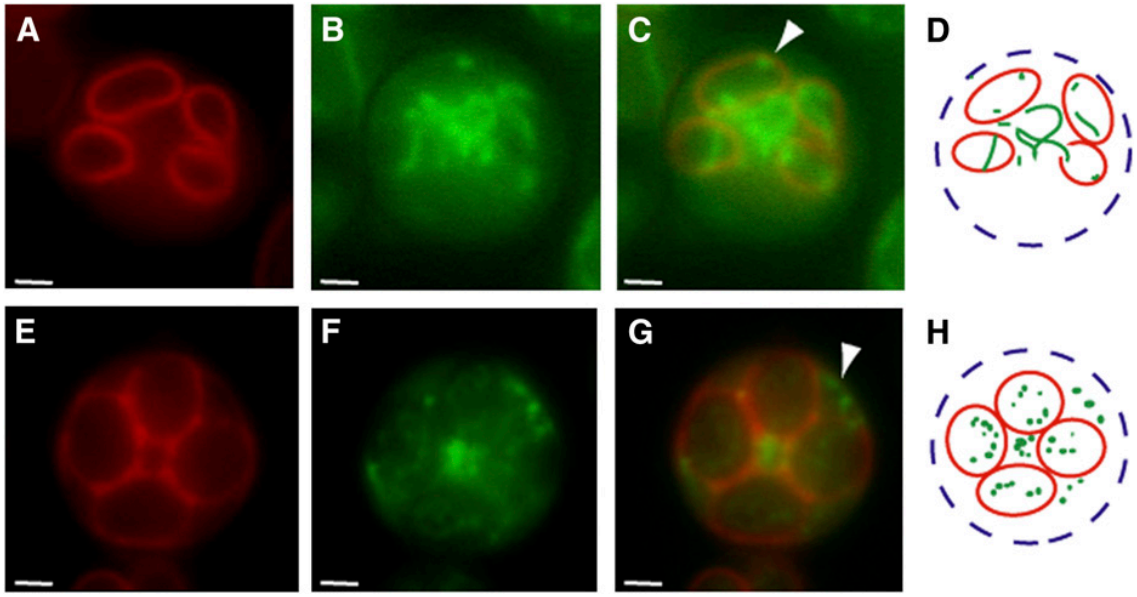

Figure 5 Segregation of mitochondria in the spore. (A) Spo20$^{51\text{-}91}$–RFP indicating the prospore membranes in a cell in meiosis II. (B) GFP-tagged *MRPS17*. (C) Merge of images in A and B. Arrowhead indicates mitochondrial material located within the prospore membrane. (D) Representation of the fluorescence image in C. Dashed line indicates the outline of the cell, red lines the prospore membrane, and green speckles the mitochondrial protein. (E) Spo20$^{51\text{-}91}$–RFP in mature spores. (F) Mrps17–GFP. (G) Merge of images in D and E. Arrowhead indicates mitochondria that have remained in the ascus. (H) Representation of the fluorescence image in G. Dashed line indicates the outline of the cell, red lines the prospore membrane, and green speckles the mitochondrial protein. Bars, 1 μm.

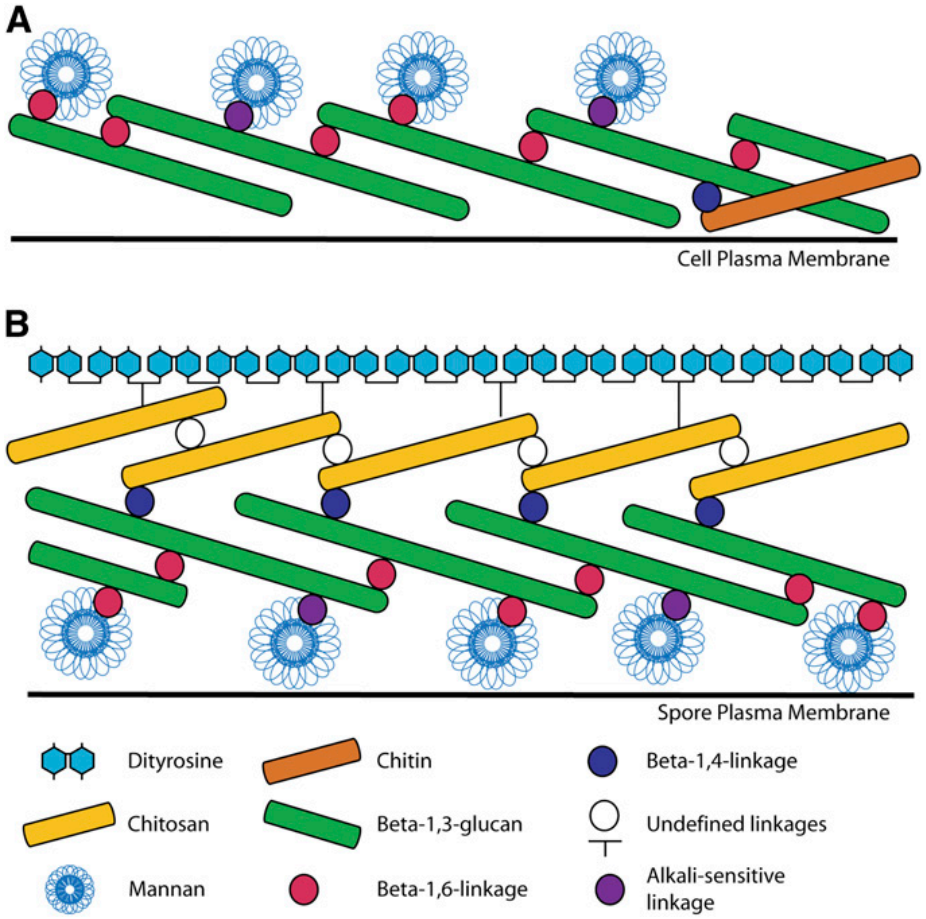

Figure 6 Model of spore wall organization. (A) Model for the vegetative cell wall showing the relationsip of three major components to the plasma membrane. (B) Model for the layered organization of the spore wall. The linkages between the mannan, β-1,3-glucan, and chitosan layers are based on work on the structure of the vegetative cell wall. The chemical linkages between chitosan chains, between dityrosine monomers, and linking the chitosan and dityrosine are unknown.

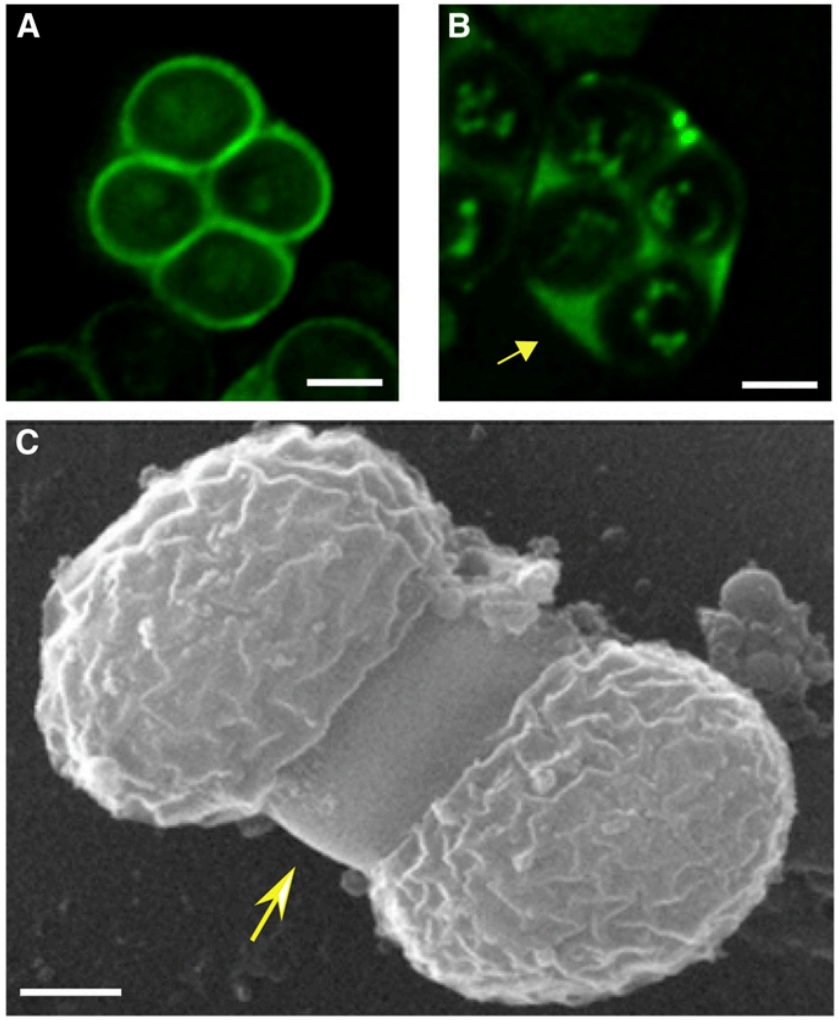

Figure 7 Features of the spore wall. (A) Localization of a secreted GFP molecule to the spore wall of wild-type spores. Bar, 2 μm. (B) Localization of the same secreted GFP in spores lacking a dityrosine layer. The arrow indicates localization of the GFP fusion to the ascal cytoplasm. Bar, 2 μm. (C) Scanning electron micrograph of a pair of spores. The arrow indicates the interspore bridge that links the two spores together. Bar, 1 μm.

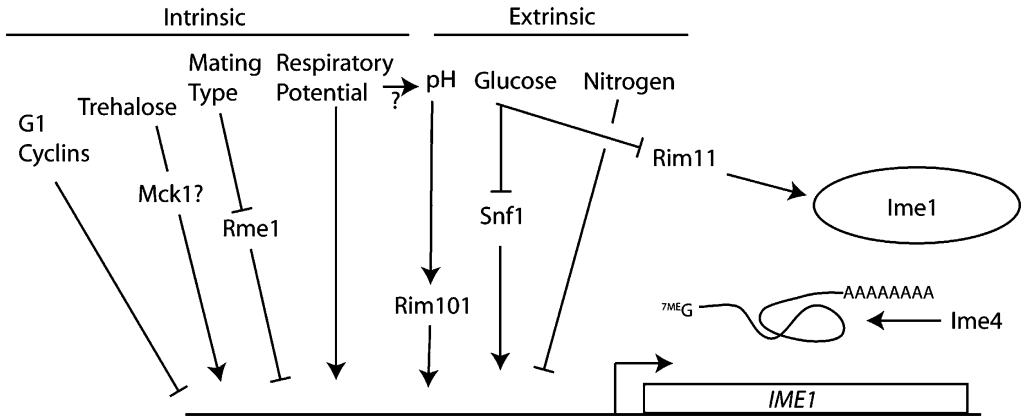

Figure 8 Factors controlling expression and activity of Ime1. Expression of *IME1* is the key event in triggering sporulation. A variety of intracellular and extracellular signals are integrated at the level of the *IME1* promoter to control gene expression and developmental choice. In addition, Ime1 activity is also controlled at the post-transcriptional and post-translational levels.

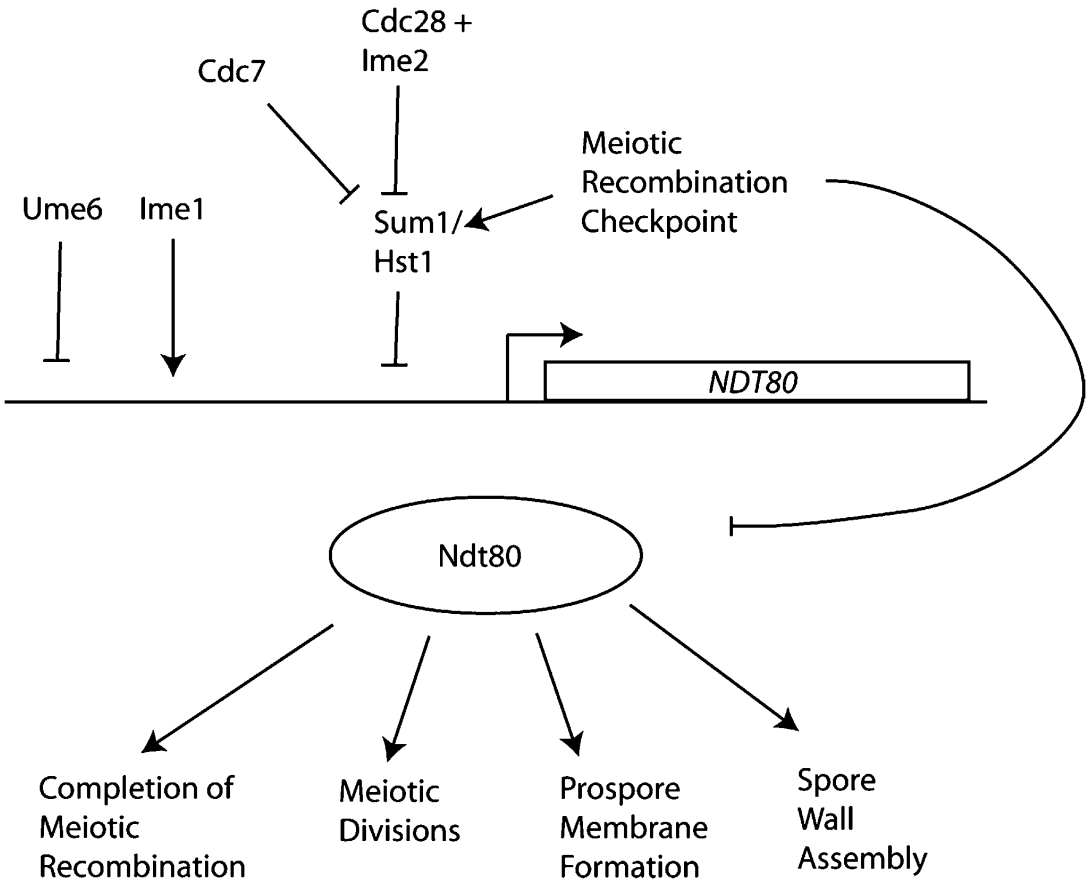

Figure 9 Inputs and outputs to Ndt80 activity. Ndt80 controls entry into the meiotic divisions. Expression is subject to nutritional, cell-cycle, and checkpoint control. Once active, Ndt80 induces multiple, independent downstream pathways.

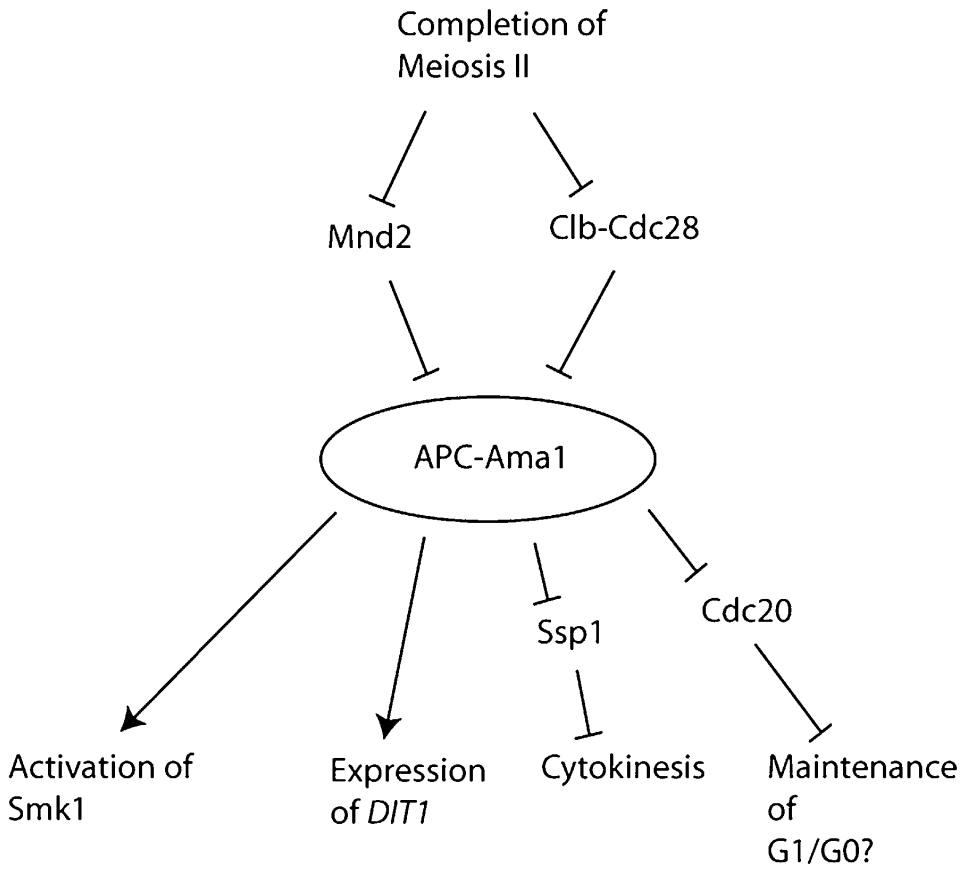

Figure 10 Coordination of meiotic exit with downstream events by APC–Ama1. The completion of meiosis leads to the upregulation of the APC–Ama1 ubiquitin ligase. This complex then triggers downstream events such as cytokinesis, spore wall assembly, and possibly entry into G1 by targeting specific substrates for degradation. Ssp1 and Cdc20 are established targets of APC–Ama1 but the substrates leading to Smk1 activation and *DIT1* expression have yet to be established.

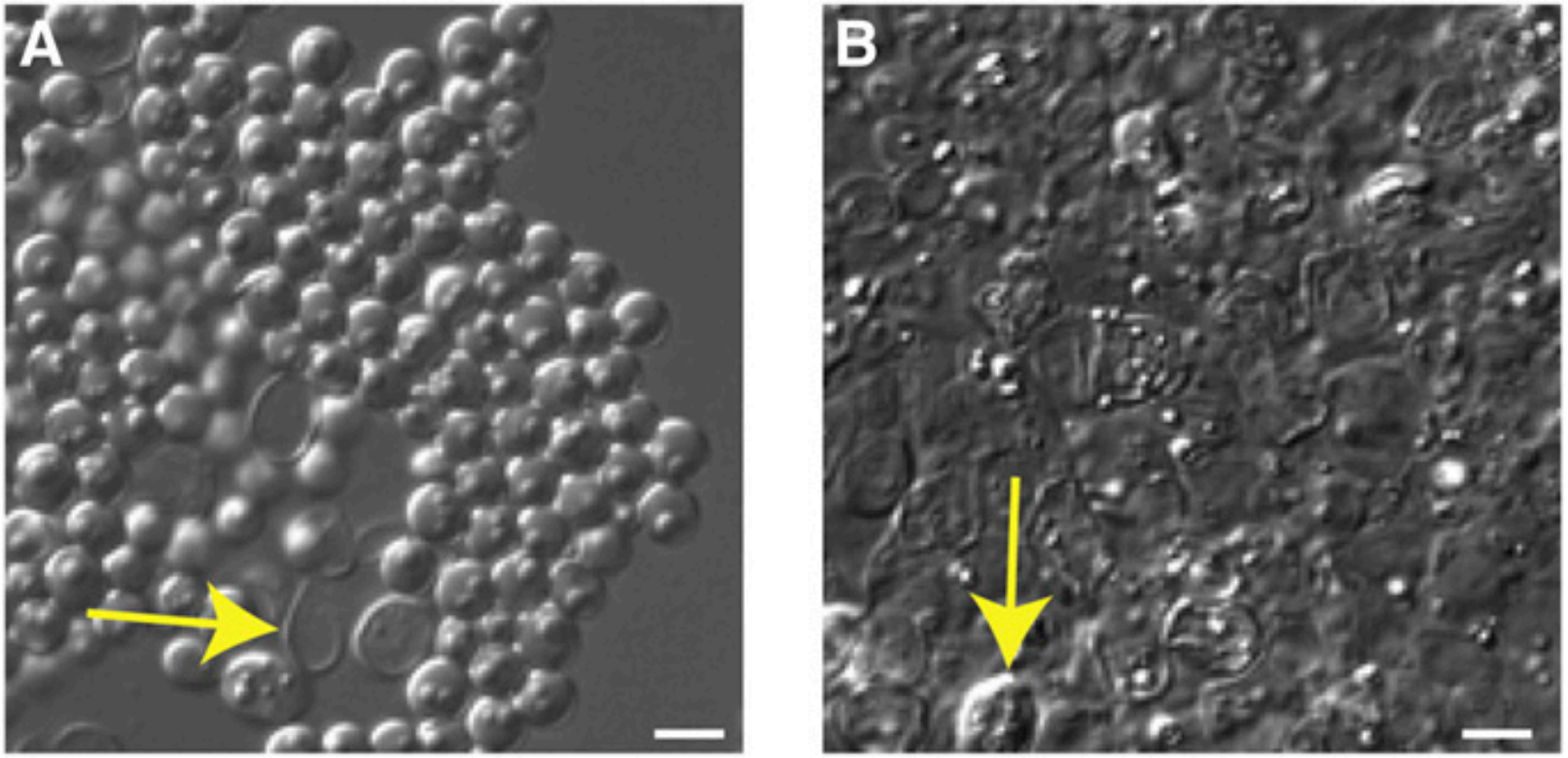

Figure 11 Spores survive passage through the insect gut. (A) Spores in the frass of *Drosophila melanogaster*. Arrow indicates a lysed vegetative cell among the spores. Bar, 4 μm. (B) Vegetative cells in the frass of *D. melanogaster*. Arrow indicates a rare intact vegetative cell among the lysed cells. Bar, 4 μm.

Acknowledgments

I thank Nancy Hollingsworth, Peter Pryciak, and members of the Neiman laboratory for comments on the manuscript and for helpful discussions. I am deeply grateful to Cindi Schwartz for her help with the tomography shown in Figure 3. I am indebted to Erin Mathieson, Susan Van Horn, and Alison Coluccio for the EM images used and to Jae-Sook Park, Hiroyuki Tachikawa, Nancy Hollingsworth, and Ed Winter for communicating results prior to publication. Work in the Neiman laboratory is supported by National Institutes of Health grants R01GM072540 and P01GM088297.