Coordinating cytokinesis and spindle disassembly during meiotic exit

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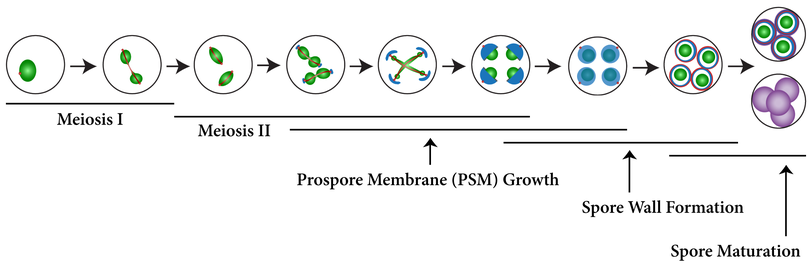
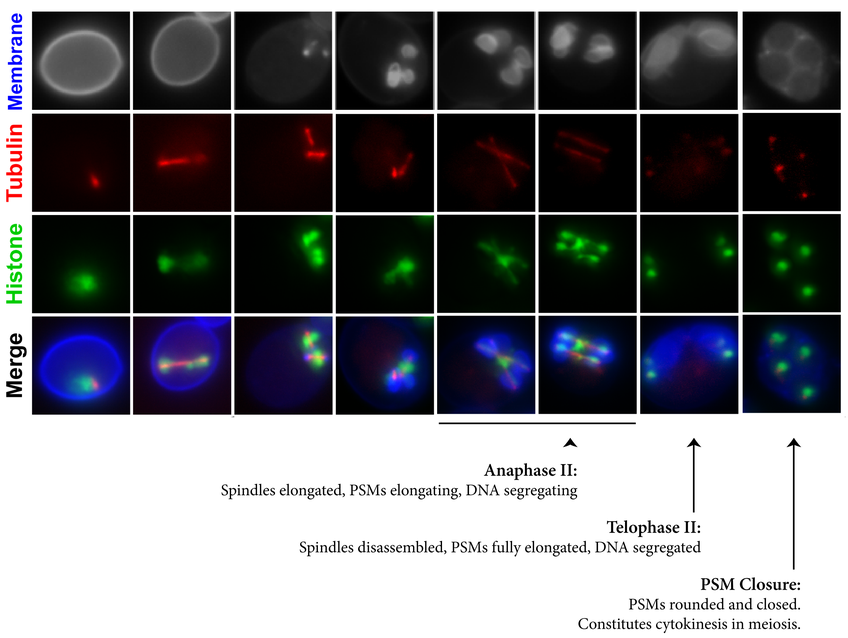
In the budding yeast Saccharomyces cerevisiae, meiosis is coupled with spore formation to create haploid gametes, a process called sporulation. Sporulation occurs under starvation conditions, and results in the remodeling of the mother cell to form four haploid spores within the ascus. Successful completion of this process requires the coordination of multiple events as cells exit meiosis to form four separate haploid gametes. These events include the timely disassembly of the spindle apparatus after meiosis II, the division of the nuclear envelope, and the closure of the prospore membranes. Previous work has shown that the sporulation specific STE20-family GCK III kinase Sps1 functions in parallel to the APC/C subunit Ama1 to promote timely prospore membrane closure, the meiotic cytokinetic event. Both sps1Δ and ama1Δ also exhibit meiosis II spindle disassembly defects. We would like to better understand how the events of meiotic exit are regulated and to better understand the relationship between spindle disassembly and cytokinesis. We have been conducting experiments to ask whether prospore membrane closure is dependent on spindle disassembly or vice versa. We have also examined the meiotic role of proteins involved in the process of spindle disassembly in mitosis. We find that the spindle midzone protein Ase1 persists on spindles after anaphase II in ama1 Δ mutants but appears to be removed normally in sps1Δ cells. This difference in Ase1 localization is consistent with our observations that sps1Δ and ama1Δ mutants exhibit distinct spindle disassembly defects, suggesting that SPS1 and AMA1 represent two mechanistically distinct pathways important for meiosis II spindle disassembly.

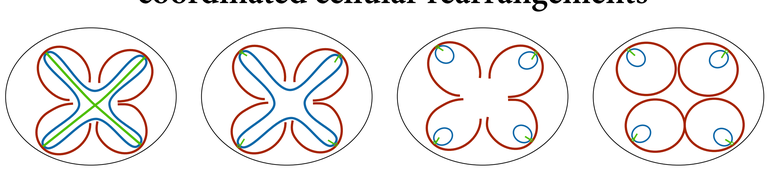
Yeast sporulation couples meiosis and spore packaging



Meiosis in sporulation

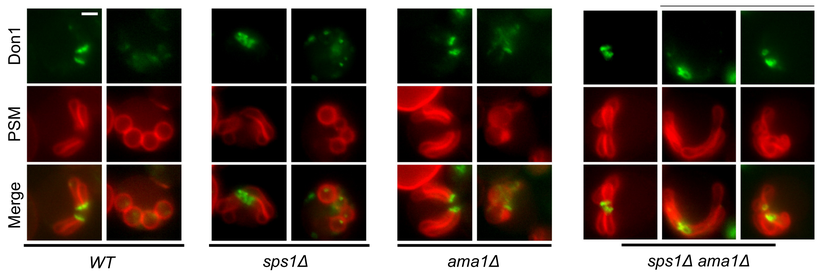


Exit from meiosis II requires multiple coordinated cellular rearrangements

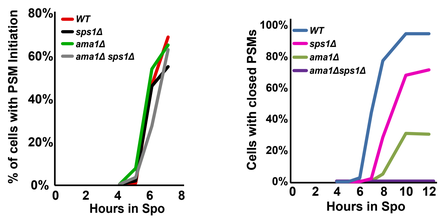


Plasma Membrane (Ascus)
Spindle / Spindle Pole Body: GFP^{Envy}-Tub1
Nuclear Envelope
Prospore Membrane: mKate2-Spo20$^{51-91}$

SPS1 and *AMA1* are in separate, parallel pathways to promote PSM closure

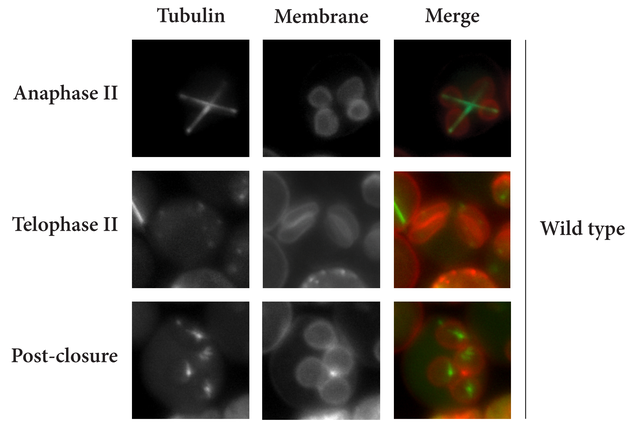


SPS1 and *AMA1* act in parallel to ensure removal of the Leading Edge Protein Complex, which includes Don1, to allow timely PSM closure.

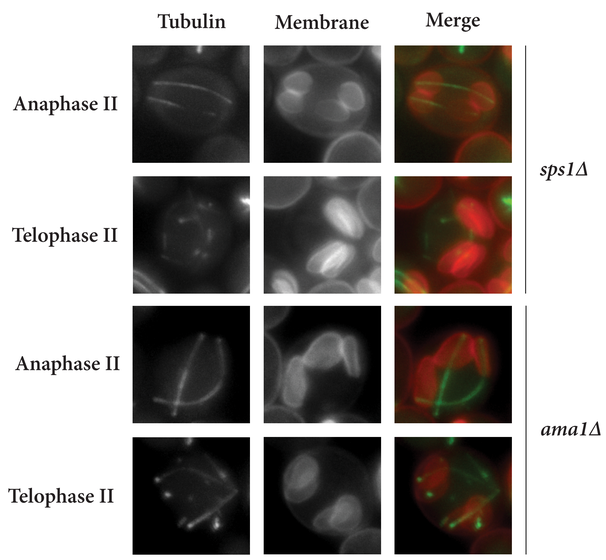


Modified from Paulissen, *et al.*, 2016

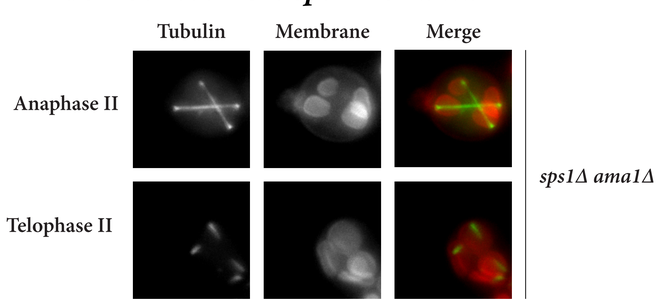
Meiotic spindles are completely disassembled prior to PSM closure



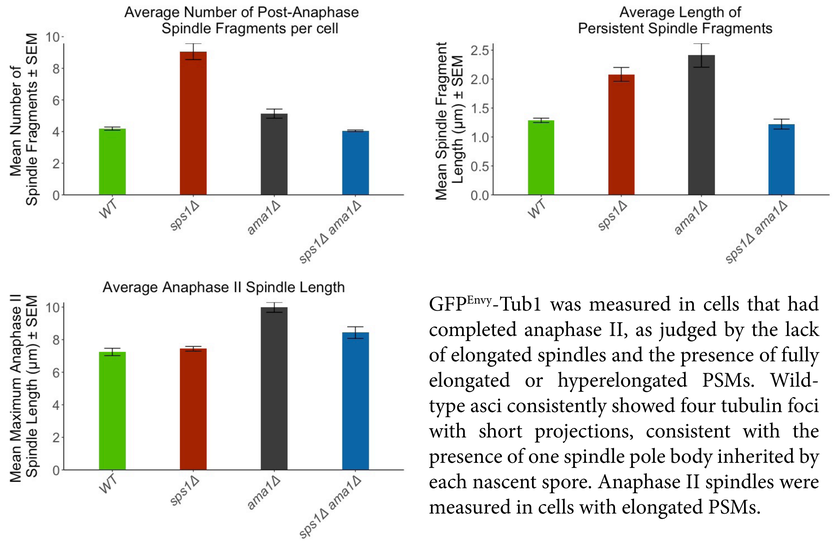
SPS1 and *AMA1* are required for proper spindle disassembly



sps1Δ ama1Δ double mutants do not resemble *sps1*Δ or *ama1*Δ



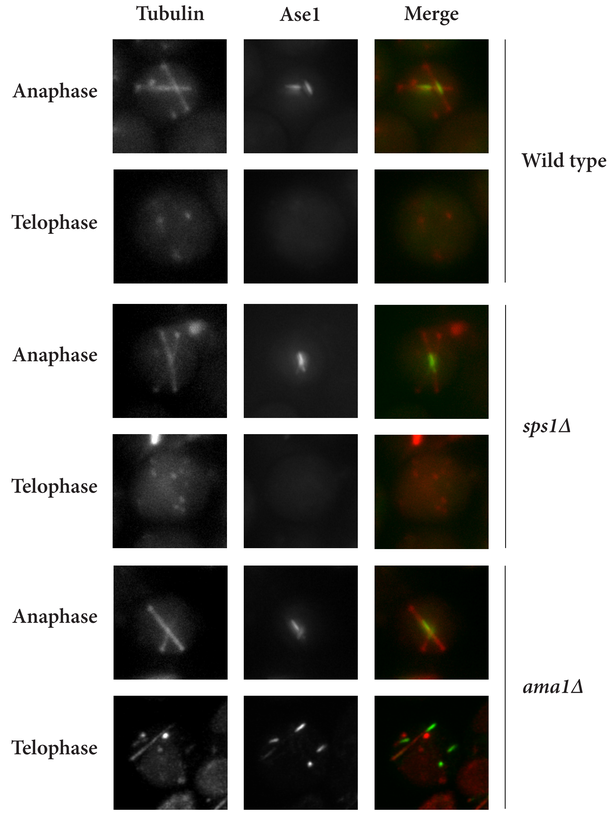
sps1Δ and *ama1Δ* exhibit distinguishable spindle disassembly defects



GFP^{Envy}-Tub1 was measured in cells that had completed anaphase II, as judged by the lack of elongated spindles and the presence of fully elongated or hyperelongated PSMs. Wild-type asci consistently showed four tubulin foci with short projections, consistent with the presence of one spindle pole body inherited by each nascent spore. Anaphase II spindles were measured in cells with elongated PSMs.

Ase1 is mislocalized after anaphase in *ama1*Δ mutants

- *ASE1* encodes a microtubule bundling protein, which binds antiparallel microtubules
- *ASE1* is required for mitotic spindle assembly and stability, as well as for establishment of the spindle midzone



Conclusions

- *sps1Δ* and *ama1Δ* have distinct spindle disassembly defects.
- Spindles in *sps1Δ* mutants "shatter" into more fragments than *ama1Δ* mutants.
- *ama1Δ* mutants exhibit anaphase II spindle hyperelongation prior to spindle breakage.
- Ama1-dependent regulation of the spindle midzone protein Ase1 may be required for proper spindle disassembly

Acknowledgements

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