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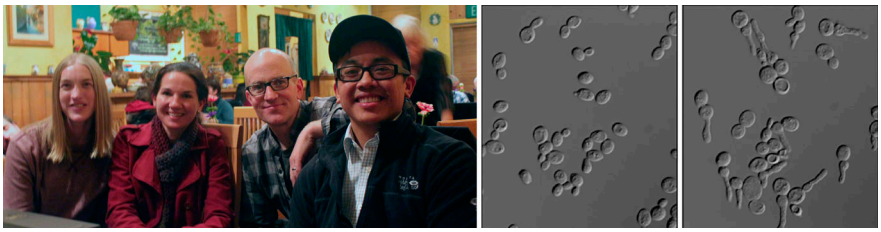
Big enough for two

Study identifies a signaling pathway that may control cell size by linking membrane transport to mitotic entry.

Focal Point



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(Left to right) Tracy MacDonough, Steph Anastasia, Doug Kellogg, Duy Nguyen, and colleagues (not pictured) uncover a signaling pathway that links membrane transport to mitotic entry in budding yeast. Cells arrest in early mitosis when signaling through the pathway is disrupted, either by blocking membrane traffic or by mutating pathway components like protein kinase C (right, compared with wild-type yeast, center). The researchers think that the pathway may allow cells to control their size by coordinating cell growth and division.

In order to remain the right size, proliferating cells must coordinate their growth and division. If cells divide before they've grown sufficiently, their daughters will be too small, whereas cells may grow too big if division is delayed. How cells balance growth and division is unclear, but Anastasia et al. identify a signaling pathway that might monitor transport of proteins and lipids to the plasma membrane and tell yeast cells when they've grown large enough to enter mitosis (1).

Cell growth requires the delivery of new material to the plasma membrane via the secretory pathway. "Membrane growth must occur at the right place and time during the cell cycle," says Doug Kellogg, from the University of California, Santa Cruz. "Yet nothing was known about how membrane trafficking and the cell cycle are linked."

Kellogg and colleagues, led by Steph Anastasia, looked for potential connections between the two processes by blocking membrane transport in budding yeast using a temperature-sensitive mutant of the vesicle docking protein Sec6 (1). "When we blocked membrane traffic, signaling to the core cell cycle regulators occurred within minutes," Kellogg recalls. "The rapidity of that response really caught our attention." Specifically, Anastasia et al. found that preventing secretion led to changes in the phosphorylation, and thus activity, of Swe1 and Mih1, the budding yeast homologues of Wee1 kinase and Cdc25 phosphastase, respectively, which regulate mitotic entry in all eukaryotes.

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To enter mitosis, Swe1 must be hyperphosphorylated and inactivated, whereas Mih1 is dephosphorylated and switched on. Disrupting membrane transport reversed these events and caused yeast to arrest in early mitosis.

Anastasia et al. then looked for proteins that might regulate Swe1 and Mih1 in response to changes in membrane trafficking. $PP2A^{Cdc55}$, a phosphatase that controls both proteins (2, 3), was a good candidate, and the researchers found that overexpressing Zds1, a regulator of $PP2A^{Cdc55}$, restored the normal phosphorylation pattern of Swe1 and Mih1 and allowed yeast to divide even when membrane transport was inhibited.

The authors then traced the signaling pathway upstream to protein kinase C (Pkc1), a protein that binds to Zds family members (4). Pkc1 promoted mitotic entry by inducing Mih1 dephosphorylation, a function that was lost in the absence of $PP2A^{Cdc55}$. This suggests that Pkc1 regulates $PP2A^{Cdc55}$, though how the kinase does this remains unknown.

Pkc1, in turn, was regulated by the GTPase Rho1, which could induce Mih1 dephosphorylation and mitotic entry as long as Pkc1 was functional. But how is this pathway, from Rho1 to mitotic entry, linked to membrane traffic? Inactive Rho1 is transported into the growing yeast bud on secretory vesicles and is then activated by a guanine nucleotide exchange factor on the plasma membrane (5).

"So we think that it's the delivery of Rho1 to the site of membrane growth, and its activation there, that signals that membrane growth is happening," explains Kellogg. According to this model, Rho1 activity and signaling to Swe1 and Mih1 would gradually increase during bud growth until a critical threshold was reached that could induce mitotic entry and cell division. Disruptions to membrane transport would shut off the pathway and prevent cells from dividing when they were too small.

"We've all been wondering how a cell knows how big it is," says Kellogg. "This model argues that a cell doesn't really measure its size—it measures the amount of growth that has occurred." Because all the components are conserved in higher eukaryotes, the pathway might operate in cells of all shapes and sizes.

This "growth-dependent signaling" model predicts that the strength of the signal is proportional to membrane growth. Kellogg and colleagues now want to test this prediction by developing a biosensor to monitor the pathway's activity during the cell cycle and under different growth conditions.

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