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Endocytosis puts exocytosis in pole position

Researchers find that endocytosis confines exocytosis to narrow zone during cell polarization.

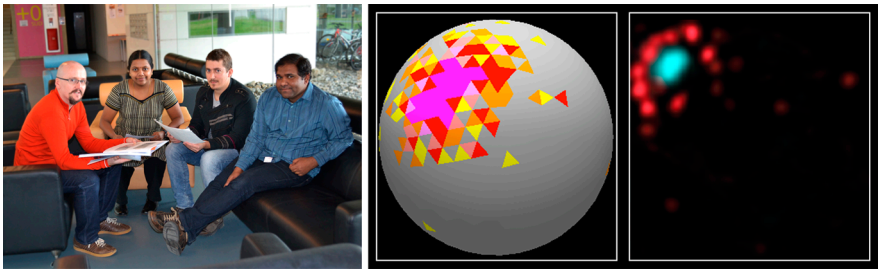
Exocytosis and endocytosis are the yin and yang of cellular transportation. By limiting where exocytosis can occur, endocytosis might help yeast cells polarize, Jose et al. reveal (1).

The pole of a budding yeast cell is where the bud sprouts when the organism reproduces asexually. Like other eukaryotes, yeast cells polarize with help from the Rho GTPase Cdc42, which amasses at the site of the future bud. Alhough researchers have probed the influence of endocytosis and exocytosis on polarity, their overall impact remains uncertain. Some studies suggest that the two processes cooperate to promote polarization, with exocytic vesicles delivering Cdc42 to the pole and endocytic vesicles recapturing any errant Cdc42 molecules that diffuse away (2, 3). Other studies indicate that endocytosis has the opposite effect, potentially draining Cdc42 from the membrane (4, 5).

Jose et al. crafted a mathematical model to investigate the consequences of these processes for yeast cell polarity. The researchers assumed that endocytic and exocytic vesicles carry Cdc42, which fosters polarization in two ways. The molecule spurs formation of actin cables along which exocytic vesicles speed to the future pole. Cdc42 also draws in the exocyst complex, promoting fusion of exocytic vesicles with the membrane. In Jose et al.'s model, exocytosis and endocytosis are locked into a feedback loop; exocytic vesicles deliver clathrin and other proteins that in turn help instigate endocytosis.

To start, the researchers used a slightly simpler version of the model in which endocytosis happened in a single step. The cells polarized, with Cdc42 focusing at one spot on the cell cortex. However, the team discovered that this outcome required separation between the zones where exocytosis and endocytosis occurred. If both activities proceeded within the Cdc42-containing region, polarization faltered.

Focal Point



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Polarity explorers (left to right) Derek McCusker, Mini Jose, Sylvain Tollis, Deepak Nair, and (not pictured) Jean-Baptiste Sibarita combined mathematical modeling with cell imaging to determine how endocytosis and exocytosis interact to establish cell polarity. The left image illustrates a result from the group's mathematical model in which the polarity-spurring protein Cdc42, penned in by endocytosis, concentrates at the cell cortex. The image of a live yeast cell on the right reveals a ring of endocytic vesicles (red) surrounding the exocytic zone (blue).

> "These activities can maintain Cdc42 in a polarized pattern on the membrane."

Jose et al. then turned to a more elaborate model that breaks down endocytosis into multiple steps, such as invagination of the membrane and vesicle release. These virtual cells also polarized, with Cdc42 clustering on the cortex. The exocytic region in the cells shrank to a small spot at the cell pole surrounded by a zone where endocytosis occurred. Using near-total internal reflection fluorescence microscopy, the researchers observed the same bull's eye arrangement in actual yeast cells. They also noticed that, as a cell polarizes, the timing of endocytosis changes so that vesicles depart on a more regular schedule.

The group's observations and simulation results suggest that endocytosis promotes polarization by "corralling" the exocytic zone, curbing the spread of polarity-inducing proteins such as Cdc42. "The striking dynamics [of endocytosis and exocytosis] were a real surprise," says senior author Derek McCusker.

The team assessed the corralling idea by tweaking their model so that endocytosis occurred all around the cell membrane. Without the endocytic barrier, all exocytic clusters in the cell broke down. Opening the corral also disrupts polarization, the researchers found when they studied cultured yeast cells with the *sla2*Δ mutation, which causes endocytosis to spread over the cell cortex. The team also observed yeast cells with several other mutations that slow endocytosis. The exocytic area enlarged in these cells, supporting the importance of corralling.

In this case, the competition between endocytosis and exocytosis appears to work to a cell's benefit. "We found that these activities can maintain Cdc42 in a polarized pattern on the membrane," says McCusker. One question that remains unanswered is whether a similar spatial relationship between endocytic and exocytic regions holds true in higher eukaryotes for cellular activities that require polarization, such as migration and wound healing. Another mystery involves the transportation of Cdc42. The model assumes that exocytic and endocytic vesicles ferry Cdc42, something researchers suspect is true but haven't confirmed because of the difficulty of tracking small, speedy vesicles. Super-resolution microscopy might soon allow scientists to verify this idea, McCusker says.

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