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Spare the Rod1, spoil transporter endocytosis

Study reveals how yeast shuffle transporter molecules in response to glucose.

Focal Point

PHOTOS COURTESY OF (LEFT TO RIGHT): SÉBASTIEN LÉON; NEIDE VIEIRA; SANDRA PAIVA; OLIVIER VINCENT; IMAGES COURTESY OF SÉBASTIEN LÉON

(Left to right) Michel Becuwe, Rosine Haguenauer-Tsapis, Sébastien Léon, Neide Vieira, Sandra Paiva, Olivier Vincent, and colleagues (not pictured) explored how the adaptor protein Rod1 helps cells endocytose transporter molecules. The yeast cells on the left have a working version of Rod1 and can remove Jen1 (green) from the plasma membrane (magenta) and direct it to the vacuole. The cells on the right carry a non-functional version of Rod1 that cannot be ubiquitylated and cannot endocytose Jen1.

When the waitress brings you soup in a restaurant, you put down your fork and pick up a spoon. Cells do something similar when they change their diet, switching the nutrient-importing proteins in their plasma membrane. Becuwe et al. show how the post-translational modification of a key adaptor protein enables cells to remove unneeded transporters (1).

Transporter proteins embedded in the plasma membrane import nutrients such as glucose and amino acids. Cells tend to match their transporters to food availability. For example, when yeast cells are growing in a medium that contains lactate, they sport the Jen1 transporter that helps absorb that molecule. But in the presence of glucose, which provides more energy, the yeast endocytose Jen1 (2) and replace it with a glucose transporter. The trigger for removal is ubiquitylation of the transporters by the enzyme Rsp5, but it's unclear how this enzyme targets the right transporter at the right time. Rsp5's interactions might hold the key. The ubiquitin ligase can latch onto molecules that carry a so-called PY motif, a group that includes the arrestin-related proteins, which researchers think are involved in transporter ubiquitylation (3, 4).

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Becuwe et al. investigated further, focusing on the arrestin-related protein Rod1. They found that Rod1 was necessary for yeast cells to endocytose Jen1 in the presence of glucose. If Rod1 was absent, cells didn't ubiquitylate Jen1 molecules, suggesting that the protein helps Rsp5 target the transporter.

To determine how cells connect Rod1 activity to glucose availability, the researchers checked Rod1's phosphorylation status, because previous work had shown that it can be phosphorylated. When yeast subsist on lactate, Rod1 is phosphorylated, the team found. But when the cells began feasting on glucose, Rod1 was rapidly dephosphorylated, which, in turn, allowed it to be ubiquitylated by Rsp5. Without this latter modification, Rod1 is non-functional, Becuwe et al. discovered. Cells expressing a version of Rod1 that can't be ubiquitylated didn't remove Jen1 in response to glucose.

The researchers filled in two other steps in the molecular circuit that controls Rod1. Previous studies have shown that the Snf1 kinase, which is part of a signaling pathway that senses food availability, phosphorylates Rod1. In the presence of glucose, PP1, a phosphate-removing enzyme, shuts down Snf1. Becuwe et al. showed that PP1 also strips phosphates from Rod1, permitting its ubiquitylation. Secondly, the team found that, when yeast are growing on lactate, phosphorylated Rod1 cozies up to one of the 14-3-3 proteins, which prevents Rod1's ubiquitylation. But when yeast switch to glucose and Rod1 sheds its phosphate groups, the 14-3-3 protein is displaced, allowing Rsp5 to ubiquitylate Rod1.

"We identified a molecule that serves as a relay between signaling and endocytosis," says senior author Sébastien Léon. Rod1 enables cells to "sense glucose availability and translate it to the endocytosis machinery." Several questions remain to be answered, however, Léon adds. For example, researchers don't understand how ubiquitylation turns on Rod1 and how the activated protein helps Rsp5 trigger endocytosis of transporters. They are also uncertain about where Rod1 does its job. Although it likely acts at the plasma membrane, it might also work within the cytoplasm, helping to ubiquitylate transporters that have already been endocytosed.

Down the road, the results could open up new avenues for cancer treatment. To fuel their metabolism, tumor cells often boost the number of glucose-absorbing GLUT transporters in their plasma membrane. Reducing expression of these transporters makes cancer cells more vulnerable to chemotherapy in vitro. Humans also have arrestin-related proteins and a version of Rsp5, which suggests that this pathway could also furnish drug targets that might alter transporter endocytosis and starve cancer cells.

1. Becuwe, M., et al. 2012. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201109113.
2. Paiva, S., et al. 2002. *Biochem. J.* 363:737–744.
3. Lin, C.H., et al. 2008. *Cell.* 135:714–725.
4. Nikko, E., and H.R. Pelham. 2009. *Traffic.* 10:1856–1867.