

## Cells use import machinery to export their goods as well

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(PhysOrg.com) -- In the bustling economy of the cell, little bubbles called vesicles serve as container ships, ferrying cargo to and from the port — the cell membrane. Some of these vesicles, called post-Golgi vesicles, export cargo made by the cell's protein factory.

Scientists have long believed that other, similar vesicles handle the reverse function, importing life-supporting nutrients and proteins through an independent process. By using a finely honed type of microscopy to more precisely examine these transactions, new research shows that the processes are not as independent as assumed: Certain molecules handle cargo moving in both directions. Like stevedores, they're involved in both loading and unloading the cell's container ships.

Jyoti Jaiswal, a research assistant professor, and Sanford M. Simon, head of the Laboratory of Cellular Biophysics at Rockefeller University, examined the most common form of cellular export process called constitutive exocytosis, a continual ferrying of goods involved in the regular life and maintenance of all eukaryotic cells. This sort of shipping was assumed to end with the vesicles fusing completely to the membrane and delivering their whole load of proteins and lipids, in contrast to the more discriminating process by which similar container ships import proteins from outside the cell — called endocytosis. But in a paper published today in Cell, Jaiswal and Simon show that some of the key molecules regulating endocytosis, such as clathrin, dynamin and actin, are also at work in exocytosis.



"In retrospect, it makes perfect sense," Jaiswal says. "But at first we thought we had to be wrong because they had been defined as endocytic molecules." Adds Simon, "Once we took a step back from the dogma, we saw that cells employ these molecules for import and export. Then everything fell into place. We should stop stereotyping molecules as dedicated for this or that purpose. It puts on the blinders. There's an advantage in biology of sometimes just looking without a hypothesis."

The researchers used a special form of microscopy — total internal reflection fluorescence microscopy — capable of focusing solely on the narrow plane in which the vesicle and membrane merge. "It's a little like the guy looking under the streetlight for his keys because it's the only place he can see, but we've actually arranged for the streetlight to be focused on exactly where we're interested," Simon says. "We get the vesicles at the point of fusion without the background noise of everything else going on inside the cell."

For the first time, Jaiswal was able to observe individual post-Golgi vesicles as they deliver cargo in their lumen and cargo carried in the membrane shell that surrounds the vesicle. This ability revealed behaviors that were not expected. The researchers showed first that the most common delivery of post-Golgi cargo is a so-called kiss-and-run exchange in which the vesicle partially merges with the membrane and delivers some, but not all, of its contents. Some vesicles — those packing neurotransmitters, for instance — are mobilized by a flood of calcium to spill their haul, signaling nearby cells. By adjusting the levels of calcium inside the cell, the researchers definitively found that calcium did not affect constitutive exocytosis of post-Golgi vesicles. Then they successively inhibited three molecules known for their membranebending role in endocytosis — clathrin, dynamin and actin. In the absence of any one of these molecules, the researchers found that the vesicles merged fully with the membrane and disgorged all their cargo, which they had shown was an aberration in exocytosis, not the rule, as



had been previously assumed. Together, the experiments demonstrate that cells employ some of the same molecules for importing and exporting cargo. "They use the same machinery for both," Jaiswal says. "This blurs the line between endocytosis and exocytosis. Perhaps we should just call it membrane trafficking."

The researchers do not yet know the function of this regulation of exocytosis. They are investigating other molecules' roles in the process to further understand the role and regulation of this process.

More information: *Cell* 137(7): 1308—1319 (June 26, 2009) Exocytosis of Post-Golgi Vesicles Is Regulated by Components of the Endocytic Machinery; Jyoti K. Jaiswal, Victor M. Rivera and Sanford M. Simon

Provided by Rockefeller University (<u>news</u>: <u>web</u>)

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