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Sortilins get a new delivery route

Lysosomal sorting receptors transport cargo to secretory granules in *Tetrahymena*.

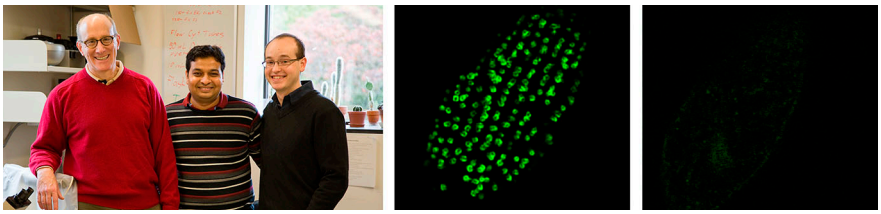
Secretory granules—cargo-laden vesicles that quickly release their contents in response to extracellular stimuli—are a widespread feature of eukaryotic cells, from the neuropeptide-containing dense-core vesicles of animal neurons to the various granules that ciliate protozoans secrete in order to capture food or defend themselves against predators. Despite their prevalence, however, relatively little is known about the biogenesis of secretory granules. Briguglio et al. reveal that a family of receptors that deliver enzymes to lysosomes are also required to sort proteins to the mucocyst granules of *Tetrahymena* (1).

Proteins are often delivered to the correct cellular compartment by sorting receptors that bind and package them into transport vesicles. Many secretory granule proteins rely on an entirely different sorting mechanism. Immature precursors condense into progressively larger aggregates as they pass through the secretory pathway until they are incorporated into granules and proteolytically processed into their mature forms, ready for exocytosis (2). But secretory granules also contain soluble, nonaggregated cargo; how these proteins are sorted is largely unknown.

In 2005, Aaron Turkewitz's lab at The University of Chicago identified a family of proteins that localize to mucocysts, the secretory granules of the ciliate *Tetrahymena thermophila* (3). Unlike other mucocyst components, these proteins didn't aggregate on their way to the granules, so, says Turkewitz, "we thought there must be some other mechanism for sorting them."

Turkewitz and his colleagues Joe Briguglio and Santosh Kumar used expression profiling to look for new components of the mucocyst sorting machinery (1). "Genes acting in a common pathway have to be coordinated with one another," Turkewitz explains. "If you know one gene in the pathway, you can ask what other genes are coregulated with it at the transcriptional level."

Focal Point



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(Left to right) Aaron Turkewitz, Santosh Kumar, and Joe Briguglio investigate how different classes of cargo proteins are sorted into mucocysts, secretory granules found in the ciliate protozoan *Tetrahymena thermophila*. The researchers find that *SOR2* and *SOR4*, two members of the sortilin family of lysosomal sorting receptors, target soluble, nonaggregating cargoes to the granules. Grt1p (green), for example, localizes to mucocysts docked at the surface of wild-type *Tetrahymena* (center) but is missorted and constitutively secreted from *SOR4*-deficient cells (right). The self-aggregating cargo Grl3p, on the other hand, is still targeted to mucocysts in the absence of *SOR4* but isn't processed into its mature, secretable form due to mislocalization of the protease cathepsin 3.

> "One class of mucocyst proteins relies on these receptors, but the other class doesn't."

Briguglio et al. searched a database of *Tetrahymena* transcriptional profiles for genes whose expression under different conditions changed in line with the expression of mucocyst cargo. This approach identified the sortilin/SOR family of transmembrane cargo receptors as potential regulators of granule biogenesis. Sure enough, deleting *SOR2* or *SOR4* inhibited the secretion of mucocyst components.

Sortilins deliver hydrolase enzymes to lysosomes in many eukaryotic species, but they have only been tentatively linked to secretory granule sorting (4). Briguglio et al. found that Sor4p bound to the soluble, nonaggregating mucocyst protein Grt1p and that this protein wasn't sorted to mucocysts in *SOR4*-deficient *Tetrahymena*. Another soluble mucocyst cargo, Igr1p, was also missorted in the absence of *SOR4*, but a classic, self-aggregating granule protein, Grl3p, was still correctly targeted. "So one class of mucocyst proteins relies on these receptors, but the other class doesn't," Turkewitz says.

However, although Grl3p was delivered to mucocysts in the absence of *SOR4*, it wasn't processed to its mature, secretion-ready form because Sor4p was required to sort the protease cathepsin 3, which cleaves Grl3p precursors, to the granules. "Having two separate pathways is a beautiful mechanism that gives *Tetrahymena* more control over mucocyst biogenesis," Turkewitz explains.

It remains to be seen if other eukaryotes also use this approach to form secretory granules. Budding yeast and humans are close relatives in comparison to evolutionarily distant ciliates like *Tetrahymena*, but several lines of evidence suggest that animal cells may also "borrow" proteins from the lysosomal sorting pathway to deliver cargo to secretory granules. "Lysosomes and secretory granules are two main classes of post-Golgi compartments," says Turkewitz. "Our results suggest that they may not be so separate from each other. The key now is to figure out how much of the lysosomal machinery is used to make secretory granules."

With this in mind, Turkewitz and colleagues now plan to investigate the function of other hits from their expression profiling screen, and, if the lysosomal and secretory granule pathways do turn out to be intimately linked, to determine where along the secretory pathway these different trafficking routes diverge.

1. Briguglio, J.S., et al. 2013. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201305086.
2. Arvan, P., et al. 2002. *Curr. Opin. Cell Biol.* 14:448–453.
3. Bowman, G.R., et al. 2005. *J. Eukaryot. Microbiol.* 52:291–297.
4. Chen, Z.Y., et al. 2005. *J. Neurosci.* 25:6156–6166.