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Rab2 directs a stop-loss program

Small GTPase supports dense core vesicle maturation in worm neurons.

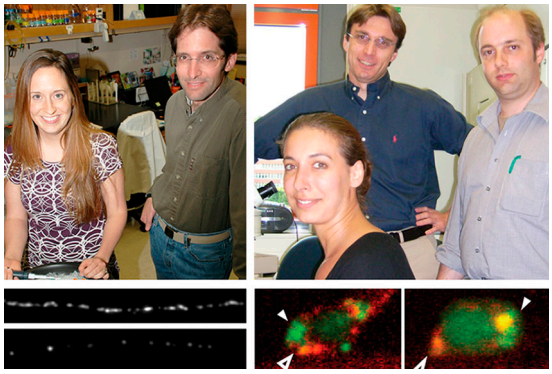
As they travel through the cell body to neuronal axons, dense core vesicles (DCVs) shed unwanted cargo and mature into compact packages of neuropeptides and other factors that modulate signaling across the synapse. Two groups reveal that the small GTPase Rab2 ensures that DCVs don't lose anything important on their journey to the nerve terminal (1, 2).

Regulated secretion of DCVs and small synaptic vesicles offers unique challenges to neurons, says Kenneth Miller from the Oklahoma Medical Research Foundation in Oklahoma City. Membranes must be transported across long distances, and the pathways must interact with the regular trafficking processes found in every eukaryotic cell. Meeting these challenges is crucial for neuronal function—*C. elegans* unable to secrete DCVs are severely paralyzed, for example (3).

Miller's group identified Rab2 (UNC-108 in worms) as a regulator of DCVs in a genetic screen (1). *unc-108* mutants suppressed a hyperactive phenotype in a similar manner to other mutants defective in DCV secretion. At the same time, Stefan Eimer's team at the European Neuroscience Institute in Goettingen, Germany, began to investigate *unc-108* mutants because of their dramatic inhibition of worm movements (2). "We're interested in neuronal membrane trafficking. Rabs control most trafficking steps and Rab2 has a very strong phenotype," says Eimer.

Both groups determined that *unc-108* mutants compromised DCV function but—to their surprise—neither neuropeptide processing nor secretion was affected. And the number and distribution of DCVs appeared normal in electron micrographs. Nevertheless, when both sets of researchers tagged neuropeptides with a fluorescent protein, they saw decreased fluorescence in the axons of mutant worms. "We were startled to see that two thirds of the fluorescence was gone," says Miller. "We initially thought the neurons must be losing neuropeptides as well, but our genetic analyses and neuropeptide immunostaining said differently."

Focal Point



Two groups of researchers find that the small GTPase Rab2 promotes the function of neuronal dense core vesicles in *C. elegans* locomotion by preventing the removal of soluble and transmembrane cargo as the vesicles mature. Left: Stacey Edwards, Kenneth Miller, and colleagues discovered that cargo was lost from dense core vesicles in Rab2 mutant axons (compare bottom to top panels). Right: Instead, as Marija Sumakovic, Stefan Eimer, Jan Hegermann, and co-workers found, the cargo (green) shifts to the endo-lysosomal system (red) in the neuron's cell bodies (compare left to right).

After being packaged into immature DCVs at the trans-Golgi network, neuropeptide precursors are cleaved by proteases. The neuropeptides then aggregate in the center of the maturing vesicle. Cargo not destined for exocytosis at the synapse is removed by a clathrin-dependent transport step to the endolysosomal system for degradation or constitutive secretion. The two groups both realized that this removal pathway was overly active in Rab2 mutants, extracting cargo that would usually be retained in wild-type DCVs—including the fluorescent label liberated from neuropeptides during proteolytic processing. Blocking traffic to endosomes with either a Rab5 mutant (2) or an inhibitor of the phospholipid PI(3)P (1) prevented the loss of this soluble cargo from the DCVs of Rab2 mutants. Miller's group demonstrated that a DCV transmembrane protein was also lost from Rab2 mutant neurons.

> "The hunt is on to find what this factor is."

The neuropeptides themselves are protected from inappropriate removal by their aggregation, so mutations in Rab2 must cause the loss of some other factors essential for DCV function and normal worm movements. "There must be something else in DCVs, apart from neuropeptides, that does the job," says Eimer. "Now the hunt is on to find what this factor is." Both Miller and Eimer speculate that this key additional cargo of DCVs could be a neurotrophin-like protein or a G protein-coupled receptor.

The two researchers also agree that there are many potential mechanisms by which Rab2 could prevent this crucial passenger from mistakenly transferring to the endosomal system. Rab2 has long been associated with transport events at the Golgi apparatus, but recent papers have connected the GTPase to endosome and phagosome trafficking (4–6). One of several possibilities, Miller notes, is that Rab2 limits either the lifetime or mass of early endosomes, preventing them from grabbing too much of a maturing DCV's contents. Eimer's group, on the other hand, found that the Rab2 effector RIC-19 is also involved in DCV biogenesis. RIC-19 contains a BAR domain, which helps curve membranes and could thereby drive a sorting process in which specific cargo is retained in a maturing DCV while unwanted content is removed. "Now that we've made this first step, we can really get into the molecular details of the maturation process," says Eimer.

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