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Exocytosis: The Many Masters of the Exocyst

Dispatch

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The exocyst is a conserved eight-subunit complex involved in the docking of exocytic vesicles. The exocyst has now been identified as an effector for five small GTPases, including Sec4, Rho1, Rho3, Cdc42 and, most recently, RalA.

It is increasingly clear that complex processes inside cells are carried out by multisubunit molecular machines. This is true of exocytosis in eukaryotic cells, where the docking of exocytic vesicles with the plasma membrane has been shown to involve a protein complex colorfully known as the 'exocyst'. The exocyst complex comprises eight proteins: Sec3p, Sec5p, Sec6p, Sec8p, Sec10p, Sec15p, Exo70p and Exo84p. It was originally identified in the budding yeast *Saccharomyces cerevisiae*, where the exocyst has been shown to be essential for exocytosis. The six 'Sec' proteins, so named because mutations in their genes inhibit secretion, were first discovered by Novick, Field and Schekman more than two decades ago in their classic genetic screen. In the early 1990s, these proteins were shown to interact physically with each other. Terbush, Novick and colleagues [1] purified the exocyst protein complex containing the six Sec proteins and two additional subunits Exo70p and Exo84p.

All of the exocyst components are hydrophilic proteins which interact with each other to form a 19.5S complex peripherally associated with the plasma membrane [2]. Mammalian homologues have been identified of all eight yeast exocyst proteins [3]. In yeast, mutant cells deficient in individual exocyst members accumulate secretory vesicles, presumably because the vesicles are not able to dock or fuse with the plasma membrane. The exocyst proteins localize to regions of active cell surface expansion: the bud tip at the beginning of the cell cycle, and the mother–daughter cell connection during cytokinesis.

The exocyst is therefore thought to be involved in directing vesicles to their precise sites of fusion [1,4,5]. Although it is clear that the exocyst plays a central role in exocytosis, little is known about how it is controlled. Given the complexity of the exocyst, one might imagine that it integrates many different inputs. Indeed, recent data have shown that many small GTPases regulate the exocyst, including members of the Rab [5], Rho [6–9] and Ral [10–13] families.

The first GTPase found to interact with the exocyst was Sec4, the founding member of the Rab family

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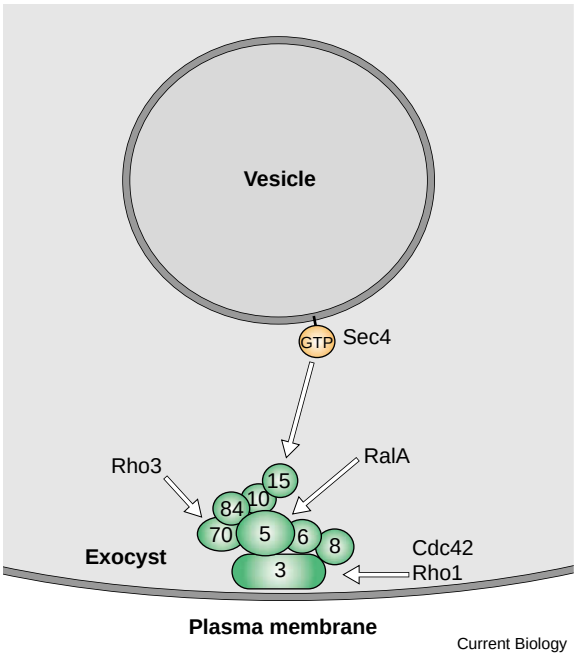

Figure 1. The eight subunits of the exocyst complex and their interactions.

The five known GTPase–exocyst associations, indicated by arrows, which are discussed in the text. (Adapted from [5].)

(Figure 1) [5]. Rabs are important regulators of all vesicular traffic events and Sec4 is essential for a post-Golgi event in yeast secretion. Genetic analysis suggested that Sec4 acts upstream of the exocyst in yeast. The exocyst component Sec15 was found to associate specifically with secretory vesicles and to interact with Sec4-GTP, which is present on the surface of the vesicular membrane. This Sec4-GTP–Sec15 interaction seems to trigger further interactions between Sec15 and other exocyst components, eventually leading to docking and fusion of secretory vesicles with specific domains of the plasma membrane.

As mentioned, the exocyst localizes specifically to regions of active secretion and cell growth, and it was important to learn how this is controlled. The second class of GTPases found to interact with the exocyst consists of members of the Rho family. In a search for mutations affecting localization of green fluorescent protein-tagged subunits of the exocyst in budding yeast, Guo *et al*. [6] identified several *rho1* mutant alleles. The effect of Rho1 on the exocyst was found not to involve a change in the organization of the actin cytoskeleton, the best-known function of Rho GTPases. Rather, Rho1-GTP interacts directly with exocyst component Sec3, which has been proposed to be a 'landmark' for defining polarized domains of the plasma membrane [4]. Another Rho family protein, Cdc42, also interacts with Sec3, and this interaction is required for the initial targeting of Sec3 to the emerging yeast bud.

Rho1 and Cdc42 both interact with the amino terminus of Sec3 and they were found to compete for Sec3 binding *in vitro*. *In vivo*, Cdc42 and Rho1 may interact with Sec3 at different stages of the yeast cell cycle [7].

While Rho1 and Cdc42 interact directly with Sec3, Rho3 was found to associate with a different exocyst subunit, Exo70 [8,9]. The Rho3–Exo70 interaction was greatly reduced when mutations were introduced into the Rho3 effector domain, and depended on the presence of Rho3-GTP [9]. The effects of mutations in the Rho3 effector domain revealed roles for the protein in regulation of actin organization, transport of exocytic vesicles to the bud, and docking of vesicles with the plasma membrane. Vesicle docking was proposed to be mediated by Exo70 [8].

The mammalian exocyst (or Sec6/8 complex) was also found to localize to areas of active exocytosis. In developing neurons, the exocyst localizes to growth cones and the tips of growing neurites [14]. In epithelial cells, the exocyst concentrates near tight junctions, suggested to be a region of active basolateral membrane addition [2], and was shown to regulate transport of vesicles between the trans-Golgi network and plasma membrane [15]. When cultured in three-dimensional collagen gels, MDCK epithelial cells form multicellular cysts and, in response to hepatocyte growth factor, tubules. This *in vitro* system provides a good model for studying the molecular pathways by which epithelial cells form these higher-order structures [16,17]. Using this system, we showed that the exocyst complex relocalizes concomitantly with the changes in cell polarity that occur when the MDCK cells form these multicellular structures [18]. Moreover, overproduction of the exocyst subunit Sec10 specifically increased the synthesis of secretory and basolateral proteins, as well as the formation of cysts and tubules, suggesting that the exocyst is centrally involved in these processes.

To date, there are no published reports linking a Rho family protein to the mammalian exocyst. Very recently, however, the mammalian exocyst has been found to interact with another GTPase, Ral. Ral is not found in yeast, suggesting that its functions are specific to multicellular organisms and their specialized cell types. Activation of Ral is one of the three signaling pathways emanating from Ras, and Ral has been implicated in the regulation of diverse cellular processes, including oncogenic transformation, endocytosis and actin cytoskeleton dynamics (see [11,12] and references therein). Ral proteins localize to the plasma membrane and to secretory or synaptic vesicle compartments. The mechanism by which Ral proteins mediate their effects is largely unknown, though they have been shown to participate in activation of phospholipase D1 (PLD1), the small GTPase ARF, the tyrosine kinase Src and the transcription factor NF-κB. Filamin and RalBP1/RLIP76 — a Rac/CDC42 GTPase-activating protein — associate directly with Ral-GTP and may be direct effectors in the Ral-mediated regulation of filopodia and endocytosis, respectively.

The interaction between RalA and the exocyst complex was first shown by Brymora *et al.* [10], who were investigating RalA signaling in neuronal cells using a pulldown assay in which recombinant GST-RalA was loaded with either GDP or GTP, bound to an affinity matrix and used to isolate RalA-binding proteins. Eight proteins were isolated that bound to RalA in a GTP-dependent manner, and these were identified by mass spectrometry as members of the exocyst complex.

Three recent papers [10–12] have confirmed the Ral–exocyst interaction by a number of criteria, and provided new insights into the functional importance of this interaction. The exocyst component Sec5 was identified as the direct target for activated Ral GTPases by two of the groups [11,12]. Moskalenko *et al.* [12] pulled out Sec5 in a yeast two-hybrid screen for Ral-GTP binding proteins, and went on to test the functional importance of the Ral–exocyst interaction in polarized epithelial cells.

Consistent with earlier evidence that the exocyst is involved in basolateral, but not apical, membrane traffic in polarized epithelial cells [2,18,19], Moskalenko *et al.* [12] found that inhibiting the interaction between RalA and Sec5 using effector domain mutant forms of RalA blocked the normal transport of several proteins to the basolateral surface and led to their mistargeting to the apical surface, without affecting the transport of normally apical proteins. This did not seem to result in a general effect on cell polarity, as the localization of endogenous E-cadherin to the lateral membrane and ZO-1 to the tight junction was unchanged. Rather, the observed effects were probably due to blocking of vesicle targeting or fusion at the basolateral surface. Indeed, inhibition of Ral activity was found to reduce the assembly of the complete exocyst complex.

Earlier work in yeast had suggested that the exocyst is involved in the interaction of secretory vesicles with actin. Sugihara *et al.* [11] — who also demonstrated that Ral interacts with the exocyst — uncovered a novel way in which the exocyst is linked to the actin cytoskeleton. They found, using anti-Sec5 antibodies in Swiss 3T3 cells, that inhibition of RalA binding to Sec5 prevented the formation of actin-rich filopodia which would otherwise be induced in the presence of certain cytokines or activated forms of RalA and Cdc42.

Finally, Polzin *et al.* [13] found that brain-specific expression of a dominant-negative form of Ral in transgenic mice influenced the regulation of the readily releasable pool of synaptic vesicles [13]. These results are consistent with those of Moskalenko *et al.* [12], who also found an effect on K^+-stimulated exocytosis in PC-12 cells [12]. Polzin *et al.* [13] also showed that Ral interacts with the exocyst, and speculate that this interaction underlies the effects on synaptic vesicles.

In summary, the exocyst has been shown to play a central role in several aspects of exocytosis and vesicle traffic, apparently being directed by interactions between specific exocyst components and different small GTP-binding proteins. Interestingly, the exocyst complex has also been implicated in other cellular activities, such as protein synthesis [18] and

mRNA splicing [20] as well as cyst and tubule formation, raising the possibility that GTPase–exocyst interactions also regulate these processes.

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