Bric-a-Brac at the Golgi

Lydia Danglot[1,2,3] and Thierry Galli[1,2,3,*]
[1]INSERM U950, Membrane Traffic in Neuronal and Epithelial Morphogenesis, Paris, F-75013 France
[2]CNRS UMR7592, Program in Development and Neurobiology, Institut Jacques Monod, Paris, F-75013 France
[3]Université Denis Diderot-Paris 7, F-75013 France
*Correspondence: thierry.galli@inserm.fr
DOI 10.1016/j.devcel.2009.06.002

Until now, Rho proteins were known as GTPases involved in cell polarity and morphogenesis. In a recent issue of *Cell*, Espinosa and coworkers show that RhoBTB3, a member of this family, is an ATPase involved in endosome-to-Golgi transport.

The Golgi apparatus is a central platform of intracellular membrane trafficking. Being next to the nucleus, it is centrally localized in most cells. It also plays a central role in the secretory pathway, between the rough endoplasmic reticulum where transmembrane and secreted proteins are produced and the plasma membrane where their surface expression or release occur. The Golgi complex also communicates with the endocytic pathway. First, endocytosed proteins can reach the Golgi apparatus from early and late endosomes. Second, a recycling pathway involving mannose 6-phosphate receptors (MPRs) allows the transport of newly synthesized lysosomal hydrolases from the Golgi complex to late endosomes via early endosomes (Johannes and Popoff, 2008). Work from many laboratories in the last two decades has shown that vesicular trafficking depends on three basic steps: the formation of a vesicular or tubular intermediate from the donor membrane, its translocation on cytoskeletal tracks, and the fusion of the vesicle or tubule with the acceptor membrane (Cai et al., 2007). Coat proteins, molecular motors, small GTPases of the Rab and Arf subfamilies, tethering factors, and SNARE proteins constitute the major players in these mechanisms. The recycling pathway of MPRs follows this very general rule. Transport of MPRs from late endosomes to the Golgi apparatus involves cytoplasmic dynein, the tethering factor GCC185, Rab6 and Arl1 GTPases, the v-SNARE VAMP3/cellubrevin, and the t-SNARE Syntaxin 10/Syntaxin 16/Vti1a (Burguete et al., 2008; Ganley et al., 2008; Reddy et al., 2006). Work from Suzanne Pfeffer's group has shown that Rab9 plays a central role in transport of MPRs from late endosomes to the Golgi apparatus. Like other Rabs, Rab9 alternates between an inactive GDP-bound form and an active GTP-bound form. A dominant-negative form of Rab9 mainly bound to GDP, Rab9 S21N, inhibits MPR recycling and lysosomal hydrolase sorting (Riederer et al., 1994).

Now Pfeffer and coworkers identify RhoBTB3 as a partner of Rab9 Q66L, a mutant mimicking the active GTP-bound form of Rab9, following a search for partners by a yeast two-hybrid screen. The interaction is very specific; no other Rab among 54 tested interacts with RhoBTB3 and the interaction is restricted to the active form of Rab9 (Espinosa et al., 2009). This interaction is particularly interesting because most Rho GTPases regulate actin and microtubules and are involved in the regulation of cell morphology and polarity, rather than trafficking. But here, the interest is even greater because RhoBTB3 is very atypical. Like RhoBTB1 and 2, RhoBTB3 includes an amino-terminal Rho domain and a C-terminal Bric-a-brac, Tramtrack, and Broad-complex (BTB) domain, a module known for mediating protein-protein interactions. The BTB domain mediates oligomerization and interaction with the Rho domain, a mechanism that prevents interaction with Cul3 ubiquitin ligase complexes and subsequent degradation (Berthold et al., 2008). Sequences just downstream of the BTB domain mediate interaction with Rab9 (Espinosa et al., 2009). The big surprise comes from the demonstration by Espinosa et al. (2009) that the Rho domain does not bind and hydrolyze GTP, but rather ATP.

What may be the role of the Rab9-RhoBTB3 interaction? Myc-tagged RhoBTB3 localizes to the Golgi apparatus (Espinosa et al., 2009). Silencing the expression of RhoBTB3 has profound effects on Golgi complex structure, which appears expanded. Antibodies against RhoBTB3 inhibit MPR transport to the Golgi in a cell-free assay and RhoBTB3 depletion enhances hexosaminidase secretion (Espinosa et al., 2009), a hallmark of MPR defects (Riederer et al., 1994). No global defects in exocytosis or endocytosis are found under these conditions. A screen for mutants of RhoBTB3 unable to bind Rab9 identified the D532E mutation. This mutant is unable to rescue the depletion of endogenous protein, suggesting that the Rab9-RhoBTB3 interaction is central to the function of RhoBTB3. Furthermore, another mutant, N138D, is unable to bind and hydrolyze ATP and also does not rescue depletion of RhoBTB3. Rab9 stimulates ATP hydrolysis by RhoBTB3, thus providing a connection between Rab9 and the Rho ATPase domain. The Rab9-dependent ATPase activity of RhoBTB3 thus appears as a new key regulation of the Golgi apparatus' homeostasis and late-endosome-to-Golgi transport.

Finally, Espinosa et al. connect RhoBTB3 with tail-interacting protein of 47 kDa (TIP47), another Rab9 effector and sorting factor that binds the cytoplasmic domains of MPRs, enabling their transport from late endosomes to the Golgi apparatus (Figure 1). RhoBTB3 and TIP47 form a large molecular weight membrane-associated complex. Rab9 and ATP stimulate the disassembly of this complex (Espinosa et al., 2009). This Rab9-dependent disassembly led the authors to propose a model in which RhoBTB3's activity would allow TIP47's uncoating. This would then allow SNARE-dependent fusion of vesicles with Golgi membrane, thus placing RhoBTB3's function close to the final stage of

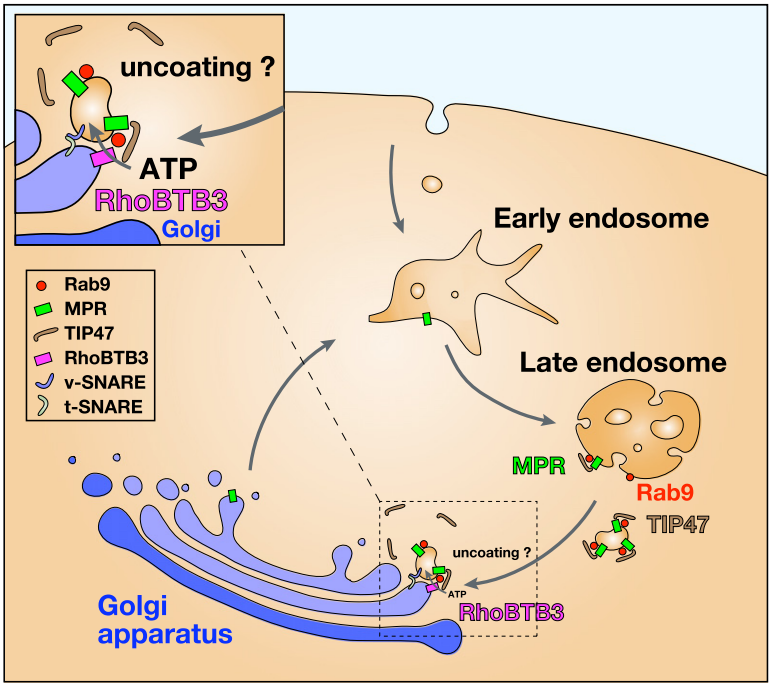

Figure 1. RhoBTB3, an ATPase in Late-Endosome-to-Golgi Transport
The transport of mannose 6-phosphate receptors (MPRs) from late endosomes to the Golgi apparatus involves Rab9, TIP47, the v-SNARE VAMP3/cellubrevin, and the t-SNARE Syntaxin 10/Syntaxin 16/Vti1a. Espinosa et al. (2009) now show that Rab9 bound to GTP interacts with RhoBTB3, an atypical Rho, which turns out to be an ATPase located in the Golgi apparatus and involved in MPR transport. The authors propose a role of RhoBTB3 in TIP47 uncoating.

late-endosome-to-Golgi-apparatus transport (Figure 1).

While this is certainly a sound hypothesis that will likely be tested in future work, the results presented by Espinosa et al. (2009) also pave the way for additional lines of investigation. For instance, what is downstream of RhoBTB3? When and where is RhoBTB3 activated? It is intriguing that GFP-tagged RhoBTB3 also labels vesicles (Berthold et al., 2008) as if it could also play a role prior to fusion at the Golgi, for instance during the transport along microtubule tracks toward the Golgi complex of $Rab9^{+}$ endosomal vesicles. Furthermore, depletion of RhoBTB3 also affects the recycling of TGN46, a protein that cycles between early endosomes and the Golgi complex. Thus RhoBTB3 could play a more general function in the fusion of retrograde vesicles, coming from early and late endosomes, at the Golgi complex. Finally, correlated low expression levels of RhoBTB3 and Cul3 are found in breast and uterine cancer, further suggesting that keeping RhoBTB3 expression at an appropriate level is important for maintaining cellular homeostasis (Berthold et al., 2008). The intriguing atypical RhoBTB3, which turned out to be quite unlike other Rho GTPases, has certainly not revealed all its secrets… yet more unsolved mysteries in membrane trafficking remain ahead (Pfeffer, 2007)!

REFERENCES

Berthold, J., Schenkova, K., Ramos, S., Miura, Y., Furukawa, M., Aspenstrom, P., and Rivero, F. (2008). Exp. Cell Res. *314*, 3453–3465.

Burguete, A., Fenn, T., Brunger, A., and Pfeffer, S. (2008). Cell *132*, 286–298.

Cai, H., Reinisch, K., and Ferro-Novick, S. (2007). Dev. Cell *12*, 671–682.

Espinosa, E.J., Calero, M., Sridevi, K., and Pfeffer, S.R. (2009). Cell *137*, 938–948.

Ganley, I., Espinosa, E., and Pfeffer, S. (2008). J. Cell Biol. *180*, 159–172.

Johannes, L., and Popoff, V. (2008). Cell *135*, 1175–1187.

Pfeffer, S.R. (2007). Annu. Rev. Biochem. *76*, 629–645.

Reddy, J., Burguete, A., Sridevi, K., Ganley, I., Nottingham, R., and Pfeffer, S. (2006). Mol. Biol. Cell *17*, 4353–4363.

Riederer, M., Soldati, T., Shapiro, A., Lin, J., and Pfeffer, S. (1994). J. Cell Biol. *125*, 573–582.