

Pfeffer Lab

Receptor Trafficking in Mammalian Cells: Three dimensional biochemistry

Background:

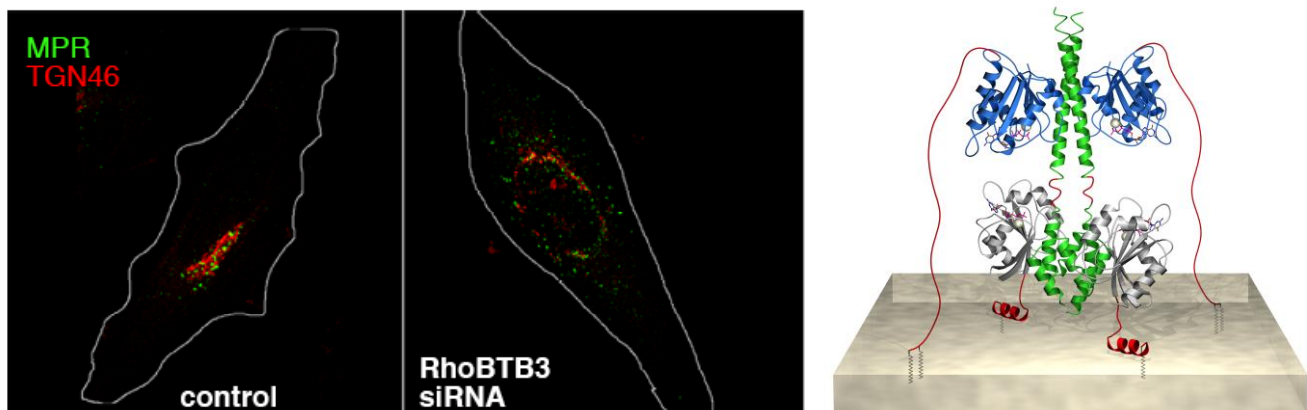
- The ability of cells to respond to extracellular signals requires cell surface receptors
- We study how receptors are delivered to the correct compartments and how their movements are regulated by protein assemblies
- Trafficking is regulated by Rab GTPases--human cells have more than 60!
- Rabs recruit proteins onto membranes that help vesicles form, move and dock with their targets

Primary Questions:

- How are receptors transported from late endosomes to the trans Golgi? This process uses Rab9, TIP47, RhoBTB3, and p40, SNAREs and motors. How do these proteins interact to carry out their functions? We study this in live cells and also in the test tube. In live cells, we use siRNA to deplete essential transport factors and study the accumulated intermediates. In vitro, we can study the process and analyze the mechanism. We also study the purified proteins to understand how they work.
- We recently showed that a long, coiled-coil protein named GCC185 is at the Golgi complex and is a likely tether that “catches” incoming vesicles. How does it work? Below (right) is a model of how the C-terminus of GCC185 (green) binds Rab6 GTPase (Blue) and Arl1 GTPase (grey). These proteins anchor the 185,000 MW protein on the Golgi. We would like to understand how this protein can “catch” incoming transport vesicles and are using a wide variety of approaches to figure this out. Is the tether fully extended? Does it have other binding sites at its N-terminus? We have much to do to better understand the mechanism of this putative vesicle tether.
- We just discovered that a Rho GTPase family is actually an ATPase. What is this fascinating protein doing at the Golgi complex? It seems to uncoat transport vesicles.
- We are undertaking an automated, genome-wide siRNA screen to look for all the proteins that when depleted from cells, block the transport of receptors from late endosomes to the Golgi complex.

Systems: cultured human/mammalian cells; yeast two hybrid

Approaches: • Protein purification and characterization, in vitro receptor trafficking, RNA interference, live cell video microscopy, large scale siRNA screening



Shown above left is the location of Mannose 6-phosphate receptors (green) in relation to the Golgi complex (red). If we deplete cells of RhoBTB3, both markers disperse. At right is the structure of GCC185's C-terminus (green) bound to 2 molecules of Rab6 (blue) and 2 molecules of the small GTPase, Arl1, in grey. We are studying how these two GTPases anchor GCC185 on the Golgi, and what RhoBTB3 ATPase is doing to help GCC185 function.