Vesicle Traffic: COPs, SNARES & Other Things

Lecture Outline

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Introduction

Vesicles from outside the cell such as phagosomes and other endosomes enter and must end up in the right place. But they are just part of the vesicular traffic in eukaryotic cells. Vesicles flow back and forth between the endoplasmic reticulum and Golgi. They travel from the Golgi to lysosomes and to the cell surface and back again. How do vesicles know where to go in the cell? How do they know when they've arrived? When they've arrived at the right place how do they know what to do? The behaviour of vesicles is determined in part by the molecules that they contain in their membranes. As we will see some of the traffic is controlled by COPs. We learned in an earlier lecture that vesicles can move along tracks of microtubules. So there are also roadways that exist, for at least some of the vesicular traffic. One current model of vesicular trafficking which uses both of these systems is the "kiss & run" hypothesis. Vesicles travel along cytoskeletal elements until they reach appropriate regions were fusion occurs under the direction of the vesicular components. While many questions still exist about precise mechanisms, much is known about vesicular trafficking and the molecules that are involved. We'll look at some of these.

Vesicle Trafficking

• Vesicular trafficking underlies a multitude of cellular functions (e.g., Vesicle trafficking mediates cell polarity, cytokinesis and cell motility)
• There are a large number of different intracellular membrane compartments and vesicles are continually moving between all of these compartments, for example:
  o Transmembrane proteins and secreted proteins are transported from one membrane compartment to another by vesicles
  o Secretory, lysosomal and membrane proteins are synthesized in the endoplasmic reticulum, transported through the Golgi apparatus and to their specific sites via vesicles
  o Molecules that are taken up at the cell membrane in endosomes and are transported through the cytoplasm in these vesicles to various fates
  o Regulated and unregulated secretion from the cells occurs; cells need to regulate the movements and fate of specific components but not others
**Vesicle Coats**
Various proteins associate with vesicles. These coat proteins serve two apparent functions: to organize and pinch off the vesicle and to concentrate the constituents targeted to that vesicle type. Here is what is known about vesicles and their coats:
- Clathrin coated vesicles (seen in lecture on Receptor Mediated Endocytosis)
- COPI-coated vesicles
- COPII-coated vesicles
- Uncoated vesicles
- As yet unidentified coats?

**COPS**
Previously we learned about clathrin-coated vesicles and where they function. Here we learn about two other proteins that coat vesicles, COPI and COPII:
- COPII coat vesicles that move retrograde from ER to Golgi
- COPI coat vesicles moving from Golgi to ER
- Need GTP binding proteins to link to membrane (e.g., ARF)

**Small GTP-Binding Proteins**
As mentioned in the lectures on signal transduction, the term G protein has been applied in this course and is used by most researchers to mean heterotrimeric GTPases that associate with the cell membrane. Many low molecular weight GTP-binding proteins also exist. Sometimes they are called monomeric G proteins to distinguish them from heteromeric G Proteins. Here are some of their attributes:
- 20-40 kDa
- Bind GTP and GDP
- Have GTPase activity
- Widely distributed in mammalian cells
- Initiate and terminate specific cell functions at specific times (i.e., act as biotimers)
- They also determine where specific processes occur
- Superfamily of more than 100 proteins (Takai et al, 2001. Physiol. Rev. 81: 153-208)

There are different groups of monomeric GTP-binding proteins:
- Grouped into five families: Ras, Rho, Rab, Sar1/Arf, and Ran
  - Ras family regulates gene expression
  - Rho family regulates cytoskeletal reorganization and gene expression
  - Rab and Sar1/Arf families regulate vesicle trafficking
  - Ran family regulates nucleocytoplasmic transport and microtubule organization
- Most cells have all of the families
- Some members show tissue-specific expression; e.g., Rab3A mediates exocytotic events thus is expressed in neurons, neuroendocrine cells, and exocrine cells
Here we will focus on Rab involvement in vesicle trafficking.

**Rab & The Rab Cycle**

Most Rab proteins regulate the targeting/docking/fusion processes in vesicle trafficking; some regulate budding. For example, Rab3A plays a key regulatory role in Ca\textsuperscript{2+}-dependent exocytosis, particularly in neurotransmitter release from nerve terminals. In yeast *S. cerevisiae*, Rab knockout mutants are characterized by the massive accumulation of secretory vesicles (Review: Takai et al, 2001. Physiological Reviews 81: 153-208). A number of Rab GTPases have been identified. Many of these have been shown to be associated with specific cellular components or vesicles and some of them have been experimentally shown to mediate specific fusion processes. This is an active and exciting area of research that we only have the time to touch upon here. The diversity of Rabs and their functions is shown in the next figure.

Like all GTP-binding proteins, there is a need to exchange GDP for GTP to activate the protein. In the monomeric GTP-binding proteins this is typically carried out in association with a membrane bound guanine nucleotide releasing protein. Here's a complete generic cycle:

- Rab-GDP in cytoplasm with lipid group unexposed
- Guanine Nucleotide Releasing Protein (GNRP) recognizes specific Rab-GDP
- Binding initiates exchange of GTP for GDP
- Conformational change in Rab-GTP exposes lipid tail
- Insertion of Rab into membrane via lipid tail (not shown) organizes coat protein
More Acronyms: Or, How They Got Their Names! Initial research on secretion revealed that NEM inhibited fusion. This lead to the discovery of NSF followed by SNAP. This finally led to the discovery of SNARES allowing models of vesicle targeting and fusion to be developed more completely. These acronyms will be used below.

- NEM: N-Ethylmaleimide (modifies SH groups)
- NSF: NEM-Sensitive Fusion protein
- SNAP: Soluble NSF Attachment Protein
- SNARE: SNAP Receptor
- v-SNARE: Vesicle SNARE
- t-SNARE: Target membrane SNARE

Complementary SNARES mediate vesicle docking
1. Budding of a vesicle from the donor membrane with vesicle-specific v-SNARE;
2. Targeting of the vesicle to the acceptor membrane with complimentary t-SNARE;
3. Docking of the vesicle to the acceptor membrane; and
4. Fusion of the vesicle with the acceptor membrane.

The following picture shows the concept of complementary snares.

In the next section we'll see how all of the above information is linked to vesicle formation, transport and targeting.

Current Model: Vesicle Formation, Transport & Fusion at Target Membrane
The model that follows incorporates all of the material we discussed above. For simplicity and clarity, the vesicle coats and uncoating process are left off. However, these occur as detailed in the lecture on receptor mediated endocytosis and at the same time the processes that are detailed here. Let's start at the donor membrane and progress to the fusion of the formed vesicle at the target membrane:

- Interaction of Rab-GDP with the GNRP has led to the exchange of GTP for GDP
- The exposed lipid tail of Rab-GTP inserts in the donor organelle membrane
- v-SNAREs accumulate leading to vesicle formation
- The vesicle moves to the target organelle
- The vesicle docks via t-SNARE recognition of the v-SNARE
- Dephosphorylation of the GTP bound to Rab releases the Rab into the cytoplasm
- Rab recycles
- The vesicle fuses with the target organelle
Remember that this process is still under active study and the type of Rab (or Arf) and the types of coat proteins will depend on the specific process.

**Docking & Vesicle Fusion**
The events leading up to vesicle fusion have been well studied. In addition to the v-SNARE in the target organelle membrane, there are other proteins that are involved in docking and fusion. Of these, SNAP25 is central to the fusion process. The following picture and points summarize the sequence.

- Vesicle docking occurs by v-SNARE-t-SNARE binding
- Conformational changes occur in the v-SNARE-t-SNARE association
- A fusion protein complex is formed with SNAP25
- The fusion protein complex disrupts the lipid bilayers leading to biomembrane fusion

The constituents of the SNARE complex and some events not detailed here have been reviewed recently (Hay, 2001. Exp. Cell Res. 271: 10-21).