Molecular Mechanisms of Membrane Fusion

Albert Lasker Basic Medical Research Award

James Rothman and Randy Schekman For discoveries revealing the universal machinery that orchestrates the budding and fusion of membrane vesicles—a process essential to organelle formation, nutrient uptake, and secretion of hormones and neurotransmitters.

Richard H. Scheller and Thomas C. Südhof For discoveries concerning the molecular machinery and regulatory mechanisms that underlie the rapid release of neurotransmitters.

The 2013 Albert Lasker Basic Medical Research Award honors two scientists for their discoveries concerning rapid neurotransmitter release. Richard H. Scheller (Genentech) and Thomas C. Südhof (Stanford University School of Medicine) identified and isolated many of this reaction’s key elements, unraveled central aspects of its fundamental mechanism, and elucidated how cells govern it with extreme precision. These advances have provided a molecular framework for understanding some of the most devastating disorders that afflict humans as well as normal functions such as learning and memory.

& finally!
Basic mechanisms for intracellular membrane trafficking

Isolation of SNAREs

SNAREs: targets of clostridial neurotoxins

Inverted SNAREs $\rightarrow$ cell fusion
SNARE-mediated fusion in vitro

Calcium and synaptotagmin in vitro

SNAREs form 4-stranded coiled-coil “core” complex

Calc-SNARE complexes

Trans-SNARE complexes

Vesicle membrane

Single, fused membrane

Target membrane

Fusion pore
During an experiment one optically measures $D(M)$ to be able to calculate the external force, $F=D(M) \cdot k$. A negative force means attraction and a positive force means repulsion. When the surfaces are in contact, they deform elastically to form a circular contact with a diameter of several $10\mu$m. In such contact, $D$ may vary only little, while the actuator continues to move. Then, the external force (or load), $F$ is increased roughly proportional to $k' \cdot M$. Thus: $F = k' \cdot M$. 

Hydration barrier to spontaneous fusion
Seeing the fusion pore with nanodiscs

SNARE proteins: one to fuse and three to keep the nascent fusion pore open, Shi Science 2012

Membrane Contact Sites (MCS)

Emr's lab: ER-PM tethering proteins in yeast: Ist2 (related to mammalian TMEM16 ion channels), the tricalbins (Tcb1/2/3, orthologs of the extended synaptotagmins) and Scs2 and Scs22 (vesicle associated membrane proteins).

Endoplasmic reticulum: one continuous network compartmentalized by synthetic vesicles Tim Lienhard and Catherine Rassoulzadegan

Structure of a lipid‐bound Extended‐Synaptotagmin indicates a role in lipid transfer Schauder et al., De Camilli & Reinisch teams.

Staging Membrane Fusion
Josep Rizo, Science 2012


Non-vesicular lipid transfer
VAP-A/E-Syt
Conclusion

How do SNAREs get to where they are supposed to be?

Each type acts at distinct locations

SNAREs and coats: possible links

- Several v-SNAREs interact with molecular coats:
  - VAMP-2-AP-2
  - VAMP4-AP1
  - VAMP7/TI-VAMP-AP-2 & AP3
- The Longin domain of Sec22, Ykt6, TI-VAMP/VAMP7 resembles a domain in AP2 subunits
The single lipidation (i.e., farnesylation) of Ykt6 shifts the conformation of the longin SNARE from a semiclosed state into a dominantly closed and fusion-inactive state through the coordinated actions of the longin domain, the SNARE core, and the lipid molecule. This autoinhibited, closed conformation of Ykt6 can be relieved by further lipidation (i.e., palmitoylation) of the protein. The results described here demonstrate that lipid molecules can actively and dynamically regulate protein activities in addition to their well-known passive membrane-anchoring roles.
Tetanus Neurotoxin-Insensitive Vesicle-Associated Membrane Protein (TI-VAMP)

- TI-VAMP/VAMP7
  - X-linked gene
  - 25kD v-SNARE
  - ubiquitous
  - Insensitive to NTs
  - N-terminal extension of 100aa called Longin domain

The Longin family

Ykt6, Sec22, SEDL, component of the transport protein particle (TRAPP) involved in endoplasmic reticulum-to-Golgi vesicle transport

Missense mutation → X-linked spondyloepiphyseal dysplasia tarda

TI-VAMP interacts with AP-3
Regulation of TI-VAMP endocytosis by Hrb
A molecular network comprising a v-SNARE, a GEF, a Rab, a Kinesin, a Golgin and a Spectraplakin is involved in transport of vesicles from center to periphery.

Burgo & al, Dev Cell 2012
Partners of VAMP7: ip and proteomics → SNAP-47

SNAP-47: cytosolic pool + ER + endosomes
SNAP-47 interacts with VAMP4, VAMP7 & VAMP8

SNAP-47 in & out the nucleus

SNAP-47 regulates distribution and function of VAMP7

SNAP-47: several atypical functions from the nucleus to the cytoplasm?