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The molecular basis for cellular secretion: SNAREs, SNARE regulators, and secretory vesicles

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Research Objectives

In order for eukaryotic cells to grow, newly synthesized proteins and lipids are delivered to the cell surface via a process known as exocytosis. My laboratory utilizes the yeast *Saccharomyces cerevisiae* as a model system to elucidate the mechanisms that underlie intracellular membrane trafficking and exocytosis. In particular, we are interested in the role of SNAREs, fusogenic proteins that are present upon both vesicle and acceptor membranes, in the steps leading to vesicle docking and fusion. One project deals specifically with the role of cell signaling in the control of exocytosis, and concerns the involvement of protein kinases and phosphatases in regulating SNARE functioning. A second project deals with proteins that bind to SNAREs (called SNARE regulators) and prevent them from assembling into the fusion complex. Finally, a third project deals with studying the actual biogenesis of secretory vesicles (SVs) and aims at understanding the molecular requirements necessary for their formation. All three projects are relevant towards understanding the processes of cell growth, hormonal secretion, and neurotransmission that are found in higher eukaryotes, yet can be demonstrated in a simple and elegant fashion using yeast.

Findings

Originally, we discovered the vesicle SNAREs (Snc1 and 2) that are required for SV docking and fusion in yeast. Their mammalian counterparts, known as VAMPs or synaptobrevins, are required for the fusion of synaptic vesicles with the presynaptic membrane. Both Sncs and VAMPs are important because they assemble into a complex with partner t-SNAREs, located on the plasma or presynaptic membranes, to drive membrane fusion (Fig. 1). In recent years, we have focused upon regulation of the SNAREs by signal transduction pathways, as well as by SNARE-binding proteins. These types of regulation are important for determining where and when SNAREs can assemble into the fusion complex, thus, regulating the delivery of lipids and proteins to the cell surface.

Role of SNARE phosphorylation in vesicle fusion

Deletion of the *SNC1,2* genes in yeast results in the accumulation of SVs and a block in cellular secretion. We then identified two other genes (*VBM1* and *VBM2*) that, when inactivated by mutation, allow cells lacking the v-SNAREs to grow and secrete normally. Our recent work has revealed that a protein phosphatase is activated directly by sphingolipid precursors that accumulate in vbm mutant cells. Activation of the phosphatase results in dephosphorylation of the Sso t-SNAREs, resulting in the formation of a t-t SNARE complex that drives fusion (Fig. 1). Thus, phosphorylated SNAREs do not assemble into SNARE complexes, while dephosphorylated SNAREs do. These findings demonstrate that a lipid-activated signaling cascade controls secretion and that SNARE

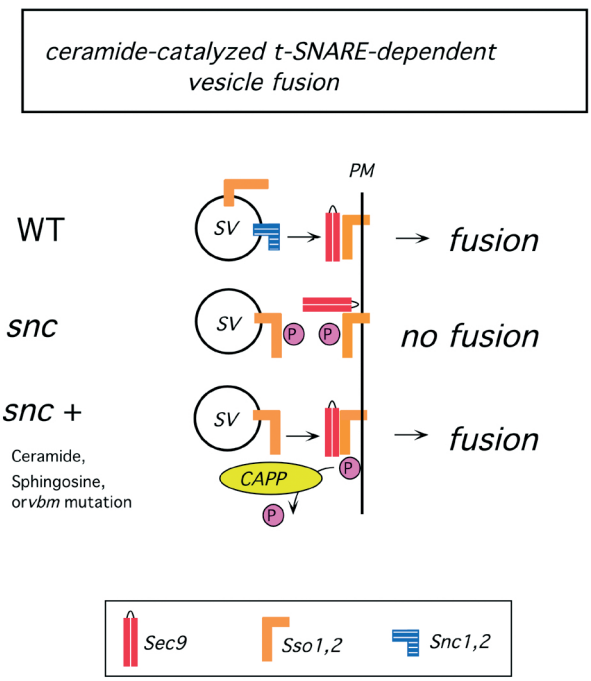

Fig. 1 *Phosphorylation and vesicle fusion. In wild-type cells (a) v- (Snc 1,2) and t-SNAREs (Sso1,2 and Sec9) assemble into a fusion complex, resulting in secretion. In snc cells (b), no v-SNAREs are present, Sso t-SNAREs are then hyperphosphorylated, and vesicle fusion is blocked. However, when snc cells are treated with ceramide, sphingoid bases, or by vbm mutation (c), Sso t-SNAREs are dephosphorylated by a phosphatase, allowing it to assemble into a 2:1 fusion complex with Sec9.*

phosphorylation and dephosphorylation play an important role in SV docking and fusion. Ongoing studies show that SNARE phosphorylation occurs at all levels of the secretory pathway (i.e., ER-Golgi). Thus, our efforts are to understand how signaling cascades coordinate cell cycle with physical growth processes.

Role of SNARE regulators in exocytosis

Earlier, we identified a v-SNARE-binding protein (Vsm1) that acts as a regulator of vesicle docking and fusion. Recently, we have shown that Vsm1 also binds to the Sso t-SNAREs, but in a phosphorylation-dependent fashion. Thus, phosphorylated Sso, which is unable to assemble with SNAREs, binds preferentially to Vsm1. We conclude that SNARE phosphorylation not only affects SNARE assembly directly, but also the association of proteins that prevent assembly.

Role of exocytic v-SNAREs in endocytosis

We have recently shown that the Snc v-SNAREs participate in endocytic, as well as exocytic, events. They mediate the docking and fusion of endocytic vesicles with endosomes, using different t-SNARE partners. Thus, these v-SNAREs confer both anterograde and retrograde transport to and from the cell surface.

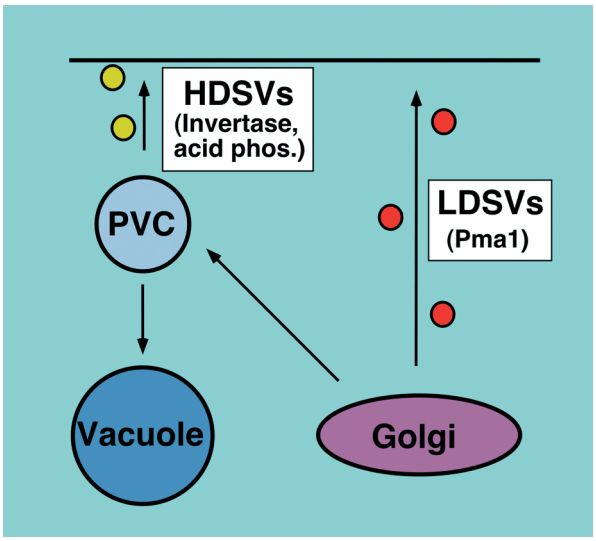

Fig. 2 *A model for SV biogenesis. Secreted proteins are trafficked from the Golgi to the surface by either LDSVs or HDSVs. Proteins secreted via the HDSV route require an intact Golgi-to-endosome (PVC) sorting pathway. Mutations that inhibit either the biogenesis or fusion of vesicles that confer Golgi-to-endosome transport abolish vacuolar protein sorting and the HDSV sorting routes. Under such circumstances, vacuolar and secreted proteins are exported from the cell via the default LDSV route.*

Biogenesis of secretory vesicles

The origin of SVs has been thought to be the Golgi. However, this has not been directly demonstrated and evidence suggests that SVs may form from another compartment. Yeast produce two types of SVs that differ in both density and content. Both low-density SVs (LDSVs) and high-density SVs (HDSVs) accumulate in cells when the vesicles are unable to fuse with the plasma membrane. We now show that mutations that block Golgi-to-endosome transport also block HDSV production. Thus, HDSV biogenesis requires transport through an intermediate compartment and is not direct (Fig. 2). We also show that HDSV biogenesis requires both clathrin and dynamin.

Selected Publications

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Marash, M. and Gerst, J.E. (2002) Phosphorylation-dependent binding of a SNARE regulator to its t-SNARE partner. (Submitted).

Acknowledgements

Our work is supported by the Israel Science Foundation, Minerva Foundation (Germany), and the Forchheimer Center for Molecular Genetics. J.E.G. holds the Henry Kaplan Chair in Cancer Research.