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The molecular basis for polarized cell growth

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Polarized cell growth is essential for a variety of cellular processes, including: growth control, differentiation, motility, and tissue formation. The establishment of cell polarity in eukaryotes involves the asymmetric organization of both the actin cytoskeleton and secretory pathway to lead to the polarized distribution of new membrane along a given axis. We are identifying the molecular requirements necessary for the transport of newly synthesized proteins and lipids to the cell surface, via secretory vesicles, as well as the role of mRNA transport and localization in the establishment and maintenance of this process.

As simple and complex organisms utilize similar strategies to deliver proteins and lipids to the cell surface, we are using the yeast, *Saccharomyces cerevisiae,* as a model system in which to study transport processes. Because yeast are genetically tractable, it allows us to identify mutations that block protein and mRNA transport in a simple and rapid fashion. Our work can be divided into three major subjects: SNARE regulation; secretory vesicle biogenesis; and mRNA transport.

First, we are studying the connection between cell signaling pathways and the control of membrane fusion events. In particular, we have shown that SNAREs, which are conserved membrane fusogens, are modified post-translationally by kinases involved in cell cycle and growth control. Phosphorylation was found to regulate both exo- and endocytosis, as well as the control of Golgi morphology. Specifically, phosphorylation of the Sso family of exocytic t-SNAREs was found to occur in the autoregulatory domain and to recruit potential inhibitors of SNARE assembly (i.e. Vsm1). In contrast, phosphorylation of the Golgi t-SNARE, Sed5, occurred downstream of the SNARE binding motif and resulted in an inhibition in Golgi-ER retrograde transport. Moreover, the dephosphorylation of Sed5 resulted in the accumulation of stacked Golgi structures not typically seen in budding yeast (see Figure 1.). Dephosphorylation in general was found to remove regulatory blocks placed on the SNAREs and allowed for the resumption of polarized growth. Ongoing work seeks to reveal the mechanism(s) by which phosphorylation and dephosphorylation control SNARE functions throughout the secretory pathway.

Second, we are determining the molecular requirements necessary for the biogenesis of secretory vesicles. These vesicles are typically thought to be derived from the Golgi, but our work and that of others suggest a more direct role for endosomal sorting compartments in the delivery of proteins and lipids to the cell surface. Yeast produce two classes of secretory vesicles, one of lower density and the other of higher density that contains soluble secreted enzymes. We have found that yeast bearing mutations that block Golgi to late endosome transport do not produce the high-density class of vesicles. Thus, protein trafficking to the late endosome is required for the biogenesis of this class of vesicles.

Third, we are examining the role of mRNA transport in the localization of proteins of the exocytic apparatus at the cell surface, as well as the mechanism by which mRNAs themselves are transported through the cell. We have recently found that mutations in the late secretory pathway (i.e. post-Golgi) strongly affect the actin cytoskeleton and, as a result, block mRNA transport (see Figure 2.). Since the late pathway utilizes the cytoskeleton for vesicle transport it suggests that the fusion events at the plasma membrane may directly control the cytoskeleton, and not just vice verse as previously thought. We are currently engaged in determining what the nature of this “exocytic signal” might be.

These studies aim at elucidating the involvement of vesicle and mRNA transport in the control of secretion and, together, aim to define the molecular events necessary for the establishment and maintenance of polarized cell growth in eukaryotes.

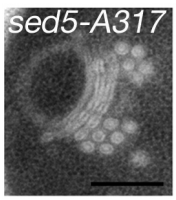

Fig. 1 *The Golgi apparatus in yeast is not structured, however, expression of a non-phosphorylated form of the Sed5 Golgi-localized t-SNARE (Sed5A317) results in the accumulation of stacked Golgi structures. Thus, Sed5 dephosphorylation may promote Golgi re-ordering following mitotic cell division in eukaryotes.*

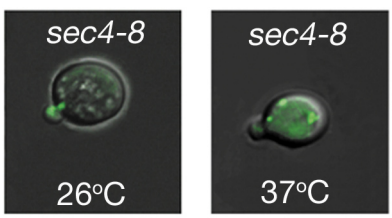

Fig. 2 *Some mRNAs are specifically localized to the growing bud in yeast. However, we have found that mutations in the secretory pathway may block mRNA transport and localization to the bud.* SEC4 *encodes a GTPase involved in vesicle docking with the plasma membrane and* ASH1 *mRNA (green) localizes properly in* sec4-8 *cells at the permissive temperature (26°C). In contrast, shifting to the restrictive temperature (37°C) blocks vesicle transport and results in the mislocalization of mRNA. Thus, the vesicular transport pathway affects mRNA transport as well.*

Selected Publications

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Weinberger, A., Kamena, F., Spang, A., and Gerst, J.E. (2004) Control of Golgi morphology and function by Sed5 t-SNARE phosphorylation. (submitted)

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