The molecular basis for polarized cell growth

Jeffrey Gerst

Stella Aronov

Rachel Kama

Antonio Castillo-Flores

Galina Gabriely

Rita Gelin-Licht

Liora Haim

Boris Slobodin

Liat Yakir-Tamag

Gadi Zipor

Einat Safran

Polarized cell growth is essential for most cellular processes including: cell division, growth control, differentiation, motility, and body plan morphogenesis. The establishment of cell polarity in eukaryotes involves the asymmetric organization of the both 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 polarized growth.

We are using the yeast, Saccharomyces cerevisiae, as a model system in which to study these processes, as both simple and complex organisms utilize similar strategies to deliver proteins and lipids to the cell surface. Because yeast are genetically tractable, it allows us to identify genes that control protein and mRNA transport in a rapid fashion. Our work can be divided into three major subjects: SNARE regulation; endosomal protein sorting and human disease; and mRNA trafficking.

First, we are studying the connection between cell signaling pathways and the control of membrane fusion events. We have shown that SNAREs, which are conserved membrane fusogens, are modified post-translationally by kinases involved in cell cycle and growth control. SNARE phosphorylation at specific residues was found to regulate both exo- and endocytosis, as well as fragmentation of the Golgi. In contrast, dephosphorylation by a conserved phosphatase restores secretion and endocytosis, and results in the ordering of the Golgi. Thus, signaling cascades that regulate the cell cycle also regulate SNARE function leading to intracellular membrane trafficking and growth control. This is important for the coordination of DNA replication and nuclear division with cell division. Ongoing work seeks to reveal the mechanisms by which SNARE functions are controlled by both phosphorylation and SNARE regulatory proteins throughout the secretory pathway.

Second, we have identified three SNARE binding proteins (Vsm1, Btn2, Gcs1) of which at least two (Btn2, Gcs1) act upon endosomal protein sorting. The human ortholog of Btn2 may be involved in Batten Disease, a lysosomal storage disorder that results in neurodegeneration in humans. We found that Btn2 encodes a Hook-like protein that localizes to late endosomes, binds to both SNAREs and the retromer coat complex, and that the deletion of *BTN2* results in the leakage of Golgi proteins to the vacuole (Figure 1). We propose that Btn2 confers protein retrieval to the Golgi from late endosomes and that defects in this process may result in Batten Disease. In the case of Gcs1, we have shown that it regulates protein recycling from early endosomes to the Golgi, while Vsm1 is a SNARE regulator that may help target SNAREs or coat proteins for degradation.

Department of Molecular Genetics

972 8 934 2106

972 8 934 4108

jeffrey.gerst@weizmann.ac.il

www.weizmann.ac.il/molgen/members/gerst.html

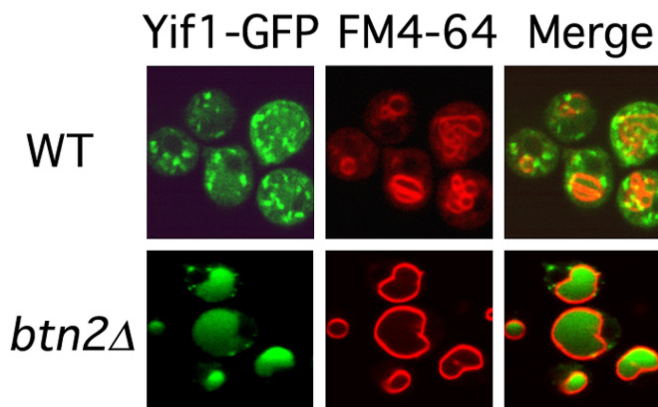

Fig 1. *Yeast as a model for Batten Disease. Yif1 is a Golgi marker that shows a dispersed pattern of labeling in wild-type yeast. In contrast, yeast lacking the BTN2 gene show a vacuolar pattern of localization (FM4-64 labels vacuolar membranes). Thus, Yif1 retrieval to the Golgi is blocked in btn2Δ cells and this phenotype may parallel defects in Batten Disease patients.*

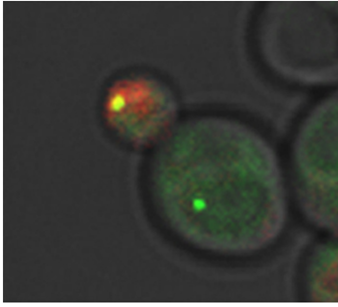

Fig 2. *mRNAs encoding polarity and secretion factors localize asymmetrically to the site of exocytosis. The gene encoding the Sec4 GTPase was tagged at the 5' end with RFP and at its 3' end with binding sites for the MS2 viral protein. When co-expressed with MS2-GFP, mRNA localization is detected by GFP while protein localization is detected by RFP. The results show that mRNA is localized to the bud prior to and during bud emergence, and that bud-localized mRNA undergoes translation. The mRNA granule in the mother cell is untranslated and used for the next round of cell division.*

Together, these studies show a direct relationship between SNARE binding proteins and endosomal protein sorting. Future studies aim at using yeast as a model system for the study of lysosomal storage disorders.

Finally, are exploring the role of mRNA trafficking in the localization of the secretory apparatus at the cell surface and subsequent polarized secretion. We have found that mRNAs encoding polarity and exocytosis factors are trafficked to sites where polarized growth eventually occurs (Figure 2.) and that mRNA trafficking is necessary to facilitate polarization. Ongoing work has demonstrated the protein factors involved in mRNA trafficking in both budding (dividing) and shmooing (mating) yeast cells. In addition, we have demonstrated a direct connection between ER transport and mRNA trafficking suggesting that these processes are interconnected in eukaryotic cells.

Together, these studies aim at elucidating the involvement of vesicle and mRNA transport in the control of polarized growth in simple eukaryotes.

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