# Intracellular protein and mRNA transport in cell growth and disease

Department of
Molecular Genetics

**Prof. Jeffrey E. Gerst**

Rachel Kama, Judith Kraut-Cohen, Noga Gadir, Vydehi Kanneganti, Liora Haim, Rita Gelin-Licht, Boris Slobodin, Liat Yakir-Tamang, Gadi Zipor

☎ 972 8 934 2106
FAX 972 8 934 4108
@ jeffrey.gerst@weizmann.ac.il
www.weizmann.ac.il/molgen/members/gerst.html

Our laboratory focuses on the importance of protein and mRNA transport in the growth of eukaryotic cells. Our work can be divided into three spheres: SNARE regulation and polarized growth; Endosomal protein sorting and human disease; and the role of intracellular mRNA trafficking in organelle biogenesis and cell growth.

Polarization is essential for many cellular processes including: cell division, growth control, differentiation, motility, and body morphogenesis. Polarity establishment in eukaryotes involves asymmetric organization of both the cytoskeleton and secretory pathway, and leads to the polarized distribution of membrane along a given axis. We principally use the yeast, *Saccharomyces cerevisiae*, as a model system since both simple and complex organisms utilize similar strategies to deliver proteins and lipids to the cell surface. Because yeast are also genetically tractable, it allows us to identify genes that control protein and mRNA transport in a rapid fashion.

First, we are studying the connection between cell signaling pathways and the control of membrane fusion events leading to cell polarization. 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 regulates exocytosis and endocytosis, as well as fragmentation of the Golgi. Thus, signaling cascades that regulate the cell cycle also control SNARE function to modulate membrane trafficking and cell growth. This is important for coordinating the processes of DNA replication and nuclear division with that of cell division. Ongoing work has also demonstrated that an exocytic signal, mediated by phosphoinositide 4, 5-bisphosphate, is released at the plasma membrane every time a secretory vesicle docks and fuses. This allows for local activation of the Cdc42 small GTPase and directly controls of actin filament formation. Thus, we have shown that active secretion regulates actin polarization in order to maintain vesicle transport to the site of growth.

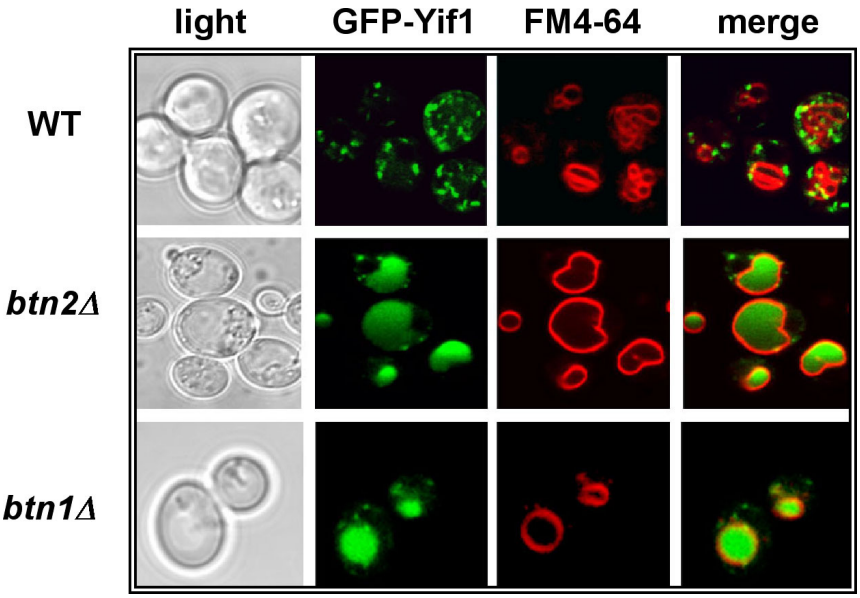
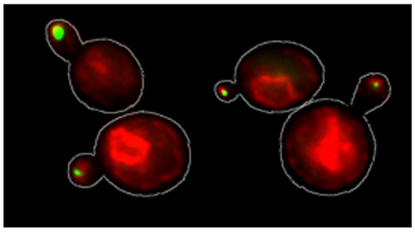



***Fig. 1*** *A Golgi protein, Yif1, is mislocalized to the vacuole in yeast lacking the Batten disease genes - BTN1 and BTN2. A plasmid expressing GFP-Yif1 was transformed into wild-type (WT), btn2Δ, and btn1Δ yeast cells. Cells were grown to mid-log phase at 26ºC prior to labeling with FM4-64 to mark the vacuole (in red) before microscopy. Merge indicates merger of the GFP (green) and FM4-64 (red) fluorescence windows. Note the dispersed labeling of the Golgi by GFP-Yif1 in WT cells, while GFP-Yif1 is mistrafficked to the vacuole in either btn2Δ or btn1Δ cells. This is because Btn1 and Btn2 act at the level of the late endosome to retrieve proteins to the Golgi, a process that may be defective in NCL patients.*

Second, we are studying yeast orthologs of proteins involved in progressive neurodegenerative disorders in humans, called *Neuronal Ceroid Lipofuscinoses* (NCLs). We initially identified Btn2 as a SNARE-binding protein and demonstrated that its removal results in defects in late endosome-Golgi protein retrieval (Figure 1). Recent results demonstrate that Btn1, which is the yeast ortholog of a human protein (called CLN3) mutated in the juvenile form of NCL (known as Batten disease), also acts upon late endosome-Golgi retrieval. Thus, using the yeast system we have pinpointed the defective process that may lead to disease onset in higher organisms. Ongoing work seeks to use yeast as a model system for the study of lysosomal storage disorders and identify potential means of therapy.

Finally, we are exploring the role of mRNA trafficking in organelle biogenesis and cell growth. We have developed novel imaging techniques that allow for the simultaneous visualization of both mRNA and its derived translation product *in vivo*, using fluorescence microscopy. We initially demonstrated that mRNAs encoding polarity and exocytosis factors are trafficked to sites where polarized growth eventually occurs (Figure 2.) and that mRNA trafficking may facilitate cell polarization. Moreover, we showed that these same mRNAs piggyback on endoplasmic reticulum membranes that are directly targeted to the site of growth (Figure 2.).



mRNA = *SRO7*
ER = Sec63-RFP

***Fig. 2*** *SRO7 mRNA is polarized to the bud tip and co-localizes with cortical ER. SRO7 mRNA (green label) in yeast can be visualized using fluorescence microscopy by employing a bacteriophage RNA-binding protein (MS2-CP) fused to green fluorescent protein (MS2-CP-GFP). MS2-CP-GFP binds directly to SRO7 mRNA that has been modified with MS2-CP binding sites inserted between the coding region of SRO7 and its 3'UTR. MS2-CP-GFP-labeled SRO7 mRNA is seen as a granule that localizes the tips of newly forming daughter cells. The co-localization of SRO7 mRNA and cortical ER membranes can be observed using Sec63-RFP as an ER marker protein (red label). Note that the GFP signal overlaps with cER present in the small buds and not with the perinuclear ER visible in mother cells. The white tracings indicate the outlines of cell shape (i.e. position of the cell wall).*

Since mRNA trafficking may play an essential role in the local translation and placement of protein, we developed a gene-tagging strategy (called m-TAG) that allows for the localization of endogenous mRNAs for the first time *in vivo*. We are employing m-TAG to localize ~10% of the mRNAs encoded by the yeast genome in order to establish a genome-wide mRNA localization map and identify the factors involved in mRNA trafficking. Other studies in the lab have allowed us to adapt the mRNA/protein tagging methodology for use in mammalian cells, as well as to develop a novel affinity purification strategy to identify RNA-binding proteins involved in mRNA trafficking.

Together, these studies aim at elucidating the involvement of both protein and mRNA transport in the control of growth in eukaryotic cells.

**Selected publications (since 2000)**


Gurunathan, S., Chapman-Shimshoni, D., Trajkovic, S., and Gerst, J.E. (2000) Yeast exocytic v-SNAREs confer endocytosis. Mol. Biol. Cell, 11, 3629-3643.

Marash, M. and Gerst, J.E. (2001) t-SNARE dephosphorylation promotes SNARE assembly and exocytosis in yeast. EMBO J., 20, 411-421.

Gurunathan, S., David, D., and Gerst, J.E. (2002) Dynamin and clathrin are required for the biogenesis of a distinct class of secretory vesicles in yeast. EMBO J., 21, 602-614.

Gurunathan, S., Marash, M., Weinberger, A., and Gerst, J.E. (2002) t-SNARE phosphorylation regulates endocytosis. Mol. Biol. Cell, 13, 1594-1607.

Gerst, J.E. (2003) SNARE regulators: Matchmakers and matchbreakers. BBA – Molecular Cell Research, 1641, 99-110.

Marash, M. and Gerst, J.E. (2003) Phosphorylation of the autoinhibitory domain of the Sso t-SNAREs promotes binding of the Vsm1 SNARE regulator in yeast. Mol. Biol. Cell, 14, 3114-3125.

Aronov, S. and Gerst, J.E. (2004) Involvement of the late secretory pathway in actin regulation and mRNA transport in yeast. J. Biol. Chem., 279, 36962-36971.

Castillo-Flores, A., Weinberger, A., Robinson, M., and Gerst, J.E. (2005) Mso1 is a novel component of the yeast exocytic SNARE complex. J. Biol. Chem., 280, 34033-34041.

Weinberger, A., Kamena, F., Kama, R., Spang, A., and Gerst, J.E. (2005) Control of Golgi morphology and function by Sed5 t-SNARE phosphorylation. Mol. Biol. Cell, 16, 4918-4930.

Robinson, M., Poon, P.P., Schindler, C., Murray, L.E., Kama, R., Gabriely, G., Singer, R.A., Spang, A., Johnston, G.C. and Gerst, J.E. (2006) The Gcs1 Arf-GAP mediates Snc1,2 v-SNARE retrieval to the Golgi in yeast. Mol. Biol. Cell, 17, 1845-1858.

Gabriely, G., Kama, R., and Gerst, J.E. (2007) Involvement of specific COPI subunits in protein sorting from the late endosome to the vacuole in yeast. Mol. Cell. Biol., 27, 526-540.

Kama, R., Robinson, M., Gabriely, G., and Gerst, J.E. (2007) Btn2, a Hook1 ortholog and potential Batten Disease-related protein, mediates sorting from late endosomes to the Golgi in yeast. Mol. Cell. Biol., 27, 605-621.

Aronov, S., Gelin-Licht, R., Zipor, G., Haim, L., Safran, E., and Gerst, J.E. (2007) mRNAs encoding polarity and exocytosis factors are co-transported with cortical ER to the incipient bud in yeast. Mol. Cell. Biol., 27, 3441-3455.

Haim, L., Zipor, G., Aronov, S., and Gerst J.E. (2007) A PCR-based genomic integration method to visualize the localization of endogenous mRNAs in living yeast. Nat. Methods, 4, 409-412.

Gerst, J.E. (2008) Message on the web: mRNA and ER co-trafficking. Trends Cell Biol., 18, 68-76.



***Acknowledgements***

Henry Kaplan Chair in Cancer Research

Supported by grants from the:

Israel Science Foundation

Minerva Foundation, Germany

***INTERNAL support***

Benoziyo Institute for Molecular Medicine

Kahn Center for Systems Biology

Center for Scientific Excellence