

# Mechanism of intracellular protein traffic

## Department of Biological Chemistry

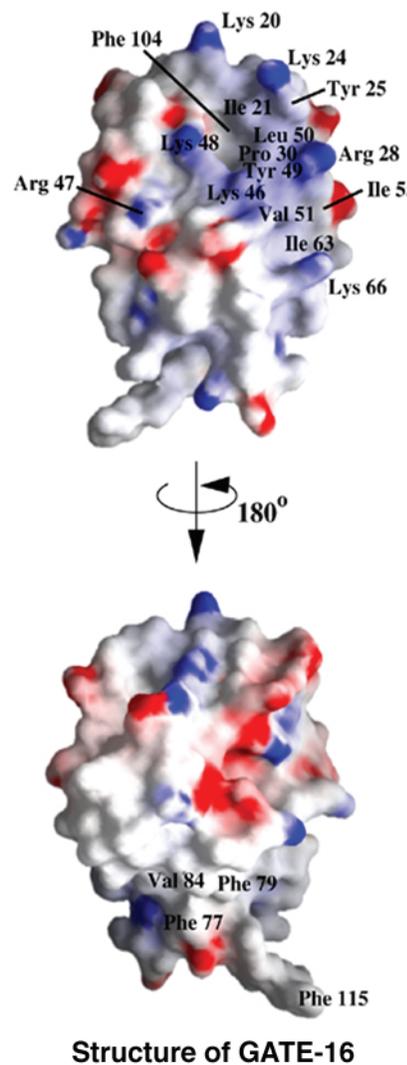
Tel. 972 8 934 3682 Fax. 972 8 934 4112  
 E-mail: zvulun.elazar@weizmann.ac.il

Intracellular vesicle traffic in all eukaryotic cells, from yeast to human, is crucial for normal cell function. Transport of proteins within the extensive network of membrane-bound compartments is highly regulated to ensure the specificity and efficiency of cargo delivery. Considerable progress has been made towards understanding the molecular basis of membrane traffic. This led to the identification of a number of traffic pathways and to the discovery of many of the proteins components that facilitate and regulate vesicle biogenesis, targeting, and membrane fusion of the vesicle with its appropriate target organelle. However, the precise molecular mechanisms that facilitate and regulate these processes remain unclear. Identification and characterization of the various regulatory factors that take part in intracellular protein transport is essential for better understanding of this complex process.

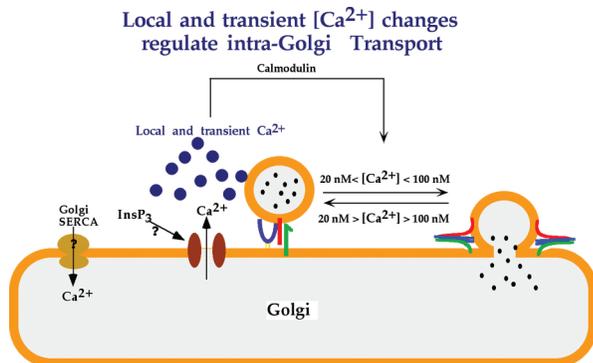
### A novel ubiquitin-like protein family involved in multiple intracellular trafficking processes

Over the past few years we have been studying questions related to the molecular mechanism of intracellular membrane transport aiming to disclose and characterize such novel factors. On the basis of transport activity in a cell-free transport assay, we have identified two novel soluble transport factors: a mammalian 16 kD protein, denoted GATE-16, and SBP56, a protein whose previous function was unknown. We have found that GATE-16 interacts with components of the membrane fusion machinery and characterized these interactions. More recently we have determined the three-dimensional structure of GATE-16 at 1.8 Å resolution (Fig. 1), and found that it belongs to a novel ubiquitin-like (UBL) protein family which appears to be involved in multiple intracellular membrane trafficking events. Members of this family include light chain-3 (LC3), a subunit of the neuronal microtubule-associated protein complex, and GABA receptor-associated protein (GABARAP), thought to promote clustering of neurotransmitter receptors. We took advantage of yeast as a model system for studying membrane trafficking, focusing on the yeast homologue of GATE-16, Aut7p. To directly examine the role of Aut7p in vivo, we knocked out the Aut7p gene in *S. cerevisiae*. The aut7 null mutant is viable and shows normal growth on YPD medium, but grows significantly slower on synthetic medium in comparison with

wild type strains. In addition, we found that under nitrogen starvation conditions, when autophagy is triggered, this mutant



**Fig. 1** Surface contours, charge distribution, and sequence conservation in GATE-16. A, Surface of GATE-16 colored according to the electrostatic distribution. Regions of basic potential are colored blue; acidic regions are in red. Conserved basic positions and partially exposed hydrophobic residues are labeled.



**Fig. 2** Proposed model for the regulation of intra-Golgi transport by calcium:  $\text{Ca}^{2+}$  efflux from the Golgi apparatus possibly via  $\text{IP}_3$ -regulated channels, transiently increases the local  $[\text{Ca}^{2+}]$  in the vicinity of the Golgi membrane. Together with calmodulin,  $\text{Ca}^{2+}$  (100nM) acts at late stages of transport, possibly in fusion, at higher  $\text{Ca}^{2+}$  concentrations (above 100 nM) intra-Golgi transport is blocked.

strain shows lower survival rates and is also defective in its ability to degrade proteins. Furthermore, Aut7p is the only protein known to date to be upregulated during autophagy. Among many secretion mutants tested, overexpression of Aut7p in cells bearing a *ts bet1-1* or *sec22-2* allele (membranal v-SNAREs which are part of the membrane fusion machinery involved in ER-to-Golgi transport) results in suppression of the growth defect phenotype at the restrictive temperature. In addition, we demonstrated that Aut7p interacts physically with two v-SNAREs, which are part of the membrane fusion machinery: Bet1p, involved in ER-to-Golgi transport, and Nyv1p, implicated in vacuolar inheritance. These findings, together with its localization and its intra-Golgi activity, suggest that Aut7p may be involved in the fusion process at different transport stages and therefore should be regarded as a general transport factor. Taken together, the studies presented here support the notion that GATE-16/Aut7p participates in multiple intracellular trafficking processes

### Regulation of intra-Golgi protein transport by calcium and calmodulin

Calcium cations play a critical role in regulating vesicular transport between different intracellular membrane-bound compartments. The role of calcium in transport between the Golgi cisternae, however, remains unclear. Utilizing a well-characterized cell-free intra-Golgi transport assay, we have recently found that changes in free  $\text{Ca}^{2+}$  concentration in the physiological range regulate this transport process. The calcium-chelating agent BAPTA blocked transport with an  $\text{IC}_{50}$  of approximately 0.8 mM. The effect of BAPTA was reversible by addition of fresh cytosol, and was irreversible when performed in the presence of a  $\text{Ca}^{2+}$  ionophore which depletes calcium from

luminal stores. We have demonstrate that intra-Golgi transport is stimulated by low  $\text{Ca}^{2+}$  concentrations (20-100 nM), but is inhibited by higher concentrations (above 100 nM). Further, we show that calmodulin antagonists specifically block intra-Golgi transport, implying a role for calmodulin in mediating the effect of calcium. Our results suggest that  $\text{Ca}^{2+}$  efflux from intracellular pools may play an essential role in regulating intra-Golgi transport.

### Selected Publications

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- Porat, A. & Elazar, Z. (2000) Regulation of intra-Golgi transport by calcium. *J. Biol. Chem.* 275, 29233-29237.
- Paz, Y., Elazar, Z. & Fass, D. (2000) Structure of GATE-16, membrane transport modulator and mammalian orthologue of autophagocytosis factor Aut7p. *J. Biol. Chem.* 275, 25445-25450.
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- Stüven, E\*, Porat, A\*, Wieland, T.E., Elazar, E., & Helms, B.J. (2001) Intra-Golgi protein transport depends on a cholesterol balance in the lipid membrane. Submitted

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