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Mechanism of intracellular protein traffic

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Intracellular vesicle traffic is crucial for normal cell function in all eukaryotic cells, from yeast to human. Transport of proteins within the extensive network of membrane-bound compartments is highly regulated to ensure the specificity and efficiency of cargo delivery. Over the past five years, our laboratory has focused on two major issues: first, the regulation of intra-Golgi transport, aiming to understand in molecular terms the mechanism by which proteins are transported and sorted within this important organelle; and second, the mechanism of autophagocytosis, a relatively poorly understood membrane trafficking pathway, responsible for delivery cytosolic proteins and organelles for degradation within lysosomes. We pursue these goals by combining *in vitro* and *in vivo* systems in yeast and mammals.

Golgi fusion and GATE-16

The mammalian Golgi is typically represented as stacks of polarized, flattened cisternae, alternating with regions rich in vesicles and tubules. The Golgi apparatus receives newly synthesized proteins and lipids from the ER, modifies them as they move along the stacks from cis to trans, and finally sorts them to multiple intracellular and extracellular destinations. A well characterized system that reconstitutes transport of proteins between early Golgi cisternae was utilized to study the molecular basis of intracellular transport. Several soluble factors were isolated as essential factors in this assay. These include NSF, SNAP, p115, and most recently Golgi-associated ATPase enhancer of 16 kD (GATE-16); the latter is a 117 amino acid protein, predominantly localized to the Golgi apparatus and peripherally bound to membranes. We showed that GATE-16 enhances the ATPase activity of NSF by its attachment to NSF. NSF/α-SNAP, in turn, stimulates the recruitment of GATE-16 to the

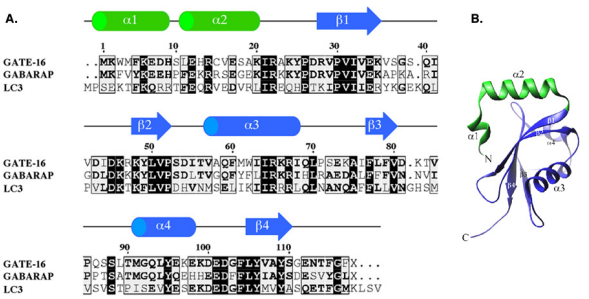

Fig.1 *(A) Sequence Alignment of mammalian GATE-16 homologues. (B) Ribbon presentation of the structure of GATE-16, based on its crystal structure, refined to 1.8 Å. The ubiquitin fold is colored cyan whereas the two additional N-terminal helices are colored green.*

unpaired GOS-28 in an ATP-dependent ATPase-independent manner, an interaction that protects the labile, unpaired GOS-28 from proteolysis.

We also demonstrated that the function of GATE-16 as a SNARE protector is essential not only for intra-Golgi transport, but also for the homotypic fusion of post-mitotic Golgi fragments. GATE-16 apparently plays a crucial role in this process, since its interaction with GOS-28 is essential to protect GOS-28 and regulate SNARE function. Thus, it seems that GATE-16 acts as a Golgi-SNARE protector in various physiological membrane fusion events. GATE-16 shares a high level of sequence identity with an expanding family of proteins that were implicated in various cellular processes associated with intracellular membrane trafficking. In yeast there is only one known homologue of GATE-16, Aut7/Apg8, which functions in membrane dynamics during autophagy. The linkage between GATE-16 and ubiquitin is evident from the crystal structure of GATE-16. The structure of GATE-16 refined to 1.8 Å shows a ubiquitin fold (the last 90 amino acids) with two additional N-terminal helices (Figure 1).

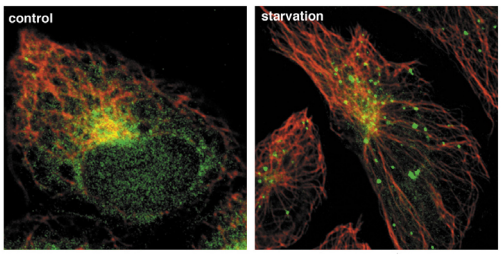

Fig.2 *GFP-LC3 is associated with autophagosomes upon nitrogen starvation. CHO cells stably transfected with GFP-LC3 were cultured for 2 h under normal growth conditions (Left panel) and in medium lacking amino acids (right panel). Red represent b-tubulin and green GFP. Vesicles stained with GFP in the left panel represent autophagosomes on their way to lysosomes.*

Autophagy and membrane trafficking

Autophagocytosis is a non-selective mechanism for sequestring of cytosolic proteins or organelles into lysosomes which is conserved from yeast to man. It operates constitutively, but can be induced under conditions of stress, in particular nutrient limitation. Autophagy vvoccurs through the formation of autophagosomes, which are specialized vesicles with double or multiple boundary membranes. During their formation, these vesicles engulf portions of cytosol or organelles such as mitochondria. The outer autophagosomal membrane fuses with the vacuole to release a single membrane-bound autophagic body into the vacuolar lumen. These autophagic bodies are subsequently degraded in a step that depends on active vacuolar lipases and other hydrolases present in the vacuolar lumen. We have recently characterized the dynamics and the cytoskeleton requirements of this process in CHO cells, utilizing GFP-tagged LC3, a specific and the only known marker for autophagosomes. We found that under normal growth conditions GFP-LC3 is predominantly localized in a perinuclear region near the microtubules organizing center and is associated, at least in part, with lysosomes located in this region. Induction of autophagy by nitrogen starvation results in the translocation of GFP-LC3 from the perinuclear region into autophagosomes within 30 – 40 minutes. These autophagosomes move along microtubules to dock and fuse with lysosomes within 15 minutes (figure 2). We show that GFP-LC3 distinct subcellular localization under different growth conditions is highly dependent on its C-terminal modification. In summary, our results indicate that under normal growth conditions LC3 exists in a defined complex in the vicinity of lysosomal membrane, resembling the pre-autophagic structure in yeast, and upon starvation, newly formed autophagosomes are delivered to lysosomes in a cytoskeletal dependent manner. We postulate that lysosomes may participate in part in autophagosomes biogenesis.

Selected Publications

Legesse-Miller, A., Sagiv, Y., Glozman, R., and Elazar, Z. (2000) Aut7p, a soluble autophagic factor, participates in multiple membrane trafficking processes. J Biol Chem, 275, 32966-32973.

Paz, Y., Elazar, Z., and Fass, D. (2000) Structure of GATE-16, membrane transport modulator and mammalian ortholog of autophagocytosis factor Aut7p. J Biol Chem, 275, 25445-25450.

Porat, A. and Elazar, Z. (2000) Regulation of intra-Golgi membrane transport by calcium. J Biol Chem, 275, 29233-29237.

Porat, A., Sagiv, Y., and Elazar, Z. (2000) A 56-kDa selenium-binding protein participates in intra-Golgi protein transport. J Biol Chem, 275, 14457-14465.

Sagiv, Y., Legesse-Miller, A., Porat, A., and Elazar, Z. (2000) GATE-16, a membrane transport modulator, interacts with NSF and the Golgi v-SNARE GOS-28. Embo J, 19, 1494-1504.

Muller, J.M., Shorter, J., Newman, R., Deinhardt, K., Sagiv, Y., Elazar, Z., Warren, G., and Shima, D.T. (2002) Sequential SNARE disassembly and GATE-16-GOS-28 complex assembly mediated by distinct NSF activities drives Golgi membrane fusion. J Cell Biol, 157, 1161-1173.

Elazar, Z., Scherz-Shouval, R., and Shorer, H. (2003) Involvement of LMA1 and GATE-16 family members in intracellular membrane dynamics. Biochim Biophys Acta, 1641, 145-156.

Scherz-Shouval, R., Sagiv, Y., Shorer, H., and Elazar, Z. (2003) The COOH terminus of GATE-16, an intra-Golgi transport modulator, is cleaved by the human cysteine protease HsApg4A. J Biol Chem, 278, 14053-14058.

Stueven, E., Porat, A., Shimron, F., Fass, E., Kaloyanova, D., Bruegger, B., Wieland, F.T., Elazar, Z., and Helms, J.B. (2003) Intra-Golgi protein transport depends on a cholesterol balance in the lipid membrane. J Biol Chem.

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