Understanding the process of intracellular protein transport whereby protein-carrying vesicles move across and between membranes is of fundamental importance in cell physiology and medicine. Autophagy is a unique membrane trafficking process essential for the major catabolic pathway by which eukaryotic cells degrade and recycle excess or defective macromolecules and organelles. This pathway is activated under environmental stress conditions as well as during certain developmental stages and has been linked to various pathological conditions. The main goal of our group is to understand the mechanism of autophagocytosis and characterize the relationship between this process and the secretory pathway while utilizing mammalian, plants and yeast systems.

We have recently reported that NSF, a major fusion factor participating in almost all intracellular processes, is attenuated under amino acid deprivation (1). This process is accompanied by the accumulation of SNARE complexes and inhibition of secretion. Our recent results indicate that such attenuation of NSF activity is essential for autophagy; in its absence the autophagic pathway is inhibited and cells do not survive even short starvation periods. Based on these findings we hypothesize that there are close relationships between the secretory pathway and autophagy, a subject we plan to explore further in the future.

Identification and characterization of the various regulatory factors that take part in intracellular protein transport is essential for better understanding of this complex process. Over the past few years we have been studying questions related to the molecular mechanism of intracellular membrane transport aiming to disclose and characterize relevant novel factors. On the basis of transport activity in a cell-free transport assay, we identified two novel soluble transport factors: a mammalian 16 kD protein, denoted GATE-16 (2), and SBP56 (3), a protein whose function was previously unknown. We found that GATE-16 interacts with components of the membrane fusion machinery and characterized these interactions. Moreover, we determined the three-dimensional structure of GATE-16 at 1.8 Å resolution (4), and identified two functional sites for protein-protein interaction (5). More recently we have shown that GATE-16 plays an important role in Golgi re-arrangement during mitosis by interacting with Gos-28, a pivotal Golgi SNARE molecule (6). In a recent collaboration with the laboratory of Graham Warren (a project funded by the Binational Science Foundation) we plan to determine the role of GATE-16 in different Golgi functions, including mitosis, and its possible role in autophagy.

GATE-16 belongs to a novel family of ubiquitin-like (UBL) proteins that have been implicated in a variety of cellular processes. Members of this family include LC3 (light-chain 3), originally identified as a subunit of the neuronal microtubule-associated protein complex, GABARAP (GABA receptor-associated protein), which was implicated in GABAA receptor trafficking and postsynaptic localization, and the yeast protein Atg8p, which plays a key role in secretion under control conditions and is essential for autophagy under starvation. Members of the UBL family undergo a unique conjugation process in which their C-terminal glycine is covalently attached to phosphatydilethanolamine by E1 and E2-like enzymes. This process is regulated by Atg4, a novel cysteine protease (7). We have recently found that the activity of Atg4 is attenuated under starvation conditions, thus providing, for the first time, an explanation for the characteristic increase in conjugated UBLs associated with this process. We further showed that this attenuation is redox-regulated in vitro and in vivo through formation and breakage of a disulfide bond between regulatory cysteines (8). These findings provide the first molecular link between autophagy and oxidative stress. In the future we expect to identify the molecules involved in this redox regulation.

We have recently utilized mammalian cells expressing GFP-LC3, a unique marker for autophagosomes, to determine the intracellular route(s) and dynamics of autophagy in living cells (9). We found that blocking of lysosomal acidification resulted in accumulation of autolysosomes both under control and starvation conditions. In this study we established...
an assay to monitor the average life-span of autophagosomes. Our findings showed that amino acid deprivation did not change the average life-span of autophagosomes but rather stimulated their rate of formation three-fold. We also demonstrated that autophagosomes are associated with microtubules and move along these tracks en route for degradation. Finally, we showed that the rate-limiting step in this pathway is fusion between autophagosomes and lysosomes, possibly due to the inhibition of NSF activity described above.

Future directions:

One of the main aims of our research is to find out the role played by ubiquitin–like proteins in the fundamental process of membrane fusion. These two lines of research have only recently been linked by the realization that the ATPases involved in membrane fusion events use ubiquitin or ubiquitin–like proteins as integral components of the process that fuses membrane bilayers. One particularly exciting possibility concerns the debate as to whether there are events downstream of SNARE-mediated interactions.

Our other major goal is to understand the relationship between the Golgi complex and stress-induced autophagy. We intend to focus on four different family members, namely Aut7p in yeast, and GATE-16, GABARAP and LC3 in mammalian cells, utilizing both in vivo and in vitro systems. The underlying hypothesis of our research is that while in the yeast Aut7p is essential predominantly for autophagocytosis, in mammals the GATE-16-related protein family members function in multiple intracellular membrane trafficking processes.

**Selected Publications**


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**Fig. 1** Autophagy in starved CHO cells during mitosis. Autophagosomes (green), lysosomes (red) microtubules (magenta) were imaged by confocal microscope.

**Fig. 2** Different stages of autophagy in the yeast Saccharomyces cerevisiae. Phagophore (left), autophagosome (middle), autophagic bodies at different fusion steps with the vacuole (right). Images were taken by high-pressure freezing followed by transmission electron microscopy.