

Regulatory mechanisms of membrane trafficking and their implications in human diseases

Intracellular membrane trafficking pathways are mediated by a network of proteins and lipids that coordinately function to efficiently and rapidly transport proteins, lipids, and sugars to their final cellular destinations. The tight regulation of membrane transport events is not only fascinating but also fundamental for normal cell function and survival. The goal of our studies is to elucidate, at a molecular level, regulatory mechanisms of membrane trafficking, and to extrapolate our knowledge to pathological situations involved in human diseases.

More specifically, our studies are focused on: (i) molecular mechanisms controlling lipid transport and their

influence on membrane trafficking events, cellular lipid homeostasis, and intracellular organelle functions, in particular, of the Golgi apparatus and the endoplasmic reticulum (ER), (ii) mechanisms controlling the Golgi-glycosylation machinery and their implication in human diseases known as Congenital disorders of glycosylation, (iii) mechanisms regulating membrane traffic in professional secretory cells, such as the insulin-producing pancreatic β cells, and their implication in diabetes.

Increasing lines of evidence suggest that lipid-transfer proteins (LTPs) play a critical role in regulation of membrane trafficking. Yet, the underlying

mechanisms of their action in intact cells remain largely unknown. We have previously found that the LTP, Nir2, which transfers phosphatidylinositol (PI)/ phosphatidylcholine (PC) between

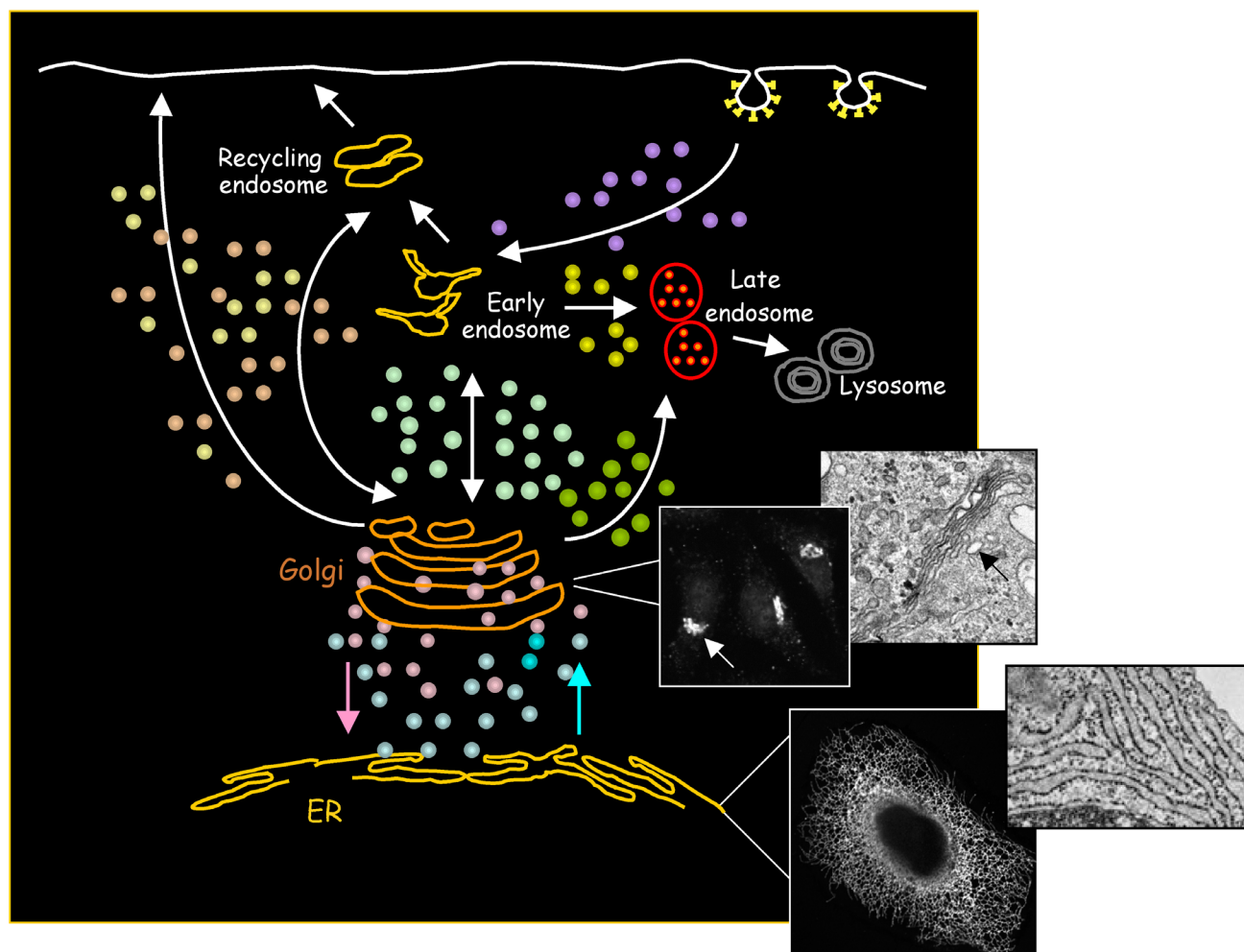


Fig. 1 The major membrane traffic pathways in eukaryotic cells.

The different organelles along the secretory and endocytic pathways are shown. The confocal images (inserts) demonstrate the localization of the VAP-B protein in the ER and of the COG complex in the Golgi apparatus. A stack of the Golgi complex as well as portion of the ER network are shown by EM micrographs.

membranes *in vitro*, is required for maintaining the structural and functional integrity of the Golgi complex by regulating the level of a key lipid, diacylglycerol (DAG), in this organelle (Litvak et al., 2005). DAG is a strongly conical component of the bilayer that induces membrane bending and the formation of highly curved intermediates, thereby facilitating membrane budding, fusion, and fission events (Lev, 2006). The level of DAG in the Golgi membrane can, therefore, directly affect Golgi-mediated transport events. Our finding that Nir2 is involved in maintaining of a critical pool of DAG in the Golgi, provides a novel mechanism for regulating membrane transport by PI/PC-transfer proteins, and demonstrates the interface between lipid homeostasis and Golgi secretory function.

Nir2 belongs to the highly conserved Nir/rdgB family of proteins (Lev, 2004). We originally isolated three human proteins of this family; Nir1, Nir2 and Nir3 (Lev et al, 1999), and have extensively studied the function of Nir2 in human cells (Litvak et al, 2002a, 2002b, 2004). More recently, we found that Nir2 interacts with the integral ER-membrane protein VAP-B. We isolated VAP-B as a Nir2-interacting protein using a proteomic approach (Amarilio et al, 2005), and further confirmed its interaction with the other two Nir proteins; Nir1 and Nir3. This interaction is mediated by a specific motif designated FFAT (double phenylalanine in an acidic tract). Interestingly, the FFAT motif has been identified in 17 distinct eukaryotic proteins, 14 of which are directly implicated in lipid-binding, -sensing or -transport, including homologs of oxysterol-binding protein (OSBP), homologs of ceramide transport protein (CERT), the Nir/rdgB proteins, and Opi1p, a transcriptional regulator of phospholipid synthesis in yeast. FFAT motif containing proteins are targeted to the ER by direct interaction with the integral ER membrane proteins of the VAP family; VAP-A and VAP-B.

The VAP are highly conserved proteins that have been identified in all eukaryotic organisms from yeast to

human. They have been implicated in regulation of a wide range of cellular processes including, membrane trafficking, lipid metabolism, the unfolded protein response (UPR), and microtubule organization (Lev et al, 2008). Recently, a single missense mutation within the human VAP-B gene, which substitutes a conserved proline residue at position 56 by a serine (P56S), was identified in three forms of familial motor neuron diseases (MNDs). This discovery has opened many questions related to the cellular functions of VAPs and the underlying mechanisms of VAP-B(P56S)-induced MNDs.

Our recent functional studies on VAPs revealed that they are involved in regulation of lipid-transport between the ER to the Golgi apparatus, possibly at the ER-Golgi membrane contact sites (MCSs), thereby affecting the lipid composition of the Golgi membranes and consequently their structural and functional properties (Peretti et al, 2008). MCSs are sites of close apposition between the ER membrane and other intracellular organelles that facilitate the transport of small molecules, such as Ca²⁺ or lipids, by a non-vesicular transport mechanism. Although MCSs have been identified in all eukaryotic organisms, their molecular composition and cellular function remain poorly understood. Nevertheless, LTPs containing dual targeting determinants for two different membrane compartments might efficiently transfer lipids at MCSs. For example, the LTPs, Nir2, OSBP and CERT, which interact with the Golgi membranes and with the ER membranes through their FFAT motif that binds VAPs, might transfer lipids at the ER-Golgi MCSs. Current ongoing studies are aimed at elucidating the molecular composition of ER-Golgi MCSs, the function of VAP proteins at these sites, as well as the molecular mechanisms of VAPs action in mammalian cells in general, and their cellular function(s) in pancreatic β cells, in particular.

Our basic interest in membrane trafficking pathways implicated in human diseases has prompted us to investigate the function of the

Conserved Oligomeric Golgi (COG) complex in membrane transport. The COG is a heterooctameric complex that has been implicated in regulating the transport, retention and/or retrieval of components of the Golgi-glycosylation machinery. Yet, the underlying mechanism of its action remains to be established. Recently, mutations in the genes encoding different subunits of the COG complex have been identified in several Congenital disorders of glycosylation (CDGs) patients. CDGs constitute a genetically heterogeneous group of diseases caused by deficient or increased glycosylation of glycoconjugates, such as glycoproteins and glycolipids. The phenotypic spectrum of these disorders ranges from severe to mild disease and from multi-system to mono-organ disease. The severe cases appear in the neonatal period, with a 20% lethality in the first year of life. Current ongoing studies are aimed at elucidating the mechanisms of COG action in mammalian cells and their involvement in CDGs.

Our studies cover a wide range of basic questions related to molecular mechanisms of membrane trafficking in mammalian cells. We apply multidisciplinary experimental approaches including, advanced biochemical and molecular biology technique, advanced imaging techniques using confocal and electron microscopy, and basic lipidology. We believe that our research will contribute to the understanding of the underlying mechanisms of certain human diseases and might lead to the development of new therapeutic approaches.

Selected publications

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Lev, S., Ben Halevy, D., Peretti, D., Dahan, N. (2008) The VAP proteins: from cellular functions to motor neuron diseases. Submitted

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