Introduction: Regulatory processes, an emerging feature in intracellular membrane traffic

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The subject of this volume is the molecular mechanism of the intracellular membrane trafficking, a central eukaryotic cell biological process. In the post genomic era, essential molecules involved in intracellular membrane/protein transport are emerging with increasing pace. The present challenge is to compile the molecular networks that govern these processes. Understanding of regulatory processes and participating molecules are likely to reveal global cellular regulatory circuits that couple membrane trafficking with other cellular functions. The part of the membrane transport machinery, which forms stabile protein complexes is rather well known already. However, the regulatory mechanisms that link these more stabile complexes to other cellular functions are only starting to emerge. This book focuses on the regulatory aspects of this process.

Continuous intracellular membrane traffic is needed for growth and maintenance of the compartmental organisation of eukaryotic cells. This trafficking is carried out by an elaborate and highly dynamic tubulo-vesicular network that ensures transport of membrane components, proteins, and lipids from their site of synthesis to their site of function. The biosynthetic pathway, also called protein secretion pathway and exocytotic pathway, leads from endoplasmic reticulum (ER) to cell surface and to the endocytotic compartments via the Golgi complex (Fig. 1). Proteins destined to plasma membrane and to the endocytotic compartments as well as those secreted to cell exterior are first translocated to the ER. In the ER, proteins undergo several types of posttranslational modifications and are folded with the help of a number of chaperones and foldases. The properly folded and oligomerised protein molecules are packaged into transport vehicles in the form of membrane vesicles that are targeted to the next station, the Golgi complex. Fusion of the vesicles with the Golgi membranes releases the proteins into the Golgi lumen, where they may undergo further modifications. The trans-Golgi network is the major sorting compartment in which the proteins are packaged into vesicles that are targeted either to the plasma membrane or to the endocytotic/lysosomal compartments. The endocytotic/lysosomal pathway is used to transport material into the cell to the endosytic compartments and to lysosomes. Basically, the same membrane transport mechanisms operate in all eukaryotic cells from yeast to mammalian neurons and at the different transport steps within the cell. Exocytosis of neurotransmitter-loaded transport vesicles bears striking similarity with molecular mechanisms of transport vesicle fusion in other cell types and even in unicellular eukaryotes like yeast. However, neurotransmitter exocytosis of synaptic plasma membrane associated vesicles is evoked by plasma membrane influx of calcium, which is triggered by membrane depolarisation. In consequence, neuronal exocytosis involves calcium-binding synaptotagmin proteins. For synaptotagmins homologues have not been identified, for example, in

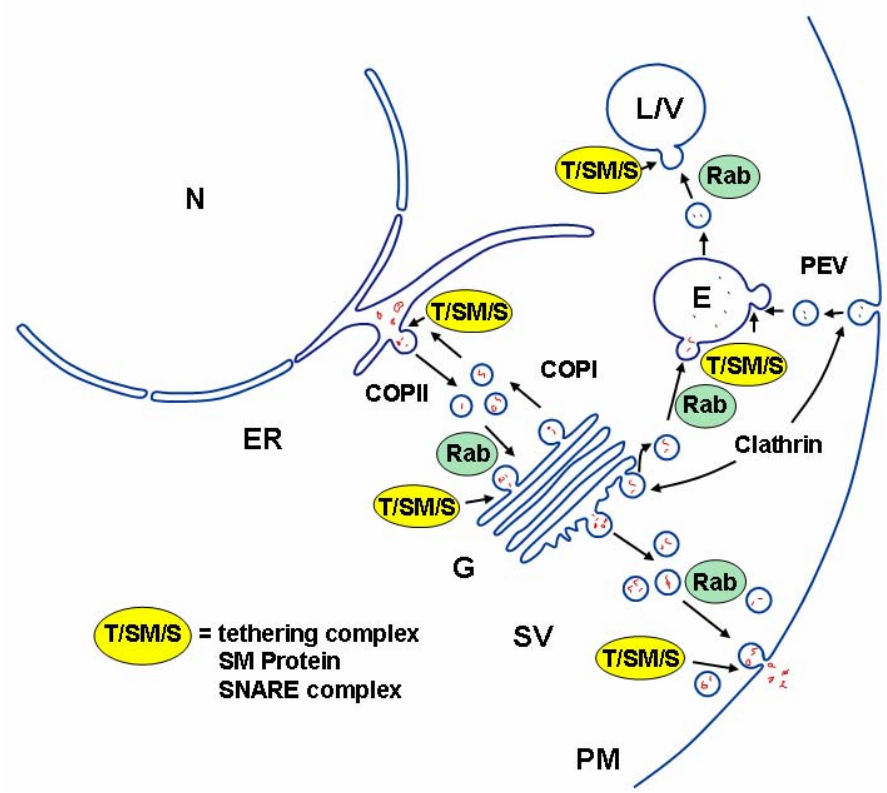

Fig. 1. Schematic presentation of the secretory pathway. Transport of proteins from ER to plasma membrane and the endosomal compartments via the Golgi complex is depicted to occur in vesicles that are budding from the donor membrane and targeted to and fused with the acceptor membrane. The sites of function of the coat complexes (COPI, COPII and Clathrin) as well as those of the Rab proteins, tethering complexes (T), SM proteins (SM), and the SNARE complexes (S) are indicated. ER: endoplasmic reticulum; G: Golgi complex; SV: secretory vesicle; PM: plasma membrane; E: early and late endosomes; L/V: lysosomal-vacuolar compartment; N: nucleus.

yeast cells. Although *in vitro* reconstitution experiments in yeast have shown Ca^{++} dependence of vesicle fusion, the Ca^{++} dependent regulation in non-neuronal cells has not yet been studied in detail.

The basic principle of the biosynthetic or secretory pathway was first proposed by Palade (1975). The outlines of the secretory pathway were elucidated in the late 1970's and early 1980's partially with the help of yeast mutants defective in protein secretion and partially with biochemical and morphological methods in yeast and animal cells. In both systems, *in vitro* reconstitution experiments proved to be excellent tools in dissecting transfer of proteins from one compartment to another. Instrumental for the mapping of different intracellular transport pathways was the pioneering yeast genetic work by P. Novick and R. Schekman for the secretory

pathway and by S. Emr and T. Stevens for the endocytic pathway (Novick and Schekman 1979; Bankaitis et al. 1986; Rothman and Stevens 1986). This, combined with the rigorous biochemical approaches pioneered by Rothman and coworkers in mammalian cells, resulted in a very rapid increase in the number of molecules active in transport and in their functional role in this process. Genetic screens are biased for essential genes, thus, being often unable to reveal the role of functionally redundant genes or non-essential regulatory molecules. Biochemical work, on the other hand, is biased toward isolation and characterisation of sufficiently stable protein complexes that withstand the experimental protocols. Nevertheless, extensive research employing a multitude of elegant genetic and biochemical approaches during the following years, culminated in a model for the transport mechanism, vesicle budding from the donor membrane followed by targeting and fusion with the acceptor membrane, which was introduced by Rothman (1994). Fine-tuning during the past ten years has resulted in a more detailed understanding of the transport pathways and the molecular machineries performing various tasks along it. Subsequent structural work on single components and even protein complexes are now bringing our knowledge of the stable protein complexes to a new level.

Vesicle budding

Transfer of proteins and membrane components from one compartment to another starts with vesicle budding from the donor membrane. A widely held view is that specific coat proteins and adaptors are instrumental in this process by aiding the vesicle budding and selecting the cargo for the vesicles. Clathrin was identified as the first vesicle coat protein. It is involved both in formation of endocytic vesicles at the plasma membrane and in vesicle formation at the trans-Golgi network. As described in the review by B. Ritter and P.S. McPherson, during the past ten years, the relatively simple model of clathrin mediated membrane budding has changed dramatically. In addition to clathrin and its adaptor protein AP2, a large number of accessory proteins have been identified that are involved in the process. The lack of suitable genetic approaches has limited the experimental approaches in mammalian cells in the past. Now, the situation has changed when the loss-of-function approaches have become possible with the introduction of the RNAi techniques. Among other modern methods, comparative genomics in the form of identification of sequence motives, and proteomics approaches have been helpful in identifying new components in this process. Non-clathrin coats, COP I and COP II, play an important role in membrane transport between the ER and the Golgi complex. Unfortunately, the chapter that dealt with these processes, transport from ER and the retrograde transport, was cancelled at such a late stage in the preparation of this book that it was not possible to get a substitution for the chapter. However, some of the other authors in this volume discuss aspects of these processes. In addition, there are some excellent recent reviews on this subject, for example, by Haucke (2003), Barlowe (2003), and Duden (2003).

Although the role of coat complexes in membrane budding is well established, there are numerous examples of membrane budding in different cellular compartments without the coat proteins. The important structural role of the lipids in vesicle formation by regulating the membrane curvature is an important aspect of membrane functionality in transport processes and is described by J.C.M. Holthuis. Certainly, lipid dynamics and their physico-chemical properties are of utmost importance in intracellular membrane traffic. In the future, it will be a great challenge to develop efficient ways to visualise lipids *in vivo* and to study lipid-protein and lipid-lipid interactions. These approaches in combination with proteomics techniques, will open completely new ways of understanding the dynamisc of membrane transport.

Vesicle targeting – regulation by Rab GTPases

Small GTP binding proteins are ubiquitous regulators of different cellular functions. The basic principle of this mode of regulation is cycling of the GTPases between GTP bound active and GDP bound inactive form of the proteins. The rab GTPases have been known to be involved in membrane transport since the 1980's when Salminen and Novick (1987) found that yeast Sec4 protein, essential for protein secretion in yeast, is a member of this protein family. The detailed functions of rab proteins are still poorly understood. Rab proteins function in all intracellular transport steps and regulate vesicle tethering and fusion, cargo selection, and cytoskeleton-dependent organelle transport. A great number of Rab accessory proteins, Rab activators, and downstream effectors have been identified but their role is even less understood than the role of the Rab proteins themselves. C.G. Burd and R.N. Collins described the current state of the art. They discuss the future challenges, which include; analysis of the diversity of specific functions of individual Rab proteins and their auxiliary factors, connection of Rab signalling with other signalling networks, for example, cell growth and differentiation and the roles of the uncharacterized Rab proteins.

Tethering complexes

Targeting of the transport vesicles to the correct intracellular localization is essential for biosynthesis and maintenance of the cell architecture. A commonly held view is that the specificity of targeting is provided by the so-called tethering factors, which can be long coiled-coil proteins or large multiprotein complexes. It is believed that the tethering factors can connect the vesicle from certain distance to the site of the following fusion event involving the SNARE proteins. W. Guo and S. Chu give a detailed account of the present knowledge on various tethering proteins and their proposed functions at different intracellular organelles and at plasma membrane. Most extensively studied tethering complexes are the TRAPP

complex mediating targeting of the ER derived vesicles to the Golgi complex and the Exocyst complex that targets Golgi-derived vesicles to plasma membrane. Both of these complexes were first identified in yeast and were later found also in mammalian cells, and were shown to have a similar function there. The tethering complexes interact with Rab proteins and in some cases have been shown to function as their downstream effectors. There are indications that the tethering proteins may have other functions in addition to the tethering *per se*. Such functions include, for example, cargo sorting and acceleration of the assembly of correctly paired SNARE proteins. It has also been proposed that one Exocyst component, Exo84, is involved in mRNA splicing. Mutations in some Exocyst subcomponents display defects in transport steps other than exocytosis suggesting that tethering components may be participating in tethering or other membrane transport functions at more than one intracellular location. Finally, recent data suggests that at least some components of the Exocyst complex interact with the ER translocation machinery and, thereby, regulate the efficiency of translation of secreted and membrane proteins suggesting a regulatory circuit adapting the translation efficiency with the cells secretory capacity. Clearly, future studies are needed to dissect all the interactions of the tethering complexes with several other cellular functions in addition or in connection to the tethering events.

SM proteins

Similar to the Rab proteins, the Sec1/Munc18 (SM) proteins participate in each targeting/fusion step inside the cell. The SM proteins are implicated in regulation of the SNARE complex formation or function at the target membrane by binding to the target membrane SNAREs. Genetic data suggest a positive role for SM-family proteins in membrane traffic regulation. However, numerous *in vitro* studies have suggested a negative regulatory role for these proteins. Thus, in spite of a multitude of experimental approaches, the SM proteins have resisted attempts to their detailed functional analysis and these controversial results have made it difficult to form a model for SM protein function. The review by M. Kauppi, J. Jäntti, and V. Olkkonen brings together the present knowledge in this field. Structural data available for some SM and SNARE proteins have revealed a remarkable diversity in the mode of SM protein binding to the cognate t-SNARE proteins. There seems to exist at least four different modes of binding between these two proteins. Also, SNARE-independent binding of Munc18 to plasma membrane has been shown to take place in epithelial cells. A model is emerging which takes into account non-SNARE proteins as regulators of the SM protein function. Candidates for such proteins are, for example, the tethering complex components and the Rab proteins as well as other less well-characterised proteins like the Mint proteins in mammalian cells. It is likely that future studies will reveal currently unidentified proteins in association with SM proteins in membrane traffic regulation.

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