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Membrane biogenesis: from mechanism to disease

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Summary

The biogenesis of membranes involves the continous flow of proteins and lipids which are selectively targeted to or retrieved from specific compartments within eukaryotic cells. While some diseases are caused by the impairment of particular protein transport pathways or mislocalization of a certain protein others may be related to altered signal transduction cascades resulting from defective endocytosis of plasma membrane receptors or other membrane trafficking defects. The implications of this hypothesis for our understanding of the proper functioning of a eukaryotic cell and for the treatment of human diseases are being discussed.

I. Overview

Unlike bacteria, eukaryotic cells are elaborately subdivided into membrane-bounded, structurally and functionally distinct compartments. Each of these organelles contains a specific set of proteins, lipids and other molecules which enables them to fulfill characteristic functions within the cell. On average, the membrane-bounded compartments together occupy nearly half the volume of a cell, and about one third of all proteins within a eukaryotic cell are membrane proteins. Thus, membrane biogenesis and organelle maintenance are major tasks which are essential for all eukaryotic cells (Palade, 1975).

During the past two decades it has become clear that a number of inherited metabolic and neurological disorders result from the mistargeting of particular proteins to an incorrect destination within the cell. As an example for this class of diseases I will describe a number of disorders resulting from defective peroxisome biogenesis.

Other pathological states pertaining to membrane trafficking however may arise from autoimmune impairment of cells expressing a particular antigen or from genetic defects resulting in the generation of an abnormal protein as exemplified by the deposition of β-amyloid protein in brains of patients suffering from Alzheimer's disease. In the second part of this chapter I will, therefore, focus my discussion on the trafficking of membranes at the nerve terminal in normal and certain pathological states.

II. Peroxisome biogenesis & dysfunction

A. How peroxisomes are formed

Peroxisomes are ubiquitous eukaryotic organelles which are involved in a variety of metabolic processes such as the scavenging and destruction of peroxides, the β-oxidation of fatty acids and the biosynthesis of ether lipids. However, unlike mitochondria and chloroplasts they do not contain their own DNA and like most other intracellular membranes cannot be formed *de novo*. Biologists and physicians alike have become increasingly interested in the biogenesis of these organelles since Goldfischer reported in 1973 that patients with the cerebro-hepato-renal syndrome Zellweger's disease lacked demonstrable peroxisomes. Until now the number of peroxisomal biogenesis disorders (PBD) has grown to sixteen which fall into eleven different complementation groups. In order to learn more about the molecular basis of these diseases investigators have studied the way by which peroxisomes import their constituent proteins from the cytosol using both mammalian cell cultures and yeast as model systems.

Protein targeting to the peroxisomal matrix is mediated by evolutionary conserved peroxisomal targeting signals (PTSs) which bind to specific PTS receptors as depicted in **Figure 1**. The majority of peroxisomal matrix proteins carries a C-terminal tripeptide (SKL or closely similar) termed PTS1. PTS2 is a conserved N-terminal nonapeptide (R/K) (L/V/I) (X_5) (H/Q) (L/A) and is used by a smaller subset of matrix proteins.

Other internally located PTSs have been identified but, as with the targeting signals of peroxisomal membrane proteins, no consensus sequence has been found (Rachubinski & Subramani, 1995).

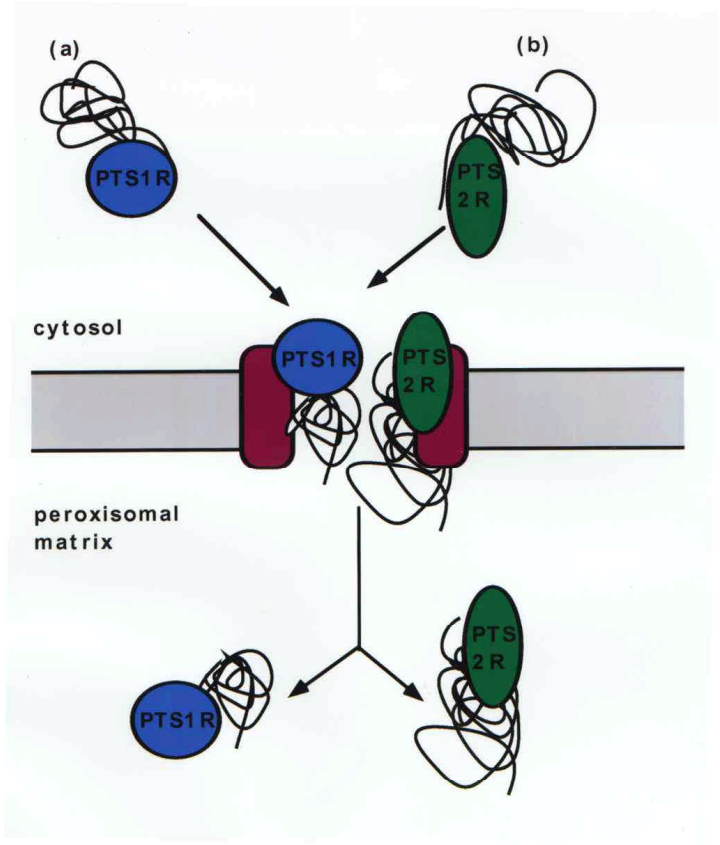

Figure 1: Hypothetical model for how proteins get imported into peroxisomes.

(**a**) Import of proteins containing the PTS1 signal for targeting to the peroxisomal matrix. PTS1R, receptor for PTS1 containing proteins.

(**b**) Import of proteins containing the PTS2 signal for targeting to the peroxisomal matrix. PTS2R, receptor for PTS2 containing proteins.

Components of a putative import channel across the peroxisomal membrane (gray) are indicated by purple rectangles.

Yeast and human cells selectively deficient in the PTS1 or PTS2 import pathway have been used to identify the PTS receptors (PTSR). PTS1 signals are recognized by the PTS1R now collectively referred to as Pex5p, a tetratricopeptide repeat (TPR) protein of 64-69 kDa. It is still unclear whether this protein is localized to the cytoplasm, the outer face of the peroxisomal membrane, or the peroxisomal matrix (van der Leij et al., 1993; Dodt et al., 1995; Szilard et al., 1995; Wiemer et al., 1995). Independent reports from three different laboratories now suggest that the src-homology domain 3 (SH3 domain) of the peroxisomal membrane protein Pex13p functions as a docking site for the mobile cytosolic PTS1R Pex5p to facilitate the delivery of PTS1 containing proteins (Elgersma et al., 1996; Erdmann & Blobel, 1996). PTS2 signals are recognized by the PTS2R termed Pex7p. Again it is unclear whether this protein resides in the cytosol or the peroxisomal matrix (Zhang & Lazarow, 1995). The clinical documentation of a series of similar human peroxisomal disorders (i.e. Zellweger syndrome, neonatal adrenoleukodystrohy, rhizomelic chondrodysplasia punctata (RCDP) etc.) has led to the identification of the human homolog of PTS1R (Dodt et al., 1995; Wiemer et al., 1995). The PTS1 and PTS2 pathways may be linked through a direct interaction between the tetratricopeptide repeat (TPR) region (a recently identified protein-protein interaction motif) of PTS1R and the WD40 repeats (another distinct protein-protein interaction motif) of PTS2R although rigorous biochemical evidence for such an interaction has not yet been reported (Rachubinski & Subramani, 1995).

Unlike most other protein translocation systems peroxisomes are capable of importing stably folded (Walton et al., 1995) or even oligomeric proteins (Glover et al., 1994; McNew & Goodman, 1994). How these proteins actually cross the membrane is unknown. One possibility is that peroxisomes contain very large pores, but no experimental evidence for the existence of such pores has been reported. Alternatively, some form of pino- or endocytosis at the peroxisomal membrane might be involved in the protein transport process. It is also possible that most peroxisomal proteins are imported into as yet unidentified peroxisomal precursors and that peroxisomes are derived from these precursors by maturation. We also know very little about the energetics of protein transport into peroxisomes although ATP hydrolysis is required for the import of proteins into the matrix (Subramani, 1996). Thus, protein translocation into peroxisomes turns out to obey somewhat different rules than the protein translocation systems of mitochondria (Haucke & Schatz, 1997) or the endoplasmic reticulum (Rapoport et al., 1996).

B. Human peroxisomal disorders

Human peroxisomal biogenesis disorders occur with a relatively high frequency of about 1/50 000 live births and are a genetically heterogenous group of autosomal, recessive, lethal diseases that fall into at least eleven distinct complementation groups as identified by cell fusion complementation analysis. Twelve out of the sixteen PBDs known to date are associated with severe neurological disability while even patients suffering from the remaining PBDs show some sort of neurological defect. These disorders can be grouped into three different classes, A,B, and C, according to the molecular defect leading to the disease. In group C the subcellular localization or activity of a single peroxisomal protein or enzyme is compromised. These disorders usually show the least severe phenotype. Patients with group A or B disorders often exhibit the presence of non-functional peroxisome ghosts which miss a few or many peroxisomal matrix proteins due to deficiencies in either the PTS1 or PTS2 or both protein import pathways (Rachubinski and Subramani, 1995). We will now turn to a more detailed analysis of the defects associated with these classes of disorders.

1. Zellweger Syndrome

Zellweger syndrome (ZS), a rare fatal disorder in newborn infants was originally described by Goldfischer et al. in 1973. It is an inherited metabolic disease associated with a number of cerebral, hepatic and renal defects and belongs to group A of the peroxisomal biogenesis disorders. Cells isolated from ZS patients have peroxisome ghosts lacking many peroxisomal matrix proteins and these patients show elevated levels of very long-chain fatty acids and are deficient in plasmalogens (ether lipids). ZS is the most severe PBD known to date and is invariably fatal. A number of similar diseases such as neonatal adrenoleukodystrophy and infantile refsum disease have been described all of which show a related but less severe phenotype compared to ZS.

It appears that a number of mutations can lead to ZS and cells belonging to these various complementation groups show differences in their capability of importing proteins via either the PTS1 or PTS2 pathways. Cells from patients in complementation group2 with ZS have mutations in their PTS1 receptor gene. Elegant studies *in vitro* have shown that the human PTS1 receptor can complement the protein import defect in these cells suggesting that the mutated PTS1 receptor is indeed the cause for the disease (Dodt et al., 1995; Wiemer et al., 1995). Thus, gene therapeutic approaches may soon provide means of treating this horrible disease.

2. Rhizomelic chondrodysplasia punctata

Rhizomelic chondrodysplasia punctata (RCDP) is a rare autosomal recessive phenotype associated with complementation group 11 of the peroxisome biogenesis disorders and is characterized by severe growth failure, profound developmental delay, cataracts, rhizomelia, and a severe deficiency in plasmalogens (Braverman et al., 1997). Cells from RCDP patients are unable to import peroxisomal thiolase, an enzyme targeted to peroxisomes via the PTS2 pathway.

Recently, the molecular defects leading to RCDP have been elucidated. Analysis of cells from RCDP patients have revealed a number of mutations within a single gene with homology to the yeast PTS2 receptor (Baverman et al., 1997; Motley et al., 1997; Purdue et al., 1997). Subsequent cloning identified this gene as the human PTS2R, Pex7 (Braverman et al., 1997; Motley et al., 1997; Purdue et al., 1997). Expression of human Pex7 in RCDP cells rescues PTS2 targeting and restores the activity of dihydroxyacetone-phosphate acyltransferase, a peroxisomal enzyme of plasmalogen synthesis (Purdue et al., 1997).

The two pathways of protein import into peroxisomes may however not be completely separate since several ZS patients with defective PTS1 receptors also show reduced amounts of PTS2 targeted enzymes. Moreover, an isoform of the human PTS1 receptor Pex5 is required for the efficient import of PTS2 targeted proteins and the tetratricopeptide repeats (TPR) of Pex5 directly interact with the WD40 domain of Pex7 in the two-hybrid system (Braverman et al., 1997). It is therefore possible that the molecular defects associated with some complementation groups of PBDs may result from a mutant receptor which not only is unable to bind its import substrates but may additionally fail to associate or cooperate with the other PTS receptor protein resulting in multiple import defects.

3. Peroxisome-to-mitochondrion mistargeting

An interesting example of a group C peroxisome biogenesis disorder which is caused by the mistargeting of a single peroxisomal protein is represented by primary hyperoxaluria type 1 (PH1). PH1 is an autosomal recessive disease associated with a normally occuring P11L polymorphism and a PH1-specific G170R mutation in the gene encoding for the homodimeric enzyme alanine:glyoxylate aminotransferase 1 (AGT) (Leiper et al., 1996). The P11L substitution creates an amino-terminal mitochondrial targeting signal which competes with its carboxy-terminal peroxisomal import signal and in vitro is sufficient to direct the protein into mitochondria. This mutation alone does not interfere with the peroxisomal targeting of AGT in living cells. AGT containing both mutations, however, is mistargeted to mitochondria both in vitro and in vivo. Recent work has now shed light on this phenomenon: the G170R mutation abolishes the ability of the protein to form homodimers in the cytosol and thereby prevent its mistargeting to mitochondria which are unable to import fully folded or dimeric proteins (Haucke and Schatz, 1997). Thus, mistargeting is due to the unlikely occuring polymorphism that generates a functionally weak mitochondrial targeting signal and a disease-specific mutation which, in combination with the polymorphism, inhibits AGT dimerization and therefore allows the protein to cross the mitochondrial membranes.

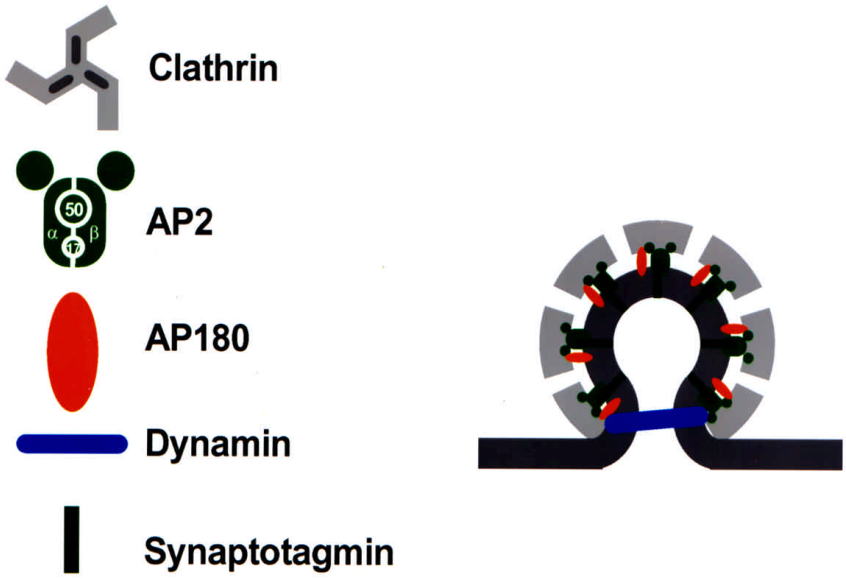

Figure 2: Components involved in the formation of a clathrin-coated bud that mediates synaptic vesicle endocytosis. The individual proteins are indicated by differentially colored symbols.

III. Membrane trafficking at the nerve terminal & disease: a putative link between synaptic vesicle endocytosis and the biology of cancer

I will now turn to the description of the mechanism by which synaptic vesicles are retrieved and recycled at the plasma membrane and discuss a number of recent observations which suggest that endocytosis, and by extrapolation also other membrane trafficking events may play an important role in regulating signal transduction pathways which in turn are intimately linked to the biology of cancer, Alzheimer's disease and other major human diseases.

A. The synaptic vesicle cycle

Synaptic vesicles (SV) are specialized secretory organelles involved in synaptic transmission in the nervous system. Upon stimulation SVs dock and fuse with the plasma membrane and release their content into the synaptic cleft. Membrane fusion occurs by a closely similar mechanism from yeast to neurons, and is mediated by specific pairing of SNARE proteins on the two membranes undergoing fusion (Ferro-Novick and Jahn, 1994). Following exocytosis, SV membranes are retrieved and reused for the generation of new SVs. This entire cycle occurs with high specificity and can be very rapid (less than one minute) (Ryan, 1996).

The most widely accepted model for how SVs are being regenerated proposes that SVs are retrieved through clathrin-mediated endocytosis (Cremona and De Camilli, 1997) involving a coat complex consisting of the heavy and light chains of clathrin, the plasma membrane-specific adaptor complex AP2 (a heterotetramer composed of α,β, μ and σ subunits) and the accessory protein AP180.

The importance of clathrin coats in SV endocytosis has recently been corroborated by genetic studies in Drosophila and C. elegans. It is still unclear what recruits this complex to the membrane, but one possibility is that synaptotagmin (Zhang et al., 1994), an abundant protein of SVs may facilitate this process by interacting with the AP2 adaptor. However, both AP2 and AP180 have been found to interact directly with membrane phosphoinositides (PIs) indicating that both lipid and protein may participate in anchoring the coat to membranes.

Vesicle fission of the mature coated bud is then effected by the recruitment and oligomerization of the GTPase dynamin to the stalk of endocytic pits. Upon hydrolysis of GTP dynamin disassembles and the clathrin-coated vesicle pinches off to eventually re-enter the pool of SVs awaiting a stimulus for another round of exocytosis. This step may be aided or regulated by the inositol 5-phosphatase synaptojanin (Mc Pherson et al, 1996), which is selectively concentrated in nerve terminals in association with endocytic intermediates of SV membranes.

Recent evidence suggests that the SH3 domain of the nerve terminal phosphoprotein amphiphysin I (Bauerfeind et al, 1997), together with its partner protein amphiphysin II plays an important role in recruiting dynamin to the invaginated endocytic pit (Shupliakov et al., 1997; Wigge et al., 1997). Through its affinity for both, dynamin and the adaptor AP2, amphiphysin may link the assembly of the clathrin coat to the formation of dynamin rings, thereby coordinating these two events leading to the generation of calthrin coated vesicles (David et al., 1996; Ramjaun et al., 1997; Wigge et al., 1997).

B. A putative link between endocytosis and the biology of cancer

Amphiphysin I is a neuron-specific protein which was originally found as a component associated with SVs (Lichte et al., 1992) and as the autoantigen in a subgroup of patients suffering from Stiff-man syndrome (SMS) (De Camilli et al., 1993). Stiff-man syndrome is a rare disease of the central nervous system characterized by painful spasms of limbs, trunk and abdominal muscles (Layzer, 1988). Group II patients are characterized by the presence of autoantibodies against amphiphysin I and all suffer from breast cancer (Folli et al., 1993). In an effort to elucidate the connection between amphiphysin I autoimmunity and cancer Floyd et al. (submitted for publication) have analyzed the expression of amphiphysin I in breast cancer tissues. Amphiphysin I was present as an alternatively spliced, overexpressed 108 kDa isoform in several breast cancer tissues and as two 128 and 108 kDa forms in the breast cancer of a SMS patient. Although it is not yet clear whether the high amphiphysin expression level is directly linked to the enhanced proliferation of the malignant cells, the observation that amphiphysin I is overexpressed in some forms of cancer supports the idea that amphiphysin family members play a role in the biology of cancer cells. It is well conceivable that overexpression of a mutant protein involved in endocytosis could alter signaling cascades initiated by endocytosed plasma membrane receptors and could thereby lead to tumorigenesis as described below.

Another link between endocytosis and signal-dependent cell proliferation has recently emerged from studies in transfected mammalian cells. First, inactivation of the clathrin- and dynamin-dependent uptake of the receptor for epidermal growth factor (EGFR) by overexpressing a mutant form of dynamin leads to enhanced proliferation of these endocytosis-defective cells (Vieira et al., 1996). The altered proliferative response is presumably due to the hyperphosphorylation of a subset of EGF-dependent signal transducing molecules suggesting an important role for EGFR signaling in establishing and controlling specific signaling pathways.

Second, Grb2, an SH3-SH2-SH3 domain containing protein involved in transducing signals from growth factor receptors (i.e. EGFR) to the Ras pathway upon stimulation with EGF transiently associates with dynamin, a GTPase involved in vesicle fission from the plasma membrane (as described above) (Wang and Moran, 1996). The transient interaction between dynamin and Grb2 is required for the internalization of the EGFR as microinjection of a peptide corresponding to the Grb2 SH3 domain blocks endocytosis. Thus, activation and termination of EGF signaling appear to be regulated by the diverse interactions of Grb2 with either signal transducing or endocytic components providing another link between endocytosis and the attenuation of signal transduction events from the plasma membrane.

IV. Perspectives

The examples described in this article are just some out of a growing number of studies on how mislocalization of certain proteins due to genetic alterations either in the protein itself or in its targeting machinery or perturbation of membrane trafficking pathways may lead to disease. Although many of the described connections between membrane traffic, complex inherited disorders, signal-mediated growth control, and pathogenesis remain mechanistically poorly understood accumulating evidence suggests that the biogenesis of membranes and the trafficking of organelles and molecules within the cell may be intimately linked to the regulatory and signal transduction networks governing the physiological state of a cell. A better understanding of this crosstalk might eventually lead to improved treatments for today's diseases including cancer, Alzheimer's disease, diabetes and others.

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