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Minireview

Biogenesis of lysosomal membranes

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Abstract

Lysosomal membrane glycoproteins are highly glycosylated proteins decorating the luminal surface of lysosomal membranes. Their biosynthetic route from the rough endoplasmic reticulum to the lysosomal compartment has been elucidated during recent years. Signals for intracellular sorting have been identified and characterized. The function of these proteins remains to be determined. Besides resident proteins the lysosomal membrane harbours at least one transient passenger, lysosomal acid phosphatase, which is sorted as a membrane-bound precursor like resident lysosomal membrane proteins and liberated from the membrane by limited proteolysis upon arrival in dense lysosomes.

Key words: Lysosomal membrane glycoprotein; Lysosomal acid phosphatase; Lysosome; Intracellular targeting; Sorting signal

1. Introduction

Lysosomes are membrane-bound organelles with an acidic internal milieu containing hydrolytic enzymes for degradation of proteins, lipids, nucleic acids and saccharides. Lysosomes are the principle site of intracellular digestion [1]. Biological materials destined for degradation in the lysosomes are either ingested by endocytosis and shuttled to the lysosomes via early and late endosomes or large particles are taken up by phagocytosis into specialized cells such as macrophages resulting in the formation of phagolysosomes [2]. In a process called autophagy, obsolete endogenous cellular structures are transfered to and degraded in the lysosomal compartment [3].

The formation of lysosomes requires an input both from the biosynthetic and the endocytic pathway. Newly synthesized lysosomal hydrolases are sorted in the *trans*-Golgi network by binding to mannose 6-phosphate specific receptors and are selectively transported to the endosomal compartment where uncoupling of ligand and receptor occurs. In the endosomes the newly synthesized lysosomal enzymes encounter the molecules on the endocytic route destined for degradation. Degradative processes are likely to occur already in endosomes. From this compartment the lysosomal enzymes and their substrates are delivered to the lysosomes where degradation is completed [4,5]. At present there is no consensus about the molecular mechanisms of formation of lysosomes. One model is based on preexisting late endosomes and lysosomes communicating via transport vesicles [6]. The alternative model postulates a gradual maturation of early endosomes to late endosomes and subsequently to lysosomes by processes of continuous fusion and fission of vesicles [7].

The membrane limiting the lysosomal compartment has multiple functions. It is responsible for acidification of the interior and sequestration of the highly active lysosomal enzymes capable of destructing cellular structures [2]. Furthermore the lysosomal membrane mediates the transport of degradation products from the lysosomal lumen to the cytoplasm and regulates the fusion and fission events between lysosomes themselves and other organelles [8,9]. In recent years several integral membrane glycoproteins of the lysosomal membrane have been described. The present minireview intends to compare these lysosomal membrane glycoproteins, their biosynthetic routes and the molecular mechanisms of their targeting.

2. Resident and transient lysosomal membrane glycoproteins

Burnside and Schneider [10] first showed in 1982 that the polypeptide composition of lysosomal membranes differs distinctly from the pattern of proteins in the plasma membrane. The investigations revealed glycoproteins with molecular weights between 90 and 110 kDa as major structural components of the lysosomal membrane. These results were confirmed and extended with a highly purified preparation of lysosomes from CHO cells. In this study glycoproteins with a molecular weight between 100 and 120 kDa were comprised for about 50% of the lysosomal membrane proteins [11]. Several glycoproteins of the lysosomal membrane have meanwhile been defined by monoclonal and polyclonal

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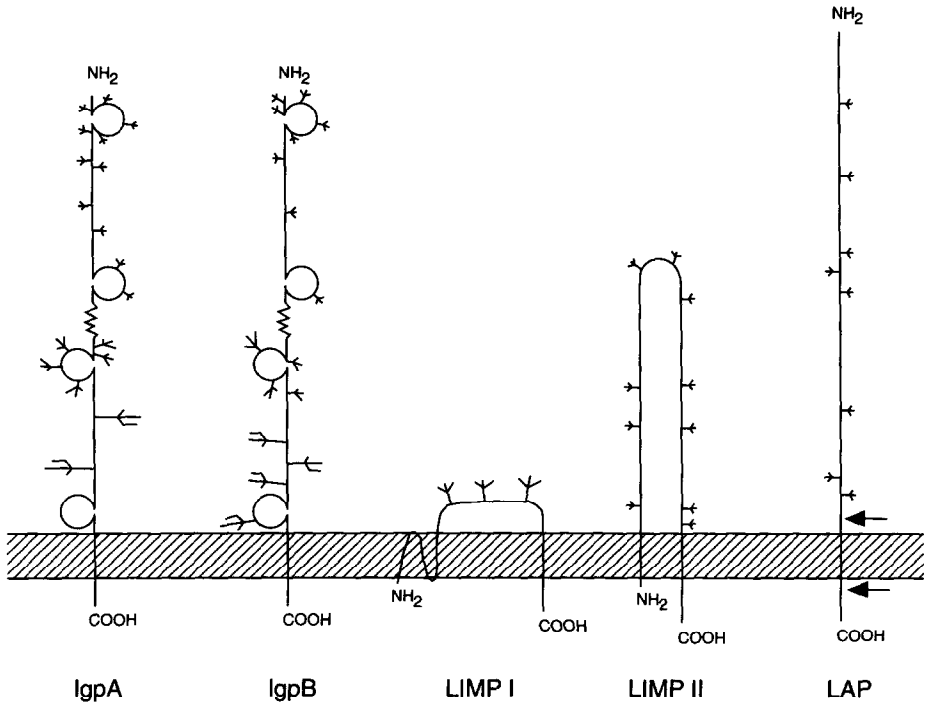

Fig. 1. Structures of resident and transient lyosomal membrane glycoproteins lgpA, lgpB, LIMP I, LIMP II and LAP. The membrane is indicated by a hatched area. Hinge regions are shown as ⩙ ; large antennary structures represent N-linked polylactosaminoglycans, small antennae represent complex type N-linked oligosaccharides. Loops are formed by disulfide bonds (only given for lgpA and B). Arrows mark putative proteolytic processing sites in LAP.

antibodies in mouse [12], rat [13–15], chicken [16] and human [17,18] cells. These lysosomal membrane glycoproteins share a number of biochemical features. They are heavily glycosylated containing predominantly Asn-linked oligosaccharides of the complex type [13–18]. The isoelectric points are very acidic (pIs between 2 and 4) due to a high content of sialic acid residues in the oligosaccharides [13,19]. The core proteins often comprise for less than 50% of the overall molecular weight [13,14,16–18].

Comparison of amino acid sequences deduced from cDNAs of murine, rat, chicken and human sources identified several types of lysosomal membrane glycoproteins. The alignment data suggest, that rat lysosomal membrane glycoprotein 120 (lgp 120) [20], which is identical with rat lgp107 [21], mouse lysosome-associated membrane protein 1 (mLAMP-1) [22], chicken lysosome–endosome–plasma membrane 100 (LEP100) [23] and human lysosome-associated membrane protein A (lamp A) [24], which has also been designated as human lamp-1 [25], are species specific versions of the same protein. This lysosomal membrane glycoprotein has been named lgp-A [5,19]. The second lysosomal membrane glycoprotein, lgp-B, has been isolated from different species: rat lgp 110 [19], which has independently been isolated by Nogushi et al. (LGP 96) [26], mouse LAMP-2 [27], which is identical with mouse lgp 110 [19] and human h-lamp-2 [25]. Lgp-A and lgp-B are conserved proteins with a marked sequence identity. LgpAs and lgpBs exhibit a higher degree of sequence identity across species boundaries than lgp-A compared to lgp-B from the same species. These correlations suggest, that the genes encoding the lysosomal membrane proteins lgp-A and lgp-B diverged from each other in evolution prior to the divergence of mammals and birds [19]. Lgp-A and lgp-B are type I membrane glycoproteins with a large N-terminal luminal domain a single transmembrane domain and a short cytoplasmic domain of 10–11 amino acids. The luminal domains contain 16–20 *N*-glycosylation sites, most of which are used. Some of these glycans are of the poly-*N*-acetyllactosamine type. Furthermore these glycoproteins contain O-linked glycans [31]. The luminal domains of lgp-A and -B can be divided into two homologous subdomains, which are separated by a proline rich hinge region. This hinge region has similarties to the immunoglobulin hinge region [24]. Both lgp-A and lgp-B have 4 pairs of cysteine residues located at conserved relative positions. Neighboring cysteines are disulfide bonded hence forming four loops [32,33] (Fig. 1).

Human lysosomal integral membrane protein I (LIMP I or CD63) [28] and rat LIMP II [29] are type III membrane glycoproteins according to the nomenclature of Wickner and Lodish [30]. LIMP I has a molecular weight of 30–50 kDa, the core peptide comprises for about 25 kDa and contains 4 transmembrane domains. The three

N-glycosylation sites in the large luminal domain are likely to be substituted with poly-*N*-acetyllactosaminyl structures. There is no homology to lgp A or lgp B [28,31]. LIMP II with a molecular weight of 74 kDa has two short cytoplasmic tails of 2–4 and 20–21 amino acid residues at the amino- and carboxy-terminus, respectively. The uncleaved signal peptide forms one of the two transmembrane domains. The main part of the protein is located on the luminal side and contains 11 potential *N*-glycosylation sites, most of which are likely to be glycosylated since the protein core has a molecular weight of about 50 kDa only [29] (Fig. 1).

Another well defined constituent of the lysosomal membrane is the precursor of lysosomal acid phosphatase (LAP). cDNA cloning and expression revealed, that lysosomal acid phosphatase (LAP) is synthesized and transported from the endoplasmic reticulum to the lysosomes as a type I membrane glycoprotein with a large N-terminal luminal domain, a single transmembrane domain and a short cytoplasmic domain of 19 amino acids. The luminal domain contains eight potential *N*-glycosylation sites, which are all used. The complex type oligosaccharides in part substituted with sialic acid residues are contributing about 16 kDa to the overall molecular weight of 63 kDa of the membrane bound LAP precursor [34–36] (Fig. 1). After arrival in dense lysosomes the luminal domain of LAP containing the active site of the enzyme is slowly released into the lysosomal matrix by proteolytic processing. Thus LAP is only a transient constituent of the lysosomal membrane.

3. Intracellular routes of lysosomal membrane glycoproteins

There is still some controversy about the routes along which lysosomal membrane glycoproteins are delivered to lysosomes. After translocation into the endoplasmic reticulum lgps, LIMPs and LAP traverse the cisternae of the Golgi apparatus, where the majority of the oligosaccharide side chains are converted to the complex type. This process is performed with comparable kinetics for all known lysosomal membrane glycoproteins. About 30 min after synthesis half of the newly synthesized molecules have reached the trans-Golgi network (TGN) as determined by aquisition of endo-β-*N*-acetylglucosaminidase H resistant oligosaccharides [14,18,35,37,38]. From the TGN the lysosomal membrane proteins may be transported directly to endosomes or indirectly via the cell surface. In the former case sorting would occur at the level of the TGN, in the latter at the level of the cell surface. From the endosomes the lysosomal membrane glycoproteins may either be delivered straightforward to dense lysosomes or may be transiently retained in endosomes and recycle between the endosomes and the cell surface. Thus lysosomal membrane glycoproteins may reach the cell surface either directly from the TGN or indirectly from endosomes. Attempts to discriminate between a direct or indirect routing to the cell surface on a kinetic basis have remained inconclusive. The fraction of a lysosomal membrane protein, that is present at the cell surface usually is low and may escape detection by some methods. The low frequency at the cell surface is sometimes taken as an argument that the biosynthetic route does not include an obligatory passage of the cell surface. This, however, may not be correct, if retrieval through internalization is rapid.

Kinetic studies revealed major differences between transport times of lgps, LIMPs and LAP from the TGN to dense lysosomes. Whereas lgpA, lgpB and LIMP II reach the lysosomal compartment with a $t_{1/2}$ of about 30 min, LIMP I reaches its final destination with a $t_{1/2}$ of 90 min and it takes 5–6 h until half of the newly synthesized LAP molecules have entered dense lysosomes [14,36,37,38]. It has been proposed, that the majority of lgpA, lgpB and LIMP II reach the lysosomes without passing the cell surface [37]. However, at least a minor portion of lgpA, lgpB and LIMP II reaches the lysosomes on the indirect route via the cell surface in human HL-60 and chinese hamster ovary cells [40,41]. The majority of endogenous lgpB, closely related to lgpA (see above), is transported via the basolateral plasma membrane of polarized MDCK cells to the lysosomes [42]. LEP100, the chicken version of lgpA, was shown to enter the plasma membrane/endosome pool and to rapidly cycle between plasma membrane and endosomes before reaching the lysosomes in expressing mouse L cells [43]. LEP-100 can even shuttle back to the plasma membrane after having reached dense lysosomes [44]. Whether or not the transport to the cell surface from endosomes or the TGN is obligatory for lgpA, lgpB and LIMP II, their rapid transport from the TGN to lysosomes strongly suggests, that their residence time in the endosome/plasma membrane pool is short.

LAP is an example of a lysosomal membrane glycoprotein, that is slowly delivered from the TGN to lysosomes (see above). The delay occurs at the level of endosomes. Rather than being delivered to lysosomes, endosomal LAP recycles on average 20 to 50 times back to the cell surface before it is delivered to lysosomes. At steady state one out of five LAP molecules in the recycling endosome/plasma membrane pool is found at the cell surface [45]. The amount of LAP located at the plasma membrane can be different from cell type to cell type. In expressing MDCK cells only 1% of the membrane bound LAP precursor is located in the basolateral plasma membrane, when these cells are grown polarized resembling an epithelium. Hardly any LAP is detectable in the apical plasma membrane under these conditions. In these polarized cells the LAP precursor is directly sorted to the basolateral cell surface and recycles between basolateral early endosomes and plasma mem-

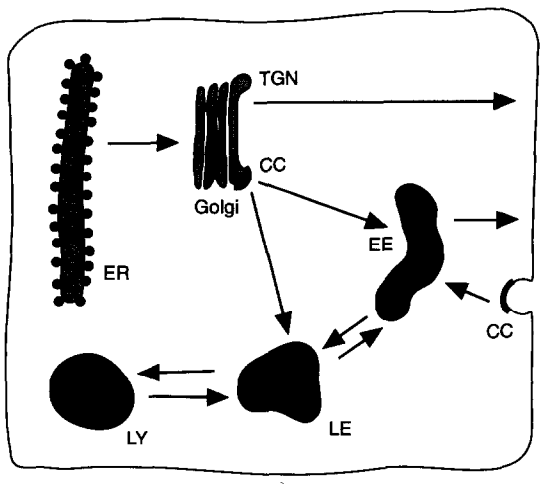

Fig. 2. Intracellular pathways of lysosomal membrane glycoporteins. Arrows indicate pathways of lysosomal membrane proteins discussed in the text. Lysosomal membrane glycoproteins can be enriched in clathrin coated membrane areas at the TGN or at the plasma membrane, which form clathrin coated vesicles for transport to the endosomal compartment. ER, endoplasmic reticulum; Golgi, Golgi-apparatus; TGN, *trans*-Golgi network; EE, early endosomes; CC, clathrin coat; LE, late endosomes; LY, lysosomes.

brane before delivery to dense lysosomes via late endosomes [46].

In Fig. 2 the intracellular pathways of lysosomal membrane proteins from the site of synthesis at the rough endoplasmic reticulum to the lysosomes are summarized schematically.

4. Targeting signals in lysosomal membrane glycoproteins

Recycling receptors accomplishing receptor-mediated endocytosis, such as low density lipoprotein (LDL)-, transferrin- and 300 kDa mannose 6-phosphate receptor, contain signals for rapid endocytosis through clathrin coated pits in their cytoplasmic domains. These signals were shown to comprise short linear arrays of four to six amino acids containing an essential tyrosine residue [47].

The short cytoplasmic domains of the lysosomal membrane glycoproteins lgpA, lgpB, LIMP I and LAP also contain a single tyrosine residue each (Fig. 3). Furthermore it has been shown for the lgps and LAP that they are rapidly endocytosed from the cell surface [41,43,45]. LAP has been shown to be internalized from the cell surface through clathrin coated pits [48]. Expression of truncated versions of lgpA and LAP lacking the short cytoplasmic domains abolished efficient lysosomal targeting and caused accumulation of the mutant proteins at the plasma membrane. The accumulation of truncated LAP at the plasma membrane prolongs the half time of transport to dense lysosomes from 6–7 h for wild-type LAP to 24 h. Substitution of the single tyrosine residue in the cytoplasmic tails of lgpA by cysteine and in LAP by phenylalanine led to an acccumulation at the cell surface as did the deletion of the entire cytoplasmic tails. Furthermore replacing the cytoplasmic domains of these two proteins by the respective domains of resident plasma membrane proteins was sufficient to redistribute the chimeric proteins to lysosomes [49–51]. These results suggest, that the cytoplasmic tails of lgpA and LAP contain sorting signals with an essential tyrosine residue for rapid endocytosis and efficient lysosomal targeting.

The 20 amino acid cytoplasmic tail of the lysosomal membrane glycoprotein LIMP II (Fig. 3) does not contain a tyrosine residue and therefore has to be directed to the lysosomes by a different signal. Domain swapping experiments performed with LIMP II revealed that substitution of the cytoplasmic domain of LIMP II by cytoplasmic domains of two resident plasma membrane proteins resulted in accumulation of the chimeras at the cell surface and the chimeras were not sorted to the lysosomes, efficiently. Moreover, transplantation of the cytoplasmic tail of LIMP II onto these two plasma membrane proteins redirected them to the lysosomal compartment [52].

Recently a Leu-Ile-X motif at the C-terminus of LIMP

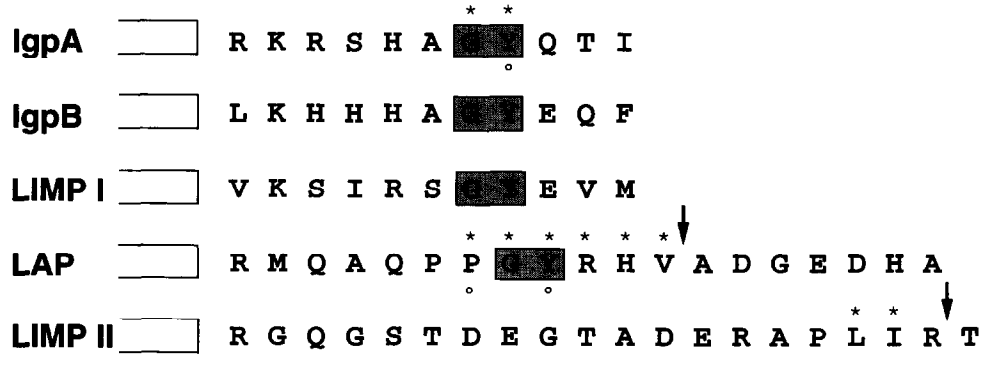

Fig. 3. Targeting signals in cytoplasmic domains of lysosomal membrane glycoproteins lgps, LIMPs and LAP. Amino acid sequences of cytoplasmic domains are given in one letter code. Residues shown to be essential for rapid endocytosis and lysosomal targeting are marked with asterisks. Conserved Gly-Tyr motifs are shaded; Pro and Tyr residues essential for basoalteral sorting of lgpA or LAP are marked with dots. Arrows mark the C-terminal portion of cytoplasmic domains, that are not required for sorting to lysosomes.

II was shown to be crucial for rapid internalization and correct sorting to the lysosomes. The peptide segment between the transmembrane domain and the Leu-Ile-X motif seems to be necessary for its proper function as an internalization signal [53]. A related di-leucine motif in the cytoplasmic tail of the C-terminus of the T-cell receptor CD 3 γ and δ chains [56] and the GLUT 4 glucose transporter [39] has been shown to be critical for their efficient internalization. Di-leucine motifs either preceeded or followed by a histidine in the cytoplasmic tails of the 46 kDa and 300 kDa mannose 6-phosphate receptor have been implicated in the sorting of these two receptors in the Golgi apparatus [54,55].

Detailed analyses of the tyrosine-containing signal in the lgpA cytoplasmic tail by substitution of one or more amino acids revealed, that substitution of the amino acid residues His, Ala and Gly preceeding the essential Tyr residue do not change the intracellular distribution. As for the Leu-Ile-X motif, the relative position of the tyrosine containing signal in the tail is important for its proper function [49]. Harter and Mellman [41] showed, that substitution of the Gly N-terminally preceeding the critical tyrosine residue in the lgpA cytoplasmic tail by Ala resulted in a partial redistribution of the mutant to the plasma membrane, albeit rapid internalization was not abolished. Exchange of the critical Tyr by Cys gave a more pronounced accumulation at the cell surface and abolished rapid internalization. These data indicate, that the Gly preceeding Tyr may be critical for direct sorting of lgpA from the Golgi to endosomes.

Amino acid residues contributing to the internalization signal in the cytoplasmic tail of LAP have been identified by investigation of two series of truncation and alanine substitution mutants [51]. The truncation mutants showed, that the 12 N-terminal residues of the cytoplasmic tail are sufficient for rapid endocytosis. The substitution mutants identified the hexapeptide ^{411}Pro-Gly-Tyr-Arg-His-Val^{416} as the LAP internalization signal (Fig. 3). Interestingly, mutations within the five C-terminal amino acids of the cytoplasmic tail can also impair internalization. This has been attributed to steric hindrance of the interaction between the sorting signal and its putative cytoplasmic receptor by the C-terminal five amino acids, which are inert in the wild-type form. Two-dimensional NMR analysis of an eighteen amino acid peptide corresponding to the cytoplasmic domain of LAP revealed that the ^{410}Pro-Pro-Gly-Tyr^{413} tetrapeptide is adopting type I β-turn structure in solution. The NMR data furthermore indicate, that nascent helices are adjacent to both sides of the turn [51,57]. The LAP hexapeptide internalization signal is therefore made up of three amino acids of the tight turn and three amino acids of the C-terminal nascent helix. Similar tight turn structures have been shown for the AsN-Pro-Val-Tyr internalization signal of the LDL receptor [58], and have been postulated for the Tyr-Thr-Arg-Phe internalization signal of the transferrin receptor [59]. These results may suggest, that tight turns are a general structural motif of tyrosine containing internalization signals. Recently, the first exception from this rule has been reported. TGN 38/41 is a type I membrane glycoprotein predominantly residing in the TGN and recycling between the TGN and the cell surface. Its cytoplasmic domain contains a Tyr-GlN-Arg-Leu tetrapeptide signal for internalization from the plasma membrane. This internalization signal does not adopt a tight turn conformation but lies within a nascent helix [60].

In polarized epithelial MDCK cells lgpA and LAP are passing the basolateral plasma membrane en route to the lysosomal compartment. In lgpA substitution of the single cytoplasmic domain tyrosine residue by alanine both blocks sorting at the TGN to the basolateral surface domain and internalization from the cell surface [61]. For LAP the basoalteral sorting determinant was investigated with the same series of cytoplasmic tail truncation and substitution mutants, which had been used for characterization of the internalization signal (see above). For basolateral sorting as for rapid internalization the 12 N-terminal amino acids of the tail were sufficient. Pro^{411} and Tyr^{413} within these aminoterminal 12 amino acids are essential for basolateral sorting (Fig. 3). These data indicate, that the determinants for basolateral sorting and internalization of LAP reside in the same segment of the cytoplasmic domain and that the structural requirements for basolateral sorting are less stringent than those for rapid internalization. Even though these two sorting signals are overlapping they can be distinguished, e.g. the basolateral sorting determinant tolerates the conservative substitution of Tyr^{413} by Phe, whereas rapid endocytosis of this mutation from the cell surface is blocked. This suggests, that different cytoplasmic receptors are involved in sorting at the TGN and at the cell surface [46]. Whereas the basolateral sorting determinants in lgpA and LAP are closely related to the tyrosine containing internalization signals, in other cases, e.g. the LDL-receptor, determinants for basolateral sorting have been identified, that are unrelated to the tyrosine containing internalization signals [61].

5. Sorting machinery for lysosomal membrane glycoproteins

Sorting of membrane proteins is thought to involve interaction of their respective cytoplasmic domains with specific cytosolic receptors. So far two types of such receptors, called adaptors, have been identified and characterized. Both are heterotetrameric protein complexes of two 100 kDa and two smaller polypeptides of about 50 and 20 kDa. HA-1 adaptors are located at the TGN, whereas HA-2 adaptors are restricted to the plasma membrane. One of the 100 kDa subunits, β-adaptin, has

been shown to bind to the cytoplasmic domain of a plasma membrane receptor and clathrin, which can assemble clathrin coats on the cytoplasmic surface of membranes. This is a prerequisite for the formation of transport vesicles [62,63].

Recently, it was shown in an in vitro system, that the cytoplasmic tail of LAP binds with high affinity to the trunk portion of the plasma membrane adaptor HA-2, but displays poor binding to the TGN adaptor HA-1. These data fit well with the observation, that newly synthesized LAP in expressing BHK-21 cells is transported from the TGN to the plasma membrane, rather than being sorted into clathrin coated vesicles at the TGN (see above). On the other hand, the cytoplasmic domain of the 46 kDa mannose 6-phosphate receptor, which is sorted from the TGN via clathrin coated vesicles to the endosomal compartment was shown to bind to both adapters with similar efficiency [64].

Cytoplasmic receptors recognizing basolateral sorting determinants in cytoplasmic tails of lysosomal membrane proteins and endocytic receptors in polarized epithelial MDCK cells are not known at present.

6. Perspectives

It has been suggested that lysosomal membrane glycoproteins form a coat on the luminal surface of the lysosomal membrane, which may protect this membrane from digestion by lysosomal acid hydrolases [19]. At present there are hardly any data as to the in vivo functions of this group of membrane glycoproteins. Not even the functions of the phosphatase LAP, which played a pivotal role in the discovery of lysosomes some 40 years ago, are known today, since its in vivo substrates remain elusive. Construction of defect mutants for lysosomal membrane glycoproteins by homologous recombination in the mouse system might pave the way towards a better understanding of their in vivo functions.

After identification and characterization of a large number of intracellular sorting signals for lysosomal membrane glycoproteins, a lot has still to be learned about their recognition by cytoplasmic receptors, which may be part of a complex sorting machinery directing each passenger protein from its place of synthesis to its respective final destination.

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