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WE DANCE ROUND *IN A RING AND SUPPOSE, BUT THE SECRET SITS IN THE MIDDLE AND KNOWS.*
ROBERT FROST

BIOLOGICAL FUNCTIONS OF LYSOSOMAL MEMBRANE-ASSOCIATED GLYCOPROTEINS

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• Lysosomes are the final repository of degradation products from the extracellular and intracellular spaces. The lysosomal membrane forms a unique vacuole participating in both endocytosis and autophagocytosis (1). It is extremely resistant to degradation by lysosomal hydrolases, maintains an acidic intralysosomal environment, transports amino acids and oligosaccharides produced by lysosomal hydrolases, in teracts and fuses with other membrane organelles, such as endosomes and phagosomes, and with the plasma membrane: (2,3).

STRUCTURE, BIOGENESIS, AND INTRACELLULAR TRAFFICKING OF LYSOSOMAL MEMBRANE-ASSOCIATED GLYCOPROTEINS

• The knowledge on the structure of lysosomal mem branes was enriched by identifying lysosomal membrane proteins unique to lysosomes. The lysosomal membrane-associated glycoproteins (Lamp-1 and Lamp-2) were ob tained from various species. The human cDNA encoding their polypeptide chains has also been isolated (4-6). Interspecies homology within each type of Lamps is high, e.g. human and mouse Lamp-1 share about 66% homology. Sequence homologies between the two groups, however, are less, human Lamp-1 being approximately 35% homologous to Lamp-2 (7). Lamps are encoded by genes localized to different chromosomes, Lamp-1 to 13q34, Lamp-2 to Xq24-25 (8). It could be speculated that the two genes were most likely produced by a duplication of a primordial gene early in evolution (6). Studies on the Lamp-2 protein family revealed that they are conserved from birds to mammals, and the diversity is generated by alternative splicing of a single Lamp-2 gene (9). Lamps contain a polypeptide core of approximately 43 kD. They are synthesized as precursor molecules (90 kD) that are processed to mature glycoproteins of approximately 110 kD (Lamp-1), and 105 kD (Lamp-2) (10, 11). The larger part of the molecule, extensively glycosylated (N- and O-glycans), is located at the luminal side of the lysosome, and is connected to a transmembrane domain extended to a short cytoplasmic tail (Fig. 1). The intraluminal domain contains two internally homologous domains separated by a region rich in proline residues. The homologous domains have eight half-cysteine residues connected in disulfide loops much like the immunoglobulin molecules. The position of the cysteine residues is conservedbetween Lamp-1 and Lamp-2 molecules, and among different species.

Several observations prompt that the mechanism of Lamp traffic to lysosomes differs from that of lysosomal enzymes (Fig. 2). By expressing Lamp-1 cDNA in COS-1 cells it was first shown that the tyrosine residue in the cytoplasmic tail is essential for the delivery of Lamp molecules to lysosomes (12). Two possible routes to reach lysosomes are discussed

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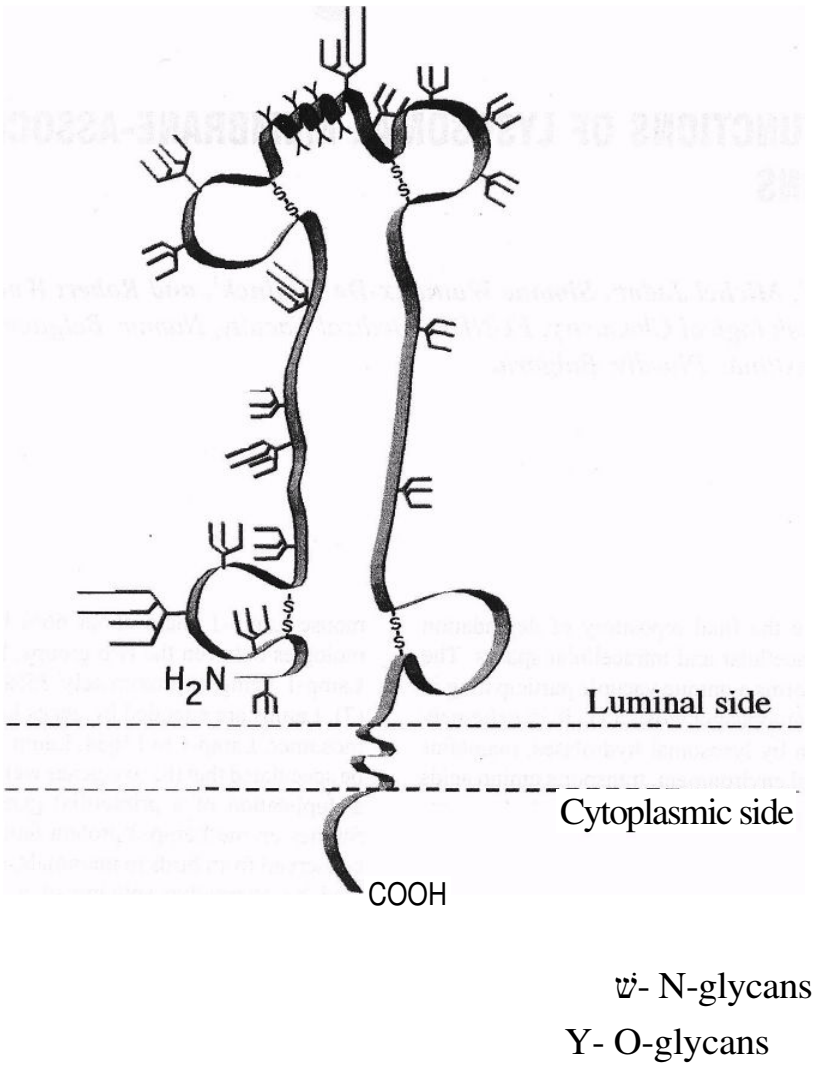

Figure 1. *Structure of Lamp-1. Lamp-2 has an almost identical configuration, (modified after Ref 1)*

for Lamps : *(i)* Lamps are sorted among other proteins at the trans-Golgi cisternae, transported to late endosomes and then to lysosomes together with newly synthesized lysosomal enzymes, and *(ii)* Lamps are first transported to the cell surface along with the secretory protein pathway and then sorted from plasma membrane proteins by selective internalization, followed by transport to dense lysosomes (13). Recent studies provide evidence that the majority of newly synthesized Lamps is directly transported to lysosomes (1), while soluble lysosomal enzymes are transported to pre-lysosomes (endosomes) by binding to the mannose-6-phosphate receptors (14).

LYSOSOMAL MEMBRANE-ASSOCIATED GLYCOPROTEINS OH THE CELL SURFACE - ADHESION MOLECULES ?

• . Although the molecular structure of Lamps is well determined, their biological role remains unclear. These molecules focus the current interest of many scientists because of the diverse biological functions suggested as well as for their peculiar appearance on the plasma membrane, unclarified yet. There has been some controversy whether or not Lamps are expressed on the cell surface. Immunoelectron microscopy failed to detect any surface Lamps, while by immuno-fluorescence labelling they were visualized on the cell sur-

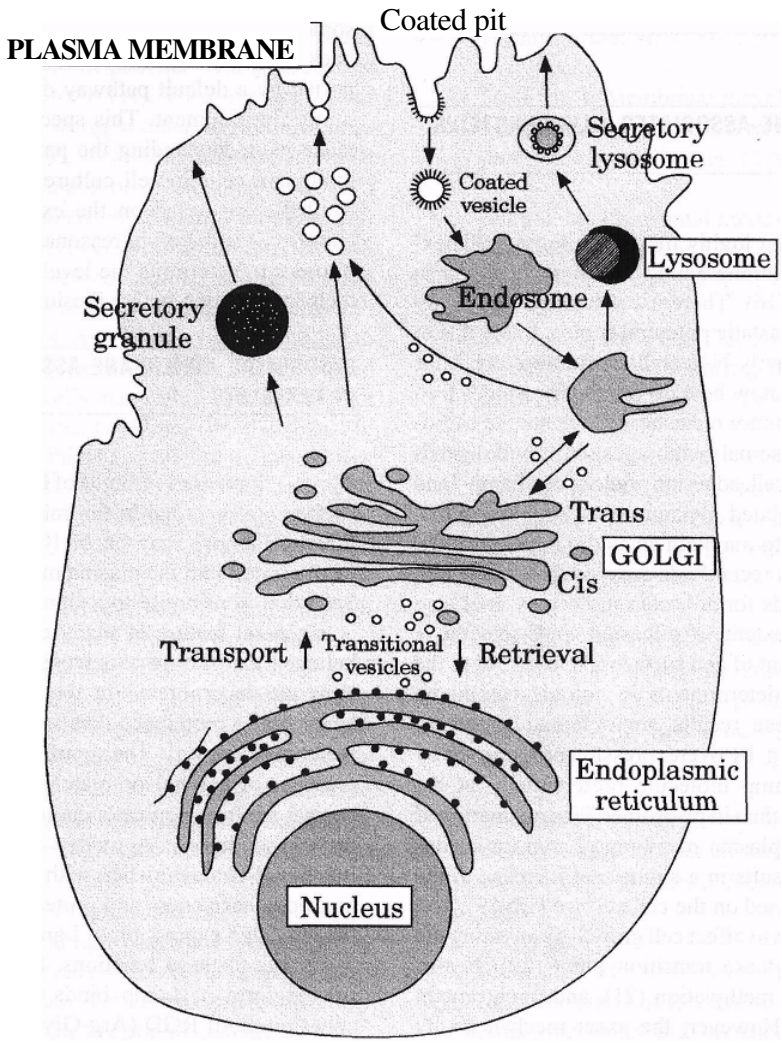

***Figure** 2. Trafficking of newly synthesized Lamp molecules. Lamps exit from the trans-Golgi and enter the endosome compartment. Most Lamps are transported to prelysosomes together with lysosomal enzymes. The majority of them enter lysosomes, but some are transported to the plasma membrane and exocytosed. A small part of Lamps are targetted to the plasma membrane probably via a default pathway. Then they return to endosomes, and through the endocytic pathway finally reach lysosomes. (modified after Ref 1)*

face in minor quantities depending on the cell type and differentiation stage (15). Only a small amount of the total number of Lamps can be detected on the cell surface - only 2% of total labelled Lamp-1 was expressed on the HL-60 cell surface (14). Considering that Lamps are the major cellular sialoglycoproteins, their expression constitues more than 50000 Lamp molecules on the plasma membrane (3). The transport kinetics of Lamp-1 to the surface of pulse-labelled biotinylated HL-60 cells showed that its maximal expression was recorded after 60-80 min of chase, after which the

amounts decreased, indicating that the molecules were internalized. The half-time for internalization was found to be 13 min (12). These experiments reveal that the exact duration of the cell cycle, the stage of differentiation, and the cell viability are important parameters to be considered when examining cell surface Lamps.

LYSOSOMAL MEMBRANE-ASSOCIATED GLYCOPROTEINS IN CANCER CELLS

• It was found that highly metastatic tumor cells ex press more plasma membrane-bound Lamp molecules than poorly metastatic ones (16). There are results strongly sug gesting that the high metastatic potential is most likely due to the increased amount of poly-N-acetyllactosamines in Lamps (16). It is interesting to know how do the sugar residues lead to a higher incidence of tumor metastasis. Such unique carbo hydrates may protect ly sosomal membranes and provide ligands on the cell surface for cell adhesion molecules. Lamp-land Lamp-2 are highly sialylated glycoproteins, suggesting that one of their functions is to maintain an acidic intralysosomal environment (17). It was recently discovered that sialyl Le^x and sialyl Le^a are ligands for adhesion molecules. It is also demonstrated that the extent of adhesion to E-selectin is proportional to the amount of cell surface Lamp-1, and to the cell surface sialyl-Le^x determinants in colonic carcinoma cells (16). Most of these results are obtained following genetic manipulations, e.g. by overexpression of Lamps or by expressing a mutant Lamp molecule preferentially at the plasma membrane rather than in lysosomes. The incubation of tumor cells having few plasma membrane Lamp molecules with sodium butyrate results in a significant increase in the amount of Lamps expressed on the cell surface (18, 19). The sodium butyrate is known to affect cell growth by arresting the cell cycle at the Gl/S phase transition point (20). It also causes changes in DNA methylation (21), and in chromatin conformation (22, 23). However, the exact mechanism by which butyrate augments surface human Lamps is not clear. We are currently investigating the mRNA level in butyrate treated and untreated cancer cells.

The augmented expression in malignant cells of some endogenous galactoside-binding lectins (galectins), suggests that these lectins may play an important role in the transformation process and in metastasis (24), e.g. cell surface galectin-3 was found to mediate homotypic cell adhesion in insect cells (25). Interestingly, the cellular ligands for galectin-3 are Lamp-1 and Lamp-2 (26). It is possible that, by interacting with galectin-3, surface Lamps facilitate both the adhesion of tumor cells to extracellular matrix proteins and the invasion process itself (18). Being ligands for E-selectin, Lamps may mediate binding of cancer cells to endothelial cells, and thus promote metastasis. Therefore, the expression of Lamps in malignant cells may depend on their surrounding matrices, and may play a role in tumor invasion and metastasis. It is interesting to know whether cell surface Lamps detected in some tumor cells are normal Lamp molecules transported back to the cytoplasm *via* internalization, or proteins trans ported by a default pathway destined to serve as ligands in tumor development. This speculation reinforces the impor tance of understanding the pattern of Lamps expression in malignant cells in cell culture and *in vivo.* Almost all data available are based on the examination of monolayer cell cultures. It is therefore reasonable to study biopsy specimens in order to determine the level of expression of Lamps, and their interactions with adhesion molecules *in situ.*

LYSOSOMAL MEMBRANE-ASSOCIATED GLYCOPROTEINS IN PLATELETS

• Increased amounts of Lamp-1 and Lamp-2 on the cell surface are recorded in thrombin-activated platelets (7, 27). Platelet Lamp-1 may be of lysosomal origin and becomes incorporated into the plasma membrane during secretion (7). Secretion in response to a signal from cellular storage pools is a general feature of many cells, including platelets. It is believed that during exocytosis the granule membrane proteins are incorporated in the plasma membrane. Lamp-1, being such a protein, could also be translocated to the plasma membrane this way. The significant and regulated cell surface presence of Lamp-1 on platelets indicates that at least some normal adult human cells could express this glycoprotein in certain conditions. Activated platelets share some surface functional characteristics with malignant cells, such as enhanced adhesiveness and protease activity. It is possible that the regulated expression of Lamps on activated platelets may contribute to these functions. It was shown that a deglyco-sylated form of Lamp binds collagen and the fibronectin adhesion motif ROD (Arg-Gly-Asp), thus suggesting a possible role in cell-matrix adhesion (28). Moreover, the amount of cell surface Lamp-2 was found to correlate with platelet activation in cancer patients with prethrombotic state: platelets, from, healthv; individuals, showed low surface levels of Lamp-2 in contrast to cancer patients having significantly elevated expression (29). Therefore, the cell membrane expression of Lamps may be an informative indicator of *in vivo* platelet activation, and thus serve as an useful parameter in identifying patients with prethrombotic disorders. On the other hand, platelets become activated *in vitro* upon storage and this leads to a decrease in the utility of concentrates stored for transfusion. Recently, a simple and effective fiowcyto-metric test was introduced to evaluate the quality of stored platelet concentrates by measuring the level of plasma membrane bound Lamp-1 and Lamp-2 (30).

LYSOSOMAL MEMBRANE-ASSOCIATED GLYCOPROTEINS IN LEUKOCYTES

• Lamp-1 and *Lamp-2* were detected on the surface of peripheral blood lymphocytes in patients with scleroderma and systemic lupus erythematosus (31). Their expression was significantly augmented following phytohemagglutinin stimu lation. Lamps likely contribute to the migration of activated leukocytes to sites of inflammation. Holcombe *et ol* (32) reported that the surface expression of Lamps on peripheral blood mononuclear cells correlated with serum interleukin-8 and soluble interleukin-2 receptor levels, shorter disease duration, and greater functional impairement in patients with systemic lupus erythematosus. As Lamps could be shuttled to the plasma membrane during cell activation, their elevated expression on mononuclear cells may reflect immune system activation in scleroderma patients. Moreover, their surface expression correlates with clinical and laboratory parameters of disease development (33), and thus may be an useful indicator of disease activity in patients with autoimmune diseases.

Dahlgren *et al* (34) showed the presence of Lamps in both neutrophil lysosomes and secretory organelles, thus speculating that Lamps may play a role in the inflammatory response. During phagocytosis, Lamp-1 and Lamp-2 become markedly concentrated around the ingested particle, and appear on the cell surface when the secretory organelles are mobilized (34). Thus, Lamps could be part of a mobilizable system designed to recognize and eliminate microbial agents, as they carry polylactosaminoglycans containing selectin ligands. Since recognition of microorganisms often involves microbial lectins (35), surface Lamps may serve as adhesion molecules facilitating the binding to bacterial lectins; type-1 fimbrated *Escherichia coli* binds to neutrophil Lamp-1 (36). These results support the data that Lamps are localized in secretory organelles of human neutrophils, suggesting that these glycoproteins may play a role on the surface of activated/ extravasated neutrophils.

CONCLUSION

• Although the molecular structure of Lamps is well determined, their biological role is still unclear. Recent results suggest that Lamp-1 and Lamp-2 could be regarded as a new family of adhesive glycoproteins participating in tumor invasion and metastasis, bacterial binding, inflammation, and platelet activation as well as in neuronal injury in status epilepticus. Future studies may contribute to the application of Lamp-1 and Lamp-2 as prognostic parameters in clinical oncology and immunology.

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