Subcellular Localization of Mannose 6-Phosphate Glycoproteins in Rat Brain*

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Transport of soluble lysosomal precursor proteins to lysosomes relies on the addition of mannose 6-phosphate (Man 6-P) residues. The nascent polypeptide chain follows the first steps of the secretory pathway, involving the co-translational addition of preformed oligosaccharide chains onto select asparagine residues. A sorting event in the Golgi apparatus allows diversion of the precursor lysosomal proteins from the secretory pathway. This process entails the two-step generation of Man 6-P on N-linked oligosaccharides and subsequent binding to Man 6-P receptors (MPRs). The MPR-lysosomal protein complexes exit the Golgi in transport vesicles, which then fuse with an acidic prelysosomal compartment (endosome). The free receptors recycle back to the Golgi, whereas the lysosomal proteins accumulate in lysosomes. In most cell types, the Man 6-P recognition marker is rapidly removed shortly after endosomal/lysosomal targeting. This explains why typically the bulk of the lysosomal proteins are found in their dephosphorylated state (for reviews, see Refs. 1–5). However, studies using immortalized cells have shown that the extent of dephosphorylation can vary considerably depending on the cell line and culture conditions (6, 7).

To investigate the fate of lysosomal enzymes in vivo, Sleat et al. (8) analyzed the level of Man 6-P-containing glycoproteins in a series of rat tissues. This study revealed that brain had significantly higher levels of Man 6-P glycoproteins than other tissues analyzed (e.g. 8-fold higher than liver). Importantly, the phosphorylation state of individual brain lysosomal enzymes was greatly elevated (e.g. on average, the amount of total activity for a given lysosomal enzyme represented by the phosphorylated form was 37% in brain compared with 0.7% in liver). This suggests that brain cells are somehow unable to efficiently process lysosomal enzymes to remove the Man 6-P modification.

There are at least three possible hypotheses regarding the mechanism for the limited dephosphorylation of brain lysosomal enzymes. First, this could be the consequence of transport of some of the lysosomal precursor proteins to a specialized, metabolically inactive compartment where they are stored for later use. Second, there could be delayed transfer of lysosomal precursor proteins from endosomes to lysosomes. Finally, the lysosomal enzymes could retain the Man 6-P modification after delivery to “classical,” metabolically active lysosomes. Comparing the steady state localization of the phosphorylated and dephosphorylated lysosomal enzymes potentially could distinguish among these three models. The first two would result in distinct subcellular locations, whereas the third would result in identical subcellular locations for the phosphorylated and dephosphorylated species.

In this report, we have investigated the subcellular localization of Man 6-P glycoproteins in rat brain using two complementary approaches. In a biochemical approach, we used a wide range of subcellular fractionation methods to compare the relative distributions of Man 6-P glycoproteins and lysosomal marker enzyme activities. In a morphological approach, we used double-label fluorescence and scanning confocal microscopy to compare the relative localization of Man 6-P glycoproteins and representative lysosomal enzymes. Taken together, our results indicate that the bulk of the brain Man 6-P glycoproteins reside in classical neuronal lysosomes, suggesting that these organelles are not able to fully process the Man 6-P signals.

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‡ The abbreviations used are: Man 6-P, mannose 6-phosphate; MPR, Man 6-P receptors; CI, cation-independent; P, microsomal fraction; M, mitochondrial fraction; L, light mitochondrial fraction; LAP, lysosomal acid phosphatase.

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EXPERIMENTAL PROCEDURES

Subcellular Fractionation—Brain and liver were obtained from adult male Wistar rats weighing 250–300 g. After dissection, brain tissue was immersed immediately in ice-cold 0.25 M sucrose and homogenized by one passage through a motor-driven Potter apparatus (1500 rpm). Fractionation of subcellular organelles by differential centrifugation was as described by de Duve et al. (9). Isopycnic centrifugations were carried out according to Beaufay et al. (10) using a Spincou model L-7–65 Ultracentrifuge and VTi65 rotor.

Lysoosomal Enzyme Assays—Enzyme measurements were performed using 4-methylumbelliferyl-β-D-galactoside substrates in the presence of 0.1% Triton X-100, incubated at 37 °C, and terminated by the addition of 2 volumes of 0.5 M glycine, 5 mM EDTA, 0.05% Triton X-100, pH 10.5. Reaction conditions were as follows (enzyme, substrate/buffer): β-galactosidase, 1 μM 4-methylumbelliferyl-β-D-galactoside, 80 mM acetate pH 4.0; α-fucosidase, 0.2 mM 4-methylumbelliferyl-α-fucoside, 80 mM citrate, pH 5.0; β-glucosidase, 5 μM 4-methylumbelliferyl-β-glucoside, 80 mM citrate, pH 4.5, 6 mM taurocholate.

Detection of Man 6-P Glycoproteins—Samples from individual fractions (representing equivalent portions of tissue homogonate) were analyzed by SDS-polyacrylamide gel electrophoresis on 10% reducing gels. Blotting and detection of the Man 6-P glycoproteins using iodinated soluble cation-independent (CI) MPR were as described (11, 12). Total Man 6-P glycoproteins were estimated using a PhosphorImager and ImageQuant 3.3 software (Molecular Dynamics, Sunnyvale, CA) by integrating the total machine counts present in each lane after correcting for background binding to nitrocellulose.

Immunoblotting—Equivalent samples (representing material present in 0.5 mg of tissue homogonate) were submitted to SDS-polyacrylamide gel electrophoresis on 8% non reducing gels. After semi-dry transfer of proteins to PVDF (Immobilon-P Millipore), membranes were probed with a rabbit polyclonal antisera against the CI-MPR kindly provided by Dr. Kurt von Figura (Gottingen, Germany) and the signal revealed using a chemiluminescence system (Boehringer-Mannheim).

Determination of Lysoosomal Enzyme Phosphorylation State—Sucrose density gradient fractions were analyzed to determine the percentage of total lysosomal enzyme activities represented by phosphorylated forms as described in (8). Samples (1 ml) were diluted into column buffer (0.15 M NaCl, 0.1% Triton X-100, 25 mM Tris-HCl, pH 7.2) and loaded onto a 1-cm3 bed volume of Affi-Gel 10-immobilized soluble CI-MPR. The column was washed with 6 ml of column buffer, mock-eluted with 2 ml of column buffer containing 10 mM glucose 6-phosphate, and eluted with 4 ml of column buffer containing 10 mM Man 6-P. The starting material, Man 6-P eluate, and the unbound material (pooled run-through, wash, and mock-eluted fractions) were adjusted to column buffer (0.15 M NaCl, 0.1% Triton X-100, incubated at 37 °C, and terminated by the addition of 2 volumes of 0.5 M glycine, 5 mM EDTA, 0.05% Triton X-100, pH 10.5. Reaction conditions were as follows (enzyme, substrate/buffer): β-galactosidase, 1 μM 4-methylumbelliferyl-β-D-galactoside, 80 mM acetate pH 4.0; α-fucosidase, 0.2 mM 4-methylumbelliferyl-α-fucoside, 80 mM citrate, pH 5.0; β-glucosidase, 5 μM 4-methylumbelliferyl-β-glucoside, 80 mM citrate, pH 4.5, 6 mM taurocholate.

RESULTS

Differential Centrifugation—We initially used a scheme of differential centrifugation to investigate the subcellular localization of the Man 6-P-containing glycoproteins in rat brain and liver. Equivalent samples were fractionated by SDS-polyacrylamide gel electrophoresis and blotted on nitrocellulose, and Man 6-P glycoproteins were visualized using iodinated soluble CI-MPR probe as described in Valenzano et al. (11). Fig. 4A shows representative blots obtained from such an experiment. The total amount of specifically bound [125I]-CI-MPR probe is 9-fold higher in brain than in liver, consistent with previous results (8). Total Man 6-P-containing glycoproteins were quantified and compared with lyosomal marker enzymes (Fig. 1B).

In liver, the Man 6-P glycoproteins have a distribution consistent with their being in prelysosomal compartments in transit to lysosomes. In this well characterized tissue, Golgi, plasma membrane, the bulk of endosomes, and fragmented endoplasmic reticulum sediment in the microsomal fraction P (14, 15). Classical lysosomes sediment in both the mitochondrial fraction M and the light mitochondrial fraction L (14). As expected, the lyosomal markers β-galactosidase, α-fucosidase, and β-glucosidase are predominantly found in M + L (92, 90, and 78%, respectively, of the total activity in M + L + P), whereas most of the Man 6-P-containing glycoproteins are found in P (59% of M + L + P) (Fig. 1B, right panels). Importantly, this different distribution clearly demonstrates that most of the liver Man 6-P glycoproteins are not in classical lysosomes.

In brain, the lysosomal markers β-galactosidase, α-fucosidase, and β-glucosidase are to a large extent found in M + L (76, 68, and 61%, respectively, of the total activity in M + L + P), although the P fraction contains considerably higher activity than in liver. In contrast to liver, the Man 6-P glycoproteins have a distribution very similar to that of the lyosomal marker enzymes (31% of the M + L + P Man 6-P-containing glycoproteins are found in P), consistent with the phosphorylated forms being present in classical lysosomes.

Isopycnic Centrifugation Analysis—Results presented in Fig. 1D raise the possibility that the brain Man 6-P glycoproteins are present in lysosomes or in nonlysosomal structures that also sediment in the M + L fraction. To help discriminate between these two possibilities, we analyzed the behavior of the Man 6-P glycoprotein-containing organelles by isopycnic centrifugation in sucrose density gradients (Fig. 2). This analysis shows that the distribution of the Man 6-P-containing organelles and the distribution of the lysosomes are very similar. This is true regardless of the source of the organelles (ML, Fig. 2, left panel; P, Fig. 2, right panel) or if the lysosomal marker enzymes are known to be targeted by the Man 6-P-dependent pathway (β-galactosidase, α-fucosidase) or by a Man 6-P-independent mechanism (β-glucosidase). In M + L, the shoulder in the distribution of β-galactosidase and α-fucosidase (fractions 8–9) and the slight shift of β-glucosidase from β-galactosidase and α-fucosidase suggests some heterogeneity in the lysosome population, whereas the Man 6-P glycoproteins seem to be present throughout these organelles. Note that in Fig. 2, left panel, the dip in the Man 6-P glycoprotein profile at fraction 10 is most likely an artifact due to poor electrotransfer.

For comparison, liver was also analyzed in parallel (Fig. 3). Most of the Man 6-P glycoproteins, found in the microsomal fraction P (see Fig. 1), are present in structures showing an equilibrium density distinct from that observed for lysosomes (Fig. 3, right panel). These organelles most likely represent light endosomes (16). The prelysosomal nature of these structures is further supported by the finding that Man 6-P glycoproteins in these lighter fractions have a M, higher than those.
**FIG. 1.** Distribution patterns of Man 6-P glycoproteins and lysosomal marker enzymes in rat brain and liver after differential centrifugation. *N*, nuclear fraction; *M*, heavy mitochondrial fraction; *L*, light mitochondrial fraction; *P*, microsomal fraction; *S*, soluble fraction. 

Panel A, 1 mg of tissue equivalents of each fraction were submitted to SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose, and probed with iodinated CI-MPR receptor. Molecular mass standards are shown in kDa. Panel B, results are presented as recommended by de Duve et al. (9), where the area is proportional to the total activity. **Ordinate**, relative specific activity (percentage of total recovered radioactivity or enzyme activity/percentage of total recovered proteins). **Abcissa**, relative protein content of fractions (cumulatively from left to right). M6PGPs, mannose 6-phosphate glycoproteins.
in the dense fractions, suggesting they are lysosomal enzyme precursors in transit to the lysosome that have not yet been proteolytically processed.

One potentially complicating factor in our analysis would be if in brain, lysosomal, and prelysosomal/endoosomal compartments have similar physical properties. To test this possibility, we used two different centrifugation-based cell fractionation procedures to compare the distribution of organelles containing the CI-MPR, a marker of prelysosomal compartments (17), with that of the lysosomal marker β-galactosidase. Following

FIG. 2. Density distribution of Man 6-P glycoproteins after isopycnic centrifugation of a brain mitochondrial (M + L) and a microsomal (P) fraction in a sucrose density gradient. Fractions from differential centrifugation were layered on top of a continuous sucrose density gradient extending from 1.09 g/ml to 1.26 g/ml. Centrifugations were performed at 240,000 × g in a Vit85 Beckman rotor for 180 min. A, Man 6-P glycoprotein profiles. B, total Man 6-P glycoprotein levels and activities of lysosomal marker enzymes measured in the same gradient. ■, mannose 6-phosphate glycoproteins; ○, β-galactosidase; ▲, α-fucosidase; □, β-glucosidase.
differential centrifugation of brain homogenate, Western blotting revealed that the CI-MPR was present mainly in the P fraction, whereas β-galactosidase was mostly in M + L (Fig. 4A). Moreover, analysis of the pooled M + L + P fractions by isopycnic centrifugation (Fig. 4B) shows that membranes associated with the CI-MPR have a lower equilibrium density than those containing β-galactosidase activity. Taken together with our previous results (Figs. 1 and 2), this indicates that most of the brain Man 6-P glycoproteins are not located in a prelysosomal/endosomal compartment.

**Fig. 3.** Density distribution of Man 6-P glycoproteins after isopycnic centrifugation of a liver mitochondrial (M + L) and a microsomal (P) fraction in a sucrose density gradient. Conditions as described in Fig. 2. ■, mannose 6-phosphate glycoproteins; ○, β-galactosidase; △, α-fucosidase; □, β-glucosidase.
These results suggest that the brain Man 6-P glycoproteins are contained in lysosomes. As an additional test of this hypothesis, we used other separation methods in an attempt to separate the Man 6-P glycoprotein-containing organelles and classical lysosomes. One method entailed fractionation of brain M + L and P fractions by isopycnic centrifugation in Nycodenz media (18). Consistent with previous results, the distributions of Man 6-P glycoproteins and lysosomal marker enzymes were quite similar (Fig. 5). The same conclusion was reached from free flow electrophoresis experiments (data not shown). Finally, we also measured additional lysosomal marker enzymes (α-glucosidase, β-mannosidase, β-glucuronidase, β-hexosaminidase, α-iduronidase, acid phosphatase, sulfatase, and cathepsin B) and obtained essentially the same distributions as those markers reported in Figs. 2, 3, and 5 (data not shown).

Release of Man 6-P Glycoproteins from Brain Organelles—Taken together, the distributions of Man 6-P glycoproteins and lysosomal marker enzymes reported in Figs. 1, 2, 4, and 5 would be best explained by a steady state co-localization of these molecules in brain lysosomes. A classical approach to assess if a molecule is located in a particular organelle is to compare the effect of membrane-disrupting treatments on the release of the molecule of interest and on known marker proteins. In particular, the osmotic properties of endocytic organelles differ between endosomes and lysosomes. We analyzed the effect of treatments known to damage the lysosomal membrane (suspension in hypotonic media (9), 37 °C incubation in isotonic glucose (19), and repeated cycles of freezing and thawing (9)) on the release of Man 6-P glycoproteins from vesicles present in brain M + L fractions. These treatments were performed in the presence of excess Man 6-P to avoid possible binding of released lysosomal enzymes to MPRs present in other membranes. After sedimentation of membranes by centrifugation, pellets and supernatants were recovered and analyzed for release of Man 6-P glycoproteins and the lysosome marker enzymes α-fucosidase and β-galactosidase. Fig. 6 shows that in all three membrane-disrupting treatments, soluble lysosomal marker enzymes and Man 6-P glycoproteins exhibit very similar release curves, indicating that they are likely to reside within the same organelle.

Lysosomal Enzyme Phosphorylation State in Sucrose Density Gradients—If, as suggested by our previous experiments, the bulk of the brain Man 6-P glycoproteins are located in lysosomes, then the phosphorylated and the dephosphorylated forms of the lysosomal enzymes would have identical distributions. To test this, detergent-solubilized fractions from a brain M + L sucrose density gradient (similar to the one shown in Fig. 2, left panel) were applied to a column of immobilized CI-MPR. Enzyme activities (α-fucosidase and β-galactosidase) were determined on samples of each fraction (total activity), on pooled flow-through and washes, and on Man 6-P eluates. The bound and unbound activities were expressed as the percentage of the total activity present in the gradient. Fig. 7 shows that a large proportion of the active α-fucosidase and β-galactosidase are retained on the column (39 and 65%, respectively), in accordance with data reported previously (8). Importantly, the dephosphorylated and phosphorylated forms of each enzyme have similar equilibrium densities, consistent with both forms being located in the same organelle.

Confocal Microscopy—In addition to subcellular fractionation, we used laser-scanning confocal microscopy to investigate the cellular location of Man 6-P glycoproteins in rat brain. This was compared with the distribution of cathepsin D and β-galactosidase, two lysosomal enzymes targeted by the Man 6-P pathway, and to lysosomal acid phosphatase (LAP), which is synthesized as a membrane protein and is targeted to the lysosome by a Man 6-P-independent pathway. The micrographs shown in Fig. 8 represent 1-μm-thick optical sections of rat cerebral cortex layer 5, an area rich in pyramidal neurons. The Man 6-P glycoproteins (red) are predominantly found in compartments in neuronal cell bodies and some processes. Comparison of the Man 6-P glycoprotein and lysosomal enzyme-labeling (green) indicates that there is some variability both in the relative staining intensity of different neurons and in the pattern of staining. For instance, the β-galactosidase antisera stains neuronal cell bodies, but in addition, there is strong staining of long apical dendritic processes that project toward the surface of the brain (Fig. 8, bottom panels). Also, within a given cell body, there appeared to be minor differences in the relative staining of individual vesicles containing Man 6-P...
glycoproteins and the different lysosomal markers (most prominent with LAP). Nonetheless, the overall staining patterns showed extensive colocalization (Fig. 8, merged panels). Similar findings were obtained on neurons in numerous areas of the brain, indicating that the majority of neuronal lysosomes contain Man 6-P glycoproteins.

**Fig. 5.** Density distribution of Man 6-P glycoproteins and lysosomal marker enzymes after isopycnic centrifugation of a brain mitochondrial (M + L) and a microsomal (P) fraction in a Nycodenz™ density gradient. Fractions were isolated by differential centrifugation and layered on top of a continuous Nycodenz density gradient extending from 1.09 g/ml to 1.26 g/ml. Centrifugations were performed at 240,000 x g in a Vti65 Beckman rotor for 180 min. A, Man 6-P glycoprotein profiles. B, total Man 6-P glycoprotein levels and activities of lysosomal marker enzymes measured in the same gradient. ■, mannose 6-phosphate glycoproteins; ○, β-galactosidase; △, α-fucosidase; □, β-glucosidase.
DISCUSSION

In this report, we have investigated the subcellular localization of Man 6-P glycoproteins in rat brain using a number of complementary approaches. The biochemical experiments consistently demonstrate co-migration of Man 6-P glycoproteins and lysosomal marker enzymes in brain subcellular compartments. However, it is possible that even using the wide range of fractionation methods employed in this study, distinct compartments may not be distinguishable. The morphological experiments provide high-resolution information but are nonquantitative, and results may be dependent on antigen accessibility considerations. The convergence of these approaches strengthens our conclusion that the bulk of brain Man 6-P glycoproteins reside within neuronal lysosomes.

It is reasonable to assume that the low phosphorylation state of lysosomal enzymes in most other tissues is due to the short half-life of the Man 6-P marker (1.4 h in mouse BW5147 cells (20)) compared with the long half-lives of lysosomal enzymes (generally days, see Refs. 21–23). Conversely, the high phosphorylation state of lysosomal enzymes in neurons could either be because of a relatively long half-life of the Man 6-P marker or, less likely, to a short half-life of the enzyme itself. A prolonged Man 6-P half-life could result from either the presence of some unusual neuron-specific carbohydrate modification that hinders the action of the dephosphorylating enzyme or from low levels of this enzyme. Further insight into this question will require identification of the endogenous enzyme responsible for removing the Man 6-P modification. This is unlikely to be the classical lysosomal acid phosphatase, as indicated by overexpression studies of LAP in cultured cells (24) or by our findings that Man 6-P glycoproteins and LAP coexist in the same neuronal compartments. In vitro studies indicate that the Man 6-P modification can be removed by

FIG. 6. Lysosome integrity and release of Man 6-P glycoproteins from brain M + L fractions. A brain M + L fraction was subjected to treatments causing disruption of the lysosomal membrane. A, hypo-osmotic rupture. The fraction was exposed to the indicated sucrose concentration 10 min at 0 °C. The sucrose concentration was re-adjusted to 0.25 M before pelleting the membranes. B, freeze-thawing. The fraction was subjected to the indicated number of cycles of freezing in liquid Nitrogen and thawing at 4 °C. C, the fraction was resuspended in iso-osmotic glucose and incubated for the indicated time at 37 °C. Following the indicated treatments, the membranes were centrifuged for 10 min at 50,000 rpm in a Beckman TLA120.1 rotor. Enzyme activities and Man 6-P glycoproteins were measured in the supernatants as described under “Experimental Procedures” and expressed as percentages of the amounts released under the same conditions in the presence of 0.05% Triton X-100. ◊, α-fucosidase; ●, β-galactosidase; △, mannose 6-phosphate glycoproteins.

FIG. 7. Phosphorylation state of lysosomal enzyme activities in a brain M + L fraction after isopycnic centrifugation on a sucrose density gradient. A brain M + L fraction was submitted to isopycnic centrifugation exactly as described in Fig. 2, left panel. Fractions were analyzed to determine the percentage of lysosomal enzymes activities represented by the phosphorylated form, as described under “Experimental Procedures.” Results are presented as percentages of the total corresponding activity recovered in the gradient. ◊, unbound; ●, bound.
purple acid phosphatase (25). If this observation is physiologically relevant, it will be of interest to determine the cellular and intracellular distribution of this enzyme in brain.

When considering the biological mechanisms and consequences of dephosphorylation of lysosomal enzymes, it is important to note that different cell lines exhibit marked differ-
ences in rates of dephosphorylation of lysosomal enzymes. For instance, Gabel et al. (26) find that some CI-MPR-deficient cell lines do not rapidly remove the Man 6-P marker (26). This may not be a direct consequence of loss of the CI-MPR, as knock out mice that lack either the CI-MPR or the CD-MPR (27) exhibit little change in overall levels of Man 6-P glycoproteins in various solid tissues (brain, liver, spleen, kidney, and lung) compared with mice that contain both MPRs. Also, in some CI-MPR-containing cell lines, dephosphorylation is dependent on cell culture conditions such as cell density, serum starvation, and the presence of growth factors such as insulin-like growth factor II (6, 7). Changes in dephosphorylation of lysosomal enzymes may also occur in vivo under certain pathological conditions. For instance, approximately one-third of all human breast carcinomas exhibit elevated levels of Man 6-P glycoproteins compared with normal breast epithelia (12), although the underlying mechanism of this is not known.

Our studies on normal brain tissue avoid nonphysiological situations that can occur in cultured cell systems and thus are biologically relevant. However, it is important to note that brain contains ~10–50-fold more glial cells than neurons, and there are a wide variety of neuronal cell types. This cellular heterogeneity complicates interpretation of the biochemical analysis in two areas. First, we have found that the phosphorylation state of different brain lysosomal enzymes varies considerably (this study and Ref. 8). It is possible that this reflects heterogeneity within a given cell type. However, given that the relative abundance of different lysosomal enzymes varies in neurons and glia (28), this would also be consistent with a situation where most of the glial-derived hydrolases lacked Man 6-P and the neuron-derived hydrolases contained Man 6-P. Second, the multiple peaks or shoulders in the profiles of the sucrose and Nycozden density gradients (this study) and in free flow electrophoresis experiments indicate that the brain Man 6-P glycoprotein-containing lysosomes are heterogeneous. This is also consistent with other studies on brain lysosomes (29, 30). This heterogeneity may occur within a given cell type (possibly reflecting either changes in endogenous proteins and lipids as lysosomes mature or degradative intermediates during digestion of different substrates), or it may be because of the presence of lysosomes derived from different cell types. Thus, it will be important to develop biologically relevant in vitro model systems to clarify these and other mechanistic questions.

The increased level of mannos 6-phosphate-containing lysosomal enzymes is not unique to rat. Elevated levels of Man 6-P glycoproteins have been observed in neurons compared with other cell types in humans and mice using bisected receptor and histochemistry, and similar results have been observed in bovine and mouse brain by blotting (31). Retention of the Man 6-P modification on neuronal lysosomal enzymes could have important functional consequences. One possibility is that retention of the Man 6-P marker serves a protective function, allowing efficient reuptake of inadvertently secreted hydrolases. This would be particularly important in brain, given that there is extensive vesicular trafficking and exocytosis in neurons, and even a small amount of mis-sorting could result in release of lysosomal enzymes and damage to surrounding tissue. Another intriguing possibility is that the Man 6-P modification is used to tether lysosomal enzymes to surface MPRs, greatly increasing their local concentration and allowing efficient degradation of extracellular material (32, 33). In this manner, the lysosomal enzymes may participate in important processes such as neuronal migration, outgrowth, and synaptic remodeling.

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