Molecular Neurobiology

ISSN0893-7648/05/32(1): 27–41/$30.00

Lysosomal Membrane Proteomics and Biogenesis of Lysosomes

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Abstract

This review focuses on events involved in the biogenesis of the lysosome. This organelle contains a diverse array of soluble, luminal proteins capable of digesting all the macromolecules in the cell. Altered function of lysosomes or its constituent enzymes has been implicated in a host of human pathologies, including storage diseases, cancer, and infectious and neurodegenerative diseases. Luminal enzymes are well-characterized, and aspects of how they are incorporated into lysosomes are known. However, little is known about the composition of the membrane surrounding the organelle or how the membrane is assembled. Our starting point to study lysosome biogenesis is to define the composition of the membrane by the use of proven methods for purification of lysosomes to near homogeneity and then to characterize membrane-associated and integral lysosomal membrane proteins. This has been achieved using advanced proteomics (electrophoretic or chromatographic separations of proteins followed by time-of-flight mass spectrometric identification of peptide sequences). To date, we have identified 55 proteins in the membrane-associated fraction and 215 proteins in the integral membrane. By applying these methods to mouse models of lysosome dysgenesis (such as BEIGE, Pale Ear, PEARL) that are related to human diseases such as Chediak-Higashi and Hermansky-Pudlak syndromes, it may be possible to define the membrane protein composition of lysosomes in each of these mutants and to determine how they differ from normal. Identifying proteins affected in the respective mutants may provide hints about how they are targeted to the lysosomal membrane and how failure to target them leads to disease; these features are pivotal to understanding lysosome biogenesis and have the potential to implicate lysosomes in a broad range of human pathologies.

Index Entries: Lysosomes; biogenesis; membrane; proteomics; mass spectrometry; mouse models.

Received 9/16/04; Accepted 10/14/04
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Introduction

In 1967, J. W. Callahan traveled to the Montreal Children's Hospital to receive a small sample of brain taken by open brain biopsy from a young girl with the same symptoms as an earlier patient described by Derry et al. *(1)*. The earlier case and the case of the young girl were the earliest described examples of the late infantile form of GM1 gangliosidosis *(2)*. In addition to demonstrating storage of GM1 ganglioside, electron micrographs of involved neurons in the brain specimen revealed the membranous cytoplasmic bodies characteristic of the gangliosidoses, but we also noted several inclusion bodies with less well-formed intra-organellar structures. At the time, we wondered whether these were transitional organelles lying somewhere between mature, functionally competent lysosomes and the membranous cytoplasmic bodies considered (even at that early time) largely end-stage residual, tertiary lysosomes. We also speculated as to whether intermediate structures might reflect a different group of lysosomes whose composition or complement of hydrolases differed from the others. In the intervening years, various lysosome-like organelles (platelet-dense granules, azurophilic granules, brush border organelles of osteoclasts, melanosomes) as well as traditional lysosomes have been defined in various tissues *(3)*. Of course, the only way to define the similarities or differences between these organelles is to isolate pure organelles, separate them into subfractions, and study their composition. At the time of De Duve and his colleagues in Leuven, this was a daunting task; however, in the face of current technologies, this task is approachable.

Lysosomes, Phagosomes, and Endosomes

Lysosomes are single-membrane-bounded organelles containing a large array of soluble and membrane-associated hydrolases with acidic pH optima capable of digesting all the macromolecules and organelles in the cell *(3)*. Lysosomal hydrolases are synthesized, *N*-glycosylated, and folded in the endoplasmic reticulum (ER; ref. *4*). In the post-ER/early Golgi, they are recognized and tagged with mannose-6-phosphate moieties, which allows their capture by one of two mannose-6-phosphate receptors (MPRs) in the trans-Golgi network (TGN). Signals in the cytosolic tail (C-tail) domain of the MPR facilitate organization of adaptor complex protein structures *(5–7)*, at which point the decorated vesicles are trafficked to and become fused with other intracellular endosomal compartments *(8)*. Similarly, other adaptor complexes recognize spatially separate, yet similar signals such as the di-leucine-type and/or Tyr-X-X-Ø (X = any amino acid; Ø = large hydrophobic amino acid) that occur in the short C-tail sequences of integral membrane proteins *(8–10)*. Lysosome maturation completes a series of these vesicular transport steps that involve all sectors of the endosome/lysosome compartment (i.e., endosome–lysosome, phagosome–lysosome, autophagic vacuole–lysosome, and plasma membrane [PM]–lysosome fusions).

Several classes of signaling proteins are implicated in these fusions, but the roles of each in sorting these vesicles to various compartments are just beginning to emerge *(5,11)*. For example, four independent assembly protein/adaptor complexes (adaptor protein [AP]1, AP2, AP3, and AP4), which are all composed of four subunits called adaptins *(9,12–14)*, and another group of adaptors, the monomeric Golgi-localized, γ-ear-containing, adenosine diphosphate (ADP) ribosylation factor-binding proteins (GGAs), localize on the cytosolic surfaces of vesicles and/or endosome membranes. AP1 (at the TGN and endosomes) or AP2 (at the PM), bind to the C-tail of a receptor protein to trigger assembly of preformed clathrin triskelion complexes into cagelike structures, thereby allowing the vesicles to associate with fusion membranes *(9)*. AP3 complexes localize more to endosomes, whereas AP4 complexes associate more with

TGN membranes; however, both appear less reliant on clathrin for targeting, suggesting that other proteins may be involved. These could include the GGA adaptors. AP3 complexes (defective in Hermansky–Pudlak Syndrome [HPS]) are associated with late endosome-to-lysosome sorting and are believed to prevent the recycling of certain lysosome membrane proteins (such as lysosome-associated membrane glycoproteins [LAMPs]) from the endosome back to either the PM or the TGN. Little is known about AP4 complexes, but some indirect evidence suggests they are involved in melanosome maturation and endosomal/lysosomal sorting *(14,15)*.

This complicated process also involves other signaling proteins, including soluble Arf proteins that become membrane-bound on binding of guanosine triphosphate (GTP) as well as Rab and soluble *N*-ethylmaleimide sensitive-factor attachment protein receptor [SNARE] proteins. At some point, vesicle–vesicle fusion is initiated, GTP is converted to guanosine diphosphate [GDP], and dissociation of the adaptor targeting complexes occurs. A TGN remnant with its bound MPR is recycled back to the TGN. The internal endosome/lysosome acidic pH is achieved through the function of an integral membrane-localized ATP–proton pump complex comprising several proteins *(6,16)*. It is not clear how or when individual proteins of the essential proton pump mechanism are acquired or at what stage the complex is assembled *(17–19)*.

Substances for digestion (cargo) are acquired into the lysosomal compartment via a series of dynamic processes (such as pinocytosis, phagocytosis, and autophagy) that also involve membrane fusion and fission processes with formation of transitional (short-lived) vesicular and tubular structures comparable to those involving TGN vesicles. Simpler molecules arising as products of digestive events are translocated via membrane transporter proteins from the intralysosomal compartment across the lysosomal membrane and are released into the cytoplasm for their re-utilization. Transportation into and out of the lysosome is often codependent on the functional H^+-ATPase–proton pump *(10,17,18)*. Following an undetermined number of vesicle fusions and cargo acquisition/digestion cycles, individual lysosomes reach a mature stage, after which they are released from cells by a process of exocytosis *(20,21)* or they are remodeled via a poorly understood recycling process *(22,23)*.

In receptor-mediated endocytosis, cargo (cytokines, lipoproteins, single proteins, hormones, and so on) bind via ligands to extracellular domains of receptors in the PM, are internalized in clathrin-coated vesicles, and elicit downstream responses (reviewed in refs. *24* and *25*). Again, the vesicles must be uncoated before membrane fusion. Many other signaling events initiated by receptors' C-domains then occur to sort those components destined for the lysosome from those that are to be recycled either back to the TGN or the PM *(26)*. After fusing with existing endosomes, receptor cargo appears temporally in smaller intraluminal vesicles, and transport signaling occurs on the C-domains of resident endosomal membrane proteins.

Autophagosomes and phagosomes are transient vesicles that sequester intra- (autophagosomes) or extracellular (phagosomes) materials largely for their controlled degradation *(27–29)*. Autophagosomes arise from purely intracellular events and are involved in the regulated turnover of intracellular components in response to the need to remodel membranes and remove organelles and/or long-lived C-proteins. Similarly, phagosomes arise from the PM via vesiculization of largely extracellular, extraneous substances (including foreign cells such as bacteria or foreign substances such as dextran particles). Lines of demarcation between autophagosomes/phagosomes and endosomes are difficult to determine precisely because their protein compositions overlap significantly. Late endosomes and lysosomes having low internal pH values and dense membranes also contain the unique phospholipid, lyso-bisphosphatidic acid *(30,31)*.

Diseases of Lysosomes Where Lysosomal Biogenesis Is Normal

Luminal Lysosomopathies

The pivotal role lysosomes play in cell metabolism is manifested by the more than 40 enzymopathies where indigestible macromolecules become stored inside the organelle, alter the cell's homeostasis, and lead to a disease state. The molecular basis of diseases directly involving the luminal (soluble) enzymes is well-advanced *(32,33)*. Typically, as shown for the GM1 and GM2 gangliosidoses (extensively studied by the Callahan and Mahuran laboratories *[32,33]*), the vast majority of the associated mutations (even missense mutations) result in no, or very low, levels of detectable mutant protein *(32,33)*. This occurs as a result of the ER's quality control system, which recognizes misfolded or unassembled proteins and destroys them, resulting in little or no mutant protein reaching the lysosome. For example, of the more than 100 mutations affecting one of the hexosaminidase A subunits (α or β), only missense mutations at one α-site (Arg178) allow near normal levels of hexosaminidase A with an inactive α-subunit to be transported to the lysosome *(33)*.

Diseases of Lysosomal Integral Membrane Proteins

Several lysosomal membrane proteins (LMPs) have been linked to diseases through functional assays, such as Sanfilippo C disease *(34)* and Cobalamin F (vitamin B12) transporter protein disease *(35,36)*, or through both transporter and direct molecular analyses, such as Niemann-Pick type C (cholesterol transporter *[37–39]*), Salla disease (Sialin, sialic acid transporter *[40,41]*), Cystinosin (Cystein transporter of Cystinosis *[42,43]*), some neuronal ceroid lipofuscinoses *(44,45)*, and LAMP2 (Danon disease *[46]*). Recently, mutations in the gene for subunit A3 of the H^+–ATPase-proton pump have been identified in patients with severe infantile malignant osteopetrosis *(47)*. Few of these proteins have been characterized directly because of the presence of multiple transmembrane domains that render them very hydrophobic and, therefore, difficult to study. LMPs have also been implicated in the function of the major histocompatibility complex (MHC) class II *(48–50)* and in human pathologies such as cancer *(51)*, drug resistance *(52)*, auto-immune diseases *(53)*, and Alzheimer's disease.

Diseases of Lysosomes Where Lysosomal Biogenesis Is Abnormal

Human Chediak–Higashi Syndrome (CHS; ref. *54*), Griscelli Syndrome *(15,55)*, and HPS *(15,55–57)* as well as about 15 separate congenic mouse diseases, represent this group *(15)*. All are autosomal recessive and are characterized by variegated pigmentation, variable immune deficiencies, platelet dysfunction, and "giant" lysosomes. The 429-kDa LYST protein, which is defective in CHS *(54,58)*, is cytosolic and believed to serve as a lysosome retention regulator by inhibiting recycling of LAMPs 1, 2, and 3 and LIMP from the late endosome/lysosome back to the TGN vesicles or to the PM *(59–61)*. HPS is heterogeneous (HPS1, 2, and 3 are known). Human HPS1 arises from mutations in a gene encoding a 79-kDa protein of unknown function, whereas adaptin β3A of the AP3 complex causes HPS2 *(55,62–64)*. Defects in the adaptin β3A of the AP3 complex provide a window on late endosome–lysosome transport. The composition of LMPs involved in these disorders is undefined. Tyrosinase, a key enzyme in melanosomes, is presumably targeted for lysosomal incorporation by the well-known dileucine signal in its C-tail but is mislocated in both CHS and HPS. Other LMPs (e.g., LAMP 1 and 2) also have these signals but are not mislocated, indicating that our understanding of lysosomal targeting is incomplete *(65–67)*. It is clear that the altered melanosomes display many of the features of lysosomes; however, the extent to which melanosomes and lysosomes are similar or different is poorly defined.

Lysosomal Membrane Proteomics

Elucidation of the protein composition of the lysosomal membrane and the mechanisms involved in the incorporation of integral membrane proteins are essential to understanding the biogenesis and functions of the lysosomal system and how these are affected in human disease. Approaching answers to these problems requires that the protein composition of the soluble and membrane compartments of the organelle be defined. This requires that lysosomes be isolated in a highly purified form and in adequate yields.

Procedures for the isolation of lysosomes from tissues usually involve differential centrifugation, but experience has shown that these preparations are contaminated to varying degrees by mitochondria and peroxisomes and, to a lesser extent, heavy microsomes, which are organelles that have the same buoyant density as lysosomes. A promising technique that was employed in the past but has not received wide attention is free-flow electrophoresis *(68–70)*. Our recent experience with this technique using cultured skin fibroblasts has been promising, but because these cells do not have an abundance of lysosomes, yields are low, thus making them less than optimal for even the most sensitive proteomics applications.

To circumvent these problems, our approach has employed Triton-filled lysosomes (Tritosomes) *(71,72)*. Tritosomes are readily purified by differential centrifugation from rat liver 4 to 5 d after a single intraperitoneal injection of the neutral detergent Triton WR-1339. We have demonstrated that these tritosomes are highly enriched in marker hydrolases by direct enzyme assays, Western blots, and acid phosphatase staining/electron microscopy with minimal contamination from mitochondria, peroxisomes, and microsomal elements *(71,72)*. Based on our data and other extensive characterization over the past 30 yr, it can be concluded that tritosomes satisfy all the biochemical and physical criteria of *bona fide* lysosomes (reviewed in ref. *3*).

Purified tritosomes can be fractioned into soluble and membrane protein components by several rounds of freezing and thawing. This yields a soluble, hydrolase-rich fraction that contains mostly luminal proteins and a membrane pellet *(71,72)*. The soluble fraction is enriched in acid hydrolases, many of which have been extensively characterized because of their involvement in the storage diseases. These proteins display a minimum of hydrophobic character and are readily separated by two-dimensional (2D) gels immobilized pH gradient-sodium dodecylsulfate polyacrylamide gel electrophoresis (IPG-SDS-PAGE). Although not studied by us, this fraction has been partially characterized in other work *(73,74)*.

We initially attempted to define the composition of the lysosomal membrane (our primary interest) based on our published methods for 2D-electrophoresis coupled with trypsin in-gel digestion and mass spectrometry-based protein identification *(71,75)*. Subjecting the whole LMP fraction to 2D-gel electrophoresis produced a multitude of well-separated protein spots, some of which occurred in isoforms (Fig. 1). However, it was clear that much of the applied material remained at the top of the gel—presumably as a result of its poor solubility and aggregation in the first electrophoresis dimension. Mass spectrometry identification of the individual spots (Table 1) revealed that the majority of the identified proteins were not integral membrane proteins but, rather, could be characterized as peripherally associated with membranes (vATPase A and B subunits, Snapα) or as soluble (arginase, transferrin).

Based on this finding, we reasoned that the membrane could be separated into a membrane-associated compartment and an integral membrane protein fraction. We adopted a technique shown to work with peroxisomes that removes peripherally bound membrane proteins. This involves a 30-min incubation of the resuspended membrane in 0.1 *M* of Na_2CO_3. The proteins extracted in this fraction (membrane-associated proteins) were readily separated by the 2D-gel system, with minimal amounts of protein aggregation at the top of

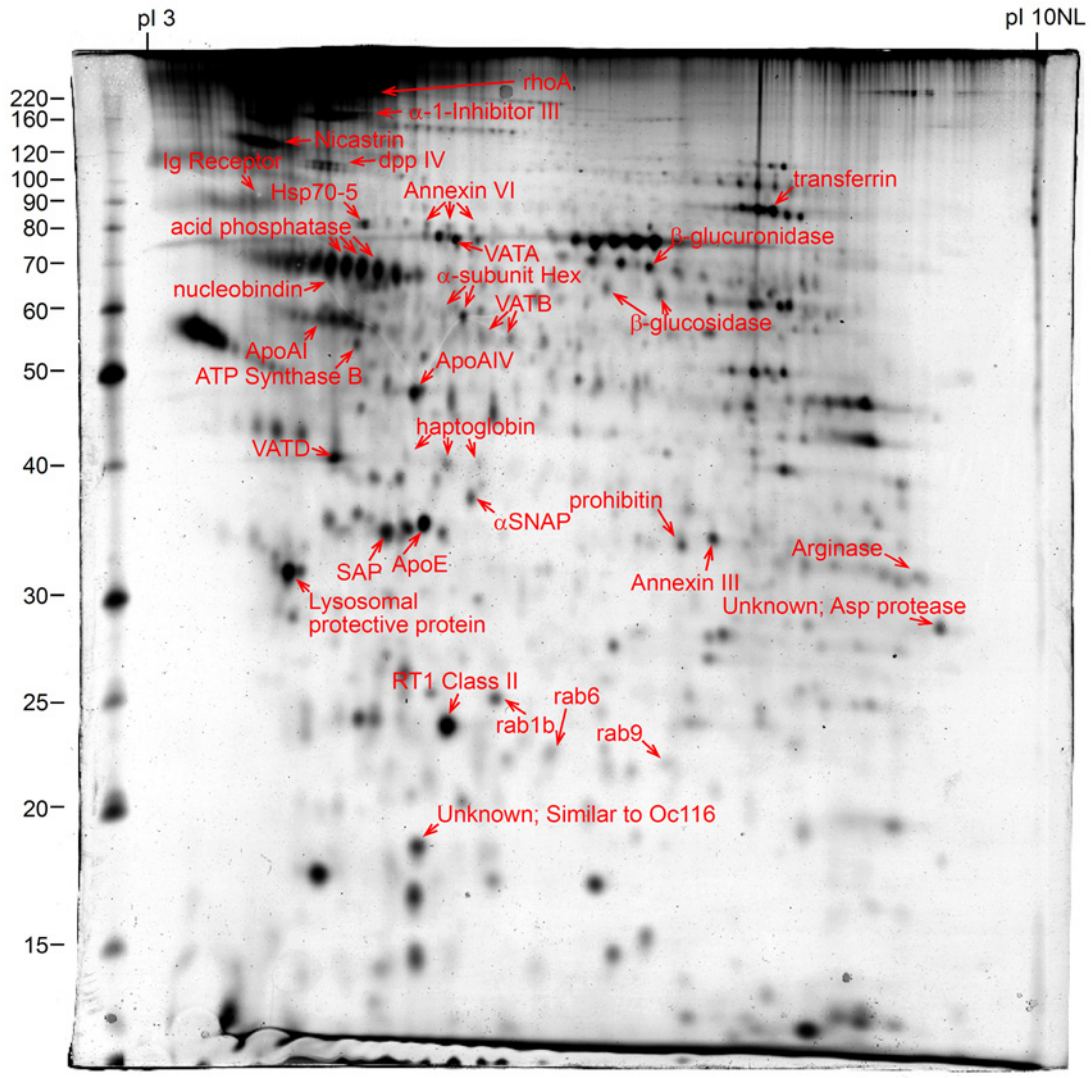

Fig. 1. 2D-IPG/SDS-PAGE of the whole-membrane protein fraction from rat liver tritosomes. Annotations of proteins identified, the approximate molecular weight (kDa), and the first dimension pI are indicated.

the gel (Fig. 2). The major spots in the membrane-associated protein fraction (Fig. 2) migrated similarly to those spots from the whole-membrane fraction (Fig. 1); indeed, the proteins identified from the two gels were similar (as annotated).

Table 1 lists 54 of the proteins identified in this fraction to date, listed according to their expected topology either on the luminal or the cytoplasmic surface of lysosomes. Typical luminal proteins include protective protein of the β-galactosidase–neuraminidase–protective protein complex; cathepsins D, B, and Z; and β-hexosaminidase α-subunit. Proteins on the cytoplasmic face of lysosomes expectedly included vATPase subunits and trafficking proteins (Rabs and Snapα). Few proteins with one or more transmembrane domains, such as Nicastrin *(71)* and dipeptidylpeptidase IV, were resolved by the 2D gels.

Table 1
Proteins Identified From the Membrane-Associated Protein Fraction (Sodium Carbonate Extractable) Sorted by Topology

Luminal	Accession	2D-Gel MW (kDa)	2D-Gel pI (approx)
Albumin	19705431	75	5–7
α-1-inhibitor III	12831225	160	4–5
Apolipoprotein A-I	6978515	60	4.5–5.5
Apolipoprotein A-IV	114008	45	4.5–5.5
Apolipoprotein E	1703338	35	4.5–5.5
β-glucosidase	6679955	60	6–7
β-glucuronidase	758260	72	6–7
β-hexosaminidase, α-subunit	232255	58	5–5.5
Calreticulin	11693172	65	3–4
Cathepsin B	203648	30	4.5–5.5
Cathepsin D	19705441	45	4–6
Cathepsin Z	34978341	35	4.5–5.5
di-*N*-acetylchitobiase	13591934	50	5–7
Dipeptidylpeptidase IV (dpp IV)	111948	90	4–5
Haptoglobin	6981042	38	4.5–5.5
Heat shock 70-kD protein 5 (glucose-regulated protein; BiP)	12835845	80	4.5–5.5
Hemopexin	16758014	65	7–8
Liver annexin-like protein	7108713	60	5–7
Lysosomal acid-phosphatase	8392842	78	4.5–5.5
Lysosomal protective protein	131082	29	3–4
Nicastrin	27819651	120	3–5
Nucleobindin	6679158	70	4.5–5.5
Protein disulfide isomerase (ER60)	1352384	65	5–7
Rat polymeric immunoglobulin Receptor	56465	80	4–5
RT1 class II histocompatability, D1 β-precursor	P18211	24	4–6
Serum amyloid P (SAP)	8392903	30	4.5–5.5
Spi2c (serine protease inhibitor 2c)	13928716	50	4–5
T-kininogen II	125521	80	3–4
Transferrin	1083816	90	6–7

Cytoplasmic	Accession	2D Gel MW (kDa)	2D-Gel pI (approx)
Aldolase B	1619606	40	7.0–8.0
Annexin III - Lipocortin III	113955	34	6–7
Annexin IV	26024200	35	4.5–5.5
Annexin VI	763181	73	5–5.5
Arginase 1	114146	35	7–9
ATPase synthase B-subunit	1374715	51	4–5
β-enolase	6978811	50	6–7
Betaine-homocysteine methyltransferase	13540663	40	7–8
Catalase	6978607	65	7–8
Fumarylacetoacetate hydrolase	8393349	40	7–8
Long-chain α-hydroxy acid oxidase	238482	65	7–8
Ornithine transcarbamylase	6679184	35	7–8
Prohibitin (BAP32)	6679299	29	5–7
rab1b	92339	25	4.5–5.5
rab6	17512290	25	4.5–5.5
Rab9	16758200	20	6–7
RhoA	6981478	220	4.5–5.5
SNAP α	18034791	35	4.5–5.5
Sorbitol dehydrogenase	8394334	40	7–8
vATPase A-subunit (VATA)	1718086	68	5–5.5
vATPase B-subunit (VATB)	17105370	55	5–5.5
vATPase D-subunit (VATD)	3955100	40	4.5–5.5
Unknown			
Unknown; similar domain in vATPase Oc116 subunit	ESTs	14	3.5–5
Unknown; similar to A. aigypti aspartate protease	ESTs	29	8–10

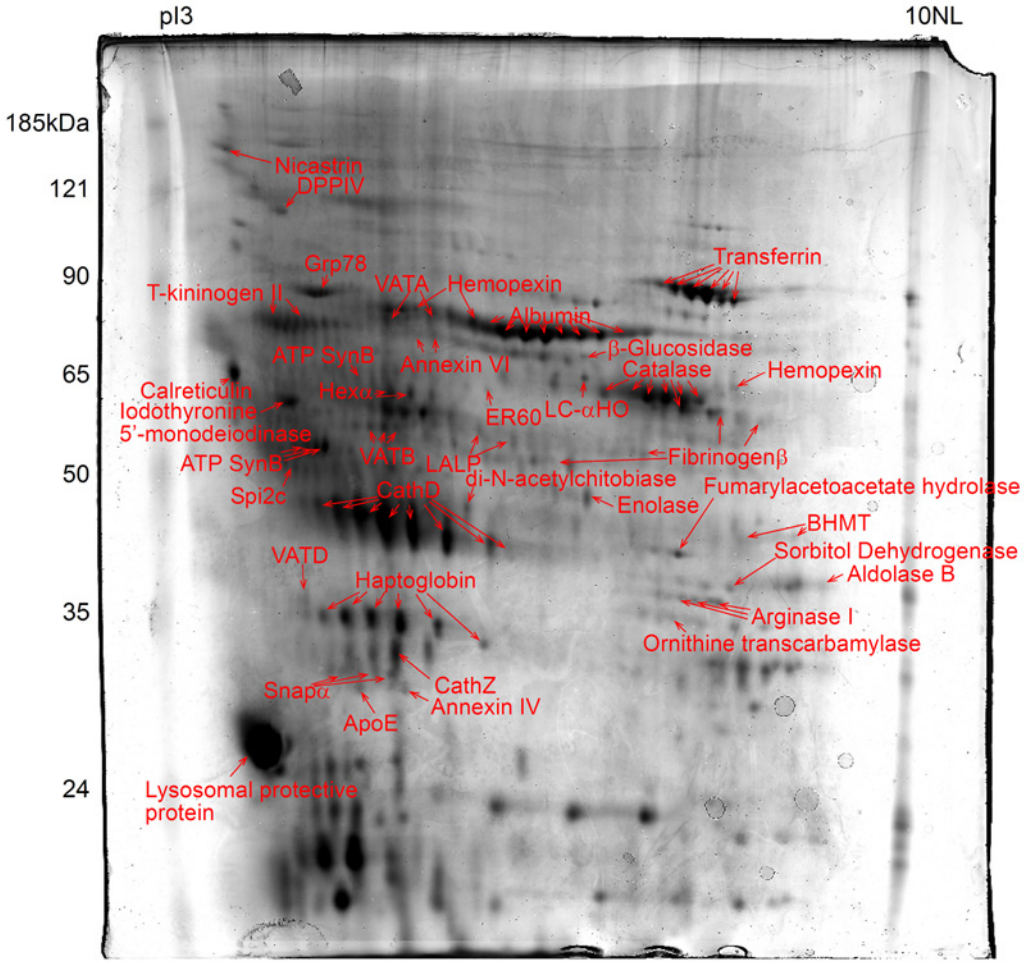

Fig. 2. 2D-IPG/SDS-PAGE of proteins of the membrane-associated protein fraction from rat liver tritosomes. Proteins released from whole tritosomal membranes by sodium carbonate treatment were separated by the 2D gel. Annotations of proteins identified, the approximate molecular weight (kDa), and the first dimension pI are indicated.

A common theme for subcellular proteomics studies is the identification of proteins that have not been previously associated with the particular subcellular compartment under investigation. After validation, these findings can provide insight regarding novel functions of the organelle or of the protein. For example, the identification of arginase in the membrane-associated fraction of the lysosome was unexpected because it is considered a C-protein *(76)*.

To determine the topology of lysosome-associated arginase, we performed a protease protection assay. Intact tritosomes were briefly (5 min) treated with proteinase K with and without detergent, followed by treatment with Western blots (Fig. 3). Arginase and rab7 (known to be cytosolic) were not protected from the protease by the membrane of the lysosome, whereas the N-terminus of Nicastrin (known to be luminal) was protected. This indicates that arginase occurs on the cytosolic surface and is not a part of the degradative luminal milieu. An intriguing functional significance of lysosomal-associated arginase is the possible existence of molecular complexes, which, as metabolons, process recycled materi-

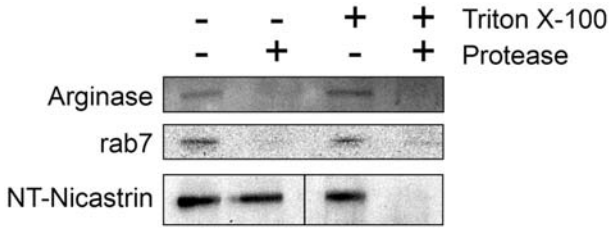

Fig. 3. Arginase exists on the cytoplasmic face of the lysosomal membrane. Intact tritosomes were incubated with or without proteinase K or detergent as indicated for 5 min. Proteins were precipitated with trichloroacetic acid and processed for immunoblotting. The proteins not protected by membrane are digested without the presence of detergent, indicating a cytoplasmic topology. The N-terminal of Nicastrin is shown as a control for the lysosomal lumen.

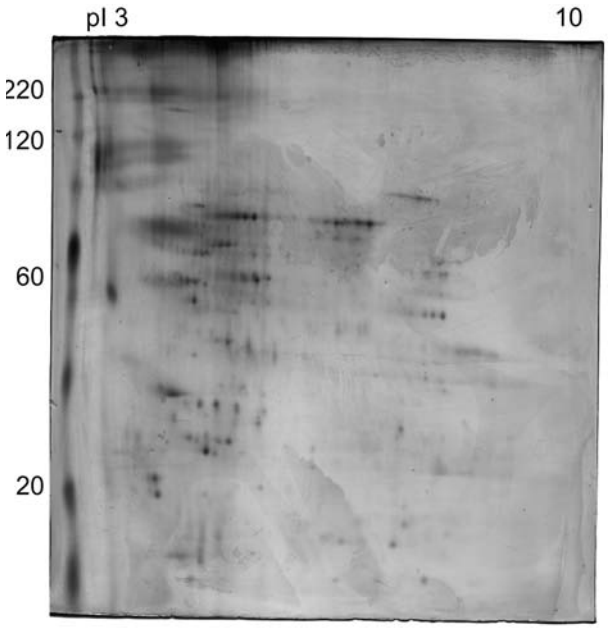

Fig. 4. 2D-IPG/SDS-PAGE of proteins from the integral membrane protein fraction of rat liver tritosomes. Note that a large amount of protein does not enter the gel, and there is a large amount of smearing. A total of 0.5 mg of protein was loaded on this gel and silver-stained. Proteins that are resolved are likely those readily found in the membrane-associated protein fraction. Approximate molecular weight (kDa) and pI range are indicated.

als as they are transported across the lysosomal membrane. Furthermore, aldolase, one of the proteins identified in this article, has been shown to modulate the assembly and activity of the vATPase proton pump, thus providing a direct link of glycolytic energy production and vATPase function *(77)*.

The integral membrane protein fraction (the material remaining after extraction with sodium carbonate) was also subjected to the 2D-gel procedure, but we employed novel detergents *(78)* and gel preparation methods *(79)* in an attempt to resolve the hydrophobic integral membrane proteins. Despite these precautions, our attempts resulted in few resolved proteins and large protein smears at the top of the gel (Fig. 4). A major drawback of this method is the poor, or no, separation of hydrophobic proteins by 2D-gels. As an alternative method, the lysosomal integral membrane protein fraction pellet was dissolved in guanidine-HCl, reduced, S-alkylated, and dialyzed against 8 *M* of urea (1% Elugent [Calbiochem])

We devised a CM-Sepharose cation-exchange column procedure to fractionate the proteins into an unbound fraction and bound proteins that were collected by stepwise elution with 20, 40, 80, and 250 m*M* of NaCl in 8 *M* of urea (1% Elugent, pH 3.5). These fractions were separated on 1D-SDS-PAGE tube gels, sliced into 2- to 4-mm sections (from high to low molecular weight), and processed for in-gel digestion and LC-MS/MS peptide identification. To date, 215 proteins have been identified in the integral membrane fraction *(80)*. The largest proteins found were the apolipoprotein b (539 kDa) and lipoprotein receptor-related protein R1 (523 kDa), whereas the smallest were vAMP8 (11.4 kDa) and ubiquitin. The proteins identified were sorted based on their generally accepted subcellular localizations (Fig. 5). Well-known endosomal/lysosomal compartment proteins (such as LAMP1, LAMP2, LIMP2, Niemann-Pick C1, and vacuolar ATP–proton pump components) constitute 19% of the total, whereas 11% (24 proteins) are unknown regarding their function and have no close homologies to

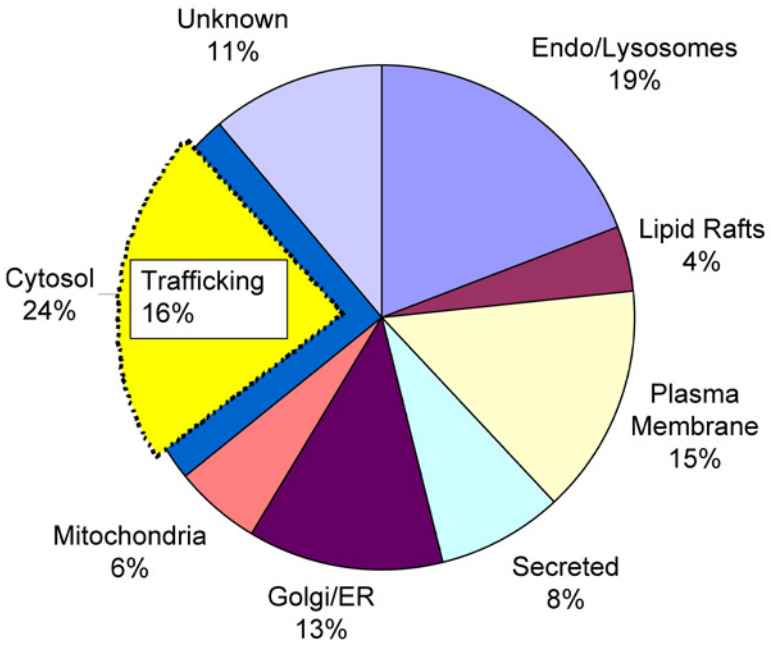

Fig. 5. Distribution of the 215 proteins identified in the integral membrane protein fraction of rat liver tritosomes (*80*). Identified proteins were sorted according to their generally accepted localizations. Proteins that were represented in the database as "unknown" or as hypothetical cDNAs were categorized if there was homology to a known protein or domain of known function.

known proteins. Proteins of the cytosol constituted 24% of the total, which includes the 16% contribution of trafficking proteins (such as Rab and SNARE proteins).

Lysosomal Membrane Proteomics and Biogenesis of Lysosomes

With a significant proportion of the proteins in the integral membrane fraction identified, it now becomes possible to determine how they got there—that is, lysosome biogenesis and maintenance. To accomplish this, a study of the lysosomal membrane isolated from the mouse models of human lysosomal biogenesis diseases such as CHS, HPS, and the very rare Griscelli Syndrome should prove informative. Similarly to humans, the mouse disorders are characterized by "giant lysosomes;" however, whereas the extent of human genetic heterogeneity encompassed in each of these distinct disorders is unknown, mutations in about 15 genes are implicated in the mouse versions of these disorders *(15,54,64,81)*. The functions of many proteins implicated in these mouse models are poorly understood, but some have been identified as adaptor complex members or subunits of biogenesis of lysosome-related organelles complexes (BLOC)-1, -2, and -3 assemblies, which are proteins that play pivotal roles in membrane fusion events *(63,82,83)*. Similarly, rab7, a major rab protein found in our studies of the lysosomal membrane whose function is necessary for late-endosome:lysosome fusion events, is mutated in the peripheral neuropathy Charcot-Marie-Tooth 2B *(84)*.

In our proteomics survey of the lysosomal membrane, we identified several rab proteins. Although it is widely believed that individual rab proteins localize to and demarcate different subcellular structures, each subcellular compartment can play host to various rab proteins,

and different rab proteins on the same vesicle may cause the creation of membrane subdomains *(85)*. Because a variety of proteins from nonlysosomal/endosomal structures were also identified in the integral membrane fraction, it is likely that these proteins exist in domain structures on the membrane as a consequence of vesicular fusion events.

Current evidence suggests that as vesicles arise from the TGN or other faces of the Golgi apparatus, they become attached to microtubule elements and are directed to the periphery of the cell *(15)*. During the transit of these microtubule-associated vesicles, sorting receptors or regulator proteins (including the rab and SNARE proteins) assemble on their surfaces, facilitating interaction with other vesicular structures in the endosomal/lysosomal lineage. It has been postulated that defects in the ability of nascent vesicles to attach to the microtubule elements characterize the variants in the HPS group, whereas the defects giving rise to CHS and Griscelli Syndrome are considered more downstream and involve protein importation into a more mature organelle. At the recipient vesicle, including late endosome/lysosome membranes, cytosolic Arf or Arf-like proteins become activated through GTP binding and attach to the cytosolic surface of the late endosome membrane *(86,87)*. Presumably, the next step is an interaction between the sorting receptor-decorated TGN vesicles and Arf-decorated acceptor to bring them into close apposition to facilitate fusion. After GTP becomes cleaved to GDP, the proteins of the complexes dissociate and are released into the cytosol. The vesicle remnants are then recycled. The role played by the lysosomal targeting regulator protein LYST, defective in CHS *(54,58)*, remains to be determined; however, a specific rab, Rab9, is believed to have a regulatory role in recycling the remnant back to the TGN *(88,89)*. There is evidence for activation of phospholipase D during fusion of the membranes, suggesting that reorganization of lipid domains occurs concurrently *(90–92)*.

In summary, lysosome proteomics using mouse models affords an excellent opportunity to describe some events in the biogenesis of lysosomes. LYST mutants (human CHS, mouse BEIGE) enable studies regarding how defective vesicle trafficking affects lysosome membrane composition, whereas the mutant adaptin β3A of the AP3 complex (mouse PEARL, human HPS2) affects studies on defective vesicle formation *(55,62–64)*. Our hypothesis is that the membranes of the "giant lysosomes" are "immature" and contain proteins not usually associated with mature lysosomes (secondary lysosomes) and/or lack many of those membrane proteins found in normal lysosomes. Identifying the composition of the "giant lysosomes" using our lysosome proteomics approach and comparing them to normal examples may help to define the roles the mutated proteins play in lysosome biogenesis.

Acknowledgments

We wish to thank Sunqu Zhang, Vanessa Blandford, and Cheryl Leung for technical assistance. This work was supported by grants to J. W. Callahan and D. Mahuran from the CIHR and Genome Canada.

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