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# Comparative bioinformatics analyses and profiling of lysosome-related organelle proteomes

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**Abstract**

Complete and accurate profiling of cellular organelle proteomes, while challenging, is important for the understanding of detailed cellular processes at the organelle level. Mass spectrometry technologies coupled with bioinformatics analysis provide an effective approach for protein identification and functional interpretation of organelle proteomes. In this study, we have compiled human organelle reference datasets from large-scale proteomic studies and protein databases for seven lysosome-related organelles (LROs), as well as the endoplasmic reticulum and mitochondria, for comparative organelle proteome analysis. Heterogeneous sources of human organelle proteins and rodent homologs are mapped to human UniProtKB protein entries based on ID and/or peptide mappings, followed by functional annotation and categorization using the iProXpress proteomic expression analysis system. Cataloging organelle proteomes allows close examination of both shared and unique proteins among various LROs and reveals their functional relevance. The proteomic comparisons show that LROs are a closely related family of organelles. The shared proteins indicate the dynamic and hybrid nature of LROs, while the unique transmembrane proteins may represent additional candidate marker proteins for LROs. This comparative analysis, therefore, provides a basis for hypothesis formulation and experimental validation of organelle proteins and their functional roles.


*Keywords:* Lysosome-related organelle (LRO); Organelle proteome; Proteomics; Bioinformatics; Protein database

## 1. Introduction

In recent years, a better understanding of complex pathways and interactions at the molecular level has changed our approach to the analysis of proteomic data. In particular, large-scale proteomic profiling of organelles and subcellular large structures has yielded valuable information about protein localization which provides functional implications to these proteins. However, organelles are no longer considered fixed entities, but rather as dynamic structures interacting with each other and remodeling themselves in response to various stimuli. Accordingly, it is unlikely that a discrete proteome can be assigned to any of the subcellular compartments. Because the concept of "pure" organelles is untenable, demonstrating the presence of organelle proteins by approaches, such as immunofluorescence or tag expression, is an important way to validate their localization [1]. Due to their dynamic nature, organelles must be analyzed under various conditions in order to understand integrated cell functions.

Complete and accurate profiling of the subcellular localization of proteins is critical for understanding their functions. However, organelle proteome characterization is challenging for several reasons. High-quality separation of organelles and large complexes without cross-contamination is technically demanding, if not impossible. Missing or incorrect identification of proteins by mass spectrometry (MS) is still common. Moreover, the same organelles in different tissues or cell types may have different profiles [2,3]. Many proteins may be associated

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with more than one organelle or subcellular compartment, and temporal and spatial regulation of organelle proteins is common. A recent study estimated that up to 39% of organelle proteins are present in multiple organelles [4]. Despite the technical challenges and the biological reality, large-scale MS proteomic profiling, coupled with separation techniques, represents the best current technology and has led to the characterization of a number of organelle proteomes, including those of mitochondria, the plasma membrane, the cytosol, the nucleus, and even subnuclear structures, such as the nucleolus (for reviews, see refs. [5–9]).

Organelle proteomic analysis combines the power of cellular biology with rapidly advancing methods and instrumentation [7]. Subcellular fractionation strategies represent the centerpiece of subcellular proteome analysis. Most fractionation procedures are designed to purify a known structure with an established experimental system and are based on conventional sequential centrifugation or density gradient centrifugation [10]. However, cellular organelles often share comparable densities, which may lead to an enrichment, but not complete purification, of the target structures. Therefore, it is important to combine several methods and strategies based on complex centrifugation and electrophoretic steps to successfully achieve high purity.

Taken together, the validation of organelle proteomic data must be performed at different levels, since highly sensitive mass spectrometers can detect trace amounts of peptides that might originate either from contaminants or from resident proteins of low abundance [7].

Bioinformatics approaches have been used for organelle proteome research in several areas, including database annotation of protein subcellular locations, prediction of subcellular location and integrated analysis of organelle proteomes. Curated protein databases, such as the UniProt Knowledgebase (UniProtKB) [11], are important repositories of our knowledge on protein subcellular locations based on experimental data of individual proteins reported in the scientific literature. Recent high-throughput experiments, such as proteomic profiling and immunofluorescence-based assays, have generated large amounts of data that can be used for database annotation of protein localization. Several specialized organelle databases, such as MitoP2 12 [12], OrganelleDB [13], and LOCATE (http://locate-human.imb.uq.edu.au/), have been developed to provide protein localization information for humans and model organisms, integrating large-scale experimental data with literature-based data. A new organelle database, the Organellar Map Database (ORMD), has recently been developed for 10 organelles/subcellular compartments of mouse liver, based on a study using Protein Correlation Profiling from MS intensity profiling [4].

Several bioinformatics methods have been developed for the prediction of protein subcellular location using machine learning and statistical approaches coupled with sequence information (for reviews, see refs. [14–18]). Although such programs are useful for automatic annotation of subcellular locations for massive amounts of sequence data, the scope of these programs is limited, focusing mostly on mitochondrial, nuclear, secretory and plasma membrane proteins. The predictive accuracy varies among different subcellular compartments. Even though the prediction program coupled with the machine learning method has been applied for deriving organelle proteome profiles [2], many membrane-enclosed organelles (e.g., lysosomes, endosomes and melanosomes) cannot be distinguished by the prediction methods.

As the technology for organelle separation and MS identification continues to improve and large amounts of proteomic data are generated, it becomes critically important to utilize bioinformatics analysis systems that integrate protein functional annotations for the systematic analysis of organelle proteomes. This facilitates the understanding of organelle biogenesis and dynamic interactions as well as the identification of candidate organelle markers for experimental validation. In this paper, we will focus on the integrated analysis of proteome profiles of lysosome-related organelles (LROs), a family of organelles that includes lysosomes, platelet dense bodies and melanosomes, to provide functional insights.

## 2. Experimental

### 2.1. Compilation of human organelle reference proteome datasets

For comparative profiling of human organelle proteomes, we compiled "reference" proteome datasets for seven LROs, the endoplasmic reticulum (ER) and mitochondria by combining data from current database annotations (UniProtKB and OrganelleDB) and published MS proteomic experiments. On the assumption that organelle proteomes may well be conserved among human and rodent species, we augmented the human proteome sets with human orthologs of known rat and mouse organelle proteins. Indeed, ~91% of all rat and mouse proteins in this study have human orthologs in UniProtKB.

### 2.2. Proteome profile analysis system: iProXpress

A systematic bioinformatics analysis of organelle proteomes was conducted using iProXpress, an integrated protein expression analysis system designed to analyze high-throughput MS proteomic and microarray gene expression experiments. The iProXpress system provides tools for protein mapping, functional annotation and profiling, as described below. A pilot version of the system has been applied to several expression studies, including the profiling of hormone-induced changes in endocrine tumor cells [19].

### 2.3. Protein mapping

As rich annotation, minimal redundancy and a high degree of data integration are critical for proteomic data interpretation, protein lists and peptide sequences generated from proteomic experiments were mapped to UniProtKB entries based on ID and peptide mapping. The Protein Information Resource (PIR) ID mapping service maps protein/gene IDs to UniProt from about 30 data sources (including NCBI identifiers such as gi, Entrez Gene and RefSeq ID). To cross-validate the ID mapping results, the peptide sequence for each mapped protein

was matched against the cross-referenced UniProt sequence to confirm the assignment. For many-to-one mapping, as is often the case for gi numbers, the mapping removes redundancy effectively.

For proteins not mapped through ID mapping, their peptide sequences were matched against the UniProtKB. In one-to-one mapping, where the peptide matches exactly one UniProtKB protein, that distinct protein will be given the assignment. In one-to-many mapping, if all the matched entries were in the same UniRef90 cluster, in which members share at least 90% sequence identity, one representative sequence was chosen. Otherwise, if the proteins belonged to different UniRef90 clusters, assignment was made with retro-inspection and manual validation of the original MS/MS protein identification results. Finally, the remaining proteins not mapped by the above steps were mapped by sequence homology. This process improved protein identification, achieving an overall protein mapping rate of over 90%.

### 2.4. Functional annotation and profiling

Following the protein mapping, a protein information matrix was generated to describe all organelle proteins based on sequence analysis and extensive annotations extracted from the iProClass database [20]. iProClass integrates information from over 90 biological databases for all UniProtKB proteins, including protein name, family classification, isoform, sequence features (domain, motif, functional site), Gene Ontology (GO) (molecular function, biological process, cellular component), function and functional category, structure and fold classification, pathway and pathway category, protein–protein interaction and complex, post-translational modification, genetics, genome, gene and protein expression, disease, literature, and taxonomy. iProClass also includes homology search results for reliable transfer of annotation from well-curated to poorly characterized proteins with evidence attribution for such homology-based inference.

The profiling analysis involves functional categorization and detailed analysis of proteins in a given organelle, as well as cross-comparison of proteins from multiple organelles based on the rich annotation in the protein information matrix to discover plausible functions. Iterative categorization and sorting of protein attributes, especially GO classes and Kyoto Encyclopedia of Genes and Genomes (KEGG) and BioCarta pathways, revealed major functional categories in the proteome. In combination with manual examination, interesting proteins found in only one type of organelle or those shared among different organelles were identified.

## 3. Results

### 3.1. Organelle proteome catalogs

As complete and accurate reference proteomes for intracellular organelles are not available, we have compiled reference datasets from published proteomes and annotated databases. These organelle proteome "catalogs" are incomplete, as proteins reported in the literature to reside in particular organelles may not be annotated in the databases and many organelle proteins are not detectable by current MS proteomic technology. Moreover, the quality of organelle purification and protein identification may vary in proteome datasets published by different groups, thus affecting the accuracy of the proteome catalog. The original MS spectra and data of the melanosome proteomes are available online in [21]. Nevertheless, the proteome datasets that we collected provide a basis for a systematic comparative analysis of LROs and may shed light on organelle functions, origins and relationships.

Our datasets contain 3290 different human proteins (UniProtKB entries) from nine organelles, as summarized in Table 1. The complete catalogs are listed in supplementary Table S1 (http://pir.georgetown.edu/iproxpress/organelle/organelle_comparison.html). Among the organelles, mitochondria and the ER have the highest numbers of proteins annotated in databases, followed by lysosomes and endosomes. Few annotated proteins are provided for LROs, where proteomics experiments are the main source of data. Human melanosomes have been extensively studied using proteomic profiling, with identification of about 1,400 proteins from all stages of melanosomes, including ~800 proteins for the early stages (I & II) and ~600 for late stages (III & IV) [21]. Mitochondria and the ER are the most studied organelles. As shown in Table 1, two recently published human mitochondrial proteomes substantially overlap with the proteome set annotated in databases, giving a combined mitochondrial proteome of over 900 proteins. In contrast, no systematic human ER proteome has been determined by proteomic profiling. The proteomic data derived from mouse ER proteomes have minimal overlap with the annotated human ER proteins.

Subcellular compartments have both shared and unique proteins. In particular, the identification of the transmembrane proteins in organellar membranes could reflect specific organellar functions. Transmembrane proteins constitute a high percentage (34–43%) of the proteomes of the ER and lysosomes, but a lower percentage (9–23%) in annotated LROs (Table 1). This may be because most LRO source data were from MS proteomics experiments, where transmembrane proteins may have been under-represented in the sample preparations. Also notably, 38–45% of ER, lysosomal and mitochondrial proteins are enzymes, while other LROs have <28% enzymes (data not shown).

### 3.2. Organelle proteome comparisons

#### 3.2.1. Comparison of LROs with mitochondria and ER

Based on the percentage of proteins found in only one type of organelle examined (~70%), the mitochondrial proteome is the most distinct from other organelles (Table 1). This is expected as mitochondria are relatively easy to isolate and have specific and unique functions. There are three possible reasons for the commonality of ~30% of mitochondrial proteins observed in one or more LROs: (1) proteins truly exist in other organelles, (2) those belonging to mitochondria but contaminating the proteomes of other organelles, and (3) those misidentified as mitochondrial proteins from proteomics experiments. An example

Table 1
Organelle proteome datasets from proteomics experiments and protein databases

| Organelles | | Organisms | Data sources | | Human protein | | Unique organelle proteins | |
|---|---|---|---|---|---|---|---|---|
| | | | Source[a] | # proteins | Total | tm[b] (%) | Total (%) | tm[b] |
| Endoplasmic reticulum | | Human | UniProtKB + OrganelleDB | 494 | 714 | 308 (43.1) | 371 (52) | 197 |
| | | Mouse | [58] | 141 | | | | |
| | | | [4][c] | 228 | | | | |
| Lysosomes (LY) | | Human | UniProtKB + OrganelleDB | 117 | 266 | 89 (33.5) | 101 (38.2) | 34 |
| | | Rat | [59] | 215 | | | | |
| Endosomes (EN) | | Human | UniProtKB + OrganelleDB | 142 | 456 | 91 (20) | 166 (36.4) | 39 |
| | | Mouse | [4] | 372 | | | | |
| Mitochondria (MT) | | Human | UniProtKB + OrganelleDB | 638 | 902 | 169 (18.7) | 624 (70) | 119 |
| | | | [60] | 615 | | | | |
| | | | [61] | 672 | | | | |
| Classic LROs | Early melanosomes | Human | [21][d] | 804 | 804 | 217 (26) | 362 (45) | 103 |
| | Late melanosomes | | | 596 | 596 | 54 (9) | 300 (50.3) | 29 |
| | Platelets dense granules (PL)[e] | Human | UniProtKB | 6 | 423 | 65 (15.4) | 177 (41.8) | 24 |
| | | | [62] | 598 | | | | |
| | Neuromelanin granules (NG) | Human | [63] | 72 | 72 | 7 (9.7) | 11 (15.3) | 0 |
| New LROs | Synaptosome (SY) | Human | UniProtKB | 34 | 174 | 25 (14.4) | 65 (37.4) | 16 |
| | | Rat | [64] | 200 | | | | |
| | | | [65] | 127 | | | | |
| | Exosome (EX) | Human | [66] | 56 | 276 | 34 (12.3) | 96 (34.8) | 12 |
| | | | [67] | 295 | | | | |

[a] UniProtKB (http://www.pir.uniprot.org/) covers >90% of proteins in OrganelleDB (http://organelledb.lsi.umich.edu/).
[b] # of proteins with at least one transmembrane (tm) domains.
[c] Also see ORMD: http://proteome.biochem.mpg.de/ormd.htm (derived from data in [4]).
[d] The total number of melanosome proteins determined is 1438, combining the data from both pigmented MNT1 and non-pigmented SK-MEL-28 melanoma cells. Shown here is the early stages (I and II) and the late stages (III and IV) melanosomes from MNT1 cell.
[e] Proteome for platelet dense body is not available, instead data from whole platelet enriched with dense body is used here. % percentage of transmembrane or unique proteins vs. total proteins in each organelle is given in parenthesis.

of the first group is hVDAC1 (UniProtKB:P21796), voltage-dependent anion-selective channel protein 1, which is identified in nearly all of these organelles. This is a well-studied mitochondrial (outer membrane) protein, but has also been confirmed in non-mitochondrial membrane organelles [22,23].

Similarly, the ER proteome is also distinct among the nine organelles, with 52% unshared proteins. Nonetheless, many ER proteins found in several organelle compartments suggest that some are truly shared. In fact, the ER proteome shares 11–27% of its proteins with the LROs, but <7% with mitochondria. This commonality of the ER and LRO proteomes is consistent with that ER proteins are involved in LRO maturation and function (Fig. 1). In particular, endosomes share ∼27% of their proteome with the ER, reflecting the dynamic exchange of protein components between these two organelles. A similar case was seen for proteins targeted to endosomes for transport to the cell surface. The identification of Rab4a and Rab4b detected only in the ER and in endosomes among the organelles examined reflects this concept. Indeed, Rab4 is known to control the rapid recycling of cargo proteins directly back to the plasma membrane from early endosomes and recycling endosomes. In particular, Rab4 regulates the intracellular sorting and distribution of transferrin receptors, low-density lipoprotein receptors, and epidermal growth factor receptors [24].

Finally, the presence of cytosolic ribosomal proteins in organelle proteomes (except the ER) may be considered as "contamination." The numbers of ribosomal proteins in the different proteome datasets are: 31 (4%) in the ER, 8 (3%) in lysosomes, 47 (10%) in endosomes, 69 (5%) in melanosomes, 2 in exosomes, 1 in platelet dense granules, and 1 in synaptosomes. The exosome and platelet datasets contain few ribosomal proteins, probably because these organelles are isolated from urine and blood, respectively, where they can be readily collected with high purity. The mitochondrial proteome contains 62 mitochondrial ribosomal proteins and is free of cytosolic ribosomal protein contamination because mitochondria usually separate well from cytosolic ribosome fractions.

### 3.2.2. *Proteins shared among LROs*

Among LROs, melanosomes have the highest number of identified proteins, many of which are shared by at least one other LRO. When compared with the melanosome proteome, neuromelanin granules (67%) and exosomes (50%) have the highest percentage of shared proteins, followed by synaptosomes (45%), platelets (43%), and lysosomes and endosomes (∼40%). This is consistent with the functional relationship between neuromelanin granules and melanosomes, and that melanosomes, exosomes, platelets and synaptosomes are all secretory organelles. In contrast, the ER has only 32% in common with melanosomes.

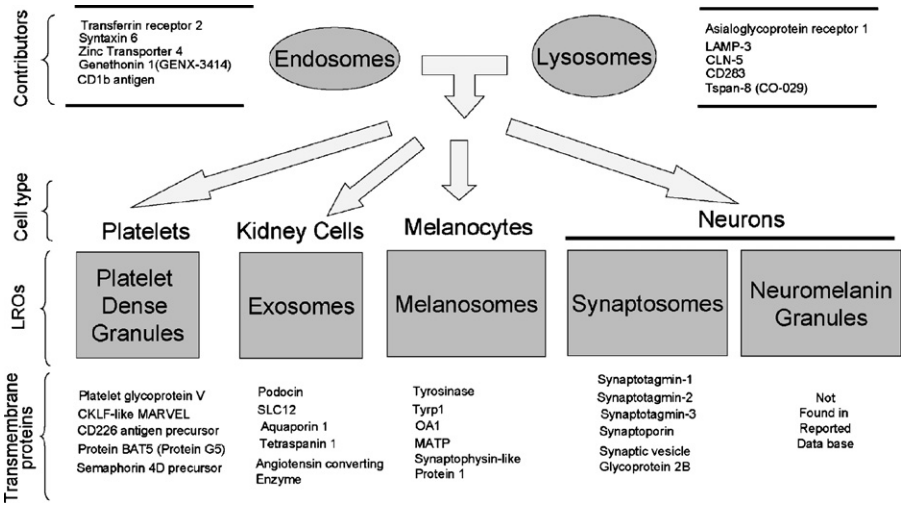


Fig. 1. Overview of mapped transmembrane proteins characteristic of LRO proteomes. LROs are dynamic organelles that distinguish them from contributors like endosomes and lysosomes (top) due to their complex transmembrane protein traffic (arrows). A cell-type specific maturation process takes place to produce specialized types of LROs suitable to perform specific functions for specific types of cells (middle). All mature LROs include an unshared transmembrane protein set (bottom) that differentiates them from their progenitors or contributors, but is still variable and dependant on the cell type.

These results indicate that LROs are more closely related with each other than with the ER.

The melanosome proteome changes significantly from the early to late stages. When compared with the proteome of early stage melanosomes, neuromelanin granules have the highest percentage of shared proteins (56%), followed by exosomes (39%), lysosomes (33%), platelets and synaptosomes (31%), endosomes (24%) and the ER (24%). When compared to late stage melanosomes, the pattern of other organelles that contain shared proteins is similar to that in early stages except for lysosomes, which along with the ER (12%) have the lowest percentage of shared proteins (14%). The higher percentage of shared proteins between lysosomes and early melanosomes compared with late melanosomes may be due to similarities these organelles share in their early stages of maturation. Here we show that late stage melanosomes are clearly different from early stage melanosomes, as reflected by the low percentage of shared proteins by the ER and late stage melanosomes. The analysis of different organelle proteomes suggests that LROs, including melanosomes, have functional and dynamic stages of maturation.

Among the LRO-specific proteins not found in the ER or mitochondria, 265 are shared by two LROs, 77 are shared by three LROs, and 17 are shared by four or more LROs. We further examined the functional roles of the 94 proteins commonly found in three or more LROs (Table 2) to determine what functions these proteins may bring to their shared compartments. This group of common proteins may represent the core component shared by LROs. A number of these proteins have GTP binding and/or transporter activities, including Ras-related proteins (Rab) and ADP-ribosylation factors (Arf), which regulate vesicular traffic and organelle structure. Also present in several LROs are proton pump protein (vATPase) subunits, which are responsible for the regulation of the internal acidic environment in these organelles, which is essential to their functions. Many other proteins have hydrolase activities, some of which are involved in protein transport such as Vesicle-fusing ATPase. In addition, the presence of cytoskeleton and motor proteins is consistent with vesicle movement in the secretory pathway. Among the 17 proteins found in 4 or more LROs, 6 are notably absent in lysosomes, namely, macrophage migration inhibitory factor (MIF) (a cytokine and an enzyme), guanine nucleotide-binding protein β subunit (transducin β chain 1), γ-actin, vacuolar ATP synthase catalytic subunit A (the ubiquitous isoform), sodium/potassium-transporting ATPase α-3 chain, and syntaxin-binding protein 1 (N-Sec1) (Table 2).

The dynamic nature of LROs is indicated by the shared presence of many Rab family proteins, which are critically involved in vesicle fusion and transport. From an estimate of ∼70 Rab family proteins encoded in the human genome, about 40 were identified in the LRO proteomes (Table 3). Interestingly, only 12 were present in 4 or more organelles. Although some of these LRO-associated Rab proteins are also found in the ER or in mitochondria, none can be cataloged as ER- or mitochondria-specific. However, several Rabs were found only in one type of LRO; for example, Rabs 7L1, 20, 34, 39B and 41 were identified only in melanosomes. From this group, Rab7L1 [25], 2004), Rab-20 and Rab-34 are associated with trafficking from the Golgi [26,27]. Rab34 interacts with Rab7 [28] and Rab20 is associated with the polarized sorting of V-ATPase, a critical component of the pH regulating machinery in melanosomes. This is consistent with the proposed route of some proteins trafficking directly from the Golgi to melanosomes [29] and the polarized sorting of elements to melanosomes [30]. In addition, Rab-27A plays a crucial role in melanosome dispersion in melanocytes [31], and Rab38 has been shown to have a specific function in melanosomes and maps to a coat color locus in mice [32], although they are also found in dense granules of platelets [33] and in ER [34]. Therefore, this group of Rabs represents a myriad of selected cargos received from different trafficking routes that together contribute to the unique functions of melanosomes, but their presence in melanosomes is the

Table 2
Proteins shared by three or more LROs (94)[a]

| UniProtKB AC—protein names | Functional categories |
|---|---|
| *P62820—Ras-related protein Rab-1A (YPT1-related protein) | |
| P51148—Ras-related protein Rab-5C (RAB5L) (L1880) | |
| P20340—Rab-6A (Rab-6) | |
| Q9NRW1—Ras-related protein Rab-6B | |
| P62491—Ras-related protein Rab-11A (Rab-11) (YL8) | |
| Q9NP72—Ras-related protein Rab-18 | |
| P62070—Ras-related protein R-Ras2 (Ras-like protein TC21) (teratocarcinoma oncogene) | |
| P61225—Ras-related protein Rap-2b precursor | |
| P63000—Ras-related C3 botulinum toxin substrate 1 precursor (p21-Rac1) | |
| P18085—ADP-ribosylation factor 4 | |
| P84085—ADP-ribosylation factor 5 | GTP binding (GO:0005525) and/or transporter activity (GO:0005215) |
| P62330—ADP-ribosylation factor 6 | |
| Q96BM9—ADP-ribosylation factor-like protein 8A (ADP-ribosylation factor-like protein 10B) | |
| Q9NVJ2—ADP-ribosylation factor-like protein 8B (ADP-ribosylation factor-like protein 10C) | |
| Q9NTK5—Putative GTP-binding protein PTD004 | |
| Q15019—Septin-2 (Protein NEDD5) | |
| O75131—Copine-3 (Copine III) | |
| P20645—Cation-dependent mannose-6-phosphate receptor precursor (CD Man-6-P receptor) | |
| Q92544—Transmembrane 9 superfamily protein member 4 | |
| Q99805—Transmembrane 9 superfamily protein member 2 precursor (p76) | |
| P11717—Cation-independent mannose-6-phosphate receptor precursor (CI Man-6-P receptor) (CI-MPR) | |
| P54920—α-Soluble NSF attachment protein (SNAP-α) | |
| P05090—Apolipoprotein D precursor (Apo-D) (ApoD) | |
| *P38606—Vacuolar ATP synthase catalytic subunit A, ubiquitous isoform | |
| *P21281—Vacuolar ATP synthase subunit B, brain isoform (EC 3.6.3.14) | |
| *P36543—Vacuolar ATP synthase subunit E (EC 3.6.3.14) (V-ATPase E subunit) | Hydrogen ion transporter activity (GO:0015078) and other ion channels |
| *P61421—Vacuolar ATP synthase subunit d (EC 3.6.3.14) (V-ATPase D subunit) (32 kDa) | |
| Q9Y5K8—Vacuolar ATP synthase subunit D (EC 3.6.3.14) (V-ATPase D subunit) (28 kDa) | |
| P20073—Annexin A7 (Annexin VII) (Synexin) | |
| P50897—Palmitoyl-protein thioesterase 1 precursor (EC 3.1.2.22) (palmitoyl-protein hydrolase 1) | |
| P45974—Ubiquitin carboxyl-terminal hydrolase 5 (EC 3.1.2.15) (ubiquitin thioesterase 5) | |
| P34059—*N*-acetylgalactosamine-6-sulfatase precursor (EC 3.1.6.4) (galactose-6-sulfate sulfatase) (GalNAc6S sulfatase) (chondroitin sulfatase) | |
| P18669—Phosphoglycerate mutase 1 (EC 5.4.2.1) (EC 5.4.2.4) (EC 3.1.3.13) | |
| P07686—β-Hexosaminidase β chain precursor (EC 3.2.1.52) (*N*-acetyl-β-glucosaminidase) | |
| P16278—β-Galactosidase precursor (EC 3.2.1.23) (lactase) (acid β-galactosidase) | |
| P10253—Lysosomal α-glucosidase precursor (EC 3.2.1.20) (acid maltase) (aglucosidase alfa) | |
| P42785—Lysosomal Pro-X carboxypeptidase precursor (EC 3.4.16.2) (prolylcarboxypeptidase) | |
| Q9UHL4—Dipeptidyl-peptidase 2 precursor (EC 3.4.14.2) (dipeptidyl-peptidase II) (DPP II) | Hydrolase activity (GO:0016787) |
| P07858—Cathepsin B precursor (EC 3.4.22.1) (cathepsin B1) (APP secretase) (APPS) | |
| P49773—Histidine triad nucleotide-binding protein 1 (adenosine 5′- monophosphoramidase) (protein kinase C inhibitor 1) (protein kinase C-interacting protein 1) (PKCI-1) | |
| Q9UBR2—Cathepsin Z precursor (EC 3.4.22.-) (cathepsin X) (cathepsin P) | |
| *P30041—Peroxiredoxin-6 (EC 1.11.1.15) (antioxidant protein 2) | |
| *Q92820—γ-Glutamyl hydrolase precursor (EC 3.4.19.9) | |
| *Q13510—Acid ceramidase precursor (EC 3.5.1.23) (acylsphingosine deacylase) | |
| P20933—*N*(4)-(β-*N*-acetylglucosaminyl)-L-asparaginase precursor (EC 3.5.1.26) (glycosylasparaginase) | |
| *P13637—Sodium/potassium-transporting ATPase α-3 chain (EC 3.6.3.9) | |
| *P62873—Guanine nucleotide-binding protein β subunit 1 | |
| P46459—Vesicle-fusing ATPase (EC 3.6.4.6) (vesicular-fusion protein NSF) | |
| P07437—Tubulin β-2 chain | |
| P13645—Keratin, type I cytoskeletal 10 (cytokeratin-10) (CK-10) (keratin-10) (K10) | |
| *P63261—Actin, cytoplasmic 2 (γ-actin) | Structural molecule activity (GO:0005198) or cell motility (GO:0006928) |
| Q16181—Septin-7 (CDC10 protein homolog) | |
| Q9BQE3—Tubulin α-6 chain (α-tubulin 6) | |
| Q9Y490—Talin-1 | |
| O15144—Actin-related protein 2/3 complex subunit 2 (ARP2/3 complex 34 kDa subunit) | |
| O14745—Ezrin-radixin-moesin-binding phosphoprotein 50 (EBP50) (Na(+)/H(+) exchange regulatory cofactor NHE-RF) (solute carrier family 9 isoform 3 regulatory factor 1) | |
| O75083—WD repeat protein 1 (actin-interacting protein 1) (AIP1) (NORI-1) | Protein binding (GO:0005515) or ATP binding (GO:0005524) |
| *P14174—Macrophage migration inhibitory factor (MIF) | |
| P27348—14-3-3 Protein theta (14-3-3 protein tau) (14-3-3 protein T-cell) (HS1 protein) | |

Table 2 (*Continued*)

| UniProtKB AC—protein names | Functional categories |
|---|---|
| P31946—14-3-3 Protein β/α (protein kinase C inhibitor protein 1) (KCIP-1) (Protein 1054) | |
| P58546—Myotrophin (Protein V-1) | |
| Q08380—Galectin-3-binding protein precursor (lectin galactoside-binding soluble 3-binding protein) | |
| *P61764—Syntaxin-binding protein 1 (Unc-18 homolog) | |
| Q15036—Sorting nexin-17 | |
| P30626—Sorcin (22 kDa protein) (CP-22) (V19) | |
| P30086—Phosphatidylethanolamine-binding protein 1 (PEBP-1) (prostatic-binding protein) | |
| P40227—T-complex protein 1 subunit zeta (TCP-1-zeta) (CCT-zeta) (CCT-zeta-1) (Tcp20) | |
| P50990—T-complex protein 1 subunit theta (TCP-1-theta) (CCT-theta) (NY-REN-15 antigen) | |
| Q99832—T-complex protein 1 subunit eta (TCP-1-eta) (CCT-eta) (HIV-1 Nef-interacting protein) | |
| P34810—Macrosialin precursor (GP110) (CD68 antigen) | |
| P13473—Lysosome-associated membrane glycoprotein 2 precursor (LAMP-2) (CD107b antigen) | |
| Q14108—Lysosome membrane protein 2 (lysosome membrane protein II) (LIMP II) | |
| *P11279—Lysosome-associated membrane glycoprotein 1 precursor (LAMP-1) | |
| *P27105—Erythrocyte band 7 integral membrane protein (Stomatin) | Other transmembrane proteins |
| *P08962—CD63 antigen (melanoma-associated antigen ME491) | |
| O15400—Syntaxin-7 | |
| O75955—Flotillin-1 | |
| P63027—Vesicle-associated membrane protein 2 (VAMP-2) (Synaptobrevin-2) | |
| *P62988—Ubiquitin 76 Homo sapiens (human) | |
| P53396—ATP-citrate synthase (EC 2.3.3.8) (ATP-citrate (pro-S-)-lyase) (citrate cleavage enzyme) | |
| P07741—Adenine phosphoribosyltransferase (EC 2.4.2.7) (APRT) | |
| P11216—Glycogen phosphorylase, brain form (EC 2.4.1.1) | |
| O75874—Isocitrate dehydrogenase [NADP] cytoplasmic (EC 1.1.1.42) | Other enzymes |
| P14618—Pyruvate kinase isozymes M1/M2 (EC 2.7.1.40) (pyruvate kinase muscle isozyme) | |
| P09104—γ-Enolase (EC 4.2.1.11) (2-phospho-D-glycerate hydro-lyase) (neural enolase) | |
| P00338—L-Lactate dehydrogenase A chain (EC 1.1.1.27) (LDH-A) | |
| P30046—D-Dopachrome decarboxylase (EC 4.1.1.84) (D-dopachrome tautomerase) | |
| P02765—α-2-HS-glycoprotein precursor (Fetuin-A) (α-2-Z-globulin) | |
| P31948—Stress-induced-phosphoprotein 1 (STI1) (Hsc70/Hsp90-organizing protein) (Hop) | |
| P61769—β-2-microglobulin precursor | |
| Q9Y696—Chloride intracellular channel protein 4 (intracellular chloride ion channel protein p64H1) | Others |
| Q9NWT0—CDNA FLJ20625 fis, clone KAT04008 (hypothetical protein C11orf59) | |
| P39019—40S ribosomal protein S19 | |
| P62753—40S ribosomal protein S6 (phosphoprotein NP33) | |

[a] The protein set only includes proteins commonly present in LROs but not in either mitochondria or the ER.
* Proteins shared by four or more LROs (total 17), the rest of the group are proteins shared by three LROs only (total 77).

consequence of dynamic interactions with the milieu and they are not structural components of those organelles.

In addition, there are two synaptosome-unique Rabs (Rab-3A and 3C), two Rabs unique to exosomes (Rab-3D and 8B) and one unique to lysosomes (Rab-7b); these Rabs may be involved with specific trafficking of proteins to those organelles. The synaptic vesicle molecule Rab-3A is also found in glomerular podocytes [35]. Rab7b is a recently identified lysosome-associated small GTPase involved in monocytic differentiation of human acute promyelocytic leukemia cells [36].

Although Rab2B is found only in melanosomes and synaptosomes, the Rab2B gene is expressed widely in kidney, prostate, lung, liver, thymus, colon, pancreas and skeletal muscle, and at low levels in the placenta [37]. Therefore, it is also expressed in other types of cells besides melanocytes and neuronal cells. Rab3D is involved in the regulation of the secretory pathway, such as in ACTH-containing dense-core granules [38] and gastric enzymogen granules [39]. Rab6B is also found in the Golgi complex [40] in addition to melanosomes, platelets and synaptosomes. Rab15 is detected in endosomes [41] and Rab38 in the ER [34], based on published information. In light of the distribution of Rab proteins among the LROs, one may postulate that some Rab proteins have common functions in many LROs, while others may have more specific functions for subsets of LROs.

### 3.2.3. *Proteins found in only one type of LRO*

Proteins found in one organelle that are not shared by other organelles in the compiled organelle reference datasets are potentially unique to that organelle. As shown in Table 4, some transmembrane proteins were found in only one type of LRO. These proteins reflect possible functional aspects of each type of LRO, and might in some cases represent specific markers for individual LROs. Although it is beyond the scope of this paper to consider all the proteins in this category, we mention a few of special interest. Analysis of unshared transmembrane proteins in LROs revealed the importance of controlling the flux of electrolytes, which control intracellular pH ($Na^+$ and $K^+$); enzyme activation (copper) or fusion of synaptic vesicles ($Ca^{2+}$). Interestingly, the control of $Ca^{2+}$ is an important

Table 3
Ras-related protein Rabs shared among LROs

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 8 | -- | -- | ER | LY | EN | ME | -- | EX | PL | SY | MT | P61106 - Rab-14 |
| 7 | m1 | m4 | ER | LY | -- | ME | -- | EX | PL | SY | MT | Q15907 - Rab-11B (GTP-binding protein YPT3) |
| 6 | m1 | m4 | ER | LY | EN | ME | -- | EX | PL | -- | -- | P61019 - Rab-2A |
| 6 | m1 | m4 | -- | LY | EN | ME | -- | EX | PL | -- | MT | P51149 - Rab-7 |
| 5 | m1 | m4 | ER | -- | EN | ME | -- | EX | -- | -- | MT | Q15286 - Rab-35 (Rab-1C) (GTP-binding protein RAY) |
| 5 | m1 | m4 | ER | -- | EN | ME | -- | EX | -- | -- | MT | P61006 - Rab-8A (Oncogene c-mel) |
| 5 | m1 | -- | -- | -- | EN | ME | -- | EX | PL | -- | MT | P61020 - Rab-5B |
| 5 | m1 | -- | -- | -- | -- | ME | -- | EX | PL | SY | MT | Q9H0U4 - Rab-1B |
| 4 | m1 | m4 | ER | -- | EN | ME | -- | -- | -- | -- | MT | P61026 - Rab-10 |
| 4 | m1 | -- | ER | -- | -- | ME | -- | EX | -- | -- | MT | Q9UL25 - Rab-21 |
| 4 | m1 | -- | -- | LY | -- | ME | -- | EX | -- | SY | -- | P62820 - Rab-1A (YPT1-related protein) |
| 4 | m1 | -- | -- | -- | -- | ME | -- | EX | PL | -- | MT | P51153 - Rab-13 |
| 3 | m1 | m4 | -- | LY | EN | ME | -- | -- | -- | -- | -- | P51148 - Rab-5C (RAB5L) (L1880) |
| 3 | m1 | -- | -- | -- | -- | ME | -- | EX | -- | -- | MT | Q9H082 - Rab-33B |
| 3 | -- | -- | ER | -- | EN | ME | -- | -- | -- | -- | -- | Q969Q5 - Rab-24 |
| 3 | -- | -- | -- | LY | EN | ME | -- | -- | -- | -- | -- | Q9NP72 - Rab-18 |
| 3 | -- | -- | -- | LY | EN | ME | -- | -- | -- | -- | -- | P20340 - Rab-6A (Rab-6) |
| 3 | -- | -- | -- | -- | EN | -- | -- | EX | -- | SY | -- | P62491 - Rab-11A (Rab-11) (YL8) |
| 3 | -- | -- | -- | -- | -- | ME | -- | -- | PL | SY | -- | Q9NRW1 - Rab-6B |
| 2 | m1 | -- | -- | -- | -- | ME | -- | -- | -- | -- | MT | Q13637 - Rab-32 |
| 2 | -- | -- | ER | -- | EN | -- | -- | -- | -- | -- | -- | P61018 - Rab-4B |
| 2 | -- | -- | ER | -- | EN | -- | -- | -- | -- | -- | -- | P20338 - Rab-4A |
| 2 | -- | -- | -- | LY | EN | -- | -- | -- | -- | -- | -- | P51151 - Rab-9A (Rab-9) |
| 2 | -- | -- | -- | -- | EN | ME | -- | -- | -- | -- | -- | Q9UL26 - Rab-22A (Rab-22) |
| 2 | -- | -- | -- | -- | EN | -- | -- | EX | -- | -- | -- | P20339 - Rab-5A |
| 2 | -- | -- | -- | -- | -- | ME | -- | -- | -- | SY | -- | Q8WUD1 - Rab-2B |
| 2 | -- | -- | -- | -- | -- | -- | -- | EX | PL | -- | -- | O00194 - Rab-27B (C25KG) |
| 2 | -- | -- | -- | -- | -- | -- | -- | EX | -- | SY | -- | P20337 - Rab-3B |
| 2 | m1 | -- | -- | -- | EN | ME | -- | -- | -- | -- | -- | P59190 - Rab-15* |
| 2 | -- | -- | ER | -- | -- | ME | -- | -- | -- | -- | -- | P57729 - Rab-38 (Antigen NY-MEL-1)* |
| 2 | m1 | m4 | -- | -- | -- | ME | -- | -- | PL | -- | -- | P51159 - Rab-27A (Rab-27) (GTP-binding protein Ram)* |
| 1 | m1 | m4 | -- | -- | -- | ME | -- | -- | -- | -- | -- | Q7Z4W7 - Rab-related GTP-binding protein |
| 1 | m1 | -- | -- | -- | -- | ME | -- | -- | -- | -- | -- | Q9NX57 - Rab-20 |
| 1 | m1 | -- | -- | -- | -- | ME | -- | -- | -- | -- | -- | Q5JT25 - Rab-41 |
| 1 | m1 | -- | -- | -- | -- | ME | -- | -- | -- | -- | -- | O14966 - Rab-7L1 (Rab-7-like protein 1) |
| 1 | -- | -- | -- | LY | -- | -- | -- | -- | -- | -- | -- | Q96AH8 - Rab-7b |
| 1 | -- | -- | -- | -- | -- | ME | -- | -- | -- | -- | -- | Q9BZG1 - Rab-34 (Rab-39) (Rah) |
| 1 | -- | -- | -- | -- | -- | ME | -- | -- | -- | -- | -- | Q96DA2 - Rab-39B |
| 1 | -- | -- | -- | -- | -- | -- | -- | EX | -- | -- | -- | Q92930 - Rab-8B |
| 1 | -- | -- | -- | -- | -- | -- | -- | EX | -- | -- | -- | O95716 - Rab-3D |
| 1 | -- | -- | -- | -- | -- | -- | -- | -- | -- | SY | -- | Q9UBK7 - Rab-like protein 2A |
| 1 | -- | -- | -- | -- | -- | -- | -- | -- | -- | SY | -- | Q96E17 - Rab-3C |
| 1 | -- | -- | -- | -- | -- | -- | -- | -- | -- | SY | -- | P20336 - Rab-3A |

Keys:

| | |
|---|---|
| ER - endoplasmic reticulum | LY - lysosomes |
| EN - endosomes | ME - melanosomes, m1=early stage, m4=late stage |
| NG - neuromelanin granules | EX - exosomes |
| PL - platelets | SY - synaptosomes |
| MT - mitochondria | N - number of organelles proteins is detected |

* Rab-15 in EN, Rab-38 in ER, and Rab-27A in PL are derived from the literature.

feature that defines the functions and responses of LROs, one example being synaptosomes. Synaptotagmins (Syts) constitute a family of membrane-trafficking proteins localized in synaptic vesicles (Syts 1 and 2) and in the plasma membrane apposing synaptic vesicles (Syt 3 and 7); they function as complementary $Ca^{2+}$ sensors during exocytosis with a hierarchy of $Ca^{2+}$ affinities (for review see [42]). Thus, synaptosomes are characterized by the presence of several Syts in their proteome due to their role in the release of neurotransmitters into the synaptic cleft. Other specific proteins, such as syntaxin 1B, which binds Syts in a $Ca^{2+}$-dependent manner [43], further suggests the involvement of $Ca^{2+}$ in the mechanism of fusion. Recently, Syt 7 has been shown to be involved in the mechanism of regulated exocytosis of lysosomes in neurite outgrowth from neurons [44]. In other LROs, $Ca^{2+}$ plays different but important roles. Thus, pigmentation appears to be regulated also by a $Ca^{2+}$ cation exchanger, SLC24-A5 [45], which our group recently validated as a melanosome protein [21].

Control of intracellular pH is another well known feature of LROs. An example of this is observed in melanosomes, which tightly regulate their internal pH. Thus, the melanosome proteome revealed a large number of proteins associated with transporter activity and ion channel control, including vacuolar ATPases, such as vacuolar ATPase S1. Inhibition of these pumps affects melanin production and melanosome-specific protein trafficking [46]. Several solute carrier (SLC) family members and chloride channel proteins 5 and 7, all associated with ion control, may play additional roles in the regulation of melanin-producing enzymes, such as tyrosinase and tyrosinase related protein 1 [47]. The role of iron in reducing the amount of dopamine oxidation intermediates and enhancing the formation of melanin (the final product of dopamine oxidation), suggests that iron can protect cells by accelerating the conver-

Table 4
Transmembrane proteins found in only one type of LRO

**Synaptosomes** (total 16)
- P21579—Synaptotagmin-1 (Synaptotagmin I) (SytI) (p65)
- Q8N9I0—Synaptotagmin-2 (Synaptotagmin II) (SytII)
- Q9H2B2—Synaptotagmin-4 (Synaptotagmin IV) (SytIV)
- Q5T7P8—Synaptotagmin-6 (Synaptotagmin VI) (SytVI)
- O43581—Synaptotagmin-7 (Synaptotagmin VII) (SytVII)
- Q86SS6—Synaptotagmin-9 (Synaptotagmin IX) (SytIX)
- Q6XYQ8—Synaptotagmin-10 (Synaptotagmin X) (SytX)
- Q8IV01—Synaptotagmin-12 (Synaptotagmin XII) (SytXII)
- Q7L0J3—Synaptic vesicle glycoprotein 2A
- Q8TBG9—Synaptoporin
- Q7L1I2—Synaptic vesicle glycoprotein 2B
- Q8TAC9—Secretory carrier-associated membrane protein 5 (secretory carrier membrane protein 5)
- Q9UI14—Prenylated Rab acceptor protein 1 (PRA1 family protein 1)
- P42658—Dipeptidyl aminopeptidase-like protein 6 (dipeptidylpeptidase VI)
- P50993—Sodium/potassium-transporting ATPase α-2 chain precursor (EC 3.6.3.9) (sodium pump 2)
- P61266—Syntaxin-1B2

**Exosomes** (total 16)
- Q13621—Solute carrier family 12 member 1 (kidney-specific Na-K-Cl symporter)
- Q8NFJ5—Retinoic acid-induced protein 3 (G-protein-coupled receptor family C group 5 member A) (retinoic acid-induced gene 1 protein)
- O43490—Prominin-1 precursor (prominin-like protein 1)
- Q9NP85—Podocin
- Q13113—PDZK1-interacting protein 1 (17 kDa membrane-associated protein) (Protein DD96)
- Q9NZH0—G-protein-coupled receptor family C group 5 member B precursor (retinoic acid-induced gene 2 protein) (RAIG-2)
- P29972—Aquaporin-1 (AQP-1) (Aquaporin-CHIP)
- P41181—Aquaporin-2 (AQP-2) (Aquaporin-CD) (AQP-CD) (water channel protein for renal collecting duct)
- P01133—Pro-epidermal growth factor precursor (EGF)
- Q10589—Bone marrow stromal antigen 2 (BST-2)
- P12821—Angiotensin-converting enzyme, somatic isoform precursor (EC 3.4.15.1)
- O60635—Tetraspanin-1 (Tspan-1) (Tetraspan NET-1)
- O15393—Transmembrane protease, serine 2 precursor (EC 3.4.21.-)
- P54710—Sodium/potassium-transporting ATPase γ chain (Na+/K+ ATPase γ subunit)
- P04439—HLA class I histocompatibility antigen, A-11 α chain precursor
- P13746—HLA class I histocompatibility antigen, A-3 α chain precursor

**Endosomes** (total 35)

**Receptors:**
- P30559—Oxytocin receptor (OT-R)
- P22888—Luteinizing hormone receptor (LHR)
- P04629—High-affinity nerve growth factor receptor precursor (EC 2.7.10.1) (neurotrophic tyrosine kinase receptor type 1)
- P00533—Epidermal growth factor receptor precursor (EC 2.7.10.1)
- P01130—Low-density lipoprotein receptor precursor (LDL receptor)
- P18825—α-2C adrenergic receptor (α-2C adrenoceptor)
- P37288—Vasopressin V1a receptor (V1aR)
- P47901—Vasopressin V1b receptor (V1bR)
- Q9UP52—Transferrin receptor protein 2 (TfR2)
- P51681—C-C chemokine receptor type 5 (C-C CKR-5)
- P21462—fMet-Leu-Phe receptor (fMLP receptor) (*N*-formyl peptide receptor) (FPR) (*N*-formylpeptide chemoattractant receptor)
- Q5VSK2—Mannose receptor, C type 1-like 1
- Q4VX17—Secretory carrier membrane protein 3
- P55073—Type III iodothyronine deiodinase (EC 1.97.1.11) (Type-III 5′deiodinase) (DIOIII) (Type 3 DI) (5DIII)
- O95210—Genethonin-1 (GENX-3414)
- Q6UWV6—Ectonucleotide pyrophosphatase/phosphodiesterase 7 precursor (EC 3.1.4.12) (E-NPP7) (NPP-7)
- P56817—β-secretase 1 precursor (EC 3.4.23.46) (β-site APP cleaving enzyme 1)
- Q8N4L2—Transmembrane protein 55A (EC 3.1.3.-) (Type II phosphatidylinositol 4,5-bisphosphate 4-phosphatase)
- Q86T03—Transmembrane protein 55B (EC 3.1.3.-) (Type I phosphatidylinositol 4,5-bisphosphate 4-phosphatase)
- Q8NFT2—Six-transmembrane epithelial antigen of prostate 2 (SixTransMembrane protein of prostate 1) (prostate cancer-associated protein 1)
- Q9UHE8—Six transmembrane epithelial antigen of prostate 1
- O43493—Trans-Golgi network integral membrane protein 2 precursor (Trans-Golgi network protein TGN51)
- O43752—Syntaxin-6
- O14662—Syntaxin-16 (Syn16)
- P29016—T-cell surface glycoprotein CD1b precursor (CD1b antigen)

**Others**:
- O15126—Secretory carrier-associated membrane protein 1 (secretory carrier membrane protein 1)
- Q6P5W5—Zinc transporter ZIP4 precursor (solute carrier family 39 member 4)
- O14863—Zinc transporter 4 (ZnT-4) (solute carrier family 30 member 4)
- O95342—Bile salt export pump (ATP-binding cassette sub-family B member 11)
- Q9UNQ0—ATP-binding cassette sub-family G member 2
- Q14849—MLN 64 protein (StAR-related lipid transfer protein 3) (StARD3) (START domain-containing protein 3)
- Q9NV92—NEDD4 family-interacting protein 2 (NEDD4 WW domain-binding protein 5A)
- Q9GZU1—Mucolipin-1 (Mucolipidin) (MG-2)
- Q9UH99—Sad1/unc-84-like protein 2 (Rab5-interacting protein)
- Q8NFT8—δ-notch-like EGF repeat-containing transmembrane protein (QPRR262)

Table 4 (*Continued*)

| | |
|---|---|
| **Lysosomes** (total 27) | |
| **Transport** (GO:0006810): | |
| O60896—Receptor activity-modifying protein 3 precursor (CRLR activity-modifying protein 3) | Q13571—Lysosomal-associated multitransmembrane protein (retinoic acid-inducible E3 protein) (HA1520) |
| O60895—Receptor activity-modifying protein 2 precursor (CRLR activity-modifying protein 2) | Q8TEZ7—Membrane progestin receptor β (mPR β) |
| O60931—Cystinosin | P07306—Asialoglycoprotein receptor 1 (ASGPR 1) (ASGP-R 1) (hepatic lectin H1) |
| O15431—High-affinity copper uptake protein 1 (hCTR1) (copper transporter 1) | Q8WVQ1—Soluble calcium-activated nucleotidase 1 (EC 3.6.1.6) |
| Q7Z2H8—Proton-coupled amino acid transporter 1 (proton/amino acid transporter 1) (Q7Z2H8) | Q8TBA6—Golgin subfamily A member 5 (Golgin-84) |
| Q99571—P2X purinoceptor 4 (ATP receptor) (P2X4) | Q14789—Golgin subfamily B member 1 (Giantin) (Macrogolgin) |
| Q9NRA2—Sialin (solute carrier family 17 member 5) (sodium/sialic acid cotransporter) | Q11201—CMP-N-acetylneuraminate-β-galactosamide-α-2,3-sialyltransferase (EC 2.4.99.4) |
| Q5T465—Solute carrier family 29 (nucleoside transporters), member 3 | P52848—Bifunctional heparan sulfate *N*-deacetylase/*N*-sulfotransferase 1 (EC 2.8.2.8) |
| | P26572—α-1,3-mannosyl-glycoprotein 2-β-*N*-acetylglucosaminyltransferase (EC 2.4.1.101) |
| **Others including antigen processing, and immune response** | P19075—Tetraspanin-8 (Tspan-8) (Transmembrane 4 superfamily member 3) (Tumor- associated antigen CO-029) |
| P13765—HLA class II histocompatibility antigen, DO β chain precursor (MHC class II antigen DOB) | O75503—Ceroid-lipofuscinosis neuronal protein 5 (Protein CLN5) |
| P01903—HLA class II histocompatibility antigen, DR α chain precursor (MHC class II antigen DRA) | O43291—Kunitz-type protease inhibitor 2 precursor (hepatocyte growth factor activator inhibitor type 2) |
| P02748—Complement component C9 precursor | O15455—Toll-like receptor 3 precursor (CD283 antigen) |
| P01730—T-cell surface glycoprotein CD4 precursor (T-cell surface antigen T4/Leu-3) | Q5T021—Protein tyrosine phosphatase, receptor type, F |
| Q9UQV4—Lysosome-associated membrane glycoprotein 3 precursor (LAMP-3) | |
| **Platelets** (total 24) | |
| **Cell adhesion (GO:0007155) and/or protein binding (GO:0005515):** | P55160—Nck-associated protein 1-like (membrane-associated protein HEM-1) |
| P16109—P-selectin precursor (granule membrane protein 140) | Q9Y2A7—Nck-associated protein 1 (NAP 1) (p125Nap1) (membrane-associated protein HEM-2) |
| P23229—Integrin α-6 precursor (VLA-6) (CD49f antigen) | O75352—Mannose-P-dolichol utilization defect 1 protein (suppressor of Lec15 and Lec35 glycosylation mutation homolog) |
| P08514—Integrin α-IIb precursor (platelet membrane glycoprotein IIb) (GPα IIb) | Q9HCN6—Platelet glycoprotein VI precursor |
| P16284—Platelet endothelial cell adhesion molecule precursor (PECAM-1) | Q9P2E5—Chondroitin sulfate glucuronyltransferase (EC 2.4.1.226) |
| P40197—Platelet glycoprotein V precursor (GPV) (CD42D antigen) | O95870—Protein BAT5 (HLA-B-associated transcript 5) (Protein G5) |
| P14770—Platelet glycoprotein IX precursor (GPIX) (CD42a antigen) | O60704—Protein-tyrosine sulfotransferase 2 (EC 2.8.2.20) (tyrosylprotein sulfotransferase-2) |
| P07359—Platelet glycoprotein Ib α chain precursor (glycoprotein Ibα) (GP-Ib α) | Q9NYV9—Taste receptor type 2 member 13 (T2R13) (taste receptor family B member 3) |
| Q15762—CD226 antigen precursor (DNAX accessory molecule 1) (DNAM-1) | Q96DZ9—CKLF-like MARVEL transmembrane domain-containing protein 5 (chemokine-like factor superfamily member 5) |
| Q92854—Semaphorin-4D precursor (leukocyte activation antigen CD100) | P42892—Endothelin-converting enzyme 1 (EC 3.4.24.71) |
| Q96AP7—Endothelial cell-selective adhesion molecule precursor | Q8TDI7—Transmembrane cochlear-expressed protein 2 |
| **Others:** | Q53XM7—VAMP (Vesicle-associated membrane protein)-associated proteins B and C |
| P42857—Neuron-specific protein family member 1 (brain neuron cytoplasmic protein 1) | |
| Q9UM47—Neurogenic locus notch homolog protein 3 precursor (Notch 3) | |
| **Melanosomes** (total 181) (partial list here) | |
| **Transporter activity (GO:0005215) and ion channel (GO:0005216):** | P27449—Vacuolar ATP synthase 16 kDa proteolipid subunit (EC 3.6.3.14) (**late stage only**) |
| Q96BI1—Organic cation transporter-like protein 2 (imprinted multi-membrane spanning polyspecific transporter-related protein 1) (solute carrier family 22 member 18) | Q8IVS1—Major facilitator superfamily domain containing 1 |
| O15438—Canalicular multispecific organic anion transporter 2 (multidrug resistance-associated protein 3) | Q9H2V7—Spinster-like protein (SPIN1 protein) |
| P11166—Solute carrier family 2, facilitated glucose transporter member 1 (glucose transporter type 1, erythrocyte/brain) | Q8NCC2—Solute carrier family 2 (Facilitated glucose transporter), member 6 (**late stage only**) |
| Q8TDB8—Solute carrier family 2, facilitated glucose transporter member 14 (glucose transporter type 14) | Q9ULQ1—KIAA1169 protein |

Table 4 (*Continued*)

Q9UP95—Solute carrier family 12 member 4 (electroneutral potassium-chloride cotransporter 1)
P55011—Solute carrier family 12 member 2 (bumetanide-sensitive sodium-(potassium)-chloride cotransporter 1)
P50443—Sulfate transporter (diastrophic dysplasia protein) (**late stage only**)
Q96QE2—Proton myo-inositol cotransporter (H(+)-myo-inositol cotransporter) (Hmit)
Q16563—Synaptophysin-like protein 1 (Pantophysin)
P53794—Sodium/myo-inositol cotransporter (Na(+)/myo-inositol cotransporter)
Q71RS6—Sodium/potassium/calcium exchanger 5 precursor (Na(+)/K(+)/Ca(2+)-exchange protein 5) (**late stage only**)
P53985—Monocarboxylate transporter 1 (MCT 1)

O15403—Monocarboxylate transporter 7 (MCT 7) (MCT 6)

O15427—Monocarboxylate transporter 4 (MCT 4) (MCT 3)

Q9HD45—Transmembrane 9 superfamily protein member 3 precursor (SM-11044-binding protein) (EP70-P-iso)
Q15904—Vacuolar ATP synthase subunit S1 precursor (EC 3.6.3.14) (V-ATPase S1 subunit) (V-ATPase S1 accessory protein) (V-ATPase Ac45 subunit) (XAP-3)

P51795—Chloride channel protein 5 (ClC-5)

P51798—Chloride channel protein 7 (ClC-7)

Q13563—Polycystin-2 (polycystic kidney disease 2 protein homolog)
Q9HBA0—Transient receptor potential cation channel subfamily V member 4 (TrpV4)
Q9Y5S1—Transient receptor potential cation channel subfamily V member 2 (TrpV2)
Q6P2P0—Membrane-associated transporter protein, isoform b

Q8N169—Solute carrier family 1 (Glial high affinity glutamate transporter), member 3
Q71UA6—Neutral amino acid transporter
Q14728—Tetracycline transporter-like protein

**Transferase activity (GO:0016740):**
P48651—Phosphatidylserine synthase 1 (EC 2.7.8.-) (PtdSer synthase 1) (PSS-1) (Serine-exchange enzyme I)
Q99735—Microsomal glutathione S-transferase 2 (EC 2.5.1.18) (Microsomal GST- 2) (Microsomal GST-II)
Q14435—Polypeptide N-acetylgalactosaminyltransferase 3 (EC 2.4.1.41) (Protein-UDP acetylgalactosaminyltransferase 3)
Q96L58—β-1,3-galactosyltransferase 6 (EC 2.4.1.134) (β 3GalT6) (galactosylxylosylprotein 3-β-galactosyltransferase)
O94766—Galactosylgalactosylxylosylprotein 3-β-glucuronosyltransferase 3 (EC 2.4.1.135) (β-1,3-glucuronyltransferase 3) (glucuronosyltransferase-I)
Q9H553—α-1,3-mannosyltransferase ALG2 (EC 2.4.1.-) (GDP-Man:Man(1)GlcNAc(2)-PP-dolichol mannosyltransferase)
P54764—Ephrin type-A receptor 4 precursor (EC 2.7.10.1) (tyrosine-protein kinase receptor SEK) P54762—Ephrin type-B receptor 1 precursor (EC 2.7.10.1) (tyrosine-protein kinase receptor EPH-2) (NET) (HEK6) (ELK)
Q16832—Discoidin domain-containing receptor 2 precursor (EC 2.7.10.1) (receptor protein-tyrosine kinase TKT)
P36897—TGF-β receptor type-1 precursor (EC 2.7.11.30) (TGF-β receptor type I) (TGFR-1) (TGF-β type I receptor) (serine/threonine-protein kinase receptor R4)

sion of dopamine oxidation intermediates to less toxic products [48].

Interestingly, exosomes contain a group of tissue-specific proteins which reflect their tissue origin (e.g., urinary exosomes from kidney, such as kidney-specific Na-K-Cl symporter, aquaporin-2 and angiotensin-converting enzyme). As expected, exosome proteomes vary depending on what type of tissue produces them. On the other hand, endosomes, which are involved in surface receptor recycling or degradation through early and late endosomes, contain several cell surface receptors, e.g., oxytocin receptor, LH/CG-R, transferrin receptor protein 2, vasopressin V1b receptor, whereas lysosomes contain several Golgi and lysosomal constituent proteins and HLA class II antigens not found in other LROs.

In melanosomes, 103 unique transmembrane proteins were found in early stages, 29 in late stages, and 25 in both early and late stages. Thus, 78 (103 minus 29) transmembrane proteins are unique to early stages and only 4 (29 minus 25) are unique to late stages, which are sulfate transporter, sodium/potassium/calcium exchange protein 5, vacuolar ATP synthase 16 kDa proteolipid subunit and solute carrier family 2 member 6. Of the total 54 transmembrane proteins identified in late stage melanosomes, 25 are shared with other organelles (17 with LROs), and 25 shared only with early stage melanosomes. These findings suggest that late stage melanosomes are more closely related to other LROs in their membrane components than are early melanosomes. This is consistent with the fact that the proteomes of most LROs are from fully mature organelles.

## 4. Discussion

The isolation of some subcellular organelles, such as nuclei, mitochondria or Golgi, has proven to be straightforward, since they differ sufficiently and do not share a common origin. However, in cases where one organelle is derived from another, such as the plasma membrane and recycling endosomes, proteins are often found in multiple locations. Recently, it was demonstrated that ∼39% of the organelle proteome can be found in multiple intracellular locations [4]. One family of organelles, LROs, share many common features. LROs co-exist with their "progenitors" in several types of cells, making it quite challenging to differentiate them. For example, in melanocytic cells, melanosomes are one type of LRO and contain several lysosome and early endosome markers in their early stages of maturation [29]. Since the biogenesis of melanosomes is still being investigated and several organelles appear to be involved in that process, one must include all sources of proteins in the analysis. The possible asso-

ciation of multivesicular endosomes with melanosomes [49] is the most challenging to deal with from the proteomics point of view. All endosomes along the degradation pathway contain multivesicular elements, including regions of early and late endosomes. In fact, multivesicular elements of late endosomes contain a complex system of internal membranes that are involved in both the degradation and the recycling of proteins and lipids [50]. Intermediates involved in the transport from early to late endosomes are called endosomal carrier vesicles to multivesicular bodies (ECV-MVB), which selectively incorporate receptors destined for late endosomes or lysosomes [51]. Proteins from those organelles could fuse or interact with early stage melanosomes to initiate melanosome biogenesis. Therefore, the proteome profile of melanosomes and other LROs reflects the dynamic retro- and antero-grade traffic between these organelles.

Proteins detected in only one type of LRO are likely to contribute to the specific function of that organelle, while those shared by one or more LROs suggest common functions among them. The promiscuous localization of the majority of proteins in LROs also reflects their common origins as well as their transient and dynamic natures. In this study, we have provided further evidence of the common biogenesis pathways of LROs. In previous studies, lysosomes, melanosomes and platelet dense bodies had been identified as LROs based on common defects seen in various diseases, such as Hermansky Pudlak syndrome and Chediak Higashi syndrome, where their various functions were significantly affected [52]. More recently, proteomic analyses have revealed other members of the LRO family (e.g., neuromelanin granules, exosomes and synaptosomes) which in retrospect is quite reasonable, based on their phenotypes and functions. Our analysis in this study underscores the common biogenesis of those organelles and their interactions. We further show that neuromelanin granules are closest to lysosomes among all LROs, since ∼10% of lysosomal proteins and ∼45% of neuromelanin granule proteins are in common.

In this study, we have conducted a systematic bioinformatics analysis for the comparative profiling of organelle proteomes using the iProXpress proteomic analysis system as the basic research infrastructure. iProXpress supports functional interpretation and discovery of high-throughput proteomic data by comprehensive mapping of proteins from disparate sources to the UniProtKB and by providing rich functional annotation for protein categorization and profiling based on salient protein properties. The compiled catalogs of LRO proteomes in this study are based on protein identification from published MS proteomic studies and the database annotations (Table 1). However, organelle purity and the accuracy of protein identification may vary among publications. Although we have verified all the melanosome protein identifications from the MS spectral data [21], there is no central MS spectra data repository for the verification of protein identifications in other publications from their original spectral data. For database annotations, while the UniProtKB annotation of protein subcellular locations is based on the literature and is of high quality, proteins may not be completely annotated because of the laborious nature of manual curation. We have curated selective groups of proteins with additional literature data, such as Rab-15, Rab-38 and Rab-27A shown in Table 3. The compiled catalogs of LRO proteomes thus serve as "reference data" for the scientific community to query and browse for answering specific questions, such as "which proteins are most often seen in both melanosomes and neuromelanin granules?" As organelle proteomic research continues, this reference datasets can be updated as a resource for the LRO research. The comparative organelle proteome profiling in this study provides some interesting concepts to consider regarding the biogenesis, interactions and functions of LROs, as summarized below.

### 4.1. Dynamic interactions of LROs

Analysis of organelles in various conditions is needed to understand the dynamic nature of integrated cell functions. An example of this was observed when researchers compared proteomic data from mitochondria isolated from different human organs, such as the brain, heart, kidney and liver, which revealed that large numbers of known mitochondrial proteins were missing from various samples. Further, a combination of RNA expression and proteomics analyses revealed that only half of all mitochondrial proteins were found in every cell type [53]. Similar results came from a quantitative proteome approach used to characterize nucleoli, which surprisingly found that this organelle's proteome changes significantly over time in response to changes in cellular growth conditions [54]. Therefore, it is essential to understand the dynamics or temporal characterization of protein flux through cellular organelles. LROs are highly dynamic organelles that have to adapt and respond to a variety of different physiological stimuli in various types of cells. LROs share vesicular traffic between them as they process proteins throughout the cytoplasm, vesicles that obviously are involved in the high number of common proteins detected. These processes require a highly complicated network of proteins that varies according to the different stimuli and conditions in which the cells exist. The large number of SNARE, Rab and RAS proteins identified is not unique to any organelle or type of cell, since they are involved in many essential trafficking processes. However, some of them have unique functions in specific types of organelles. For example, Rab6, which is involved in the retrograde transport of proteins from the Golgi to the ER, is identified in this study in several related-LROs, while Rab27a (another common Rab) has a very specific function in melanocytes where it is critical for melanosome movement and transfer. Thus, the vesicular transport systems involved are quite common in the various LROs, but the cargo they carry can vary widely depending on the tissue-specific expression of that cargo. Another important aspect to consider is that proteomics analysis cannot differentiate isoforms of proteins, which may also vary dramatically in different organelles and types of cells, and which may have quite different functions. For example, Rab5 is detected in many LROs but one cannot determine if that corresponds to isoform A, B and/or C, which are phosphorylated differently [55]. Further, proteomics analysis cannot determine whether their associations in specific clusters of proteins

are similar or different between various intracellular organelles [56,57].

### 4.2. Uniqueness of LRO functions

The fact that LROs derive the bulk of their components from a wide range of cellular organelles is obvious. The uniqueness of their individual functions is no doubt related to the expression of a limited number of specific proteins found in each type of LRO. As an example, one could consider melanosomes, whose proteome contains several unique proteins known to exist only in melanosomes but not in other organelles (presently ∼1% out of ∼700 proteins). The specific function of melanosomes (i.e., pigmentation) results from a limited number of specific proteins that are responsible for their structure, their biosynthesis of melanin and their transport/transfer to neighboring cells (keratinocytes in the skin). The final goal of LROs is a common one, i.e., secretion (be that trafficking to other compartments within cells or to the extracellular environment). Therefore, the bulk of proteins in LROs may be involved in common housekeeping duties, i.e., organellar structure and/or cargo trafficking that are essential for those common functions.

### 4.3. Maturation/secretion of LROs

While various LROs are highly similar in structure (as amorphous rounded vesicles) in their early stages, each type matures in some way as it prepares to fulfil its specific cellular function(s). In this study, we have obtained data from mature organelles in each instance, since they are the more easily purified forms of organelles. It can be quite challenging to purify and characterize the early, relatively amorphous forms of LROs, since they have highly similar vesicular structures. In most instances, even the mature forms of the organelles are not easy to purify to homogeneity. Exceptions to this are pigmented organelles, e.g., neuromelanin granules and melanosomes. The latter go through an elaborate maturation process which involves the delivery and processing of important structural proteins (e.g., Pmel17) to the early stage which allows subsequent phenotypic and structural changes in the organelle. Following that, enzymatic and other regulatory components (e.g., tyrosinase and Rab27a) are delivered which allow the biosynthesis of pigment and also the transport of melanosomes to the cell periphery for eventual secretion. Comparison of the proteome complement of other subcellular organelles in various stages of maturation will provide important clues about critical processes involved in that process.

In conclusion, this approach to analysis of the proteomes of subcellular organelles provides a fascinating insight into their biogenesis and dynamic interactions. Future study will no doubt further reveal important processes involved in regulating their structures and functions.

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