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Organellar Proteomics Reveals Golgi Arginine Dimethylation[D]

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The Golgi complex functions to posttranslationally modify newly synthesized proteins and lipids and to sort them to their sites of function. In this study, a stacked Golgi fraction was isolated by classical cell fractionation, and the protein complement (the Golgi proteome) was characterized using multidimensional protein identification technology. Many of the proteins identified are known residents of the Golgi, and 64% of these are predicted transmembrane proteins. Proteins localized to other organelles also were identified, strengthening reports of functional interfacing between the Golgi and the endoplasmic reticulum and cytoskeleton. Importantly, 41 proteins of unknown function were identified. Two were selected for further analysis, and Golgi localization was confirmed. One of these, a putative methyltransferase, was shown to be arginine dimethylated, and upon further proteomic analysis, arginine dimethylation was identified on 18 total proteins in the Golgi proteome. This survey illustrates the utility of proteomics in the discovery of novel organellar functions and resulted in 1) a protein profile of an enriched Golgi fraction; 2) identification of 41 previously uncharacterized proteins, two with confirmed Golgi localization; 3) the identification of arginine dimethylated residues in Golgi proteins; and 4) a confirmation of methyltransferase activity within the Golgi fraction.

INTRODUCTION

Organelles are membrane-bound compartments that function by interacting with cytoplasmic and luminal soluble proteins making the protein composition of each organelle dynamic. The Golgi complex is the central organelle of the secretory pathway and functions to posttranslationally modify newly synthesized proteins and lipids and sort them for transport to their sites of function (Palade, 1975). However, other Golgi functions require interactions that are less clearly understood. The Golgi interacts with the cytoskeleton to maintain its perinuclear localization within the cytoplasm and to facilitate its dispersal during cell division (Lowe *et al*., 1998; Allan et al., 2002; Colanzi *et al*., 2003). Interactions with the endoplasmic reticulum (ER) are reported at both the entry and exit faces of the organelle (Ladinsky *et al*., 1999; Marsh *et al*., 2001; Ward *et al*., 2001). Furthermore, many signaling pathways intersect at the Golgi, and signaling molecules may transiently associate with Golgi membranes (Donaldson and Lippincott-Schwartz, 2000; Van Lint *et al*., 2002). These unresolved questions regarding nonclassical functions of the Golgi have stimulated several organellar proteomic analyses with the goal of comprehensively identifying its protein components (Taylor *et al*., 2000; Wu *et al*., 2000; Bell *et al*., 2001).

The number of genes that are expressed within a given cell type is estimated to be ~10,000 (Huber *et al*., 2003). However, proteins are further covalently modified to mediate the complex functional interactions, thus increasing the complexity of proteomic analyses. To simplify the analysis of cellular compartments, enrichment strategies should precede proteomic analysis. Organellar proteomics combines subcellular fractionation with mass spectrometry and provide a powerful approach to identify the protein complement of each subcellular compartment. Classically, organellar proteomics has used gel electrophoresis technology (both one-dimensional and two-dimensional) followed by the identification of resolved proteins by mass spectrometry. However, technical limitations resulting in reduced identifications of transmembrane proteins and posttranslational modifications have led to the development of alternative "nongel" strategies (Wu and Yates, 2003). Previously reported Golgi proteomes reflect these limitations resulting in 73 (Taylor *et al*., 2000), 45 (Wu *et al*., 2000), and 81 (Bell *et al*., 2001) protein identifications and no information on posttranslational modifications. Recent progress in proteomic technology has enabled more comprehensive high-throughput profiling strategies of enriched organellar fractions, resulting in hundreds of proteins identifications, some of which were previously uncharacterized (Andersen *et al*., 2003; Mootha *et al*., 2003). These studies, although impressive, also lack the identification posttranslational modification sites. Clearly, proteomic strategies capable of identifying both soluble and membrane proteins as well as some

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posttranslational modifications will provide improved insights into organellar function.

Multidimensional protein identification technology (MudPIT) facilitates the goal of establishing more comprehensive organellar proteomes (Washburn *et al*., 2001). MudPIT has been optimized for the analysis of covalent modifications (MacCoss *et al*., 2002a; Wu *et al*., 2003) as well as membrane proteins (Washburn *et al*., 2001; Wu *et al*., 2003). MudPIT minimizes the bias against particular classes of proteins by first digesting the proteins into an even more complex mixture of peptides. These peptides are separated by microcapillary multidimensional chromatography interfaced directly with a tandem mass spectrometer by using electrospray ionization (Link *et al*., 1999). The peptide sequences and posttranslational modifications are determined by comparing experimentally acquired fragmentation spectra against theoretical spectra predicted from protein or nucleotide sequence information by using SEQUEST (Eng *et al*., 1994). Using DTASelect (Tabb *et al*., 2002), the resulting peptide sequences are reassembled back into protein identifications. This approach of identifying proteins and modifications is robust and facilitates proteomic profiling studies (Florens *et al*., 2002; Schirmer *et al*., 2003; Westermann *et al*., 2003).

Here, we describe an organellar proteomic analysis in which a stacked Golgi fraction was profiled using MudPIT. More than 400 proteins (421) were identified with a minimum of five independent peptide identifications per protein (>99% empirical confidence). Use of stringent criteria allowed us to identify 1) abundant proteins within the enriched fraction, including those with known and unknown functions and 2) sites of arginine dimethylation on proteins with high sequence coverage. Golgi localization was confirmed for two of the unknown membrane proteins, and interestingly, one of these is a predicted methyltransferase and is dimethylated on R230. Methyltransferase activity was subsequently confirmed in the Golgi fraction, and multiple Golgi and ER proteins were found to be arginine methylated in vitro. This organellar profiling study resulted in the generation of a new hypothesis regarding the role of methylation in the Golgi and serves to facilitate a better understanding of Golgi functions.

MATERIALS AND METHODS

Materials

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise noted. Enzymes were purchased from Roche Applied Science (Indianapolis, IN). The arginine-dimethylated synthetic peptide LEWQPPPFR*WLPVGPH (* designates the site of arginine dimethylation) was purchased from Global Peptide Services (Fort Collins, CO). Male Sprague-Dawley rats (6 wk old, ~250 g) were purchased from Harlan (Indianapolis, IN). All methods involving animals were approved by the institutional Animal Research Committee, accredited by the American Association for Accreditation of Laboratory Animal Care.

Isolation of Stacked Golgi Fraction

Rats were treated with cycloheximide to reduce total proteins in transit through the Golgi. Cycloheximide (50 mg/kg) was administered intraperitoneally 4 h before being sacrificed by halothane inhalation. Livers were removed from groups of 20 rats, and enriched Golgi fractions were prepared as described previously (Taylor *et al*., 1997). Briefly, livers were finely minced and resuspended at a ratio of 1 g of minced liver:1 ml of homogenization buffer (0.5 M phosphate-buffered sucrose containing 100 mM KH_2PO_4/K_2HPO_4, pH 6.8, 5 mM $MgCl_2$, and 4 μg each of proteolytic inhibitors chymostatin, leupeptin, antipain, and pepstatin). The sample was homogenized using a Polytron PT10/35 (Brinkmann, Westbury, NY) with one pass for 45 s moving from the top of the tube slowly to the bottom of the tube. The homogenate was centrifuged at low speed (1500 $\times$ *g* for 10 min at 4°C). The resulting postnuclear supernatant (PNS) was loaded in the middle of a sucrose step gradient (steps of 1.3 and 0.86 M sucrose were overlaid with the PNS, followed by a 0.25 M layer). The gradient was centrifuged at high speed (100,000 $\times$ *g* for 1 h at 4°C). The SII fraction (collected at the 0.5/0.86 M interface) was adjusted to 1.15 M sucrose, placed at the bottom of a second step gradient, and overlaid with steps 1.0, 0.86, and 0.25 M. The enriched Golgi fraction was collected at the 0.86/0.25 M interface. Protein concentrations of fractions were determined using DC protein assay (Bio-Rad, Hercules, CA).

Sample Digestion

The enriched Golgi fraction was digested to peptides using two different protocols. One protocol was the CNBr/formic acid method: Golgi samples (1 mg) were pelleted at 16,000 $\times$ *g* for 30 min at 4°C. The supernatant was discarded and the pellet was resuspended in 50 μl of 500 mg/ml CNBr in 90% formic acid and incubated in the dark in the fume hood overnight (Washburn *et al*., 2001). The pH of the sample was adjusted to 8.5 and then adjusted to 8 M urea, reduced (solution was adjusted to 25 mM dithiothreitol and incubated at 55°C for 20 min), and alkylated (solution was cooled to room temperature and adjusted to 100 mM iodoacetamide and incubated in the dark for 20 min). Endoproteinase Lys-C was added at a 1:500 (mass:mass) enzyme:substrate ratio and incubated at 37°C overnight in a Thermomixer (Brinkmann). The sample was then adjusted to 4 M urea and 1 mM $CaCl_2$. Modified trypsin was added at a 1:100 (mass:mass) enzyme:substrate ratio and incubated at 37°C overnight in a Thermomixer. The second protocol was the high pH/Proteinase K method: Golgi samples (1 mg) were pelleted at 16,000 $\times$ *g* for 30 min at 4°C. The supernatant was discarded, and the pellet was homogenized in 1 ml 0.2 M Na_2CO_3, pH 11 with five passes through an insulin syringe and incubated on ice for 1 h. The membrane sample was then adjusted to 8 M urea, reduced (solution is adjusted to 25 mM dithiothreitol and incubated at 55°C for 20 min), and alkylated (solution was cooled to room temperature and adjusted to 100 mM iodoacetamide and incubated in the dark for 20 min). Proteinase K (5 μg) was added to the sample and incubated at 37°C for 3 h in a Thermomixer. An additional aliquot of Proteinase K (5 μg) was added and incubated at 37°C for 1.5 h. The reaction was quenched with formic acid to 5% final concentration and microfuged at 16,000 $\times$ *g* at 4°C for 15 min to remove any insoluble particulates.

MudPIT Analysis

Protein digests were pressure-loaded onto a fused silica capillary desalting column containing 5 cm of 5-μm Aqua C18 material (Phenomenex, Ventura, CA) and washed as described previously (Wu *et al*., 2003). The desalted peptides were then eluted onto the back-end of a triphasic chromatography column consisting of 7 cm of 5-μm Aqua C18 material (Phenomenex), 3 cm of 5-μm Partisphere strong cation exchanger (Whatman, Clifton, NJ), and 3 cm of 5-μm hydrophilic interaction chromatography material (PolyLC). The column was then placed in-line with a Surveyor quaternary high-performance liquid chromatography (HPLC) pump (ThermoElectron, San Jose, CA) and analyzed using a 12-step separation described previously (Wu *et al*., 2003). The HPLC pump was operated at a flow rate of 100 μl/min and was split to obtain flow through the column of ~100–400 nl/min. As peptides eluted from the microcapillary column, they were electrosprayed directly into an LCQ-Deca mass spectrometer (ThermoElectron) with the application of a 2-kV spray voltage applied distally to the waste of the HPLC split as described by Martin *et al*. (2000). A cycle of one full-scan mass spectrum (400–1400 *m/z*) followed by three data-dependent tandem mass spectrometry (MS/MS) spectra at a 35% normalized collision energy was repeated continuously throughout each step of the multidimensional separation. Application of the mass spectrometer scan-functions and HPLC solvent gradients were controlled by the Xcaliber data system (ThermoElectron).

Data Analysis

MS/MS spectra were analyzed using the following software analysis protocol. 2to3 determined the charge state (+2 or +3) of multiply charged peptide spectra and deleted poor quality spectra. Each MS/MS spectrum after 2to3 was searched against the RefSeq protein database containing the RefSeq rat, mouse, and human sequences concatenated into a single fasta file using SEQUEST (Eng *et al*., 1994). The MS/MS spectra were then researched to consider modifications of 1) +14 on R (methylation) and 2) +28 on R (dimethylation). All searches were parallelized and run on either the Yates Lab Beowulf computer cluster consisting of 34 1.2-GHz Athlon computer nodes or The Scripps Research Institute SGI cluster. All searches were performed without any enzyme specificity. The program DTASelect was used to filter peptide sequences from +1, +2, and +3 charged peptide precursors with normalized SEQUEST XCorr scores >0.3 (MacCoss *et al*., 2002b) and $\Delta C_n >$ 0.1, to assemble the peptide sequences into proteins and to remove redundant protein sequences (Tabb *et al*., 2002). To minimize false positives and to identify abundant proteins within the Golgi fraction, only proteins with five or more peptides exceeding the peptide filters were considered. The MS/MS spectra from the modified peptides were filtered with DTASelect and manually evaluated using criteria reported previously (Link *et al*., 1999; Wu *et al*., 2003).

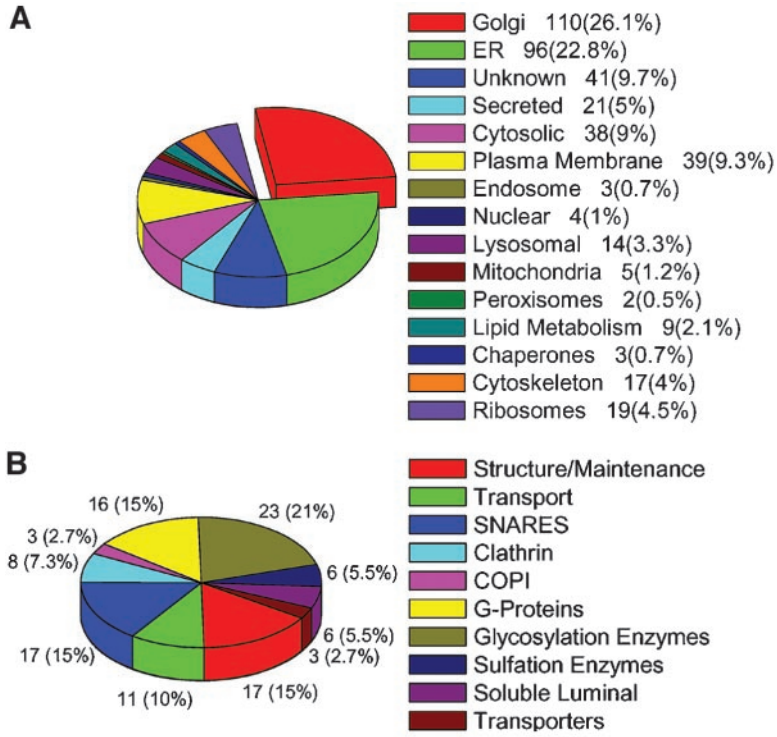

Figure 1. Functional classification of the Golgi proteome. (A) Overall profile of organelle and functional categories of protein identified from a stacked Golgi fraction. (B) Golgi-specific proteins fall in eight different functional classes and include 64% predicted membrane proteins.

Plasmids

cDNAs (corresponding to #1/gi 27229118 and #2/gi 21703704 in Table 2) were acquired from Open Biosystems (Huntsville, AL) and sequenced using the in-house facility at the University of Colorado Health Sciences Center. Both full-length open reading frames were cloned into pEGFP-N2 (BD Biosciences Clontech, Palo Alto, CA). The #2/gi 21703704 open reading frame also was cloned into pGEX-6P-1, and the fusion protein was expressed and isolated using Bulk glutathione *S*-transferase purification module (Pharmacia, Peapack, NJ). The expressed unknown protein was cleaved from glutathione *S*-transferase by using PreScission Protease and collected for in-house immunization of rabbits.

Cell Culture, Transfection, and Microscopy

Normal rat kidney (NRK) cells were plated on coverslips and grown in 10% fetal calf serum in DMEM media plus Pen/Strep. Cells were transfected using LipofectAMINE 2000 (following manufacturer's instructions), fixed with 4% paraformaldehyde in phosphate-buffered saline, permeabilized in 0.2% Triton X-100, and blocked in 0.2% gelatin in phosphate-buffered saline. For immunofluorescence, both primary and secondary antibodies (Alexa Fluor 488 [green] and 594 [red]; Molecular Probes, Eugene, OR) were incubated each for 30 min at 37°C, and coverslips were mounted in Moviol onto slides. Cells were visualized with an Axiovert 200 (Carl Zeiss, Jena, Germany) at 63×.

Methyltransferase Assay

The in vitro methylation assay was carried out according to Lin *et al.*, 2000. Briefly, 45 μg of stacked Golgi fraction in the presence and absence of 0.2% TX-100, was incubated with 4 μCi of [^{3}H]*S*-adenosylmethionine (SAM) in 25 mM Tris, pH 7.5, 1 mM EDTA, 1 mM EGTA at a final volume of 60 μl for 60 min at 37°C in the presence of a cocktail of protease inhibitors. The reaction was stopped by addition of SDS-PAGE sample buffer. Proteins were resolved on a 12% polyacrylamide gel. The gels were infiltrated with Amplify (Amersham Biosciences, Piscataway, NJ), dried, and exposed to Kodak X-OMAT for 30 d.

In-Gel Trypsin Digestion

In-gel digests were performed as described previously (Taylor *et al.*, 2000). Briefly, gel slices were excised and washed with 100 mM ammonium bicarbonate for 20 min. Proteins were reduced with 3 mM dithiothreitol/100 mM ammonium bicarbonate for 20 min at 55°C. After cooling to room temperature, iodoacetamide was added to 6 mM final concentration and incubated in the dark for 15 min at room temperature. The aqueous solution was discarded, and the gel slice was washed in 50% acetonitrile/100 mM ammonium bicarbonate for 20 min. Each gel slice was cut into 1-mm^3 pieces, dried, and reswelled with 0.2 μg of modified trypsin/25 mM ammonium bicarbonate overnight at 37°C. Peptides were extracted from the gel with 100 μl of 60% acetonitrile/0.1% trifluoroacetic acid for 20 min. The supernatants were lyophilized and the peptides were reconstituted in 10 μl of 5% formic acid immediately before analysis by mass spectrometry.

RESULTS

Proteomic Survey of an Enriched Stacked Golgi Fraction

Stacked Golgi fractions were enriched from rat livers and characterized as described previously (Taylor *et al.*, 1997). Golgi samples were prepared for proteomic analysis by using two methods reported to optimize for membrane proteins (Washburn *et al.*, 2001; Wu *et al.*, 2003). The peptide mixtures resulting from the digestions were analyzed using MudPIT. Briefly, the complex peptide samples were resolved and mass analyzed by multidimensional chromatography coupled to MS/MS. The acquired tandem mass spectra were then searched against a RefSeq protein database containing rat, mouse, and human sequences by using SEQUEST (Eng *et al.*, 1994). Protein identifications required five or more peptides to minimize false positives and to identify abundant proteins within the Golgi fraction. Using these stringent criteria, 421 proteins were identified. Most of these proteins are currently annotated in databases and were sorted into categories based on reported localizations and functions. Figure 1A provides an overview of all identified proteins in the Golgi fraction by subcellular localization and/or functional classifications. Bona fide Golgi and ER proteins are listed in Table 1, whereas proteins belonging to other classifications are available in Supplementary Table 1. Although not included in Table 1, many of the proteins listed in Supplementary Table 1 are essential for Golgi function, for example, cytosolic and cytoskeletal proteins. Others are molecules in transit, either to be secreted or en route to other organelles. Because it is well documented that no cell

Table 1. Golgi and ER Protein Indentification

	Accession Number[a]	Swiss-Prot AC[b]	Gene Name	Protein Identification	Species	% Coverage	TMDs[c]	Literature Reference
	Structure and Maintenance							
1	gi|20302065|	Q63714	None	Giantin	rat	54.6	**1 IN**	Linstedt AD, Hauri HP. (1993). Mol. Biol. Cell *4*, 679-693.
2	gi|15826852|	Q8N4D6	None	Golgi complex associated protein 1, 60kDa; Giantin-associated protein	human	26.7	**1 IN**	Sohda M, Misumi Y, Yamamoto A, Yano A, Nakamura N, Ikehara Y. (2001). J. Biol. Chem. *276*, 45298-45306.
3	gi|9507177|	P41542	VDP	p115, transcytosis associated protein	rat	29	**1 OUT**	Barroso M, Nelson DS, Sztul E. (1995). Proc. Natl. Acad. Sci. USA *92*, 527-531.
4	gi|12018260|	Q62839	GOLGA2	Golgi autoantigen, golgin subfamily a, 2, *cis*-Golgi matrix protein GM130	rat	59.2	0 IN	Nakamura N, Rabouille C, Watson R, Nilsson T, Hui N, Slusarewicz P, Kreis TE, Warren G. (1995). J. Cell Biol. *131*, 1715-1726.
5	gi|31982330|	P55937	GOLGA3	Golgi autoantigen, golgin subfamily a, 3, Golgin-160	mouse	24	0 IN	Fritzler MJ, Hamel JC, Ochs RL, Chan EK. (1993). J. Exp. Med. *178*, 49-62.
6	gi|20127150|	Q91VW5	GOLGA4	Golgi autoantigen, golgin subfamily a, 4, Golgin-245	mouse	8.8	0 IN	Fritzler MJ, Lung CC, Hamel JC, Griffith KJ, Chan EK. (1995). J. Biol. Chem. *270*, 31262-31268.
7*	gi|7305095|	Q9QYE6	GOLGA5	Golgi autoantigen, golgin subfamily a, 5, Golgin-84	mouse	28.7	**1 IN**	Bascom RA, Srinivasan S, Nussbaum RL. (1999). J. Biol. Chem. *274*, 2953-2962. Erratum J. Biol. Chem. *274*, 12950.
8	gi|9506751|	O35254	GRASP65	GRASP65, golgi peripheral membrane protein p65	rat	52.5	0 IN	Barr FA, Puype M, Vandekerckhove J, Warren G. (1997). Cell *91*, 253-262.
9*	gi|20301956|	Q9R064	None	GRASP55, golgi reassembly stacking protein 2	rat	36.1	0 IN	Shorter J, Watson R, Giannakou ME, Clarke M, Warren G, Barr FA. (1999). EMBO J. *18*, 4949-4960.
10	gi|16758338|	O88618	FTCD	58 kDa microtubule-binding protein (p58); formiminotransferase cyclodeaminase	rat	21.3	**1 OUT**	Bashour AM, Bloom GS. (1998). J Biol Chem. *273*, 19612-19617.
11*	gi|20301976|	P19814	None	TGN38, *trans*-Golgi network protein 1	rat	19.9	**1 IN**	Luzio JP, Brake B, Banting G, Howell KE, Braghetta P, Stanley KK. (1990). Biochem. J. *270*, 97-102.
12	gi|13027434|	Q9CRA5	GOLPH3	*trans*-Golgi protein GMx33 (alpha); Golgi phosphoprotein 3	rat	46.6	0 IN	Wu CC, Taylor RS, Lane DR, Ladinsky MS, Weisz JA, Howell KE. (2000). Traffic *1*, 963-975.
13	gi|22122661|	Q8R088	2010204I15RIK	*trans*-Golgi protein GMx33 (beta); Similar to Golgi phosphoprotein 3	mouse	24.2	0 IN	Bell AW et al. (2001). J. Biol. Chem. *276*, 5152-5165.
14	gi|12963631|	Q8NHE5	None	Golgi membrane protein SB140; Rab GTPase interacting protein	mouse	20.6	**5 OUT**	Strausberg RL et al. (2002). Proc. Natl. Acad. Sci. USA *99*, 16899-16903.
15	gi|8393450|	Q62638	MG160	MG-160; Golgi apparatus protein 1	rat	51.3	**2 IN**	Gonatas JO, Mourelatos Z, Stieber A, Lane WS, Brosius J, Gonatas NK. (1995). J. Cell. Sci. *108*, 457-467.
16	gi|6679809|	Q9Z1E1	FLOT1	Flotillin 1	mouse	30.8	**1 OUT**	Bickel PE, Scherer PE, Schnitzer JE, Oh P, Lisanti M, Lodish HF. (1997). J. Biol. Chem. *272*, 13793-13802.
17	gi|13929186|	Q9Z2S9	FLOT2	Flotillin 2; reggie1-1	rat	14.5	**1 OUT**	Lang M, Lommel S, Jung M, Ankerhold R, Petrausch, Laessing U, Wiechers MF, Plattner H, Stuermer CA. (1998). J. Neurobiol. *37*, 502-523.
	Transport							
18	gi|7657138|	O00461	GPP130	Golgi phosphoprotein 4; 130 kD Golgi-localized phosphoprotein	human	26.7	**1 OUT**	Linstedt A., Mehta A, Suhan J, Reggio H, Hauri HP. (1997). Mol. Biol. Cell *8*, 1073-1087.
19	gi|14916479|	P24668	M6PR	Mannose-6-phosphate receptor, cation-dependent	mouse	23	**1 OUT**	Ma ZM, Grubb JH, Sly WS. (1991). J. Biol. Chem. *266*, 10589-10595.
20	gi|6981078|	Q63002	M6P/IGF2R	Mannose-6-phosphate receptor, cation-independent; insulin-like growth factor 2 receptor	rat	18.7	**2 IN**	Ludwig T, Tenscher K, Remmler J, Hoflack B, Lobel P. (1994). Gene *142*, 311-312.
21	gi|33598920|	O15126	SCAMP1	SCAMP 1; secretory carrier membrane protein 1	human	35.7	**4 IN**	Singleton DR, Wu TT, Castle JD. (1997). J. Cell Sci. *110*, 2099-2107.
22	gi|6755404|	O35609	SCAMP3	SCAMP 3; secretory carrier membrane protein 3	mouse	27.8	**4 IN**	Singleton DR, Wu TT, Castle JD. (1997). J. Cell Sci. *110*, 2099-2107.
23*	gi|13384724|	Q9DCK9	RNP24	p24B; *cis*-Golgi protein	mouse	57.7	**2 IN**	Dominguez M, Dejgaard K, Fullekrug J, Dahan S, Fazel A, Paccaud JP, Thomas DY, Bergeron JJ, Nilsson T. (1998). J. Cell Biol. *140*, 751-765.
24*	gi|16758214|	Q63584	TMP21	Tmp21: *cis*-Golgi protein TM protein (p23)	rat	50.7	**2 IN**	Blum R, Feick P, Puype M, Vandekerckhove J, Klengel R, Nastainczyk W, Schulz I. (1996). J. Biol. Chem. *271*, 17183-17189.
25	gi|16758758|	Q62902	ERGIC53	ER-Golgi intermediate compartment 53 kDa protein	rat	58.8	**2 IN**	Lahtinen U, Hellman U, Wernstedt C, Saraste J, Pettersson RF. (1996). J. Biol. Chem. *271*, 4031-4037.
26	gi|9507019|	Q62991	None	RSLY1P, Sly1	rat	36.9	0 OUT	Peterson MR, Hsu SC, Scheller RH. (1996). Gene *169*, 293-
27	gi|4758416|	Q92538	GBF1	GBF1: Golgi-specific brefeldin A resistance factor 1; BFA-resistant GEF1	human	16.7	**1 OUT**	Claude A, Zhao BP, Kuziemsky CE, Dahan S, Berger SJ, Yan JP, Armold AD, Sullivan EM, Melancon P. (1999). J. Cell Biol. *146*, 71-84.
28	gi|6978561|	Q64535	TP7B	Copper-transporting ATPase 2; Wilson disease-associated protein	rat	10.8	**9 OUT**	Wu J, Forbes JR, Chen HS, Cox DW. (1994). Nat. Genet. *7*, 541-545.
	SNARES							
29	gi|6981614|	Q64357	VAMP2	Vesicle-associated membrane protein 2 (VAMP-2); Synaptobrevin 2	rat	62.9	**1 IN**	Archer BT 3rd, Ozcelik T, Jahn R, Francke U, Sudhof TC. (1990). J. Biol. Chem. *265*, 17267-17273.
30	gi|16923936|	Q64271	VAMP3	Vesicle-associated membrane protein 3 (VAMP-3); Synaptobrevin 3	rat	49.5	**1 IN**	Che YH, Yamashita T, Tohyama MJ. (2002). Chem. Neuroanat. *24*, 147-152.
31	gi|13928870|	Q9Z270	VAPA	Vesicle-associated membrane protein A; 33 kD-VAMP-binding protein	rat	23.1	**1 IN**	Nishimura Y, Hayashi M, Inada H, Tanaka T. (1999). Biochem. Biophys. Res. Commun. *254*, 21-26.
32	gi|11177880|	Q9Z269	VAPB	Vesicle-associated membrane protein B	rat	22.2	**1 IN**	Nishimura Y, Hayashi M, Inada H, Tanaka T. (1999). Biochem. Biophys. Res. Commun. *254*, 21-26.
33	gi|12831221|	O89116	VTI1A	SNARE Vti1a-beta protein	rat	52.1	**1 IN**	Antonin W, Riedel D, von Mollard GF. (2000). J. Neurosci. *20*, 5724-5732.
34	gi|7949165|	Q91XH6	VTI1B	Vesicle transport through interaction with t-SNAREs 1b homolog	mouse	28.9	**1 IN**	Antonin,W., Fasshauer,D., Becker,S., Jahn,R. and Schneider,T.R. (2002). Nat. Struct. Biol. *9*, 107-111.
35	gi|13928982|	Q08851	STX5A	Syntaxin 5a	rat	39.9	**1 OUT**	Hui N, Nakamura N, Sonnichsen B, Shima DT, Nilsson T, Warren G. (1997). Mol. Biol. Cell *8*, 1777-1787.
36	gi|13928908|	Q9Z2Q7	STX8	Syntaxin 8; syntaxin-like protein 3l35 (enriched early endosome)	rat	38.1	**1 IN**	Antonin W, Fasshauer D, Becker S, Jahn R, Schneider TR. (2002). Nat. Struct. Biol. *9*, 107-111.
37*	gi|16758358|	Q62931	GOSR1	Golgi SNAP receptor complex member 1; *cis*-Golgi SNARE (p28)	rat	54.8	**1 IN**	Subramaniam VN, Peter F, Philp R, Wong SH, Hong W. (1996). Science *272*, 1161-1163.
38	gi|13928950|	O35165	GOSR2	Golgi SNAP receptor complex member 2; membrin	rat	31.6	**1 IN**	Hay JC, Chao DS, Kuo CS, Scheller RH. (1997). Cell *89*, 149-158.
39	gi|18034791|	P54921	SNAPA	Soluble NSF attachment protein alpha	rat	51.98	0 IN	Clary DO, Griff IC, Rothman JE. (1990). Cell *61*, 709-721.
40	gi|4505331|	Q99747	SNAPG	Soluble NSF attachment protein gamma	human	19.9	0 IN	Clary DO, Griff IC, Rothman JE. (1990). Cell *61*, 709-721.
41	gi|31543750|	Q9Z2P6	SNAP29	Synaptosomal-associated protein, 29kD. SNAP-29	rat	29.2	0 IN	Steegmaier M, Yang B, Yoo JS, Huang B, Shen M, Yu, S, Luo Y, Scheller RH. (1998). J. Biol. Chem. *273*, 34171-34179.
42	gi|13489067|	Q9QUL6	NSF	NSF; N-ethylmaleimide sensitive factor	rat	20.4	0 IN	Beckers CJ, Block MR, Glick BS, Rothman JE, Balch WE. (2002). Nature *339*, 397-398.
43	gi|4759086|	O08547	SEC22L1	Vesicle trafficking protein sec22b	human	54	**2 OUT**	Hay JC, Chao DS, Kuo CS, Scheller RH. (1997). Cell *89*, 149-158.
44	gi|16924008|	Q62891	RSEC22	Sec22 homolog	rat	20.9	**4 IN**	Hay JC, Hirling H, Scheller RH. (1996). J. Biol. Chem. *271*, 5671-5679.
45	gi|9506425|	Q62896	BET1	BET1 homolog; Golgi vesicular membrane traffic protein p18	rat	36.4	**1 OUT**	Joglekar AP, Xu D, Rigotti DJ, Fairman R, Hay JC. (2003). J. Biol. Chem. *278*, 14121-14133.
	Clathrin							
46	gi|9506497|	P11442	CLTC	Clathrin, heavy polypeptide (Hc)	rat	29	0 IN	Brodsky FM, Chen CY, Knuehl C, Towler MC, Wakeham DE. (2001). Annu. Rev. Cell Dev. Biol. *17*, 517-568.
47	gi|6671553|	O35643	AP1B1	AP-1, beta 1 subunit; beta-prime adaptin	mouse	13.5	0 IN	Robinson MS, Bonifacino JS. (2001). Curr. Opin. Cell Biol. *13*, 444-453.
48	gi|8392872|	P52303	AP1B1	Adaptor protein complex AP-1, beta 1 subunit	rat	18.2	0 IN	Robinson MS, Bonifacino JS. (2001). Curr. Opin. Cell Biol. *13*, 444-453.
49	gi|6753070|	P22892	AP1G1	Adaptor protein complex AP-1, gamma 1 subunit	mouse	8.2	0 IN	Robinson MS, Bonifacino JS. (2001). Curr. Opin. Cell Biol. *13*, 444-453.
50	gi|6671557|	P35585	AP1M1	Adaptor protein complex AP-1, mu subunit 1	mouse	28.1	0 IN	Robinson MS, Bonifacino JS. (2001). Curr. Opin. Cell Biol. *13*, 444-453.
51	gi|21313640|	Q9DBG3	AP2B1	Adaptor protein complex AP-2, beta 1 subunit	mouse	19.5	0 IN	Robinson MS, Bonifacino JS. (2001). Curr. Opin. Cell Biol. *13*, 444-453.

(*continued*)

Table 1 continued.

52	gi|13929192|	P19804	NME2	Nucleoside diphosphate kinase	rat	28.9	0 IN	Baillat G, Gaillard S, Castets F, Monneron A. (2002). J. Biol. Chem. *277*, 18961-18966.
53	gi|7661968|	Q14677	EPN4	Epsin 4. enthoprotin	human	23.5	0 IN	Wasiak S, Legendre-Guillemin V, Puertollano R, Blondeau F, Girard M, de Heuvel E, Boismenu D, Bell AW, Bonifacino JS, McPherson PS. (2002). J. Cell Biol. *158*, 855-862.
	COPI							
54	gi|4758030|	P53621	COPA	alpha-COP	human	12	0 IN	Waters MG, Serafini T, Rothman JE. (1991). Nature *349*, 248-251.
55	gi|11120716|	O35142	COPB2	beta prime-COP	rat	9.3	0 IN	Csukai M, Chen CH, De Matteis MA, Mochly-Rosen D. (1997). J. Biol. Chem. *272*, 29200-29206.
56	gi|22122433|	Q91W48	ARCN1	delta-COP	mouse	28.6	0 IN	Waters MG, Serafini T, Rothman JE. (1991). Nature *349*, 248-251.
	G-Proteins							
57	gi|4758988|	P11476	RAB1A	Rab1A	human	64.4	0 IN	Zahraoui A, Touchot N, Chardin P, Tavitian A. (1989). J. Biol. Chem. *264*, 12394-12401.
58	gi|21313162|	Q9H0U4	RAB1B	Rab1B	mouse	47.3	0 IN	Touchot N, Zahraoui A, Vielh E, Tavitian A. (1989). FASEB Lett. *256*, 79-84.
59	gi|10946940|	P53994	RAB2	Rab2	mouse	27.8	0 IN	Ayala J, Touchot N, Zahraoui A, Tavitian A, Prochiantz A. (1990). Neuron *4*, 797-805.
60	gi|19923231|	P20340	RAB6	Rab6A	human	40.9	0 IN	Martinez O, Schmidt A, Salamero J, Hoflack B, Roa M, Goud B. (1994). J. Cell Biol. *127*, 1575-1588.
61	gi|13027392|	P09527	RAB7	Rab7	rat	62.8	0 IN	Feng Y, Press B, Wandinger-Ness A. (1995). J. Cell Biol. *131*, 1435-1452.
62	gi|33695095|	O88386	RAB10	Rab10	human	14.5	0 IN	Chen YT, Holcomb C, Moore HP. (1993) Proc. Natl. Acad. Sci USA *90*, 6508-6512.
63	gi|14249144|	Q15907	RAB11B	Rab11B	rat	29.8	0 IN	Lai F, Stubbs L, Artzt K. (1994). Genomics *22*, 610-616.
64	gi|16758368|	P35287	RAB14	Rab14	rat	34.9	0 IN	Elferink LA, Anzai K, Scheller RH. (1992). J. Biol. Chem. *267*, 5768-5775.
65	gi|10880989|	P35293	RAB18	Rab18	human	42.7	0 IN	Lutcke A, Parton RG, Murphy C, Olkkonen VM, Dupree P, Valencia A, Simons K, Zerial M. (1994). J. Cell Sci. *107*, 3437-3448.
66	gi|8394133|	O35963	RAB33B	Rab33B	mouse	20.5	0 IN	Zheng JY, Koda T, Fujiwara T, Kishi M, Ikehara Y, Kakinuma M. (1998). J. Cell Sci. *111*, 1061-1069.
67	gi|16923986|	Q9QUI0	ARHA	RhoA	rat	58.5	1 IN	Hoshijima M, Kondo J, Kikuchi A, Yamamoto K, Takai Y. (1990). Brain Res. Mol. Brain Res. *7*, 9-16.
68	gi|11968136|	P01121	ARHB	RhoB	rat	38.8	1 IN	Kuroda S, Kikuchi A, Takai Y. (1989). Biochem. Biophys. Res. Commun *163* 674-681.
69	gi|9625037|	P35238	ARHG	RhoG	human	35.1	0 IN	Vincent S, Jeanteur P, Fort P. (1992). Mol. Cell Biol. *12*, 3138-3148.
70	gi|4757952|	P21181	CDC42	Cdc42	human	52.4	0 IN	Johnson DI, Pringle JR. (1990). J. Cell Biol. *111*, 143-152.
71	gi|13591955|	P04897	GNAI2	GTP-binding protein (G-alpha-i2)	rat	23.4	1 IN	Itoh H et al. (1986). Proc. Natl. Acad. Sci. USA *83*, 3776-3780.
72	gi|20357529|	P11016	GNB2	GTP-binding protein, beta-2 subunit: G(I)/G(S)/G(T) beta subunit 2	human	18.8	0 IN	Gao B, Gilman AG, Robishaw JD. (1987). Proc. Natl. Acad. Sci. USA *84*, 6122-6125.
	Glycosylation Enzymes							
73	gi|13929462|	P15291	B4GALT1	UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase 1, glycoprotein-4-beta-galactosyltransferase 2	human	8	1 IN	Hansske B, Thiel C, Lubke T, Hasilik M, Honing S, Peters V, Heidemann PH, Hoffmann GF, Berger EG, von Figura K, Korner C. (2002). J. Clin. Invest. *109*, 725-733.
74	gi|10181158|	Q9QY13	B4GALT3	UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase 1, glycoprotein-4-beta-galactosyltransferase 3	mouse	28.6	1 IN	Bettenhausen B, Gossler A. (1995). Genomics *28*, 436-441.
75	gi|12621124|	Q8JJ05	C1GALT1	UDP-galactose:N-acetylgalactosamine-alpha-R beta 1,3-galactose transferase	rat	54.3	1 IN	Ju T, Cummings RD, Canfield WM. (2002). J. Biol. Chem. *277*, 169-177.
76	gi|34328221|	Q7TND1	GALNT1	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 1	mouse	36	1 IN	Ten Hagen KG, Bedi GS, Tetaert D, Kingsley PD, Hagen FK,Balys MM, Beres TM, Degand P, Tabak LA. (2001). J. Biol. Chem. *276*, 17395-17404.
77	gi|21426841|	Q99ME1	GALNT2	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 2	mouse	44.2	1 IN	Hagen FK, Ten Hagen KG, Beres TM, Balys MM, VanWuyckhuyse BC, Tabak LA. (1997). J. Biol. Chem. *272*,
78	gi|7657112|	O08832	GALNT4	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 4	mouse	13.3	0 OUT	Hagen FK, Ten Hagen KG, Beres TM, Balys MM, VanWuyckhuyse BC, Tabak LA. (1997). J. Biol. Chem. *272*,
79*	gi|6678788|	P45700	MAN1A1	Mannosidase 1, alpha; mannosyl-oligosaccharide alpha-1,2-mannosidase	mouse	48.2	1 IN	Lal A, Schutzbach JS, Forsee WT, Neame PJ, Moremen KW. (1994). J. Biol. Chem. *269*, 9872-9881.
80	gi|6754620|	P39098	MAN1A2	Mannosidase 1, beta	mouse	42.6	4 OUT	Herscovics A, Schneikert J, Athanassiadis A, Moremen KW. (1994). J. Biol. Chem. *269*, 9864-9871.
81	gi|4758698|	Q16706	MAN2A1	Mannosidase 2, alpha 1	human	18.6	1 IN	Misago M, Liao YF, Kudo S, Eto S, Mattei MG, Moremen KW, Fukuda MN. (1995). Proc. Natl. Acad. Sci. USA *92*, 11766-
82	gi|5540100|	P49641	MAN2A2	Mannosidase, alpha, class 2A, member 2; mannosidase, alpha type II-X	human	6.3	1 IN	Misago M, Liao YF, Kudo S, Eto S, Mattei MG, Moremen KW, Fukuda MN. (1995). Proc. Natl. Acad. Sci. USA *92*, 11766-
83	gi|18158455|	O35390	ENMAN	Endo-alpha-mannosidase	rat	22.8	0 OUT	Spiro MJ, Bhoyroo VD, Spiro RG. (1997). J. Biol. Chem. *272*, 29356-29363.
84	gi|9910280|	Q9NYU2	None	UDP-glucose ceramide glucosyltransferase-like 1	human	6	1 IN	Aoki K, Ishida N, Kawakita M. (2003). J. Biol. Chem. *278*, 22887-22893.
85*	gi|31981620|	Q8CIC9	MGAT1	Mannoside acetylglucosaminyltransferase 1	mouse	56.4	1 IN	Nozaki H, Matsuzawa T, Nakamura T, Arai I, Urashima T. (2003). Biochim. Biophys. Acta *1649*, 140-145.
86	gi|16758392|	Q09326	MGAT2	Mannosyl (alpha-1,6-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase	rat	50.2	1 IN	D'Agostaro GA, Zingoni A, Moritz RL, Simpson RJ, Schachter H, Bendiak B. (1995). J. Biol. Chem. *270*, 15211-15221.
87	gi|20128770|	P70624	UGT1A7	UDP glycosyltransferase 1 family, polypeptide A7	rat	14.3	2 IN	Grove AD, Kessler FK, Metz RP, Ritter JK. (1997). J Biol. Chem. *272*, 1621-1627.
88*	gi|16915936|	Q9UQ53	None	Mannosyl (alpha-1,3-)-glycoprotein beta-1,4-N-acetylglucosaminyltransferase 2	human	31.3	2 IN	Yoshida A, Minowa MT, Takamatsu S, Hara T, Ikenaga H, Takeuchi M. (1998). Glycoconj. J. *15*, 1115-1123.
89	gi|8393371|	Q9WTS2	FUT8	Fucosyltransferase 8. (alpha (1,6) fucosyltransferase); alpha-1,6-fucosyltransferase	mouse	19.5	2 OUT	Hayashi H, Yoneda A, Asada M, Ikekita M, Imamura T. (2000). DNA Seq. *11*, 91-96.
90	gi|22219438|	Q9QW64	SIAT1	Sialyltransferase 1 (beta-galactoside alpha-2,6-sialyltransferase)	rat	57.9	0 OUT	Weinstein J, Lee EU, McEntee K, Lai PH, Paulson JC. (1987). J. Biol. Chem. *262*, 17735-17743.
91	gi|6677957|	P54751	SIAT4A	Sialyltransferase 4A (beta-galactosidase alpha-2,3-sialyltransferase)	mouse	25.6	1 IN	Martin LT, Marth JD, Varki A, Varki NM. (2002). J. Biol. Chem. *277*, 32930-32938.
92	gi|6981540|	P97877	None	Sialyltransferase 8C (GT3 alpha 2,8-sialyltransferase)	rat	13.9	1 IN	Zeng G, Gao L, Yu RK. (1997). Gene *1997*, 131-134.
93	gi|10946990|	Q9JMG2	COSMC	Core 1 beta3-galactosyltransferase-specific molecular chaperone	human	26.6	1 IN	Ju T, Cummings RD. (2002). Proc. Natl. Acad. Sci. USA *99*, 16613-16618.
94	gi|11995464|	Q9EPI0	XT-II	Xylosyltransferase 2	rat	29.1	1 IN	Gotting C, Kuhn J, Zahn R, Brinkmann T, Kleesiek K. (2000). J. Mol. Biol. *304*, 517-528.
95	gi|21326465|	Q8K464	None	UDP-glucuronate decarboxylase 1	rat	31.9	1 IN	Moriarity JL, Hurt KJ, Resnick AC, Storm PB, Laroy W, Schnaar RL, Snyder SH. (2002). J. Biol. Chem. *277*, 16968-
	Sulfation Enzymes							
96	gi|7305591|	O70281	TPST1	Protein-tyrosine sulfotransferase 1	mouse	44.6	1 IN	Ouyang Y, Lane WS, Moore KL. (1998). Proc. Natl. Acad. Sci. USA *95*, 2896-2901.
97	gi|6678421|	O88856	TPST2	Protein-tyrosine sulfotransferase 2	mouse	21.3	1 IN	Ouyang YB, Moore KL. (1998). J. Biol. Chem. *273*, 24770-
98	gi|13242253|	Q02353	HSST1	N-deacetylase/N-sulfotransferase (heparan glucosaminyl) 1	rat	46.9	1 IN	Hashimoto Y, Orellana A, Gil G, Hirschberg CB. (1992). J. Biol. Chem. *267*, 15744-15750.
99	gi|7106357|	P52850	HSST2	N-deacetylase/N-sulfotransferase (heparan glucosaminyl) 2	mouse	10.1	1 IN	Aikawa J, Grobe K, Tsujimoto M, Esko JD. (2001). J. Biol. Chem. *276*, 5876-5882.
100	gi|6754244	Q8R3H7	HS2ST1	Heparan sulfate 2-O-sulfotransferase 1	mouse	24.2	1 IN	Rong J, Habuchi H, Kimata K, Lindahl U, Kusche-Gullberg M. (2000). Biochem. J. *346*, 463-468.
101	gi|15277317|	Q9EPS3	GLCE	Glucuronyl C5-epimerase. heparan sulfate-glucuronic acid C5-epimerase	mouse	17	1 IN	Crawford BE, Olson SK, Esko JD, Pinhal MA. (2001). J. Biol. Chem. *276*, 21538-21543.
	Soluble Luminal							
102	gi|16758210|	Q63083	NUCB1	CALNUC. Nucleobindin 1	rat	63.2	1 OUT	Weiss TS, Chamberlain CE, Takeda T, Lin P, Hahn KM, Farquhar MG. (2001). Proc. Natl. Acad. Sci. USA *98*, 14961-
103	gi|11072106|	Q9JI85	NUCB2	NEFA precursor	rat	70.7	1 OUT	Morel-Huaux VM, Pypaert M, Wouters S, Tartakoff AM, Jurgan U, Gevaert K, Courtoy P. (2002). Eur. J. Cell Biol. *81*, 87-100.
104	gi|18426836|	Q91ZS3	CAB45	45 kDa calcium-binding protein	rat	41.1	1 IN	Zhu Y, Wang M, Lin H, Luo. (2001). J. Breast Cancer Res. Treat. *69*, 29-38.
105	gi|6978879|	P04276	DBP	Vitamin D-binding protein	rat	30	0 OUT	Cooke NE. (1986). J. Biol. Chem. *261*, 3441-3450.

(*continued*)

Table 1 continued.

106	gi|6679809|	Q9Z1E1	FLOT1	Flotillin 1	mouse	30.8	1 OUT	Bickel PE, Scherer PE, Schnitzer JE, Oh P, Lisanti MP, Lodish HF. (1997) J. Biol. Chem. *272*, 13793-13802
107	gi|13929186|	Q9Z2S9	FLOT2	Flotillin 2; reggie1-1	rat	14.5	1 OUT	Lang DM, Lommel S, Jung M, Ankerhold R, Petrausch B, Laessing U, Wiechers MF, Plattner H, Stuermer CA (1998) J Neurobiol *37*, 502-523.
	Transporters							
108	gi|21687224|	Q96NC3	None	solute carrier family 30 (zinc transporter), member 6, hypothetical protein FLJ31101	human	14.5	7 IN	Huang L, Kirschke CP, Gitschier J (2002). J. Biol. Chem *277* 26389-26395.
109	gi|18777797|	Q64566	ATP2C1	Calcium-transporting ATPase type 2C, member 1	rat	15.7	10 IN	Gunteski-Hamblin AM, Clarke DM, Shull GE (1992) Biochemistry *31*, 7600-7608.
110	gi|34328278|	Q9DBH5	LMAN2	Vesicular integral-membrane protein VIP36	mouse	26.6	1 OUT	Harrison SM, Dunwoodie SL, Arkell RM, Lehrach H, Beddington RS. (1995) Development *121*, 2479-2489
	Endoplasmic Reticulum							
1	gi|6978739|	P11711	CYP2A1	Cytochrome P450 2a1	rat	30.3	1 IN	Matsunaga T, Nomoto M, Kozak CA, Gonzalez FJ. (1990). Biochemistry *29*, 1329-1341.
2	gi|6978741|	P15149	CYP2A2	Cytochrome P450 2a2	rat	35.4	1 IN	Matsunaga T, Nomoto M, Kozak CA, Gonzalez FJ (1990) Biochemistry *29*, 1329-1341.
3	gi|15147326|	P11509	CYP2A6	Cytochrome P450 2a6	human	16.6	1 IN	Yoshida R, Nakajima M, Nishimura K, Tokudome S, Kwon JT Yokoi T. (2003). Clin. Pharmacol. Ther. *74*, 69-76.
4	gi|19526798|	Q8VCW9	CYP2A12	Cytochrome P450 2a12	mouse	9.8	1 IN	Iwasaki M, Lindberg RL, Juvonen RO, Negishi M. (1993) Biochem. J. *291*, 569-573.
5	gi|13699809|	Q16696	CYP2A13	Cytochrome P450 2a13	human	15.4	1 IN	Saito S, Iida A, Sekine A, Kawauchi S, Higuchi S, Ogawa C, Nakamura Y. (2003) J. Hum. Genet. *48*, 249-270.
6*	gi|9506529|	P08683	CYP2C11	Cytochrome P450 2c11	rat	66	3 IN	Biagini C, Cellier C. (1996). Biochem. Biophys. *326*, 298-305
7	gi|13994166|	P11510	CYP2C12	Cytochrome P450 2c12	rat	9.2	1 IN	Zaphiropoulos PG, Mode A, Strom A, Moller C, Fernandez C, Gustafsson JA. (1988). Proc. Natl. Acad. Sci. USA *85*, 4214-
8	gi|13699816|	P33260	CYP2C18	Cytochrome P450 2c18	human	20.4	1 IN	Finta C, Zaphiropoulos PG. (2000). Genomics *63*, 433-438
9	gi|19924039|	P19225	CYP2C22	Cytochrome P450 2c22	rat	42.7	4 OUT	Emi Y, Chijiiwa C, Omura T. (1990). Proc. Natl. Acad. Sci. USA *87*, 9746-9750.
10	gi|13929204|	P24470	CYP2C23	Cytochrome P450 2c23	rat	40.3	0 OUT	Karara A, Makita K, Jacobson HR, Falck JR, Guengerich FP, DuBois RN, Capdevila JH. (1993). J. Biol. Chem. *268*, 13565-13570.
11	gi|31982435|	Q91WN9	CYP2C29	Cytochrome P450 2c29	mouse	19.4	0 OUT	Matsunaga T, Watanabe K, Yamamoto I, Negishi M, Gonzalez FJ, Yoshimura H. (1994). Biochim. Biophys. Acta *1184*, 299-
12	gi|8393233|	P05179	CYP2C7	Cytochrome P450 2c39	rat	36.5	0 OUT	Kimura H, Yoshioka H, Sogawa K, Sakai Y, Fujii-Kuriyama Y (1988). J. Biol. Chem. *263*, 701-707.
13*	gi|6978747|	P10634	CYP2D2	Cytochrome P450 2d2	rat		1 IN	Matsunaga E, Umeno M, Gonzalez FJ. (1990). J. Mol. Evol *30*, 155-169.
14	gi|6753582|	P24456	CYP2D10	Cytochrome P450 2d10	mouse	14.7	1 IN	Ichikawa T, Itakura T, Negishi M. (1989). Biochemistry *28*, 4779-4784.
15	gi|19924041|	Q64680	CYP2D18	Cytochrome P450 2d18	rat	19.4	1 IN	Mizuno D, Takahashi Y, Hiroi T, Imaoka S, Kamataki T, Funae Y. (2003). Biochim. Biophys. Acta *1627*, 121-128.
16	gi|12836461|	Q9DBJ5	CYP2D26	Cytochrome P450 2d26	mouse		2 OUT	Carninci P, Shibata Y, Hayatsu N, Sugahara Y, Shibata K, Itoh M, Konno H, Okazaki Y, Muramatsu M, Hayashizaki Y. (2000). Genome Res. *10*, 1617-1630.
17	gi|13928734|	P05182	CYP2E	Cytochrome P450 2e1	rat	27.6	3 IN	Gong P, Cederbaum AI, Nieto N. (2003). J. Biol. Chem *278*, 29693-29700.
18	gi|23463305|	P05183	CYP3A2	Cytochrome P450 3a2	rat	27.6	3 IN	Rekka E, Evdokimova E, Eeckhoudt S, Labar G, Calderon PB. (2002). Biochem. Pharmacol. *64*, 633-643.
19	gi|21955148|	Q64581	CYP3A18	Cytochrome P450 3a18	rat	28.3	4 IN	Nagata K, Murayama N, Miyata M, Shimada M, Urahashi A,Yamazoe Y, Kato R. (1996). Pharmacogenetics *6*, 103-111
20	gi|6681121|	O35728	CYP4A14	Cytochrome P450 4a14	mouse	15.4	2 OUT	Heng YM, Kuo CS, Jones PS, Savory R, Schulz RM,Tomlinson SR, Gray TJ, Bell DR. (1997). Biochem. J. *325*, 741-749.
21	gi|9665225|	P33274	CYP4F1	Cytochrome P450 4f1	rat	43.7	2 OUT	Chen L, Hardwick JP. (1993). Biochem. Biophys. *300*, 18-23.
22	gi|6978751|	Q64654	CYP51A1	Cytochrome P450 51a1	rat	9.9	2 OUT	Aoyama Y, Funae Y, Noshiro M, Horiuchi T, Yoshida Y (1994) Biochem. Biophys. Res. Commun. *201*, 1320-1326.
23	gi|13928780|	P00388	POR	Cytochrome P450 (cytochrome) oxidoreductase	rat	43.7	1 IN	Porter TD, Beck TW, Kasper CB. (1990). Biochemistry *29*, 9814-9818.
24	gi|16507237|	P11021	HSPA5/GRP78	Heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa); BiP	human	62.3	1 OUT	Munro S, Pelham HR. (1986) Cell *46*, 291-300.
25	gi|5729877|	P11142	HSP73	Heat shock cognate protein 70: heat shock 70kD protein 8	human	41.3	0 OUT	Rensing SA, Maier UG. (1994). J. Mol. Evol. *39*, 80-86.
26	gi|6981324|	P04785	PDIA1	Protein disulfide isomerase (Prolyl 4-hydroxylase, beta polypeptide)	rat	59.1	0 OUT	Edman JC, Ellis L, Blacher RW, Roth RA, Rutter WJ. (1985) Nature *317*, 267-270.
27*	gi|8393322|	P11598	PDIA3	Protein disulfide isomerase A3, ERp60	rat	56.2	1 IN	Wyse B, Ali N, Ellison D H. (2002). Am. J. Physiol. Renal Physiol. *282*, F424-F430.
28	gi|5031973|	Q15084	PDIA6	Protein disulfide isomerase-related protein	human	20	1 OUT	Kikuchi M, Doi E, Tsujimoto I, Horibe T, Tsujimoto Y (2002) J Biochem. *132*, 451-455.
29	gi|16758848|	P52555	ERP29	ERp29	rat	39.2	0 IN	Sargsyan E, Baryshev M, Szekely L, Sharipo A, Mkrtchian S (2002). J. Biol. Chem. *277*, 17009-17015.
30	gi|19072792|	Q9D1Q6	ERP44	ERp44	mouse	48.5	0 OUT	Anelli T, Alessio M, Mezghrani A, Simmen T, Talamo F, Bachi A, Sitia R. (2002). EMBO J. *21*, 835-844.
31	gi|6755863|	P08113	GRP94	ERp99	mouse	49.4	1 OUT	Mazzarella RA, Green M. (1987) J. Biol. Chem *262*, 8875-
32	gi|7706495|	Q9UBS4	ERJ3	ER-associated Hsp40 co-chaperone: hDj9; ERi3	human	19	1 IN	Ohtsuka K, Hata M. (2002). Cell Stress Chaperones *5*, 98-112
33	gi|6753304|	P19324	HSP47	Heat shock protein 47	mouse	34.8	1 IN	Koide T, Aso A, Yorihuzi T, Nagata K. (2000). J. Biol. Chem *275*, 27957-27963.
34	gi|12831229|	Q80X75	CAB140	Calcium binding protein, 140 kDa; 170 kDa glucose regulated protein GRP170 precursor	mouse	23.2	0 IN	Naved AF, Ozawa M, Yu S, Miyauchi T, Muramatsu H, Muramatsu T. (1995). Cell Struct. Funct. *20*, 133-141.
35	gi|17865351|	P55072	VCP	ATPase activity of p97, valosin-containing protein	rat	64.9	0 OUT	Zhang L, Ashendel CL, Becker GW, Morre DJ (1994) J Cell Biol. *127*, 1871-1883.
36	gi|8392935|	P11507	ATP2A2	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2; ER and SR	rat	18.3	11 IN	Lompre AM, de la Bastie D, Boheler KR, Schwartz K (1989) FEBS Lett. *249*, 35-41.
37	gi|11693172|	P18418	CALR	Calreticulin	rat	61.1	1 OUT	Fliegel L, Burns K, MacLennan DH, Reithmeier RA, Michalak M (1989). J. Biol. Chem. *264*, 21522-21528.
38	gi|6671664|	P35564	CANX	Calnexin	mouse	33.2	1 OUT	Wada I, Rindress D, Cameron PH, Ou WJ, Doherty JJ 2nd, Louvard D, Bell AW, Dignard D, Thomas DY, Bergeron JJ (1991) J. Biol. Chem. *266*, 19599-19610.
39	gi|11120720|	P70580	PGRMC1	Membrane associated progesterone receptor component 1, 25-DX	rat	46.8	2 OUT	Selmin O, Lucier GW, Clark GC, Tritscher AM, Vanden Heuvel JP, Gastel JA, Walker NJ, Sutter TR, Bell DA. (1996) Carcinogenesis *17*, 2609-2615.
40	gi|6981486|	P07153	RPN1	Ribophorin I	rat	23.8	2 IN	Kreibich G, Marcantonio EE, Sabatini DD (1983) Methods Enzymol. *96*, 520-530.
41	gi|13928974|	P25235	RPN2	Ribophorin II	rat	25.8	3 OUT	Kreibich G, Marcantonio EE, Sabatini DD (1983) Methods Enzymol. *96*, 520-530.
42	gi|6681153|	O54734	DDOST	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase 48 kDa subunit	mouse	33.8	2 IN	Yamagata T, Tsuru T, Momoi MY, Suwa K, Nozaki Y, Mukasa T,Ohashi H, Fukushima Y, Momoi T. (1997) Genomics *45*, 535 540
43	gi|31982666|	Q99MP2	SSR1	Translocon-associated protein alpha, TRAP alpha	mouse	15	2 IN	Hartmann E, Gorlich D, Kostka S, Otto A, Kraft R, Knespel S, Burger E, Rapoport TA, Prehn S (1993) Eur. J. Biochem. *214*, 375-381.
44	gi|13385134|	Q9CYN2	SPC25	RIKEN cDNA 5730406I15; 25-kDa subunit microsomal signal peptidase complex	mouse		2 IN	Strausberg RL et al (2002) Proc. Natl. Acad. Sci. USA *99*, 16899-16903.
45	gi|13994184|	O88941	None	Glucosidase 1	rat	13.3	1 IN	Khan FA, Varma GM, Vijay IK (1999) Glycobiology *9*, 797-
46	gi|6679891|	O08794	G2AN	Alpha glucosidase 2, alpha neutral subunit	mouse	12.1	2 OUT	Arendt CW, Ostergaard HL (1997) J. Biol. Chem *272*, 13117-13125.
47	gi|6679465|	O08795	PRKCSH	Alpha glucosidase 2, beta subunit	mouse	23.6	0 OUT	Arendt CW, Ostergaard HL (1997) J. Biol. Chem *272*, 13117-13125.
48	gi|24497519|	P33908	MAN1A1	Mannosidase, alpha, class 1A, member 1: Man9-mannosidase	human	28.3	2 OUT	Tremblay LO, Campbell Dyke, N, Herscovics A (1998) Glycobiology *8*, 585-595
49	gi|6005808|	Q9UKM7	MAN1B1	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 4	mouse	12.7	4 OUT	Gonzalez DS, Karaveg K, Vandersall-Nairn AS, Lal A, Moremen KW (1999) J. Biol. Chem *274*, 21375-21386

(*continued*)

Table 1 continued.

50	gi|13928768|	Q63108	None	Carboxylesterase 1	rat	43.9	**1 IN**	Robbi M, Van Schaftingen E, Beaufay H. (1996). Biochem. J. *313*, 821-826.
51	gi|21717659|	Q91YG2	None	Carboxylesterase 3	rat	56.3	0 OUT	Wallace TJ, Kodsi EM, Langston TB, Gergis MR, Grogan WM. (2001). J. Biol. Chem. *276*, 33165-33174.
52	gi|6679805|	P45878	FKBP2	FK506 binding protein 2	mouse	50	**1 OUT**	Hendrickson BA, Zhang W, Craig RJ, Jin YJ, Bierer BE,Burakoff S, DiLella AG. (1993). Gene *134*, 271-275.
53	gi|16716609|	Q91ZD0	DHCR24	3-beta-hydroxysterol delta-24 reductase	mouse	19.2	**3 IN**	Waterham HR, Koster J., Romeijn GJ, Hennekam RC, Vreken P, Andersson HC, FitzPatrick DR, Kelley RI, Wanders R. (2001). J. Am. J. Hum. Genet. *69*, 685-694.
54	gi|10120490|	Q9QZH8	None	Arylacetamide deacetylase, homologous with hormone-sensitive lipase	rat	20.9	**3 IN**	Trickett JI, Patel DD, Knight BL, Saggerson ED,Gibbons GF, Pease RJ. (2001). J. Biol. Chem. *276*, 39522-39532.
55*	gi|6978813|	P07687	EPHX1	Epoxide hydrolase 1 (microsomal xenobiotic hydrolase)	rat	49.4	0 OUT	Falany CN, McQuiddy P, Kasper CB. (1987). J. Biol. Chem. *262*, 5924-5930.
56	gi|6981050|	P27364	None	3-beta-hydroxy-delta(5)-steroid dehydrogenase	rat	45	**1 OUT**	Zhao HF, Rheaume E, Trudel C, Couet J, Labrie F, Simard J. (1990). J. Endocrinology *127*, 3237-3239.
57	gi|19924087|	P23457	AKR1C9	3-alpha-hydroxysteroid dehydrogenase	rat	23.6	0 OUT	Usui E, Okuda K, Kato Y, Noshiro M. (1994). J. Biochem. *115*, 230-237.
58	gi|8393570|	P16232	HSD11B1	11-beta-hydroxysteroid dehydrogenase 1	rat	31	0 OUT	Waddell BJ, Hisheh S, Krozowski ZS, Burton P. (2003). J.Endocrinology *144*, 3101-3106.
59	gi|14091750|	Q9Z1B9	HSD17B12	17 beta-hydroxysteroid dehydrogenase type 3	rat	12	**3 IN**	Strausberg RL et al. (2002). Proc. Natl. Acad. Sci. USA *99*, 16899-16903.
60	gi|16716609|	Q91ZD0	DHCR24	24-dehydrocholesterol reductase; 3-beta-hydroxysterol delta-24 reductase	mouse	19.5	**3 IN**	Waterham HR, Koster J, Romeijn GJ, Hennekam RC, Vreken P. (2001). Am. J. Hum. Genet. *69*, 685-694.
61	gi|19424208|	P50169	RDH1	Retinol dehydrogenase type I	rat	19.6	0 OUT	Chai X, Zhai Y, Napoli JL. (1996). Gene *169*, 219-222.
62	gi|6978847|	P36365	FMO1	Flavin-containing monooxygenase 1	rat	40.4	**1 OUT**	Lattard V, Lachuer J, Buronfosse T, Garnier F, Benoit E. (2002). Biochem. Pharmacol. *63*, 1453-1464.
63	gi|16758174|	Q9EQ76	FMO3	Flavin-containing monooxygenase 3	rat	44.8	**1 OUT**	Lattard V, Lachuer J, Buronfosse T, Garnier F, Benoit E. (2002). Biochem. Pharmacol. *63*, 1453-1464.
64*	gi|21426797|	Q8K4C0	FMO5	Flavin containing monooxygenase 5	rat	41.1	**2 OUT**	Lattard V, Lachuer J, Buronfosse T, Garnier F, Benoit E. (2002). Biochem. Pharmacol. *63*, 1453-1464.
65	gi|11560046|	P00173	CYB5	Cytochrome b5	rat	85.8	0 OUT	Yoo M. (1997). Biochem. Biophys. Res. Commun. *236*, 641-
66	gi|7106309|	P97464	EXT1	Exostoses (multiple) 1	mouse	8.5	**1 IN**	Lohmann DR, Buiting K, Ludecke HJ, Horsthemke B. (1997). Cytogenet. Cell Genet. *76*, 164-166.
67	gi|9055216|	Q9WVL6	EXT3	Exostoses (multiple) 3	mouse	8.9	**1 IN**	Carninci P, Hayashizaki Y. (1999). Meth. Enzymol. *303*, 19-44.
68	gi|31543694|	Q8R0X7	SGPL1	Sphingosine phosphate lyase 1	mouse	6	**4 OUT**	Zhou J, Saba JD. (1998). Biochem. Biophys. Res. Commun. *242*, 502-507.
69	gi|21703732|	Q91W90	TXNDC5	Thioredoxin	mouse	20.4	0 OUT	Okazaki Y et al. (2002). Nature *420*, 563-573.
70	gi|6857787|	O35465	FKBP8	FK506 binding protein 8 (38 kDa)	mouse	21.4	**1 IN**	Pedersen KM, Finsen B, Celis JE, Jensen NA. (1999). Electrophoresis *20*, 249-255.
71	gi|6679465|	O08795	PRKCSH	Alpha glucosidase II, beta-subunit	mouse	23.6	0 IN	Arendt CW, Ostergaard HL. (1997). J. Biol. Chem. *272*, 13117-13125.
72	gi|19705453|	P08011	MGST1	Microsomal glutathione S-transferase 1	rat	58.7	**3 OUT**	DeJong JL, Morgenstern R, Jornvall H, DePierre JW, Tu CP. (1988). J. Biol. Chem. *263*, 8430-8436.
73	gi|18373323|	P97886	UGT1	UDP glycosyltransferase 1 family, polypeptide A6	rat	34	**2 IN**	Ikushiro S, Emi Y, Iyanagi T. (2002). Biochemistry *41*, 12813-12820.
74	gi|11560022|	P36510	UGT2A1	UDP-glucuronosyltransferase 2A1 precursor	rat	9.1	**2 IN**	Lazard D, Zupko K, Poria Y, Nef P, Lazarovits J, Horn S, Khen M, Lancet D. (1991). Nature *349*, 790-793.
75	gi|13928718|	P08541	UGT2B2	UDP-glucuronosyltransferase 2B2 precursor	rat	68.7	**1 OUT**	Jackson MR, Burchell B. (1986). Nucleic Acids Res. *14*, 779-
76	gi|14010881|	P36511	UGT2B12	UDP-glucuronosyltransferase 2B12 precursor	rat	50.8	**1 OUT**	Green MD, Clarke DJ, Oturu EM, Styczynski PB, Jackson MR, Burchell B, Tephly TR. (1995). Arch. Biochem. Biophys. *322*, 460-468.
77	gi|4507819|	P54855	UGT2B15	UDP-glucuronosyltransferase 2B15 precursor	human	8.3	**2 IN**	Iida A, Saito S, Sekine A, Mishima C, Kitamura Y, Kondo K, Harigae S, Osawa S, Nakamura Y. (2002). Hum. Genet. *47*, 505-510.
78	gi|23463309|	P08542	UGT2B3	UDP-glucuronosyltransferase 2B3 precursor	rat	50.4	**1 OUT**	Mackenzie PI. (1987). J. Biol. Chem. *262*, 9744-9749.
79	gi|6678501|	P17717	UGT2B5	UDP-glucuronosyltransferase 2B5 precursor	mouse	37	**1 OUT**	Kimura T, Owens IS. (1987). Eur. J. Biochem. *168*, 515-521.
80	gi|13195672|	P58158	B3GAT3	Beta-1,3-glucuronyltransferase 3 (glucuronosyltransferase I)	mouse	23.6	**1 OUT**	Strausberg RL et al. (2002). Proc. Natl. Acad. Sci. USA *99*, 16899-16903.
81	gi|13929028|	P30839	ALDH3A2	Aldehyde dehydrogenase family 3, subfamily A2	rat	53.9	**3 IN**	Miyauchi K, Masaki R, Taketani S, Yamamoto A, Akayama M, Tashiro Y. (1991). J. Biol. Chem. *266*, 19536-19542.
82	gi|13162326|	Q9ES38	BAL	Bile acid CoA ligase	rat	52	**1 IN**	Falany CN, Xie X, Wheeler JB, Wang J, Smith M, He D, Barnes S. (2002). J Lipid Res. *43*, 2062-2071.
83	gi|8392962|	Q91X34	BAAT	Bile acid-Coenzyme A: amino acid N-acyltransferase	rat	31.2	**2 OUT**	Furutani M, Ari S, Higashitsuji H, Mise M, Fukumoto M, Takano S, Nakayama H, Imamura M, Fujita J. (1995). Biochem. J. *311*, 203-208.
84	gi|11560006|	P10867	GULO	L-gulono-gamma-lactone oxidase	rat	37.3	0 OUT	Nishikimi M, Kawai T, Yagi K. (1992). J. Biol. Chem. *267*, 21967-21972.
85	gi|20302049|	P20070	DIA1	NADH cytochrome b-5 reductase, diaphorase	rat	70.1	0 OUT	Pietrini G, Carrera P, Borgese N. (1988). Proc. Natl. Acad. Sci. USA *85*, 7246-7250.
86	gi|13929176|	Q9JLT5	WFS1	Wolfram syndrome 1; Wolfram syndrome 1 (wolframin)	rat	7.1	**9 IN**	Takeda K, Inoue H, Tanizawa Y, Matsuzaki Y, Oba J, Watanabe Y, Shinoda K, Oka Y. (2001). Hum. Mol. Genet. *10*,
87	gi|19557691|	O15260	SURF4	Surfeit 4; surfeit locus protein 4; surface 4 integral membrane protein	human	7.4	**5 OUT**	Duhig T, Ruhrberg C, Mor O, Fried M. (1998). Genomics *52*, 72-78.
88	gi|11968126|	O88541	CYPB	Cyclophilin B	rat	50.5	**1 IN**	Kainer DB, Doris PA. (2000). Hypertension. *35*, 958-964.
89	gi|20302024|	Q63617	ORP150	Oxygen regulated protein (150kD)	rat	25.8	0 IN	Kuwabara K, Matsumoto M, Ikeda J, Hori O, Ogawa S, Maeda Y. (1996). J. Biol. Chem. *271*, 5025-5032.
90	gi|40807491|	P33121	FACL2	Long-chain fatty-acid-coenzyme A ligase 1	human	8.9	**1 IN**	Ghosh B, Barbosa E, Singhl. (1995). Mol. Cell. Biochem. *151*, 77-81.
91	gi|16758398|	O88813	FACL5	Long-chain fatty-acid-coenzyme A ligase 5	rat	41.1	**1 IN**	Lewin TM, Kim JH, Granger DA, Vance JE, Coleman RA. (2001). J. Biol. Chem. *276*, 24674-24679.
92	gi|13929034|	P97524	FACVL1	Very-long-chain-fatty-acid-CoA ligase	rat	43.1	0 OUT	Uchiyama A, Aoyama T, Kamijo K, Uchida Y, Kondo N, Ori T, Hashimoto T. (1996). J. Biol. Chem. *271*, 30360-30365.
93	gi|21426787|	Q8K4Y7	SRAPY	Apyrase	rat		**1 IN**	Failer BU, Braun N, Zimmermann H. (2002). J. Biol. Chem. *277*, 36978-36986.
94	gi|13195636|	Q9D1M0	SEC13R	Sec13 related protein	mouse	27.6	0 OUT	Strausberg RL et al. (2002). Proc. Natl. Acad. Sci. USA *99*, 16899-16903.
95	gi|16924008|	Q62891	RSEC22	Sec22 homolog	rat	20.9	**4 IN**	Hay JC, Hirling H, Scheller RH. (1996). J. Biol. Chem. *271*, 5671-5679.
96	gi|5454042|	Q15436	SEC23A	Sec23 homolog A	human	30.2	0 OUT	Paccaud JP, Reith W, Carpentier JL, Ravazzola M, Amherdt M,Schekman R, Orci L. (1996). Mol. Biol. Cell *7*, 1535-1546.

[a] National Center for Biotechnology Information (NCBI) Accession Number

[b] Swiss Prot Accession Number

[c] Prediction of transmembrane domains using HMMTOP (Version 2.0). The number represents the number of predicted transmembrane domains and the 'in' and 'out' represent the predicted localization of the N-

* Arginine dimethylated (refer to Table 3)

Table 2. Unknown Protein Identification

	Accession no.[a]	Protein description	Species	% Coverage	TMDs[b]
1*	gi|27229118|	Q9DD20[#]-putative methyltransferase	Mouse	34.0	1
2	gi|21703704|	Q8VCS2[#]-Chr 17, Wayne State University 94	Mouse	18.2	5
3	gi|29150272|	RIKEN cDNA 1110003H02	Mouse	27.6	0
4	gi|13386156|	RIKEN cDNA 0610005A07	Mouse	29.4	0
5	gi|31981046|	RIKEN cDNA 0610039N19	Mouse	29.8	0
6	gi|13385678|	RIKEN cDNA 1200007D18	Mouse	34.1	2
7	gi|19527236|	RIKEN cDNA 1110014L17	Mouse	46.3	2
8	gi|13385718|	RIKEN cDNA 2400003B06	Mouse	53.7	0
9	gi|19526900|	RIKEN cDNA 2010200I23	Mouse	11.0	2
10	gi|21313538|	RIKEN cDNA 1810037C20	Mouse	36.5	1
11	gi|13385912|	RIKEN cDNA 2310034L04	Mouse	60.0	0
12	gi|21313316|	RIKEN cDNA 2510039O18	Mouse	18.8	2
13	gi|21313032|	RIKEN cDNA 2900024C23	Mouse	17.8	1
14	gi|31559920|	RIKEN cDNA 4633402C03	Mouse	9.4	2
15	gi|13385750|	RIKEN cDNA 4833420E20	Mouse	11.1	5
16	gi|13277372|	RIKEN cDNA 6330583M11	Mouse	34.1	0
17	gi|21312890|	RIKEN cDNA 3230402M22 gene	Mouse	36.9	0
18	gi|21361757|	Hypothetical protein PRO0971	Human	28.1	0
19	gi|40255240|	Hypothetical protein FLJ10276	Human	22.6	0
20	gi|21361732|	Hypothetical protein FLJ11099	Human	9.5	6
21	gi|40254928|	Hypothetical protein FLJ11274	Human	10.7	7
22	gi|21362102|	Hypothetical protein FLJ13576	Human	14.1	9
23	gi|8923392|	Hypothetical protein FLJ20421	Human	19.2	1
24	gi|18677735|	Hypothetical protein MGC12103	Human	19.8	0
25	gi|23956396|	Hypothetical protein MGC27952	Mouse	23.5	0
26	gi|22122511|	Hypothetical protein MGC30562	Mouse	9.2	1
27	gi|13385022|	Hypothetical protein, MGC7041	Mouse	26.9	1
28	gi|9506865|	Hypothetical protein MNCb-5081	Rat	42.4	0
29	gi|21361757|	Hypothetical protein PRO0971	Human	28.1	0
30	gi|21489985|	Hypothetical protein RMT-7	Rat	14.8	1
31	gi|21703824|	Similar to KIAA0475 gene product	Mouse	40.6	1
32	gi|7706278|	Breast cancer antigen 84	Human	17.5	2
33	gi|13384938|	Serologically defined breast cancer antigen 84	Mouse	16.7	2
34	gi|15808990|	Carcinoma related gene, F-LANa	Mouse	25.5	4
35	gi|19527026|	Expressed sequence AA959742	Mouse	29.3	1
36	gi|19923442|	CGI-100 protein	Human	18.8	2
37	gi|11067391|	Hypothetical protein FLJ10525	Rat	6.5	3
38	gi|19924091|	Synaptic glycoprotein SC2	Rat	16.9	4
39	gi|7657176|	Transmembrane protein 4	Human	36.6	0
40	gi|20531765|	CLLL6 protein; hypothetical protein from clone 24774	Human	68.9	0
41	gi|24418883|	Hypothetical protein DKFZp434F2322	Mouse	29.8	1

* Arginine dimethylated (refer to Table 3).

[#] Swiss prot accession number.

[a] National Center for Biotechnology Information (NCBI) accession number.

[b] Prediction of transmembrane domains by using HMMTOP (Version 2.0).

fractionation technique produces a "pure" preparation, proteins from other organelles such as mitochondria and perixosomes may be present as minor contaminants of the Golgi fraction. These would not be detectable by other less sensitive techniques. Therefore, because verification of the functional significance of these proteins in the Golgi requires both localization studies and functional assays, to be conservative, we listed these proteins in their most widely accepted categories.

Of the 421 proteins identified in the Golgi fraction, 110 are previously documented resident Golgi proteins (Table 1). Of these, 70 proteins (64%) are predicted transmembrane proteins (HMMTOP software, version 2.0). Multiple families of Golgi proteins are well represented (Figure 1B). Importantly, previous Golgi proteomes reported very few Golgi transferases (for glycosylation and sulfation) (Taylor *et al*., 2000; Wu *et al*., 2000; Bell *et al*., 2001). In this study, 23 glycosylation (21%) and six sulfation (5%) enzymes were identified. Furthermore, three of the transferases involved in glycosylation were later shown to be arginine dimethylated (see below; Table 3).

Identification of Novel Putative Golgi Proteins

Of the proteins identified in the Golgi fraction, 41 were proteins with no previously reported functions (Table 2). To establish whether these were Golgi residents or proteins from other organelles, it was necessary to determine their intracellular localization. For practical purposes, we selected two of the unknown predicted transmembrane proteins (protein #1/Q9DD20/putative methyltransferase and protein #2/Q8VCS2) from Table 2. Sequence analysis of Q9DD20 suggested a 20-amino acid N-terminal transmembrane domain and a domain with high sequence similarity to the SAM binding domain KOG4300 in the Conserved Domain Database at National Center for Biotechnology Information (Figure 3A, yellow and green shaded areas, re-

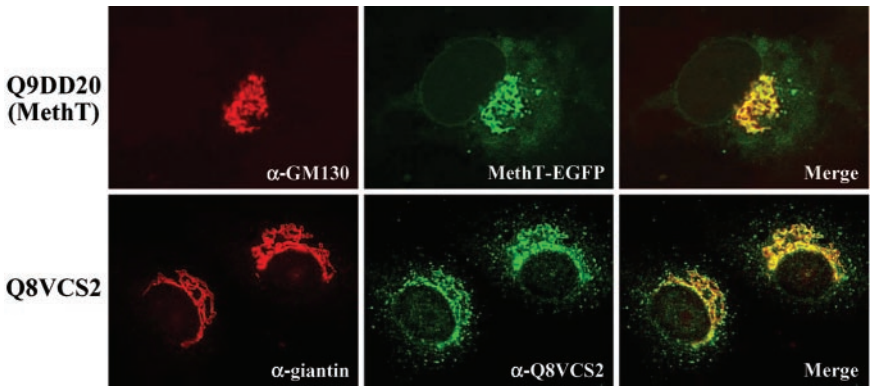

Figure 2. Subcellular localization of proteins of unknown function identified by subcellular proteomics. Fluorescence detection of GFP and antibody staining was performed in NRK cells. Top, immunofluorescence shows the localization of *cis*-Golgi protein GM130 (red, left). The fluorescence from the expression of MethT-EGFP (protein #1 in Table 2/Q9DD20) (green, middle) is shown to overlap with GM130. Bottom, double immunofluorescence shows the colocalization of *cis*-Golgi marker giantin (red, left) and Q8VCS2 (protein #2 in Table 2) (green, middle). Both unknown proteins are highly concentrated in the perinuclear region.

spectively). SAM is the donor molecule for most methyltransferases. Therefore, this domain prediction suggested that the protein may be a putative methyltransferase (Schubert *et al*., 2003). Corresponding cDNAs were acquired for both unknown proteins, fused with GFP and expressed in NRK cells. When expressed in NRK cells, colocalizations with various markers were used to determine their subcellular localization. Both proteins localized to the *cis*-Golgi judged by their colocalization with known Golgi markers (Figure 2). The top panels show that the presumptive EGFP-tagged methyltransferase (MethT-EGFP, Q9DD20) colocalizes with the *cis*-Golgi protein GM130. The bottom panels show that an antibody against Q8VCS2 colocalizes this protein with the *cis*-Golgi marker giantin. These results confirm that two of the 41 unknown proteins are Golgi localized.

Identifications of Arginine Dimethylated Proteins in the Golgi Proteome

To search for methylation of arginine residues in the proteome, the MS/MS spectra were researched against the subset database to consider modifications of 1) +14 on R (methylation) and 2) +28 on R (dimethylation). A subset database including only rat sequences was used to expedite SEQUEST differential modification searches. Eighteen proteins were identified to be arginine dimethylated in the Golgi proteome and of these, 10 were confirmed Golgi proteins, including the methyltransferase Q9DD20 (Table 3). Protein arginine methylation is a posttranslational modification that results in the addition of monomethyl or dimethyl groups to the guanidino group of arginine. As expected, most of the proteins represented are the abundant proteins with high sequence coverage. The methylated proteins are not all Golgi localized but reside in multiple subcellular organelles. The methylated residues are localized on protein domains reported to be either lumenal or cytoplasmic. This implies that there are at least two methyltransferases necessary for these modifications and that a SAM transporter is present in the Golgi membrane.

Interestingly, the putative methyltransferase, was identified to be dimethylated at Arginine-230 by 12 independent

Table 3. Dimethyl-Arginine Modified Proteins

	Accession no.[a]	Protein identification	Modification site	Localization[b]
Golgi				
1	gi|27229118|	Putative transmembrane methyltransferase	R230	Cytoplasmic
2	gi|16758214|	Integral membrane protein Tmp21-I (p23)	R171 & R176	Lumenal
3	gi|13384724|	p24B, *cis*-Golgi protein	R102	Lumenal
4	gi|20301975|	TGN38, *trans*-Golgi network protein 1	R74	Lumenal
5	gi|7305095|	Golgi autoantigen, golgin subfamily a, 5, Golgin-84	R27 & R88	Cytoplasmic
6	gi|16758358|	Golgi SNAP receptor complex member 1; *cis*-Golgi SNARE (p28)	R102	Cytoplasmic
7	gi|31981620|	Mannoside acetylglucosaminyltransferase 1	R41 & R46	Lumenal
8	gi|16915936|	Mannoayl (α-1,3-)-glycoprotein β-1,4-*N*-acetylglucosaminyltransferase 2	R8 & R17	Lumenal
9	gi|6678788|	Mannosidase 1, α	R106 & R168	Lumenal
10	gi|20301956|	GRASP55, golgi reassembly stacking protein 2	R30 & R47	Cytoplasmic
ER				
11	gi|6978747|	Cytochrome P450 2d2	R333 & R487	Cytoplasmic
12	gi|9506529|	Cytochrome P450, 2c29	R144	Cytoplasmic
13	gi|6978813|	Epoxide hydrolase 1 (microsomal xenobiotic hydrolase)	R295	Lumenal
14	gi|8393322|	Protein disulfide isomerase A3, ERp60	R329 & R404	Lumenal
15	gi|21426797|	Flavin containing monooxygenase 5	R5	Lumenal
Secretory				
16	gi|20330802|	Transferrin	R42	Lumenal
Endosome				
17	gi|18017593|	Endosomal membrane protein, EMP70, member 2	R55 & R90	Lumenal
18	gi|19111162|	Endosomal membrane protein, EMP70, member 3	R61 & R116 & R 206	Lumenal

[a] National Center for Biotechnology Information (NCBI) accession number.

[b] Localization of modified protein domain.

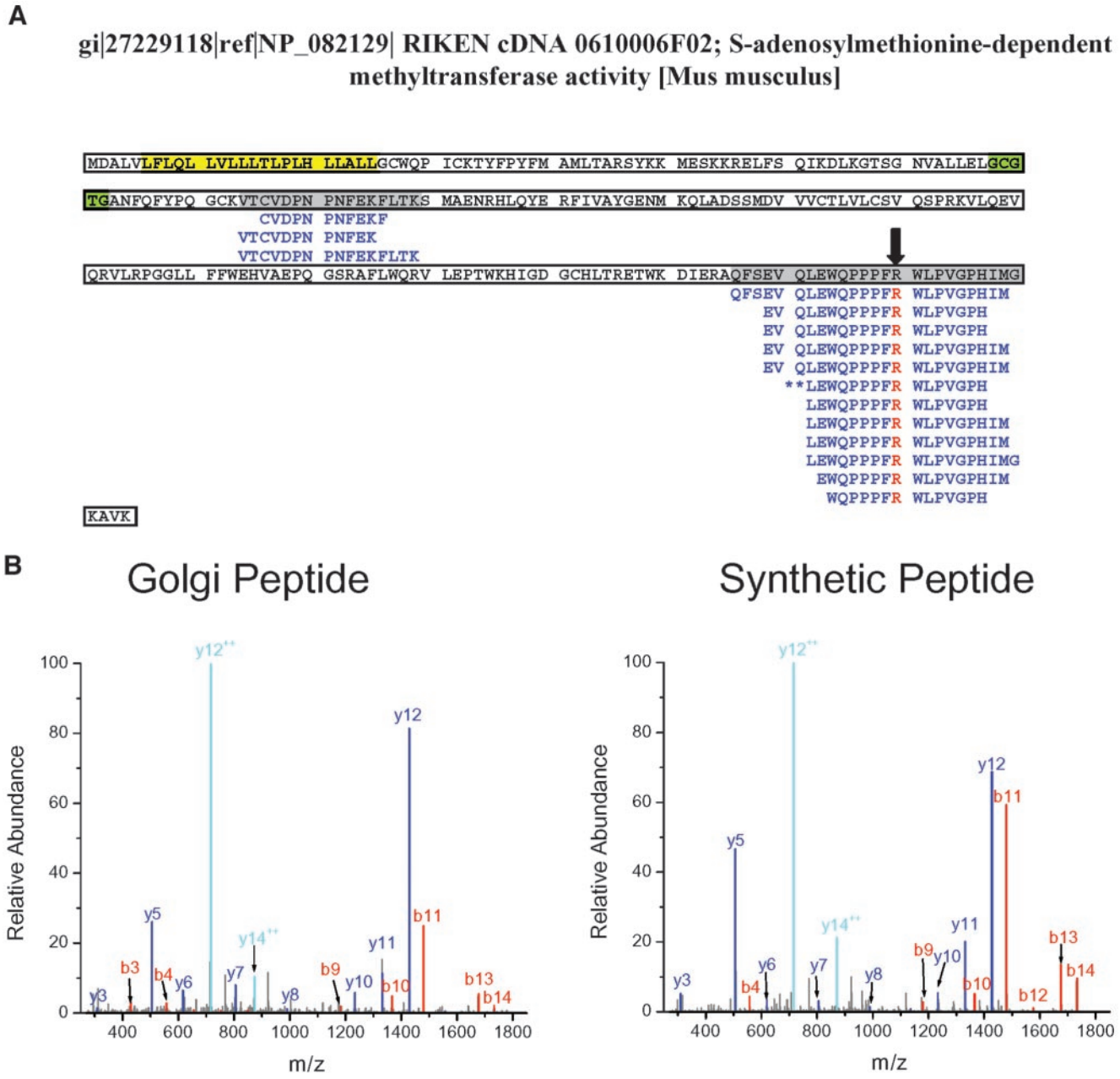

Figure 3. Identification of arginine dimethylation on putative methyltransferase. The protein identified by gi 27229118 NP_082129. RIKEN cDNA 061000 was selected from the list of proteins of unknown function for further analysis. (A) Protein sequence is displayed in boxed text. Shaded regions indicate coverage by identified peptides (gray), the predicted TMD (yellow), and the predicted SAM binding domain (green). Peptides are displayed below the protein sequence in blue text. Modified peptides are displayed in bold blue text, and modification site is indicated on the protein sequence with an arrow. The modified peptide tagged with ** was synthesized to confirm the modification site. (B) Spectrum of the dimethylarginine modified peptide acquired from Golgi MudPIT analysis is displayed on the left. Spectrum of the synthetic dimethylarginine-modified peptide is displayed on the right. Spectra are annotated using Roepstorff and Fohlman nomenclature (Roepstorff and Fohlman, 1984).

overlapping peptides (Figure 3A, bolded blue peptides). Arg-230 is located near the C terminus of the protein and the localization is predicted to be cytoplasmic. To verify the modification, one of the identified arginine dimethylated peptides, LEWQPPPFR*WLPVGPH (modified residue labeled with an asterisk), was synthesized. Tandem mass spectra were collected from the synthetic peptide and compared against the spectrum acquired in the Golgi sample (Figure 3B). The spectra from the Golgi fraction and the synthetic peptide are nearly indistinguishable. Both spectra contain prominent y5, y12, and b11 fragment ions resulting from the favored fragmentation at the N-terminal side of proline residues.

Little is known about the functional and regulatory implications of arginine methylation of nonnuclear proteins, but recent evidence has indicated a role in Golgi function. The drug ilimaquinone, which inhibits the synthesis of the methyl donor SAM, vesiculates the Golgi complex and

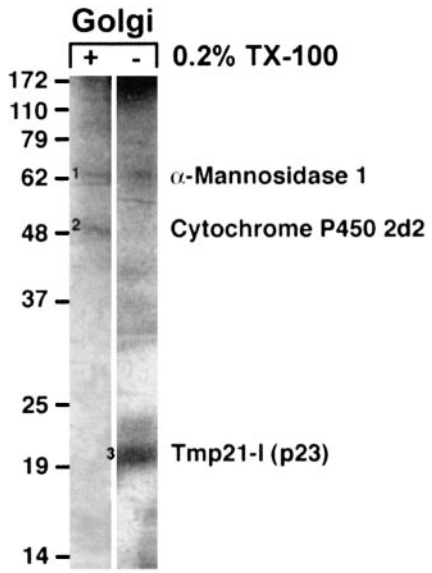

Figure 4. Methyltransferase activity is detected in the stacked Golgi fraction. Stacked Golgi fractionated from rat liver was incubated with [^{3H}]SAM in the presence and absence of 0.2% TrintonX-100 and resolved by SDS-PAGE. The gel was infiltrated with fluorographic reagent, dried, and exposed to film. Different labeled bands were detected in the presence and absence of detergent. The labeled bands were excised, digested with trypsin, and identified by mass spectrometry.

blocks secretion (Takizawa *et al*., 1993; Radeke *et al*., 1999). Addition of SAM can reverse the ilimaquinone effect (Casaubon and Snapper, 2001). Together with our proteomic evidence for arginine dimethylation of multiple Golgi proteins, these results suggest that the Golgi has endogenous methyltransferase activity. To test this prediction, the enriched stacked Golgi fraction was incubated with [^{3}H]SAM plus/minus TX-100 (Lin *et al*., 2000). The resulting samples were resolved by SDS-PAGE, and the dried gels exposed to film. More than 10 bands were labeled (our unpublished data). These were excised from the dried gels and in-gel digested with trypsin for protein identification by mass spectrometry. Three proteins from the original list of dimethylated Golgi proteins (α-manosidase 1/#1, cytochrome 450 2d2/#2, and Tmp23/#3) (Table 3) were identified in these bands (Figure 4). These data support the hypothesis that select Golgi and ER membrane proteins are modified by dimethylation of arginine residues.

DISCUSSION

We have performed a proteomic survey of an enriched stacked Golgi fraction from rat liver and have analyzed the results in the context of publicly available annotations. Our proteomic analysis is a survey of the abundant proteins within the Golgi fraction. All protein identifications have high confidence by using a minimum of five independent peptides for identification. Because the likelihood of obtaining multiple peptide identifications per protein is greater for highly abundant proteins (Gao *et al*., 2003), these criteria favor the analysis of the most abundant proteins within the fraction. This approach was used to optimally identify abundant proteins of unknown function within the fraction. Approximately 10% of the identified proteins in this Golgi fraction are unknowns (41 proteins listed in Table 2) and represent a rich source of candidates to explore novel functions or integrate known functions of this organelle.

Many known ER proteins were identified in the Golgi fraction. The ER physically adheres to multiple *trans*-Golgi cisternae (Novikoff, 1964; Ladinsky *et al*., 1999; Marsh *et al*., 2001), and this interaction is postulated to be the site of transfer of ceramide to sphingomyelin synthase, which facilitates the synthesis of sphingolipid for sorting and exit at the *trans*-Golgi (van Meer and Lisma, 2002; Munro, 2003; Hanada *et al*., 2003). As expected, the ER remains attached to the stacked Golgi during the fractionation protocol (our unpublished data), and ER proteins account for 22% of the total proteins identified. Other proteins fall into categories that are functionally linked to the Golgi or in transit through the Golgi. These proteins have been listed in separate categories. Twenty-six percent of the total proteome are bona fide, literature confirmed, Golgi proteins with the transferases forming the largest group. Because the Golgi proteome will vary in different cells and tissues and in different functional states, this study serves as a baseline to be compared in future studies.

A number of proteins in the isolated Golgi fraction were posttranslationally modified with dimethylation of arginine residues. One of these methylated proteins has a predicted SAM binding domain, suggesting that it is a methyltransferase. All methyltransferases are thought to directly transfer the methyl group from SAM to a substrate/acceptor and SAM binding domains have a high degree of homology (Schubert *et al*., 2003). Methylation of histones for chromatin regulation and gene silencing is a well-studied example. DNA is also methylated and abnormal methylation patterns on DNA are a nearly universal finding in cancer, making this an actively studied area today (Laird, 2003). Interestingly, from the point of view of our study, most methylated nuclear proteins are modified in the cytosol, and known methyltransferases are cytosolic (Friesen *et al*., 2001a,b). In contrast to methylated nuclear proteins, however, little is known about methylation of proteins elsewhere in the cell or the functional and regulatory implications of this modification (McBride and Silver, 2001). This protein seems to be a member of a novel methyltransferase family, and our data suggests that there are two methyltransferases (cytosolic and lumenal) as well as a SAM transporter in the Golgi membrane. Because little is known about methylation of ER and Golgi proteins, this represents an exciting new area of study.

The concept arising from the nuclear methylation data is that methylation (especially of arginines) promotes specific protein–protein interactions required in assembly of functional complexes (Friesen *et al*., 2001a,b). We know little about functional complexes formed with the proteins in the Golgi proteome. One of the big quandaries in the field is that arginine methylation seems to be irreversible. Methylases have never been identified, neither as an activity nor as an enzyme. However, most investigators believe that methylation is regulatory and that methylases will be uncovered (Bannister *et al*., 2002).

Compelling data suggest that methylation of Golgi proteins is important and physiologically relevant. Many of the golgins, a group of matrix-like and tethering proteins, are direct targets in autoimmune disease (Shields and Arvan, 1999; Doyle and Mamula, 2002; Nozawa *et al*., 2002). The question to be addressed is are the golgins methylated when they become targets for the autoimmune reaction? The theory that methylation is a factor in autoimmune disease is based on numerous reports from

the multiple sclerosis field and other diseases involving demyelination. At the outset of the disease, myelin basic protein becomes symmetrically arginine dimethylated and, in parallel, phosphorylation is greatly reduced or absent (Kim *et al*., 2003). Although many golgins are reversibly phosphorylated, there is no information available about their methylation or coordinated methylation/phosphorylation. In the histone field, it is postulated that coordinated dimethylation and phosphorylation regulate gene silencing. Allis and colleagues have proposed the hypothesis that binary switches of dimethylated and reversibly phosphorylated neighboring residues in defined cassettes can regulate protein–protein interaction (Fischle *et al*., 2003). These models provide a very compelling argument for studying methylation of Golgi proteins.

The data obtained from these studies will be further mined to obtain information on other posttranslational modifications. Goals for the future will be to make organellar proteomics a robust and high-throughput tool that will facilitate the understanding of global changes in protein expression and modification that occur with cellular function and disease states.

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