REVIEW ARTICLE

MECHANISMS OF DISEASE

Mendelian Disorders of Membrane Trafficking

Maria Antonietta De Matteis, M.D., and Alberto Luini, M.D.

From the Telethon Institute of Genetics and Medicine (M.A.D.M., A.L.) and the Institute of Protein Biochemistry, Consiglio Nazionale delle Ricerche (A.L.) — both in Naples, Italy; and Consorzio Mario Negri Sud, Santa Maria Imbaro, Italy (M.A.D.M.). Address reprint requests to Dr. De Matteis at the Telethon Institute of Genetics and Medicine, Via Pietro Castellino 111, 80131 Naples, Italy, or at dematteis@tigem.it.

N Engl J Med 2011;365:927-38.

Nearly all the molecules that are expressed in mammalian cells reach their correct intracellular locations by virtue of sophisticated transport-and-delivery systems. Central among these is the intracellular membrane-transport apparatus, which is designed to ferry most of the transmembrane proteins and nearly all the secreted proteins — about a third of the human proteome — from their site of synthesis, the endoplasmic reticulum, to their final destinations.

Membrane transport is responsible for controlling the size, shape, and molecular composition of most cellular organelles, including the plasma membrane, and for mediating the secretion of thousands of cargo species, including hormones, growth factors, antibodies, matrix and serum proteins, digestive enzymes, and many more. To carry out this enormous task, the system relies on a large ensemble of organelles, including the endoplasmic reticulum, the Golgi complex, and the endolysosomal stations, and on an underlying molecular machinery that is estimated to comprise more than 2000 proteins.[1] It is no surprise, then, that alterations to membrane transport, either genetic or otherwise, are associated with many diseases. Here, after a brief overview of the pathways, strategies, and mechanisms of membrane transport, we focus on mendelian disorders that arise from defects of the membrane-transport machinery.

PATHWAYS OF MEMBRANE TRAFFICKING

The main morphologic and functional features of the secretory and endocytic pathways were initially sketched out by the pioneers of modern cell biology in the 1960s and 1970s.[2] Since then, this picture has grown enormously in richness and complexity, and the underlying molecular machinery has been unraveled through approaches that are based on yeast genetics and biochemical identification of the relevant components in mammals.[3,4]

The transport of newly synthesized secretory proteins begins at their site of synthesis, the endoplasmic reticulum, a network of dynamically interconnected membrane tubules and cisternae (Fig. 1). Proteins are cotranslationally inserted into the lumen of the endoplasmic reticulum, where they are glycosylated and folded by a complex machinery that includes the chaperone proteins.[5] Folding is essential, and when it cannot be completed, proteins are degraded by the degradation system associated with the endoplasmic reticulum.[6] Moreover, if unfolded proteins accumulate in the endoplasmic reticulum, as they do under certain stress conditions, the unfolded-protein response ensues. The unfolded-protein response is a compensatory reaction that results primarily in an increase in the production of the folding-machinery proteins but can also influence different cell functions and lead to cell death or survival (see Glossary).[7]

Figure 1 (facing page). Membrane-Trafficking Pathways.

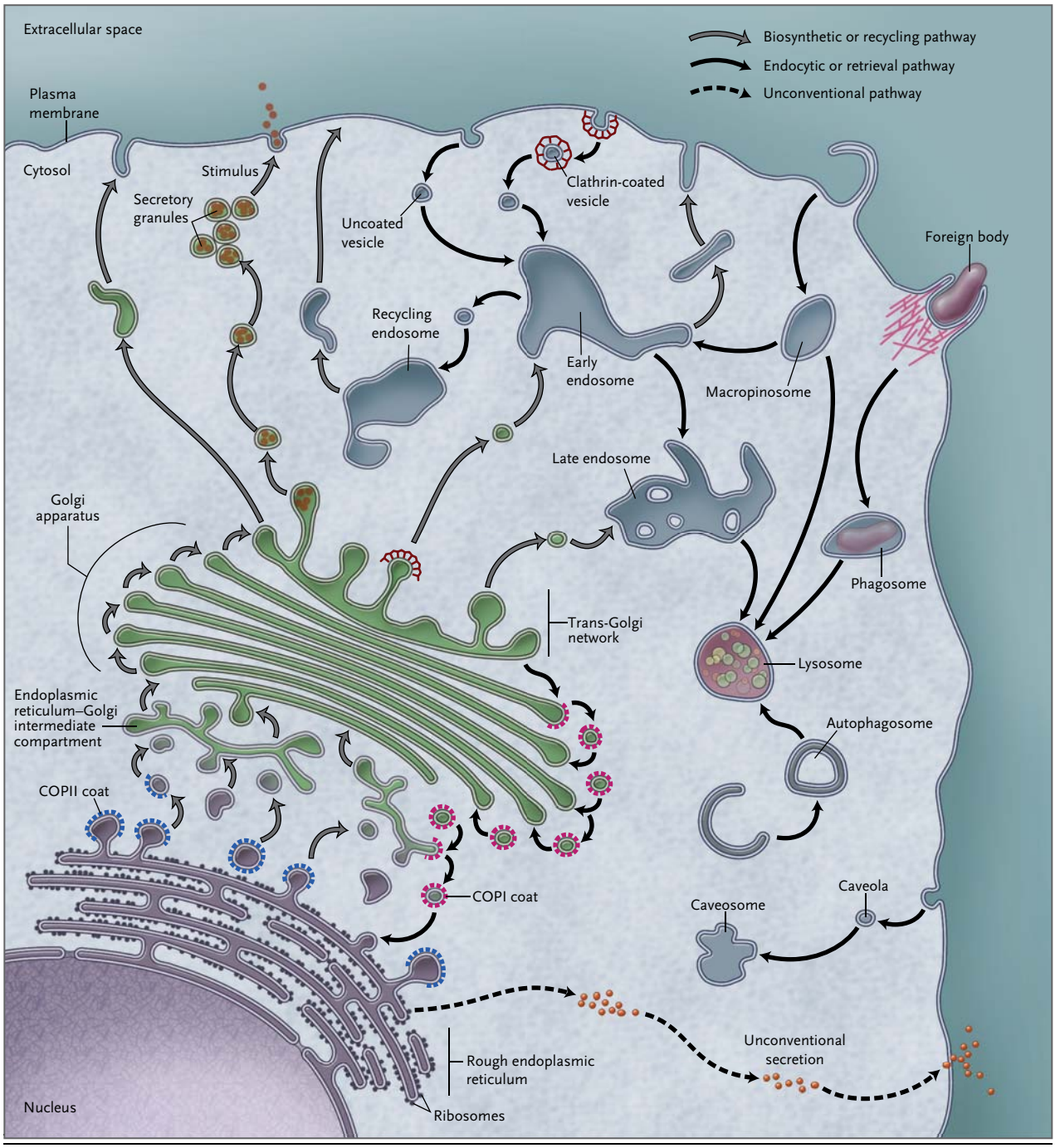
Shown are the main trafficking pathways along the secretory and endocytic pathways. The transport of newly synthesized proteins starts from the endoplasmic reticulum, where, after folding, the proteins are sorted into budding vesicles that are generated through the coat protein complex II (COPII). The vesicles then move to the endoplasmic reticulum–Golgi intermediate compartment, from which the cargoes are transported to the Golgi complex. In the Golgi complex, the cargoes enter the cis-Golgi network and proceed toward the trans-Golgi network, and the machinery proteins are returned to the endoplasmic reticulum in a manner that is dependent on coat protein complex I (COPI). At the trans-Golgi network, the different cargoes are packaged in different vesicles, which then carry them to their final destinations, such as the lysosomes, the plasma membrane, or the secretory granules in specialized cells. Most membrane proteins undergo endocytosis, which occurs through both clathrin-dependent and clathrin-independent pathways. Macropinosomes are large internalized membrane units, whereas in specialized cells, phagosomes mediate the internalization of large objects, which are then digested in the lysosomes. The endocytic carriers converge in the early endosomes, where the cargo proteins are sorted toward several destinations: the plasma membrane, the recycling endosomes, the trans-Golgi network, or the late endosomes. From the late endosomes, some cargoes move to the Golgi complex, and others are transferred to lysosomes for degradation. The autophagy pathway is also shown, through which damaged components of cells are enveloped in specialized membranes and degraded in lysosomes, and the unconventional pathway of secretion, through which cytosolic or membrane proteins reach the plasma membrane without having to pass through the Golgi complex.

After folding, proteins enter the exit sites of the endoplasmic reticulum, where they are sorted into either small or large pleomorphic budding vesicles that are generated through the membrane-bending properties of coat protein complex II (COPII)[8] (Fig. 1). All vesicles then detach from the endoplasmic reticulum through membrane fission and move to the endoplasmic reticulum–Golgi intermediate compartment (ERGIC).[8] From there, carriers containing secretory cargoes are transported forward to the Golgi complex. This step requires another coat complex, COPI,[9] and includes the translocation of the carriers along microtubules mediated by motor proteins.[10,11] From the cis pole of the Golgi, the secretory cargoes proceed toward the trans pole, whereas the machinery proteins that participate in the formation of anterograde carriers must be returned to the endoplasmic reticulum for another round of transport. This recycling is the task of COPI-dependent vesicles that form from both the ERGIC and the Golgi complex.[12]

Once in the Golgi complex, cargo proteins must traverse this organelle, which is composed of a series of interconnected stacks of four to six flat membranous cisternae and of tubular–saccular networks located at the cis and trans poles of the stacks. The main functions of the Golgi complex are to transport and chemically process cargo proteins and lipids, activities that mostly involve glycosylation. The mechanism of cargo transfer through the Golgi complex is composite and appears to involve the process of cisternal progression–maturation for large supramolecular cargoes, as well as other mechanisms for different cargo classes (Fig. 2).[13-19]

After passing through the Golgi complex and reaching the trans-Golgi network, different cargoes are packaged in specialized membranous carriers, within which they are shipped out to their respective destinations, such as the lysosomes or the plasma membrane.[20] Most proteins that are destined for the lysosomes (lysosomal enzymes) contain a mannose-6-phosphate tag and are sorted by the mannose-6-phosphate receptor into vesicles that are coated with a further protein complex, which is based on clathrin.[21] Other cargoes move to the plasma membrane (or to their specific basolateral or apical domains in polarized cells) within large, apparently uncoated pleomorphic carriers that form at the trans-Golgi network.[20] Also, in certain specialized cells, selected cargo proteins are greatly condensed into secretory granules that accumulate in the cytoplasm until their secretion is triggered by specific signals. Thus, there are several types of transport vesicles, all of which are formed by the fissioning of membrane buds from donor membranes, undergo translocation by microtubule-based motors, and dock onto and fuse with their acceptor membranes (Fig. 3).[22-27]

Once at the cell surface, most membrane proteins undergo endocytosis, a fundamental process that is involved in many functions, including control of the composition of the plasma membrane, cell signaling, and uptake of essential nutrients. There are several types of endocytic carriers, which differ in the proteins they transport, in their mor-



phologic features and dynamics, and in their underlying molecular mechanisms.[28] The best-characterized carriers are the clathrin-coated vesicles, the caveolin-coated vesicles, and the macropinosomes (pleomorphic carriers that can engulf large volumes of extracellular fluid) (Fig. 1). Phagosomes are similar to macropinosomes, and in specialized cells (e.g., macrophages) they mediate the internalization of large objects (typically bacteria), which are then digested in the lysosomes.

Most endocytic carriers then converge in the early endosomes, a vacuolar–tubular sorting station from which cargo proteins are sorted and

Glossary

Anterograde trafficking: Trafficking across the secretory stations from the endoplasmic reticulum toward the plasma membrane or the lysosomes. The main intermediate stations are the intermediate compartment, the Golgi complex, the trans-Golgi network, and the endosomes.

Phosphoinositides: A group of membrane lipids that undergo cycles of phosphorylation and dephosphorylation through organelle-specific phosphoinositide (PI) kinases and PI phosphatases, which leads to distinct subcellular distributions of the individual PI species. Since specific PIs control the correct timing and location of many trafficking events, they are key determinants of organelle identity.

Rab proteins: A large family of small GTPases that control and coordinate a multiplicity of basic events (including motility and fusion of vesicles) through the recruitment of effector proteins (e.g., tethering factors, kinases, phosphatases, and motors). Individual Rabs are located in specific compartments, and by regulating the incoming and outgoing traffic, they participate in the control of the identity of these compartments and in the spatiotemporal regulation of trafficking.

Reticulon proteins: Conserved proteins residing mainly in the endoplasmic reticulum and influencing trafficking between the endoplasmic reticulum and the Golgi complex, vesicle formation, and membrane morphogenesis. In mammals, four reticulon genes have been identified, RTN1 through RTN4.

Retrograde trafficking: Trafficking in the direction opposite to that of anterograde trafficking. Its function is often, but not always, to recycle machinery proteins from distal to proximal compartments of the secretory pathway.

Unfolded-protein response: A response in the endoplasmic reticulum to the accumulation of unfolded proteins in its lumen through the activation of an adaptive response, which is aimed at coping with the increased load in the endoplasmic reticulum and activates intracellular signal transduction pathways. These induce the remodeling of the secretory apparatus and have a major effect on signaling pathways, controlling cell survival and apoptosis. (For additional details, see the Supplementary Appendix, available with the full text of this article at NEJM.org.)

delivered to several destinations. These destinations include the plasma membrane again; the recycling endosomes, another important sorting station from which cargo proteins can either return to the plasma membrane or move into the trans-Golgi network; and the late endosomes (the last endocytic station), from which some cargoes move to the Golgi complex and others are transferred to lysosomes for degradation[29] (Fig. 1). Another organelle that can fuse directly with lysosomes is the autophagosome. Autophagy is a process by which damaged cytosolic and organellar components are enveloped in specialized membranes and targeted for lysosomal degradation.[30]

Thus, a conspicuous feature of the mammalian transport apparatus is its great complexity. There are more transport strategies, types of vesicles, and trafficking pathways than was expected until only a few years ago. Also, each anterograde trafficking step is counterbalanced by one or more recycling steps, and most of the various endocytic stations appear to be interconnected bidirectionally.[31] Moreover, certain specialized cells host uniquely differentiated organelles (e.g., secretory granules in endocrine and exocrine cells, melanosomes in melanocytes, lytic granules in immune cells, and dense granules in platelets), and at least in some cells (and potentially in all) there are unconventional secretion pathways through which a number of soluble cytosolic proteins can be transported directly to the extracellular space and some transmembrane proteins can be transported to the cell surface without passing through the Golgi complex[32] (Fig. 1).

A consequence of this multiplicity is a remarkable degree of redundancy and functional plasticity of the transport systems. This redundancy can partially compensate for certain genetic defects, and it can do so more efficiently in some cells than in others, depending on cell-specific requirements, which results in the selective vulnerability of certain tissues.

Another important issue is how the overall trafficking system maintains its homeostasis in the face of the rapid membrane fluxes that constantly change the size and composition of the transport organelles, or compartments. Among several possible mechanisms, one that has been recently explored relies on signaling circuits located on the trafficking organelles themselves that sense the passage of traffic and rapidly react to restore the balance across the compartments.[33]

MENDELIAN DISEASES OF MEMBRANE TRAFFICKING

MECHANISTIC BASIS

During the past decade, the increasingly rapid discovery of genes that are linked to human diseases has revealed that several such genes are involved in membrane trafficking. Efforts are now being more specifically directed toward understanding how disease manifestations can be mechanistically explained through our basic knowledge of the trafficking machinery and toward exploiting this new knowledge of the molecular basis of genetic syndromes to obtain insights into the organization of the trafficking processes.

Mendelian diseases of membrane trafficking arise from mutations in genes that encode either cargo proteins or components of the biosynthetic and trafficking machinery. Among these genes, those that encode cargo proteins are more widely represented because they are more numerous and because many cargoes are tissue-specific and not essential for the survival of an embryo.[34] On the

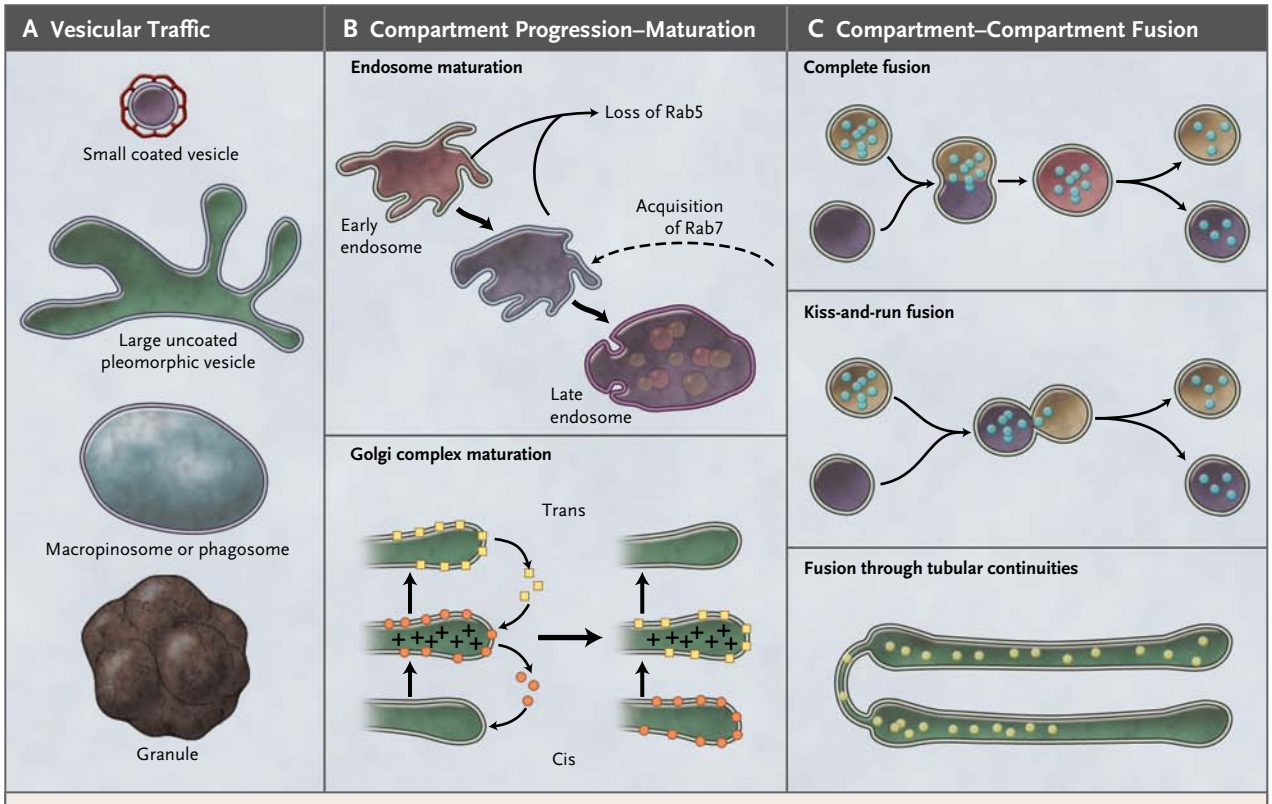


Figure 2. Transport Strategies in Membrane Trafficking.

Panel A shows vesicular trafficking, which remains central to our understanding of membrane transport. It is now clear that there are several types of vesicular carriers, including several types of small coated vesicles and large uncoated pleomorphic vesicles; large endocytic vesicles, such as macropinosomes and phagosomes; large, regulated, dense granules in specialized exocrine and endocrine cells; and synaptic vesicles in neurons (not shown). Panel B shows compartment progression and maturation, which applies to trafficking between the early and late endosomes,[13] transport through the Golgi complex,[12,14] and the maturation of phagosomes into phagolysosomes.[15] According to the maturation concept, traffic compartments change composition (i.e., mature) in lockstep with their progression along the transport pathway. For instance, early endosomes mature into late endosomes by losing a certain class of Rab (i.e., Rab5) and acquiring another class of Rab (i.e., Rab7). This process, called Rab conversion, is central to endosomal maturation. For the Golgi complex, at each maturation step, each cisterna loses its characteristic resident enzymes to the preceding cisterna (orange circles) and acquires enzymes from the more distal cisterna (yellow squares). The progression–maturation process begins when cargo molecules (black crosses) reach the cis-Golgi from the endoplasmic reticulum in carriers that coalesce to form a new cis cisterna. This new cisterna then matures by receiving medial and then trans-Golgi proteins from the older cisterna, while exporting cis and then medial Golgi proteins to the younger cisterna. Meanwhile, the cisterna progresses through the stack. In the final stage of maturation, the maturing cisterna becomes an element in the trans-Golgi network that breaks down into anterograde and retrograde transport carriers. Panel C shows direct compartment fusion, which applies to several transport steps. The transfer of cargo from late endosomes to lysosomes for degradation is based on the direct fusion of these two organelles. This fusion can be transient ("kiss and run") or complete (formation of a hybrid organelle).[16] In both cases, the cargo is transferred into the lysosomal lumen for degradation, and with complete fusion, the cargo transfer must be followed by resegregation of the two organelles.[16] A kiss-and-run process has also been described for rapid fusion of synaptic vesicles with the synaptic membrane[17] and for transient fusion between phagosomes and endosomes.[18] In fusion through tubular continuities, cargo transport is based on diffusion-mediated soluble-cargo flux through intercisternal continuities. Tubular continuities joining successive Golgi cisternae have been shown and might allow the diffusive passage of cargo molecules between cisternae (typically, soluble proteins) (light green circles).[19] Transport directionality is achieved through the arrival of cargo at the cis-Golgi and the departure of cargo from the trans-Golgi network. This mechanism, however, is still awaiting full functional verification.

other hand, mutations in genes that encode ubiquitous transport-machinery proteins are more likely to be lethal. Nevertheless, several of these mutations have been found to be involved in mendelian diseases, and more continue to be reported. Probably some of these mutations can, under favorable conditions, be partially compensated for by the plasticity of the transport systems. Table 1 provides a list of monogenic diseases that are caused by mutations in genes encoding compo-

Figure 3 (facing page). The Toolbox of Transport with Elementary Processes and Machinery.

Proteins or lipids that are present in the same organelle need to be sorted, or segregated, into different carriers, for shipping out to different destinations. Sorting is therefore usually associated with the budding of a carrier.[20] Panel A shows cargo sorting and membrane bending. There are different sorting mechanisms, including binding of a transmembrane cargo protein with a cytosolic coat component through specific sorting motifs in the cargo[20] (as in the case of the mannose-6-phosphate receptor). Soluble cargoes can bind to a transmembrane adapter, through which they can link to a cytosolic adapter. Sorting can also depend on cargo glycosylation (as in the case of cargoes binding to LMAN1 [ERGIC53]) or (at least partially) on the affinity of a cargo for membrane domains of a suitable lipid composition.[20] As for membrane bending, this can be driven by both lipids and proteins. Lipids can bend membranes in two ways: by generating transmembrane asymmetry and through the geometry of the lipid molecules themselves.[22] Proteins can bend membranes in two main ways: by inserting a hydrophobic portion into one leaflet of the membrane bilayer, thereby generating membrane asymmetry, or by mechanically forcing membranes to curve. The clathrin coat and the coat protein (COP) complexes I and II bend membranes into a round shape 50 to 100 nm in diameter (small round vesicles). Caveolin also generates vesicular shapes (caveolae). Other proteins can bend membranes into tubules; the dynamins are proteins involved in membrane fission that form helical rings around forming tubules,[22] and the dynamin-related family of the atlastins, like the reticulons and REEP1, acts at the endoplasmic reticulum.[22] All these proteins induce positive curvature (i.e., a convex cytosolic surface). However, bending can also occur inwardly. For instance, vesicles can bud into the lumen of late endosomes.[23] Finally, simple mechanical pulling of membranes by cytoskeleton-based motors can result in the formation of membrane tubules.[22] A budding carrier normally undergoes fission, as shown in Panel B, before translocating to the successive compartment. If fission is delayed, elongated carriers, and possibly tubular continuities across two compartments, are formed. Membrane fissioning can be mediated through several molecular mechanisms.[24] The best-characterized of these is driven by the large GTPase dynamin, which forms helical rings around the necks of forming vesicles and cleaves them mechanically by constricting or stretching its own helix.[24] After fission, membrane carriers move through the cytosol to reach their target compartment through vesicle translocation, as shown in Panel C. Vesicles bind to microtubule-based (kinesin and dynein) or actin-based (myosin) motors through a variety of adapters and are carried to their final destination by these motors.[10] There is a large variety of kinesins[11] and myosins,[25] each of which has a remarkable (although not absolute) degree of selectivity for different vesicular carriers or pathways. Panel D shows vesicle docking and tethering, which occur when a carrier that is approaching its acceptor compartment is first tethered to it through specialized proteins or protein complexes. Some coiled-coil proteins, called golgins, appear to have docking functions,[26] and a number of protein complexes have docking or regulatory roles at various stages of the trafficking pathway (e.g., the TRAPP [transport protein particle] complex has a role in trafficking between the endoplasmic reticulum and the Golgi complex, whereas the COG [conserved oligomeric Golgi] complex operates in enzyme trafficking within the Golgi complex).[26] Panel E shows vesicle fusion, which occurs when docking is followed by the fusion of the carrier membrane with that of the target organelle. Fusion is directly mediated by the specialized SNARE (soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor) proteins in a process that appears to bring together opposing membranes forcefully, through the pairing and fastening of specialized SNARE domains.[27]

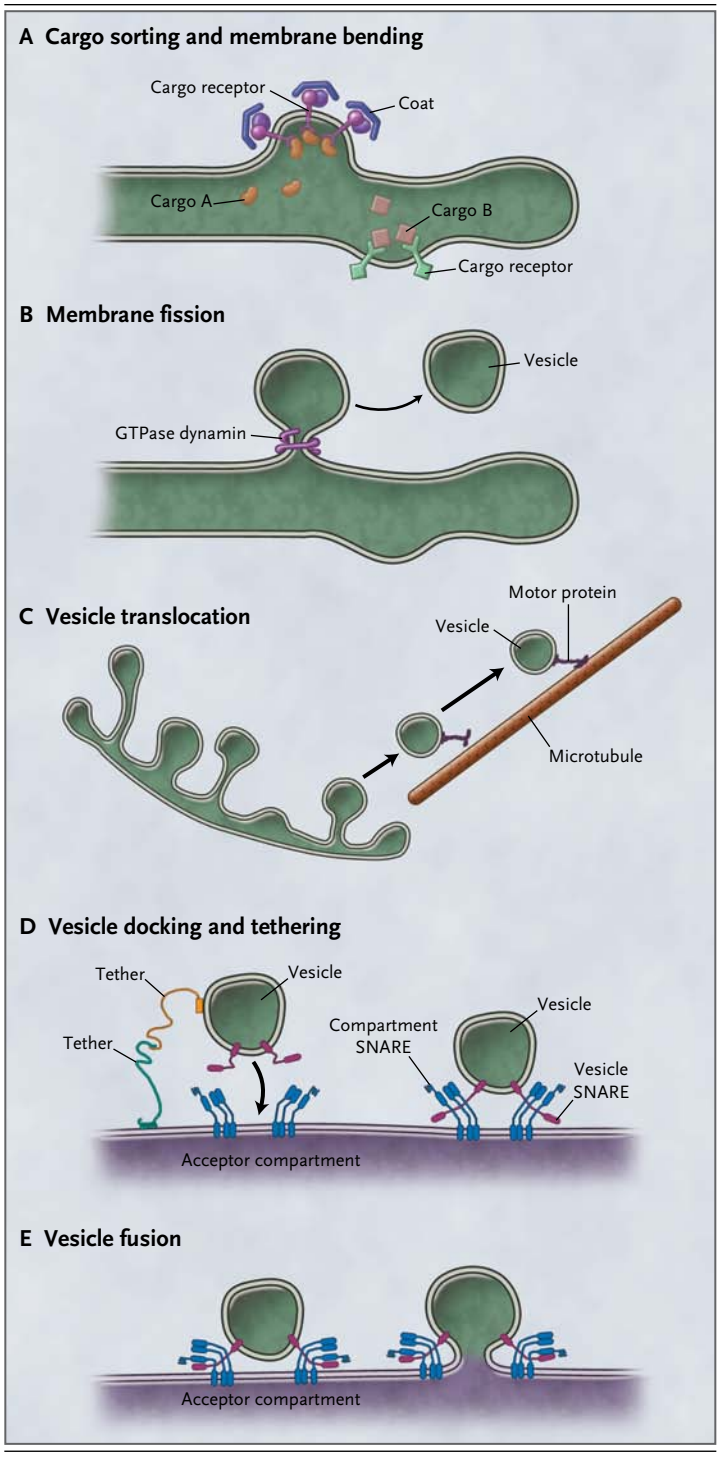
An interactive table showing genes associated with membrane-trafficking diseases is available at NEJM.org

nents of membrane-trafficking machinery (also see the interactive table, available at NEJM.org).

CARGO PROTEINS AND MACHINERY COMPONENTS

The distinction between cargo proteins and trafficking-machinery components can also be useful for the analysis of the mechanisms by which defective transport-related genes can lead to clinical manifestations. When a cargo protein is mutated, the pathogenetic chain of events that is set in motion can involve either a loss of function of the mutated cargo protein, because of truncation or early degradation (e.g., a channel protein, cystic fibrosis transmembrane conductance regulator [CFTR][35]) or a gain of function because of the accumulation of the mutated cargo protein in a given compartment, which would usually be the endoplasmic reticulum. This accumulation can activate the unfolded protein response.[7] If the load of misfolded cargo exceeds the capacity of the compensatory mechanisms activated through the unfolded-protein response, the response becomes maladaptive and triggers cell damage and death. This happens, for instance, in various disorders of myelinating cells, in which mutations in genes encoding the abundant peripheral myelin protein zero are responsible for a dominant form of Charcot–Marie–Tooth disease, called CMT1B, caused by the accumulation of the protein in the endoplasmic reticulum, activation of the unfolded-protein response, and toxicity in Schwann cells.[36]

For mutations in the machinery proteins, a central question is how defects in conserved ubiquitous housekeeping components can give rise to manifestations that are often specific to an organ or a tissue. In a few instances, the answer is that the defective genes are predominantly expressed as specific isoforms in the affected tis-



sues (as is the case in muscle dystrophies linked to defects in caveolin 3, the muscle-specific isoform of caveolin[37]). In many other cases, however, the reason for this selective tissue vulnerability appears to lie in the high demand for the defective genes in the tissues that then become damaged.

There appear to be two general explanations for this tissue specificity. The first is the presence of special tissue-specific cargoes, which might require high levels and full function of a particular trafficking component to be correctly transported. This occurs, for instance, in cells such as osteocytes or chondrocytes and intestinal cells, which secrete oversized cargoes. These cargoes include procollagen type I or II (rigid protofibrils measuring 300 nm in length) for osteocytes or chondrocytes and chylomicrons (particles measuring up to 1 μm in diameter) for intestinal cells. Here, mutations in the ubiquitous COPII component Sec23a or in the transport protein particle (TRAPP) complex subunit TRAPPC2 (which is involved in trafficking between the endoplasmic reticulum and the Golgi complex) can selectively affect osteocytes and chondrocytes, resulting in craniolenticulo-sutural dysplasia[38] and spondyloepiphyseal dysplasia tarda,[39] respectively. Along the same lines, mutations in the Sar1B GTPase that controls the COPII cycle can affect the secretion of chylomicrons in enterocytes and cause Anderson's disease (also called chylomicron retention disease).[40] Presumably, the same molecular defects can be compensated for in other cells and tissues by redundant mechanisms that can handle regular, but not special, cargo types.

Another reason for the tissue specificity of symptoms relates to a requirement for very efficient trafficking in tissues that require high transport rates for their function. Here, a defect without consequence for other cells might result in functional collapse, as can be seen in a number of cases: for cells that transport very large amounts of cargo at some stage of their life cycle, such as Schwann cells during myelination, which can selectively express genetic defects of ubiquitous trafficking components, such as MTMR2, MTMR13, FIG4, and SH3TC2, resulting in the demyelinating forms of Charcot–Marie–Tooth disease (CMT4) (Table 1, and interactive table). Also included are cells that require very high rates of internalization and recycling of plasma-membrane components, such as proximal tubular cells in the kidney, which must reabsorb essential components from the ultrafiltrate and which suffer from genetic defects of components of the endosomal system (as in many inherited forms of renal Fanconi's syndrome, including Lowe's syndrome), and cells that require very efficient long-range transport and communication, such as motor neurons,

Table 1. Genes Associated with Membrane-Trafficking Diseases, According to Their Underlying Role in Functionally Related Processes.*

Location and Gene	Disease	MIM Number	Function
Endoplasmic reticulum			
SAR1B	Chylomicron retention disease	246700	GTPase
SEC23A	Cranio-lenticulo-sutural dysplasia	607812	Coat
TRAPPC2	Spondyloepiphyseal dysplasia tarda	313400	Tethering
SPG4	Spastic paraplegia type 4	182601	Microtubule regulator
SPG31	Spastic paraplegia	610250	Morphogenesis of the endoplasmic reticulum
ATL1	Spastic paraplegia	182600	Morphogenesis of the endoplasmic reticulum
Endoplasmic reticulum–Golgi intermediate compartment and Golgi complex			
LMAN1	Combined factor V and VIII deficiency	227300	Cargo receptor
MCFD2	Combined factor V and VIII deficiency	227300	Cargo receptor
COG1	Congenital disorder of glycosylation type IIg	611209	Tethering
COG7	Congenital disorder of glycosylation type IIe	608779	Tethering
COG8	Congenital disorder of glycosylation type IIh	611182	Tethering
SCYL1BP1	Gerodermia osteodysplastica	231070	GTPase activator
FGD1	Aarskog–Scott syndrome	305400	GTPase activator
TRIP11	Achondrogenesis type 1A	200600	Microtubule binding
TRAPPC2	Spondyloepiphyseal dysplasia tarda	313400	Tethering
Plasma membrane			
DNM2	Charcot–Marie–Tooth disease, dominant intermediate type B; myopathy, centronuclear myopathy	606482; 160150	Bending or fission
CAV1	Congenital generalized lipodystrophy	612526	Coat
DYSF	Muscular dystrophy	253601	Fusion
CAV3	Muscular dystrophy	607801	Coat
Endosomes or lysosomes			
KIF5A	Spastic paraplegia type 10	604187	Motor
SPG4	Spastic paraplegia type 4	182601	Microtubule regulator or cytokinesis
SPG8	Spastic paraplegia type 8	603563	Endosome morphogen
SPG6	Spastic paraplegia type 6	608145	Bone morphogenetic protein signaling
SPG11	Spastic paraplegia type 11	610844	Bone morphogenetic protein signaling
SPG15	Spastic paraplegia type 15	612012	Cytokinesis
SPG20	Spastic paraplegia type 20 (Troyer's syndrome)	275900	Signaling by bone morphogenetic protein receptor and epidermal growth factor receptor
SPG21	Spastic paraplegia type 21 (Mast's syndrome)	248900	Unknown
BIN1	Centronuclear myopathy	255200	Tubulating protein
MTMR14	Centronuclear myopathy	160150	Phosphoinositide phosphatase
MTM1	X-linked myotubular myopathy	310400	Phosphoinositide phosphatase
AP3B1	Hermansky–Pudlak syndrome type 2	608233	Coat adapter
BLOC1S3	Hermansky–Pudlak syndrome type 8	203300	Lysosome biogenesis
DTNBP1	Hermansky–Pudlak syndrome type 7	203300	Lysosome biogenesis
MYO5A	Griscelli's syndrome type 1	214450	Motor

Table 1. (Continued.)

Location and Gene	Disease	MIM Number	Function
RAB27A	Griscelli's syndrome type 2	607624	GTPase
LYST	Chediak–Higashi syndrome	214500	Unknown
CHMP2B	Frontotemporal dementia	600795	Component of endosomal sorting complex required for transport
OCRL	Lowe's syndrome; Dent 2 disease	309000; 300555	Phosphoinositide phosphatase
ClCN5	Dent 1 disease	30009	Chloride channel
FGD4	Charcot–Marie–Tooth disease type 4H	609311	GTPase activator
FIG4	Charcot–Marie–Tooth disease type 4J; amyotrophic lateral sclerosis type 11	611228; 612577	Phosphoinositide phosphatase
MTMR13 or SBF2	Charcot–Marie–Tooth disease type 4B2	604563	Phosphoinositide phosphatase
MTMR2	Charcot–Marie–Tooth disease type 4B1	601382	Phosphoinositide phosphatase
RAB7A	Charcot–Marie–Tooth neuropathy type 2	600882	GTPase
SH3TC2	Charcot–Marie–Tooth disease type 4C	601596	Unknown
Synaptic vesicles and secretory granules			
KIF1B	Charcot–Marie–Tooth disease type 2A1	118210	Motor
STXBP1	Early infantile epileptic encephalopathy type 4	612164	Fusion
SYN1	Epilepsy	300491	Tethering or release of synaptic vesicles
SYN2	Susceptibility to schizophrenia	181500	Tethering or release of synaptic vesicles

* An interactive table is available at NEJM.org. MIM denotes Mendelian Inheritance in Man database.

which are particularly sensitive to defects in proteins involved in different steps of membrane trafficking (as is the case in hereditary spastic paraplegias).

LESSONS ON THE ROLE OF TRANSPORT PROTEINS

Our understanding of mendelian diseases can benefit from knowledge of the transport machinery. However, the reverse is also true: important lessons on the physiological functions of transport proteins can be derived from the study of disease genes. Classic examples are the combined deficiency of coagulation factors V and VIII and mucolipidosis II (also called inclusion-cell disease). Here, studies of the factors V and VIII combined deficiency helped to reveal the physiological role in transport of the protein ERGIC53 (also called lectin mannose-binding 1). After it was discovered that a mutation in this protein is the cause of factors V and VIII deficiency, a series of studies revealed that ERGIC53 functions as a chaperone in protein transport from the endoplasmic reticulum to the Golgi complex for a specific subgroup of secreted proteins that includes these two coagulation factors.[41] As for mucolipidosis II, Hickman and Neufeld observed in 1972 that lysosomal enzymes from patients with inclusion-cell disease "failed to reach their lysosomal destination."[42] Subsequent studies indicated that this disorder is caused by a defect in the Golgi enzyme that phosphorylates a specific mannose on lysosomal hydrolases. These observations helped in gaining an understanding of the key role of the mannose-6-phosphate receptor in the transport of these hydrolases from the Golgi complex to the lysosomes.[43]

Other, more recent examples of this type of molecular lesson involve entire groups of mendelian disorders that share overlapping clinical phenotypes, even though they arise from mutations in different genes. These syndromes have highlighted the existence of complex molecular networks or pathways that include distinct but functionally converging genes. A paradigmatic example has come from a genetically heterogeneous group of inherited neurologic disorders that are characterized by progressive spasticity and weakness of the lower limbs. These disorders, which are caused by corticospinal motor neuron axonopathy, are the hereditary spastic paraplegias.[44] They have

autosomal dominant, recessive, and X-linked inheritance. To date, 20 genes have been identified, half of which are involved in membrane trafficking along the exocytic and endocytic pathways (Table 1, and Fig. 1 in the Supplementary Appendix). The remainder are involved in mitochondrial functions, myelination, lipid metabolism, and DNA repair.[22]

More than 50% of patients with hereditary spastic paraplegia carry mutations in one of three genes: spastin (*SPG4*), receptor-expression-enhancing protein 1 (*SPG31* or *REEP1*), or atlastin-1 (*SPG3A*). Spastin encodes an ATPase with a microtubule-severing activity that has different splice variants with different subcellular localizations, including the endosomes and the endoplasmic reticulum. Notably, spastin interacts with the other hereditary spastic paraplegia protein, REEP1. REEP proteins, and the structurally related reticulon proteins, have a major morphogenetic role at the endoplasmic reticulum[45] because of a conserved domain of approximately 200 amino acids with two hydrophobic segments that form a hairpin in the membrane and have membrane-bending properties. Through this domain and its ability to oligomerize, the REEP and reticulon proteins can shape membranes of the endoplasmic reticulum into tubules.[45] Intriguingly, spastin also interacts with the third major hereditary spastic paraplegia protein, atlastin. These collective observations led to the hypothesis that atlastin itself might have a role in the morphogenesis of the endoplasmic reticulum. This disease-inspired hypothesis turned out to be correct and revealed that atlastin is involved in the generation of the tubular endoplasmic-reticulum network, since it mediates homotypic fusion of tubules in the endoplasmic reticulum[45,46] (Fig. 1 in the Supplementary Appendix). Finally, in a further tightening of the relationships among atlastin-1, spastin, and REEP1, these three proteins have recently been reported to interact with one another.[47]

This emerging scenario supports a convergent mechanism of disease in the many forms of hereditary spastic paraplegia that involve a defect in the formation of the endoplasmic reticulum tubular network. This might be particularly detrimental for long spinal neurons, since the endoplasmic reticulum is a conduit for many important small molecules with signaling or structural roles (e.g., calcium and lipids). Thus, the pervasiveness and continuity of the endoplasmic-reticulum network might well be essential in these extremely elongated cells, whereas such a network may be at least partially dispensable in smaller cells.

As in such examples, other cases can be identified in which information that is gathered from genetic diseases might reasonably lead to the discovery of converging molecular pathways in the near future.[48] One such case is inherited renal Fanconi's syndrome, a common clinical manifestation of a heterogeneous group of genetic disorders that are characterized by dysfunction of renal proximal tubular cells. These cells reabsorb more than 90% of nutrients, vitamins, and low-molecular-weight proteins present in the ultrafiltrate. This reabsorption of nutrients and proteins relies on efficient endocytic recycling of the multiligand receptor megalin, which captures its ligands in the ultrafiltrate, internalizes them through clathrin-dependent endocytosis, delivers them to the endolysosomes, and then recycles back to the apical surface of the cell for another round of transport.[49] The endocytic system of these cells is subjected to a very heavy burden, and a drop in its efficiency can cause low-molecular-weight proteinuria, one of the hallmarks of renal Fanconi's syndrome. Such a decline in efficiency might arise from defects in this endocytic receptor, megalin; in its associated receptor, cubilin; or in the machinery associated with their endocytosis and recycling.[48] For instance, impaired trafficking of megalin has been suggested to occur in Dent's disease, a proximal renal tubulopathy characterized by low-molecular-weight proteinuria, nephrocalcinosis, and hypercalciuria. This disease is caused by mutations in *CLCN5*, which encodes the renal chloride–proton antiporter,[50] which in turn controls the acidification and recycling activity of endosomal compartments. Moreover, it has been shown that some forms of Dent's disease (Dent 2) appear to also derive from mutations in *OCRL1*, which encodes an endosome-associated phosphatidylinositol 4,5-bisphosphate 5-phosphatase. *OCRL1* was originally discovered as the causative gene in Lowe's syndrome, a more serious disease that is characterized by proximal renal tubular dysfunction and by congenital cataracts and mental retardation.

The reasons that such different clinical outcomes (Dent 2 and Lowe's syndrome) can stem from mutations in *OCRL1* remain to be defined, with two likely hypotheses being that compensatory genes (e.g., *INPP5B*, encoding inositol polyphosphate 5-phosphatase) or alternative initiation

codons in *OCRL* downstream of nonsense mutations might be activated in a tissue-specific way in patients with Dent 2.[51] However, the overlap of the renal phenotypes caused by *OCRL* and *CLCN5* mutations allows the prediction that these two genes participate in a common molecular pathway that controls endosomal trafficking of the multiligand receptor megalin.[48]

SUMMARY

It is reasonable to hope that our basic knowledge of membrane trafficking will continue to provide insights into the pathogenesis of mendelian diseases and that studies of these diseases will continue to enhance our understanding of the membrane-trafficking system. In particular, it will be of great interest in this context to learn how to place the genes that are involved in trafficking-related diseases into coherent pathogenetic pathways.

Regrettably, the wealth of new insights into the molecular defects in membrane-trafficking disorders has not yet led to a proportionate availability of effective therapies. However, in the past few years, the potential of mendelian diseases to drive the process of drug development has been recognized.[52,53] An example in the field of membrane transport is cystic fibrosis. Effective modulators of the folding, trafficking, and activity of CFTR (the chloride channel that is mutated in cystic fibrosis[35]) have been found through high-throughput screening that was aimed at identifying pharmacologic treatments for this disease. Some of these modulators (e.g., VX-809) are now being tested in clinical trials.[54] In addition, interest in the pathways affected in mendelian disorders is being raised further by the recognition that efforts to develop drugs for their treatment might also prove useful in common diseases in which the same pathways might have a pathogenetic role, such as type 2 diabetes and Alzheimer's disease.[52,53]

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank Giovanni D'Angelo for many important discussions; Brunella Franco, Sandro Banfi, Daniela Corda, and Nicola Brunetti for their critical reading of the manuscript; and C.P. Berrie for editorial assistance.

REFERENCES

1. Gilchrist A, Au CE, Hiding J, et al. Quantitative proteomics analysis of the secretory pathway. Cell 2006;127:1265-81.
2. Mellman I, Warren G. The road taken: past and future foundations of membrane traffic. Cell 2000;100:99-112.
3. Schekman R. SEC mutants and the secretory apparatus. Nat Med 2002;8:1055-8.
4. Rothman JE. The machinery and principles of vesicle transport in the cell. Nat Med 2002;8:1059-62.
5. Ellgaard L, Helenius A. Quality control in the endoplasmic reticulum. Nat Rev Mol Cell Biol 2003;4:181-91.
6. Vembar SS, Brodsky JL. One step at a time: endoplasmic-reticulum-associated degradation. Nat Rev Mol Cell Biol 2008;9:944-57.
7. Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. Nat Rev Mol Cell Biol 2007;8:519-29.
8. Watson P, Stephens DJ. ER-to-Golgi transport: form and formation of vesicular and tubular carriers. Biochim Biophys Acta 2005;1744:304-15.
9. Béthune J, Wieland F, Moelleken J. COPI-mediated transport. J Membr Biol 2006;211:65-79.
10. Allan VJ, Schroer TA. Membrane motors. Curr Opin Cell Biol 1999;11:476-82.
11. Hirokawa N, Noda Y, Tanaka Y, Niwa S. Kinesin superfamily motor proteins and intracellular transport. Nat Rev Mol Cell Biol 2009;10:682-96.
12. Glick BS, Nakano A. Membrane traffic within the Golgi apparatus. Annu Rev Cell Dev Biol 2009;25:113-32.
13. Rink J, Ghigo E, Kalaidzidis Y, Zerial M. Rab conversion as a mechanism of progression from early to late endosomes. Cell 2005;122:735-49.
14. Bonfanti L, Mironov AA Jr, Martínez-Menárguez JA, et al. Procollagen traverses the Golgi stack without leaving the lumen of cisternae: evidence for cisternal maturation. Cell 1998;95:993-1003.
15. Desjardins M, Griffiths G. Phagocytosis: latex leads the way. Curr Opin Cell Biol 2003;15:498-503.
16. Luzio JP, Pryor PR, Bright NA. Lysosomes: fusion and function. Nat Rev Mol Cell Biol 2007;8:622-32.
17. Rizzoli SO, Jahn R. Kiss-and-run, collapse and 'readily retrievable' vesicles. Traffic 2007;8:1137-44.
18. Duclos S, Diez R, Garin J, et al. Rab5 regulates the kiss and run fusion between phagosomes and endosomes and the acquisition of phagosome leishmanicidal properties in RAW 264.7 macrophages. J Cell Sci 2000;113:3531-41.
19. Trucco A, Polishchuk RS, Martella O, et al. Secretory traffic triggers the formation of tubular continuities across Golgi sub-compartments. Nat Cell Biol 2004;6:1071-81.
20. De Matteis MA, Luini A. Exiting the Golgi complex. Nat Rev Mol Cell Biol 2008;9:273-84.
21. Braulke T, Bonifacino JS. Sorting of lysosomal proteins. Biochim Biophys Acta 2009;1793:605-14.
22. Blackstone C, O'Kane CJ, Reid E. Hereditary spastic paraplegias: membrane traffic and the motor pathway. Nat Rev Neurosci 2011;12:31-42. [Erratum, Nat Rev Neurosci 2011;12:118.]
23. Hanson PI, Shim S, Merrill SA. Cell biology of the ESCRT machinery. Curr Opin Cell Biol 2009;21:568-74.
24. Lenz M, Morlot S, Roux A. Mechanical requirements for membrane fission: common facts from various examples. FEBS Lett 2009;583:3839-46.
25. Loubéry S, Coudrier E. Myosins in the secretory pathway: tethers or transporters? Cell Mol Life Sci 2008;65:2790-800.
26. Lupashin V, Sztul E. Golgi tethering factors. Biochim Biophys Acta 2005;1744:325-39.
27. Südhof TC, Rothman JE. Membrane fusion: grappling with SNARE and SM proteins. Science 2009;323:474-7.
28. Doherty GJ, McMahon HT. Mechanisms of endocytosis. Annu Rev Biochem 2009;78:857-902.
29. Pryor PR, Luzio JP. Delivery of endocytosed membrane proteins to the lysosome. Biochim Biophys Acta 2009;1793:615-24.
30. Mizushima N, Levine B, Cuervo AM, Klionsky DJ. Autophagy fights disease through cellular self-digestion. Nature 2008;451:1069-75.
31. Russell MR, Nickerson DP, Odorizzi G. Molecular mechanisms of late endosome morphology, identity and sorting. Curr Opin Cell Biol 2006;18:422-8.

32. Nickel W, Rabouille C. Mechanisms of regulated unconventional protein secretion. Nat Rev Mol Cell Biol 2009;10:148-55. [Erratum, Nat Rev Mol Cell Biol 2009; 10:234.]
33. Pulvirenti T, Giannotta M, Capestrano M, et al. A traffic-activated Golgi-based signalling circuit coordinates the secretory pathway. Nat Cell Biol 2008;10:912-22.
34. Winter EE, Goodstadt L, Ponting CP. Elevated rates of protein secretion, evolution, and disease among tissue-specific genes. Genome Res 2004;14:54-61.
35. Rowe SM, Miller S, Sorscher EJ. Cystic fibrosis. N Engl J Med 2005;352:1992-2001.
36. Lin W, Popko B. Endoplasmic reticulum stress in disorders of myelinating cells. Nat Neurosci 2009;12:379-85.
37. Dowling JJ, Gibbs EM, Feldman EL. Membrane traffic and muscle: lessons from human disease. Traffic 2008;9:1035-43.
38. Boyadjiev SA, Fromme JC, Ben J, et al. Cranio-lenticulo-sutural dysplasia is caused by a SEC23A mutation leading to abnormal endoplasmic-reticulum-to-Golgi trafficking. Nat Genet 2006;38:1192-7.
39. Gedeon AK, Colley A, Jamieson R, et al. Identification of the gene (SEDL) causing X-linked spondyloepiphyseal dysplasia tarda. Nat Genet 1999;22:400-4.
40. Jones B, Jones EL, Bonney SA, et al. Mutations in a Sar1 GTPase of COPII vesicles are associated with lipid absorption disorders. Nat Genet 2003;34:29-31.
41. Nichols WC, Seligsohn U, Zivelin A, et al. Mutations in the ER-Golgi intermediate compartment protein ERGIC-53 cause combined deficiency of coagulation factors V and VIII. Cell 1998;93:61-70.
42. Hickman S, Neufeld EF. A hypothesis for I-cell disease: defective hydrolases that do not enter lysosomes. Biochem Biophys Res Commun 1972;49:992-9.
43. Kornfeld S. Trafficking of lysosomal enzymes in normal and disease states. J Clin Invest 1986;77:1-6.
44. Salinas S, Proukakis C, Crosby A, Warner TT. Hereditary spastic paraplegia: clinical features and pathogenetic mechanisms. Lancet Neurol 2008;7:1127-38.
45. Shibata Y, Hu J, Kozlov MM, Rapoport TA. Mechanisms shaping the membranes of cellular organelles. Annu Rev Cell Dev Biol 2009;25:329-54.
46. Orso G, Pendin D, Liu S, et al. Homotypic fusion of ER membranes requires the dynamin-like GTPase atlastin. Nature 2009;460:978-83. [Erratum, Nature 2010; 464:942.]
47. Park SH, Zhu PP, Parker RL, Blackstone C. Hereditary spastic paraplegia proteins REEP1, spastin, and atlastin-1 coordinate microtubule interactions with the tubular ER network. J Clin Invest 2010;120:1097-110.
48. Vicinanza M, D'Angelo G, Di Campli A, De Matteis MA. Function and dysfunction of the PI system in membrane trafficking. EMBO J 2008;27:2457-70.
49. Christensen EI, Verroust PJ, Nielsen R. Receptor-mediated endocytosis in renal proximal tubule. Pflugers Arch 2009;458: 1039-48.
50. Picollo A, Pusch M. Chloride/proton antiporter activity of mammalian CLC proteins ClC-4 and ClC-5. Nature 2005;436: 420-3.
51. Hichri H, Rendu J, Monnier N, et al. From Lowe syndrome to Dent disease: correlations between mutations of the OCRL1 gene and clinical and biochemical phenotypes. Hum Mutat 2011;32:379-88.
52. Brinkman RR, Dubé MP, Rouleau GA, Orr AC, Samuels ME. Human monogenic disorders — a source of novel drug targets. Nat Rev Genet 2006;7:249-60.
53. Fishman MC, Porter JA. A new grammar for drug discovery. Nature 2005;437: 491-3.
54. Jones AM, Helm JM. Emerging treatments in cystic fibrosis. Drugs 2009;69: 1903-10.