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Golgi Glycosylation and Human Inherited Diseases

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The Golgi factory receives custom glycosylates and dispatches its cargo to the correct cellular locations. The process requires importing donor substrates, moving the cargo, and recycling machinery. Correctly glycosylated cargo reflects the Golgi's quality and efficiency. Genetic disorders in the specific equipment (enzymes), donors (nucleotide sugar transporters), or equipment recycling/reorganization components (COG, SEC, golgins) can all affect glycosylation. Dozens of human glycosylation disorders fit these categories. Many other genes, with or without familiar names, well-annotated pedigrees, or likely homologies will join the ranks of glycosylation disorders. Their broad and unpredictable case-by-case phenotypes cross the traditional medical specialty boundaries. The gene functions in patients may be elusive, but their common feature may include altered glycosylation that provide clues to Golgi function. This article focuses on a group of human disorders that affect protein or lipid glycosylation. Readers may find it useful to generalize some of these patient-based, translational observations to their own research.

Cargo sorting and glycosylation are the major jobs of the Golgi apparatus. Because 1%–2% of the translated genome affects glycan (sugar chain) biosynthesis and/or binding, it is not surprising that humans have mutations in genes involved in glycan synthesis covering known pathways (Eklund and Freeze 2006). Here we focus on glycosylation disorders mostly discovered within the last 10–12 years (Lowe 2005; Freeze 2006; Jaeken and Matthijs 2007; Clement et al. 2008; Coman et al. 2008; Foulquier 2009; Freeze and Schachter 2009; Guillard et al. 2009). Many disorders that result from biosynthetic defects in the endoplasmic reticulum (ER) also require mention, but the focus remains on those that affect Golgi composition, structure, and homeostasis. The glycan biosynthetic pathways are described briefly here; other articles cover them in more detail.

Human glycosylation disorders assume many guises and their phenotypic expression in model systems and humans is not easy to predict based on cell biology alone; they often assault multiple organ systems. Recent meetings identified areas of consensus and controversy in the Golgi field (Donaldson and McPherson 2009; Emr et al. 2009). Add an assurance that many Golgi-related genetic disorders will be identified in the near future. Discoveries will confirm, extend, and modify our current

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concepts of Golgi function with new hypotheses, controversies, and, in time, consensus. An understanding of glycosylation will benefit basic scientists in many biological disciplines, but more importantly for patients, it may suggest disease markers and potential therapies.

OVERVIEW OF GLYCOSYLATION PATHWAYS

There are seven major ER-Golgi glycan biosynthetic pathways each defined by the nature of the sugar-protein or sugar-lipid bond. Human disorders occur in each of them. By far, the majority is in the N-glycosylation pathway (GlcNAc-Asn), especially those defects found in the ER. Protein O-glycosylation is more diverse. Ser/Thr residues are linked to glycans through N-acetylgalactosamine, xylose, mannose, or fucose (GalNAc, Xyl, Man, and Fuc, respectively). Each of these has pathway-specific glycosyltransferases that extend the chains. In many cases, terminal sugars are added by more promiscuous transferases that service different pathways (Stanley and Cummings 2009). Glycosphingolipids link glucose (Glc) to membrane-embedded ceramide and the glycan chain is then extended using pathway specific enzymes. Glycophosphatidylinositol (GPI) anchors are initially made in the ER transferred to protein and remodeled in the Golgi. Both glycosphingolipids and GPI anchors are enriched in detergent-insoluble, cholesterol-containing lipid rafts initially assembled in the Golgi and later found on the plasma membrane (Kinoshita et al. 2008; Fujita et al. 2009; Westerlund and Slotte 2009). Recently, defects have been identified in glycosphingolipid glycosylation (SIAT9-CDG, Amish infantile epilepsy) and two defects in glycosylphosphatidylinositol anchor biosynthesis (*PIGM*-CDG, and *PIGV*-CDG, glycosylphosphatidylinositol deficiency). A common feature of all Golgi glycosylation pathways is that nucleotide sugar donors must be transported into the Golgi using a series of transporters with different sugar preferences. Not surprisingly, defects in these carriers can affect multiple glycosylation pathways. Table 1 summarizes the ER-Golgi pathway glycans and their general functions.

ER LOCATION

Monosaccharide substrates are activated through a series of sugar specific pathways involving kinases, epimerases, and mutases that eventually generate multiple high-energy nucleotide sugar donors (Freeze and Elbein 2009) that can be used directly when acceptor proteins, lipids, or glycans face the cytoplasm. When they face the Golgi lumen, the activated donors require protein-mediated transport into the Golgi.

N-linked glycosylation begins in the ER by a step-wise assembly of a 14-sugar glycan $Glc_3Man_9GlcNAc_2$ on a PP-dolichol precursor. Initially, the half-completed precursor ($Man_5GlcNAc_2$-PP-dolichol) faces the cytoplasm, but it flips to the luminal side using a flippase. The glycan is completed therein using Dol-P-Man and Dol-P-Glc before oligosaccharyl transferase directs en bloc transfer of the completed glycan to the polypeptide chain emerging from the ribosome into the ER lumen. The protein-bound glycan is then trimmed. Two outermost glucose residues are removed

Table 1. Classification of the major glycan–protein linkages

Glycan type	Linkage	Typical proteins	Cell type
N-linked	GlcNAc-β-Asn	Cell surface receptors and secreted proteins	All cell types
O-linked	GalNAc-α-Ser/Thr	Secreted and cell surface mucins	Cell surface, gastrointestinal and reproductive tracts
O-linked	Man-α-Ser/Thr	α-Dystroglycan	Muscle tissue, nerve cells
O-linked	Xyl-β-Ser	Chondroitin/dermatan sulfate & heparan sulfate/heparin	Extracellular matrix, cartilage

quickly and the third is used as a quality control assurance check. Following its permanent removal, a single Man unit is cleaved clearing the protein for movement to the Golgi. This brief description harbors 18 distinct human glycosylation disorders even prior to the traffic reaching the Golgi (Freeze 2006; Jaeken and Matthijs 2007; Haeuptle and Hennet 2009).

Specific chaperones in the ER help escort proteins into the Golgi and genetic disorders have been found in a few of these as well. One, called COSMC, escorts an O-GalNAc pathway-specific β-galactosyltransferase to its site of action in the Golgi (Ju et al. 2008). A combined loss of coagulation factors V and VIII results from mutations in a transmembrane mannose-binding lectin in the ER-Golgi intermediate compartment (ERGIC-53), and multiple coagulation factor deficiency 2 (MCFD2) its soluble calcium-binding protein partner (Spreafico and Peyvandi 2009; Nishio et al. 2010).

O-fucosylation of selected proteins that carry tandem EGF modules such as Notch or others with thrombospondin type I repeats occurs in the ER, and may have both chaperone and glycan initiating roles (Leonhard-Melief and Haltiwanger 2010; Takeuchi and Haltiwanger 2010). The different protein modules select which of two fucosyltransferases to use and which glycan extensions occur in the Golgi.

Glycosphingolipid synthesis begins in the ER with the synthesis of GlcβCer and all subsequent extensions occur in the Golgi. Mannose-rich GPI-anchors are also constructed in the ER where they are transferred to proteins. Their lipid remodeling may be a signal for their export to the Golgi (Bonnon et al. 2010; Rivier et al. 2010).

GENETIC DISORDERS IN THE MACHINERY

We define the glycosylation machinery as those proteins that participate in the assembly of glycans of one or more pathways. Like most other glycosylation disorders, these are nearly all autosomal recessive. The defective genes are divided by pathway and listed in Table 2. Selected ones are highlighted below.

N-LINKED PATHWAY

Glycan trimming and reconstruction are unique to the N-glycosylation pathway as discussed in Stanley (2011). Mutations in the first α-glucosidase (glucosidase I) prevent the removal of the first glucose, but a backup *cis*- to medial Golgi endo-α-mannosidase is up-regulated (Volker et al. 2002) and cleaves an intact tetrasaccharide from the glycoproteins. This allows further processing to occur, and in contrast to many other N-glycosylation disorders, the typical serum glycoprotein structures such as those seen on transferrin are not altered. In fact, the disorder was only identified from the accumulation of the tetrasaccharide (Glcα1,2Glcα1, 3Glcα1,3Manα1,2) in the urine. The enzyme is transported from ER to Golgi in COP II vesicles. (Torossi et al. 2010).

Given the multiple glycosyltransferases involved in N-glycan processing and branching, this portion of the pathway might be expected to show many defects. Surprisingly, only two are known. One (CDG-IIa) results from essentially total loss of β-1,2-N-acetylglucosaminyltransferase (MGAT2) function. This enzyme is required for formation of multiantennary glycans and further processing into complex N-glycans. The few patients with this N-glycan-specific deficiency have severe psychomotor and growth retardation along with distinct abnormal facial features and thoracic deformity (Schachter and Jaeken 1999). The other defect (CDG-IId) is caused by mutation in β1,4 galactosyltransferase-I. The single patient had severe neurologic disease, myopathy, and clotting defects (Hansske et al. 2002). A series of other known β1,4 galactosyltransferases with different substrate preferences were insufficient to overcome this lesion, pointing to this enzyme as the major one required for N-glycan processing. This transferase is also responsible for extending GlcNAcβ1,3Fuc glycans necessary for fringe modulation of Notch signaling, mentioned below (Chen et al. 2001). This example underscores that mutations in a single transferase can affect multiple glycosylation pathways.

A special case of a Golgi transferase deficiency is I-cell disease or Mucolipidosis II,

Table 2. Golgi-related glycosylation disorders

	Gene function	Gene	OMIM	Clinical presentation
N-glycan disorders				
MGAT2-CDG (CDG-IIa)[a]	N-acetylglucosaminyltransferase II	*MGAT2*	212066	Mental retardation, dysmorphism, seizures
I-cell disease	GlcNAc-1-P transferase	*GNPTA*	252500	Severe developmental abnormalities
Multiple pathway disorders				
SLC35C1-CDG (CDG-IIc)[a]	GDP-fucose transporter	*FUCT1*	266265	Severe psychomotor retardation, hypotonia, elevated peripheral neutrophils
B4GALT1-CDG (CDG-IId)[a]	β1,4-galactosyltransferase	*B4GALT1*	607091	Hypotonia, spontaneous hemorrhage, Dandy–Walker malformation
COG7-CDG (CDG-IIe)[a]	Conserved oligomeric Golgi subunit 7	*COG7*	608779	Early death, dysmorphism, hypotonia, seizures, hepatomegaly, recurrent infections, cardiac failure, excessive skin
SLC35A1-CDG (CDG-IIf)[a]	CMP-sialic acid transporter	*SLC35A1*	605634	Normal transferrin, thrombocytopaenia, abnormal platelet glycoproteins
COG1-CDG (CDG-IIh)[a]	Conserved oligomeric Golgi subunit 1	*COG1*	606973	Mild MR, hypotonia, growth retardation, progressive microcephaly, hepatosplenomegaly
COG8-CDG	Conserved oligomeric Golgi subunit 8	*COG8*	611182	MR, hypotonia, encephalopathy
COG5-CDG	Conserved oligomeric Golgi subunit 5	*COG5*	—	Moderate mental retardation with cerebellar atrophy, hypotonia
COG4-CDG	Conserved oligomeric Golgi subunit 4	*COG4*	—	Mild mental retardation, mild dysmorphia, epilepsy, recurrent respiratory infections, mild ataxia
COG6-CDG	Conserved oligomeric Golgi subunit 6	*COG6*	—	Early fatality, severe neurological impairment, seizures, vomiting, intracranial bleeding,
ATP6V0A2-CDG	Golgi pH regulator	*ATP6V0A2*	219200	Cutis laxa, wrinkly skin, connective tissue weakness, large fontanelle, variable mental retardation

Continued

Table 2. *Continued*

	Gene function	Gene	OMIM	Clinical presentation
Achondrogenesis type 1A	Golgi structure	*GMAP210*	200600	Soft skull bones, short ribs that fracture easily, extremely short limbs, and lack normal ossification in the spine and pelvis
Gerodermia Osteodysplastica	Golgi structure	*SCYL1BP1*	231070	Wrinkley lax skin, osteoporosis, variable growth retardation
Congenital dyserythropoietic anemia (CDA II)	ER-Golgi protein trafficking	*SEC23B*	224100	Anemia, jaundice, splenomegaly, gall bladder disease
Cranio-lenticulo-sutural dysplasia (CLSD)	ER-Golgi protein trafficking	*SEC23A*	607812	Facial dysmorphisms, sutural cataracts and skeletal defects
O-mannose disorders (dystroglycanopathy)				
Walker–Warburg syndrome	O-mannosyltransferase	*POMT1, POMT2*	268870	Death in infancy, severe muscle weakness, diminished psychomotor development, abnormal neuronal migration ocular abnormalities
Muscle–eye–brain disease (MEB)	O-mannosyl GlcNAc transferase	*POMGnT1*	253280	Severe muscle weakness, mental retardation, epilepsy, neuronal migration disorder, ocular abnormalities
Fukuyama-type congenital muscular dystrophy (FCMD)	Putative glycosyltransferase	*Fukutin*	253800	Severe proximal and axial weakness, mental retardation, epilepsy and abnormal neuronal migration
Congenital muscular dystrophy type 1C (MDC1C)	Fukutin-related protein, a putative glycosyltransferase	*FKRP*	606612	Hypotonia, impaired motor development with respiratory muscle weakness
Congenital muscular dystrophy type 1D (MDC1D)	Putative glycosyltransferase	*LARGE*	608840	Muscular dystrophy with severe mental retardation
Glycophosphatidyl inositol disorders				
Autosomal Recessive GPI Anchor Deficiency	1st mannosyltransferase in GPI biosynthesis	*PIGM*	610273	Venous thrombosis and seizures
Hyperphosphatasia mental retardation (HPMR) syndrome	2nd mannosyltransferase in GPI biosynthesis	*PIGV*	239300	Hyperphosphatasia, mental retardation, and distinct facial characteristics

Continued

Table 2. *Continued*

	Gene function	Gene	OMIM	Clinical presentation
Glycosphingolipid disorders				
Amish infantile epilepsy	Sia2,3Galβ1,4Glc-Cer synthase (GM3)	*SIAT9*	609056	Neurological decline with tonic-clonic seizures and arrested development
Glycosaminoglycan synthesis disorders (O-xylose)				
Multiple hereditary exostoses	Heparan sulfate copolymerase	*EXT1, EXT2*	133701	Bony outgrowths
Ehlers–Danlos syndrome progeroid form	Xylosylprotein β-1,4-galactosyltransferase	*B4GALT7*	604327 130070	Connective tissue abnormalities with loose skin, hypotonia and developmental delay
Diastrophic dysplasia achondrogenesis	Anion (sulfate) transporter	*DTDST*	600972 606718	Premature calcification, scoliosis, club foot
Spondylo-epimetaphyseal dysplasia	3′-phosphoadenosine-5′-phosphosulphate synthase (PAPS)	*ATPSK2*	603005	Abnormal skeletal development and linear growth
Spondylo-epimetaphyseal dysplasia (Omani type)	Chondroitin 6-O-sulfotransferase	*CHST3*	143095 608637	Normal intelligence, reduced adult height, progressive kyphoscoliosis, joint dislocations, cardiac involvement, mild brachydactyly, camptodactyly, and microdontia
Macular corneal dystrophy	GlcNAc-6-sulfotransferase	*CHST6*	605294	Progressive corneal opacity.
Adducted thumb-clubfoot syndrome	N-acetylgalactosamine 4-O-sulfotransferase 1	*CHST14*	601776	Congenital contractures of thumbs and feet with joint instability, facial clefting, coagulopathy, abnormal skin, heart, kidney, or intestinal defects
Schneckenbecken dysplasia	UDP-GlcA / UDP-GalNAc Golgi transporter	*SLC35D1*	610804	Severely shortened long bones with bowing of limb bones and unossified vertebral bodies
O-GalNAc disorders				
Familial tumoral calcinosis	GalNAc transferase	*GALNT3*	211900	Large calcium deposits in both skin and tissue
Tn syndrome	Chaperone of β1,3GalT	*COSMC*	230430	Hematological abnormalities including anemia, leucopenia, thrombocytopaenia

Continued

Table 2. *Continued*

	Gene function	Gene	OMIM	Clinical presentation
O-fucose disorders				
Peters plus syndrome	β-1,3 glucosyltransferase specific for O-fucose on thrombospondin type 1 repeats	*B3GALTL*	261540	Mental retardation with prenatal growth delay, short stature, brachymorphism, short limbs, and eye abnormalities

[a]Data adapted from Jaeken et al. 2009.

which can arguably be called the first N-glycosylation-specific disorder to be solved in 1981 (Reitman et al. 1981; Kollmann et al. 2010). It was categorized as a "lysosomal storage disorder" because cells accumulated inclusions of undegraded material, but in contrast to most such disorders with single enzyme deficiencies, the patients' cells lacked multiple lysosomal enzymes. Instead, patients' plasma or media from cultured cells accumulated multiple mislocalized, lysosomal enzymes suggesting a shared deficiency in localization. Although lysosomal enzymes share little sequence homology, they contain features that allow recognition by an oligomeric protein complex that transfers GlcNAc-1-P from UDP-GlcNAc to selected mannose units on high mannose type glycans on those enzymes (Kornfeld 1992). Subsequently a specific α-N-acetylglucosaminidase, an "uncovering enzyme," cleaves that phosphodiester bond to generate one or two terminal mannose-6-P residues, which are ligands for Man-6-P receptors in the Golgi. This recognition marker allows for the delivery of the enzymes to the lysosome and the pH-dependent dissociation of the receptor and its recycling to the Golgi. Two receptors with different binding specificities can recognize the phosphorylated enzymes and with some preference for their different protein ligands. I-cell disease results from mutations in the GlcNAc-1-P phosphotransferase, not the "uncovering enzyme" (Kollmann et al. 2010).

This is the best example of protein-specific glycosylation that relies on structural features of the protein acceptor to allow high-affinity binding to modify glycan chain. Even simple mannose derivatives can serve as an acceptor for GlcNAc-1-P transferase reaction using this enzyme, but with 1000-fold lower efficiency (Lang et al. 1985).

TRANSPORTERS OF ACTIVATED DONORS

The activated nucleotide sugars made in the cytoplasm (or nucleus for CMP-sialic acid) must be transported into the Golgi lumen to glycosylate proteins and lipids. A series of membrane embedded, non-energy-dependent transporters are antiporters that import the nucleoside diphosphates and return the spent nucleoside monophosphates back to the cytoplasm (Liu et al. 2010). The transporters are present from yeast to mammalian cells, and are mostly localized to the Golgi. They can be highly substrate specific or show selectivity for a limited series of substrates for multiple glycosylation pathways (Hiraoka et al. 2007; Caffaro et al. 2008).

Congenital disorder of glycosylation-IIc is caused by mutations in the GDP-Fucose transporter. Patients have severe retardation and chronic infections. Some N- and O-glycans were hypofucosylated, but loss of a fucosylated glycan, sialyl Lewis^X, results in high levels of circulating neutrophils even in the absence of infections (Marquardt et al. 1999; Yakubenia et al. 2008). This glycan is required for leukocyte rolling prior to their extravasation into the tissues. For some patients, supplementing their diet with fucose corrected this deficiency. It is likely that another fucose transporter exists,

but this has not been shown (Hellbusch et al. 2007). It is possible that another known transporter also uses GDP-fucose, but less efficiently (Liu et al. 2010).

A defect in the CMP-sialic acid transporter was reported in one patient who showed developmental delay and giant megakaryocytes (Martinez-Duncker et al. 2005).

Schneckenbecken dysplasia is caused by mutations in the dual substrate Golgi transporter for UDP-glucuronic acid and UDP-N-acetylgalactosamine that provides precursors for chondroitin sulfate synthesis (see below) (Hiraoka et al. 2007). Surprisingly, this protein appears to be localized in the ER. Loss-of-function mutations cause severe skeletal dysplasia with very short long bones (Hiraoka et al. 2007).

O-MANNOSE PATHWAY

For years, clinicians characterized mutations that cause Duchenne and Becker muscular dystrophies. They also identified a set of rare muscular dystrophies with variable clinical severity that often involved neurological abnormalities and eye defects, while other patients were neurologically normal (Jimenez-Mallebrera et al. 2005; Muntoni et al. 2008; Chandrasekharan and Martin 2010). This broad spectrum of disorders is caused by defects in a recently appreciated glycosylation pathway that is primarily focused on α-dystroglycan (αDG), a peripheral membrane component of the dystrophin–glycoprotein complex (DGC) located in muscle, nerve, heart, and brain. Relatively little work has been performed in studying the Golgi in muscular dystrophies, but see review by Percival and Froehner (2007).

The severe muscular dystrophies including muscle–eye–brain (MEB) disease, Fukuyama-type congenital muscular dystrophy (FCMD), Walker–Warburg syndrome, and limb–girdle muscular dystrophy, (Table 2). These mutations also appear to affect neuronal migration in the developing brain, thus accounting for the combined effects on muscle and brain development. The first sugar, Mannose-α-Ser/Thr, is added in the ER by POMT1/POMT2 complex and it is elongated in the Golgi by a β1,2 GlcNAc transferase (POMGNT1). Mutations in these genes decrease or eliminate enzyme activity. Mutations in fukutin (FKTN) and fukutin related protein (FKRP) also cause muscular dystrophy. These proteins have features and signatures of glycosyltransferases, but the specific reactions and acceptor substrates are unknown. Another protein called "Large" (because of its size) has two glycosyltransferase domains, but, again, the donor and acceptors are unknown (Barresi et al. 2004; Kanagawa et al. 2004). Besides the glycosyltransferase homology, a key tool in grouping these disorders is a set of monoclonal antibodies that recognize an undefined O-mannose-based glycan (Martin 2007). All of these disorders decrease the apparent molecular weight of αDG to the same extent and abolish antibody reactivity.

An important insight into the glycan structure and, therefore, genes and possible biosynthetic enzymes was that LARGE-dependent antibody-reactive glycans contain Man-6-P in a diester linkage to an unknown molecule. In the absence of LARGE-dependent modification of the glycan, Man-6-P exists as a monoester (Yoshida-Moriguchi et al. 2010). Why is this significant? Addition of Man-6-P to αDG does not use the lysosomal enzyme pathway that employs high-mannose type glycan acceptors. Here Mannose-O-Thr/Ser glycans are the acceptors. This glycan can be further extended with GalNAc or with Gal and Sia (Yoshida-Moriguchi et al. 2010). The latter can also be branched. O-mannose glycans account for one-third of all O-linked species in the brain (Yuen et al. 1997), but the majority is probably on other proteins because brain-specific ablation of αDG has little effect on the amount of these glycans. One study suggests that LARGE may act on the O-mannose, complex N-glycans, and mucin O-GalNAc glycans of αDG (Aguilan et al. 2009), so the story may be more complex.

O-XYL PATHWAY: GLYCOSAMINOGLYCANS

Xylβ-serine-linked glycans on selected proteins produce glycosaminoglycans (GAG) that share a common core tetrasaccharide

GlcAβ1,3Galβ1,3Galβ1,4Xylβ. These linkage glycans are elongated into either chondrotin- and dermatan-sulfate composed of alternating (GalNAcβ1,4GlcAβ1,3) disaccharides or into heparan sulfate and heparin with alternating (GlcNAcα1,4GlcAβ1,4). Both polymers are variably sulfated and glucuronic acid (GlcA) units are epimerized to iduronic acid (IdU). The polymerization and modification occurs exclusively in the Golgi. Disorders in these pathways often cause skeletal- and chondrodysplasias.

Substrate Limitation

Sulfate ions must be imported into the cell because sulfate is not salvaged from degraded glycans. Several allelic clinical chondrodysplasias (diastrophic dysplasia, achondrogenesis type IB, neonatal osseous dysplasia I, and autosomal recessive multiple epiphyseal dysplasia), result from defective sulfation because of mutations in the sulfate ion transporter, encoded by *DTDST* (also known as *SLC26A2*) (Dawson and Markovich 2005). In spondyloepimetaphyseal dysplasia (SEMD), mutations occur in gene is *ATPSK2*, encoding the 3′-phosphoadenosine 5′-phosphosulfate (PAPS) synthase (Sugahara and Schwartz 1979), the activated donor substrate for sulfation. A mouse model of this disease shows reduced limb length and axial skeletal size. In SEMD, there is progressive reduction in the size of the columnar and hypertrophic zones in the epiphyseal growth plates (ul Haque et al. 1998).

Disorders in Chain Initiation

Mutations in *B4GALT7* cause the progeria variant of Ehlers–Danlos syndrome. The gene product (β1,4-Galactosyltransferase) (Okajima et al. 1999) adds the first Gal residue to xylose to the linkage region of GAG chains (Lindahl 1966) and only a few patients have been described (Quentin et al. 1990; Okajima et al. 1999; Faiyaz-Ul-Haque et al. 2004; Gotte and Kresse 2005). A dermatan sulfate proteoglycan from one patient's fibroblasts contained only xylose (Quentin et al. 1990).

Disorders in Chain Elongation—Hereditary Multiple Exostosis

The most common and well-studied GAG-related disorder is hereditary multiple exostosis (HME), caused by mutations in the synthetic machinery used for heparan sulfate (HS). It is one of the few autosomal dominant diseases and has a prevalence of about 1:50,000 (Schmale et al. 1994). More specifically, HME is caused by missense or frameshift mutations in either gene *EXT1* or *EXT2* that encode HS polymerases (Zak et al. 2002). HME patients develop bony outgrowths at the growth plates of the long bones. Normally the growth plates contain well-ordered chondrocytes in various stages of development, embedded in a collagen-chondroitin sulfate containing matrix (Zak et al. 2002). In HME patients, the outgrowths are composed of disorganized chondrocytes that must be surgically removed. These patients are at higher risk to develop osteosarcomas (Schmale et al. 1994) and some are diagnosed on the autistic spectrum (Li et al. 2002).

EXT1 and *EXT2* appear to form a protein complex in the Golgi. Partial loss of one allele of either gene appears sufficient to cause MHE, which is unusual because most glycan biosynthetic enzymes are produced in excess.

Loss of HS synthesis, and the concurrent decrease in tissue HS, probably leads to an abnormal distribution of HS-binding growth factors. These include several members of the FGF family, and morphogens, such as hedgehog (Hh), decapentaplegic (Dpp), and wingless (Wg) (Esko and Selleck 2002). In *Drosophila*, loss of HS disrupts Hh, Wg, and Dpp pathways (Bornemann et al. 2004). Embryonic lethality and failure to gastrulate occur in mice that are null for either *Ext* gene (Lin et al. 2000; Stickens et al. 2005). However, *Ext2* heterozygous animals are viable. These develop a single visible exostosis on the ribs in about a third of the litters (Stickens et al. 2005). Hh signaling in these animals is normal because no difference was detected in the protein distribution based on immunohistochemistry, thus not explaining the phenotype. *Ext1* heterozygotes also develop

exostoses, but the penetrance is strain-dependent, also seen within different families who carry the same mutations, suggesting a profound modifier gene effect.

Macular corneal dystrophy is caused by a deficiency in a tissue-specific sulfotransferase (CHST6), corneal N-acetylglucosamine-6-sulfotransferase (GlcNAc6ST), which is responsible for the production of corneal keratan sulphate (Akama et al. 2000). Unsulfated keratan chains are poorly soluble and their eventual precipitation disrupts the collagen network, leading to thinning and loss of transparency of the corneal stroma.

Mutations in another sulfotransferase gene (*CHST14*) are responsible for the addition of 4-O-sulfate to GalNAc residues in dermatan sulfate (DS) (Dundar et al. 2009). Its loss causes facial dysmorphism, adducted thumbs, clubbed feet, joint instability, short stature, and coagulopathy. Several patients died of respiratory insufficiency. Patient fibroblasts were missing highly flexible DS chains, but had an overabundance of less flexible chondroitin sulfate (CS), which likely affected collagen bundle formation or maintenance. The first biosynthetic step of converting CS to DS is the epimerization of glucuronic acid in CS to iduronic acid, and it is easily reversible. The sulfotransferase appears to "lock in" the commitment toward DS synthesis, but without it, reverse epimerization regenerates CS (Miyake et al. 2010). This discovery stresses the importance of dermatan sulfate in human development and matrix maintenance.

O-FUCOSE DISORDERS

Mutations in the β1,3-glucosyltransferase, *B3GALTL*, result in the autosomal recessive disorder Peters plus syndrome that is characterized by developmental delay, short stature, craniofacial defects and most frequently by anterior eye-chamber abnormalities (Lesnik Oberstein et al. 2006). When the genetic defect for Peters plus syndrome was identified, the precise function for B3GALTL was unknown. Based on protein sequence homology the protein was hypothesized to be a galactosyltransferase, but shown experimentally to be a glucosyltransferase (Kozma et al. 2006; Sato et al. 2006). Later, Hess et. al. determined that B3GALTL was involved in the addition of a glucose to O-linked fucose to form glucose-β1,3-fucose disaccharide associated specifically with thrombospondin type-1 repeats (Hess et al. 2008).

Thrombospondin type-1 repeats (TSRs) are a class of protein motifs similar to epidermal growth factor-like (EGF) repeats and are comprised of a cysteine rich domain ~40–60 amino acids involved in several biological processes such as proper coagulation, cell migration, neurogenesis, and angiogenesis (Tucker 2004).

Although TSRs are structurally and functionally similar to EGF repeats, separate and distinct enzymes carry out the modification. Addition of fucose to an EGF repeats, as in the case of NOTCH, is performed solely by protein O-fucosyltransferase 1 (POFUT1). Whereas fucose modification of TSR is only performed by protein O-fucosyltransferase 2 (POFUT2) and further extended by B3GALTL to yield a unique disaccharide glucose-β1,3-fucose (Luo et al. 2006).

It is suggested that O-fucosylation of TSR-containing proteins regulates the proper folding of secreted proteins and dysfunction would lead to accumulation of misfolded proteins and diminished secretion (Ricketts et al. 2007). Analysis of several TSRs containing proteins from Peters plus patients revealed a complete lack of O-fucosylation, supporting Peters plus syndrome as a glycosylation disorder (Hess et al. 2008).

O-GALNAC DISORDERS

Familial tumoral calcinosis (FTC) is an autosomal recessive disorder characterized by painfully large calcium deposits, hyperphosphatemia, and debilitating secondary side affects such as recurrent infection. Genome wide marker analysis of two large unrelated families helped to identify the glycosyltransferase, *GALNT3*, as the causative gene mutated in FTC (Topaz et al. 2004).

GALNT3 belongs to a large family of UDP-GalNAc transferase localized to the *cis*-Golgi and catalyzes the transfer of N-acetylgalactosamine to a serine or threonine residue allowing for the initiation of mucin O-glycosylation.

Although there are several genes with high sequence homology, it appears GALNT3 possesses a unique acceptor substrate specificity not seen in other family members which can clearly be seen in its ability to glycosylate both fibronectin and HIV gp120 (Matsuura et al. 1988; Bennett et al. 1999).

Mutations in the O-glycosylated protein, Fibroblast growth factor 23 (FGF23), result in another form of FTC. Two separate groups identified cases in which *GALNT3* was normal, but potential O-glycosylated serine residues in FGF23 were mutated. They subsequently showed that mutagenesis of either serine residue dramatically impaired O-glycosylation and for the first time proved certain mutations in FGF23 resulted in FTC (Larsson et al. 2005; Bergwitz et al. 2009).

Another O-GalNAc deficiency is the rare autoimmune disease, Tn syndrome. It is caused by somatic mutations in the X-linked gene, *COSMC* and is characterized by various hematological abnormalities (Crew et al. 2008). COSMC is a chaperone for β1,3 galactosyltransferase and is necessary for the formation of the glyco-structure, T-antigen (Ju and Cummings 2002). T-antigen is an O-linked Galβ1,3GalNAc that acts as a precursor for most mucin O-glycans and can be further extended into several other structures. Mutations in COSMC result in the formation of a truncated structure known as Tn Antigen that is simply an O-linked linked GalNAc (Ju and Cummings 2005; Ju et al. 2008).

GLYCOSPHINGOLIPID DISORDERS

Autosomal recessive Amish infantile epilepsy is associated with developmental delay, seizures, and blindness. Linkage analysis of a large Amish family identified a nonsense mutation in *SIAT9* resulting in a stop codon and consequently truncated protein (Simpson et al. 2004). SIAT9 is a sialyltransferase that makes ganglioside GM3 (Siaα2,3Galβ1,4Glc-ceramide) from lactosylceraminde (Galβ1,4Glc-ceramide). Hence, it is not surprising that patients with the truncated SIAT9 showed accumulation of non-sialylated plasma glycosphingolipids, and of GM3 precursors and lacked downstream GM3-dependent products. Interestingly, GM3 synthase null mice do not duplicate the human disorder because of an alternative biosynthetic pathway (Yamashita et al. 2003).

GLYCOSYLPHOSPHATIDYLINOSITOL ANCHOR DISORDERS

Inherited GPI deficiency (IGD) is characterized by venous thrombosis in addition to seizures and is caused by a mutation in the mannosytransferase *PIGM* that is required for the addition of the first mannose to the core glycosylphosphatidylinositol (GPI) (Maeda et al. 2001). In two unrelated consanguineous families, Almeida and colleagues identified a shared promoter mutation in a key SP1 transcription factor binding site that dramatically reduced the expression of *PIGM* leading to a defect in GPI anchor synthesis (Almeida et al. 2006).

In a follow-up paper involving the same patients, Almeida and colleagues showed patient lymphoblast treated with sodium butyrate completely restored normal *PIGM* transcript level as well as cell surface GPI expression. More importantly, treating the patient with sodium phenylbutyrate, a histone deacetylase inhibitor, resulted in a dramatic improvement after only two weeks, as measured by a lack of seizure activity and improved motor skills (Almeida et al. 2007).

Mutations in *PIGV* that encode the second mannosyltransferase of GPI anchors cause hyperphosphatasia mental retardation syndrome (a.k.a. Mabry syndrome, OMIM 239300, Rasmussen 1975; Krawitz et al. 2010). Patients with these mutations have greatly increased plasma alkaline phosphatase, a GPI-anchored protein, along with unusual facial features, variable seizures, and muscular hypotonia (Horn et al. 2010). Surface expression of GPI-anchors themselves and anchored proteins were reduced in patients' leukocytes. PIGV-deficient CHO cells can be complemented with wild-type human allele, but not with the mutant.

Somatic mutations in *PIGA* limit the first step of GPI synthesis resulting in partial or

complete loss of GPI-linked membrane proteins and are the underlying cause of Paroxysmal nocturnal hemoglobinuria (PNH), an acquired hematopoietic disorder characterized by unusual blood cell populations and intravascular hemolysis (Takeda et al. 1993).

FACTORY ORGANIZATION, SHIPPING, AND RECEIVING

It is not surprising that mutations in the glycosylation hardware disrupt patients' glycosylation pathways. Some enzymes in glycosylation pathways form complexes in the Golgi (Opat et al. 2000; Pinhal et al. 2001; Sprong et al. 2003; Young 2004; Maccioni 2007; Hassinen et al. 2010), which presumably increases efficiency of substrate modification. However, mutations in genes that disrupt Golgi organization, structure, and trafficking of either cargo or resident proteins also cause disorders that affect glycosylation. Perhaps this should be expected because drugs that alter Golgi integrity and protein trafficking also alter glycosylation. Therefore, altered glycosylation may be a surrogate indicator of the functional importance of a Golgi-related protein. Many disease-causing mutations in specific proteins (Howell et al. 2006) cause protein misfolding, retention in the ER, and proteasomal degradation. Examples include, α1-antitrypsin deficiency, autosomal dominant polycystic kidney disease, cystic fibrosis, Charcot-Marie-Tooth disease, congenital sucrase-isomaltase deficiency, Fabry's disease, familial hypercholesterolemia, and osterogenesis imperfecta (Vogt et al. 2005; Howell et al. 2006). An unusually high proportion (1.4%) of such mutations in proteins traveling the ER/Golgi circuit actually create inappropriate N-glycosylation sites. Some of these are pathological. Their delivery to the membrane is normal (Vogt et al. 2005; Vogt et al. 2007), but the presence of the glycan actually impairs function. Alternatively, the mutations may prevent the assembly into multisubunit complexes such as in the case of fibrillin1 in Marfan's syndrome (Raghunath et al. 1995; Lonnqvist et al. 1996).

It is more challenging to find the appropriate cell/tissue or specific protein that shows the alteration and identifies a mechanism. Serum proteins (hepatocyte and plasma cell-derived) are convenient markers, but they may not always indicate defective glycosylation. Impairments may only appear in selected tissues where the "glycosylation demand" is high.

COGs AND GLYCOSYLATION

Conserved oligomeric Golgi (COG) complex is composed of eight individual subunits, COG1–8. Mutations are known for six subunits, making it the best known of the trafficking defects (Ungar 2009). The complex exists in two lobes or subcomplexes. Lobe A (COG1–4) and Lobe B (COG 5–8) are bridged by a COG1/COG8 heterodimer that links the different lobes. COG1 and COG2 were identified as the defective genes in *ldlB* and *ldlC* mutant CHO lines, respectively, that were defective in the surface localization of the LDL receptor. These lines had incomplete N- and O-linked glycans and abnormal Golgi architecture. The COG complex localizes to the *cis*- and medial Golgi and is found at the tips and along the rims of the cisternae and on vesicles, making it a likely tethering factor (Vasile et al. 2006). See Figure 1.

The COG complex is found from yeast to mammals and interacts with other trafficking proteins. For instance, COG3 coprecipitates with β-COPI and in yeast, COG2 interacts with γCOPI. When COG3 is depleted using siRNA knockdown, vesicles containing glycosylation enzymes, GPP130 (*cis*-Golgi) and the SNAREs GS15 and GS28 accumulate. COGs appear to be important for retrograde trafficking because COG depletion cannot move cell surface bound bacterial toxins (e.g., Shiga and AB) through the Golgi to the ER (Smith et al. 2009).

SM (SEC1/MUNC18) protein, Sly1, interacts directly with (COG4) which also interacts with Syntaxin5 using different binding sites. Blocking COG4-Sly1 interaction impairs pairing of SNAREs involved in intra-Golgi transport thereby markedly attenuating Golgi-to-ER retrograde transport (Laufman et al. 2009). The interaction between p115 and COG2 is essential for Golgi ribbon reformation after the disruption of the ribbon by p115

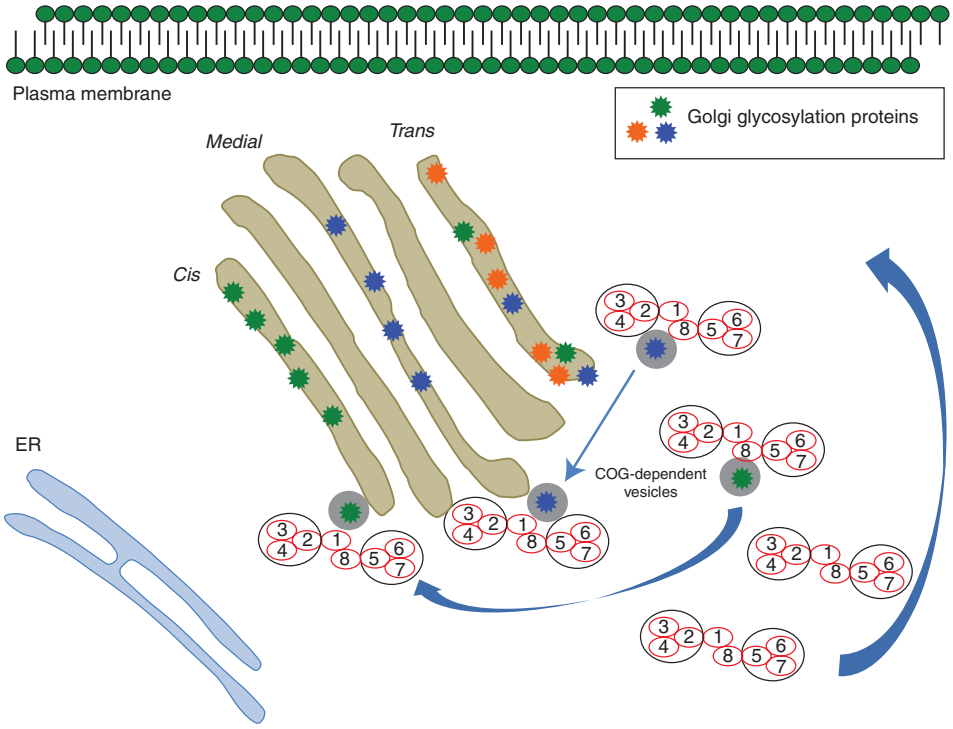

Figure 1. Role of the conserved oligomeric Golgi (COG) complex in Golgi localized retrograde transport. In focus is how the COG complex transports various mislocalized Golgi glycosylation proteins back to their proper destinations and then returns after redirecting the vesicles.

siRNA knockdown or brefeldin A treatment (Sohda et al. 2007).

In a broad screen of Rab binding factors mammalian COG6 bound to GTP-restricted Rab1, Rab6, and Rab41 and COG4 preferentially interacts with Rab30-GTP (Fukuda et al. 2008).

The first sib patients with a COG-based trafficking disorder showed defective N- and O-glycosylation caused by splice mutations in COG7 (Wu et al. 2004). The patients had perinatal asphyxia, feeding problems, growth retardation, hypotonia, enlarged cholestatic liver, epileptic seizures, cerebral atrophy, and severely shortened limbs with absence of long bone epipheses. Another curious feature was an accumulation of excessive, wrinkled, doughy-like skin. Both patients died of infections and cardiac insufficiency. Six additional COG7-deficient patients with this mutation showed similar severe phenotypes (Morava et al. 2007; Ng et al. 2007). One patient with a different splice site mutation had no dysmorphic features and prolonged survival (Zeevaert et al. 2009a). A COG1-deficient patient was identified with generalized hypotonia, but with normal strength, failure to thrive, small hands and feet, and short stature (Foulquier et al. 2006) Cerebellar and cerebral abnormalities were milder than in COG7 patients. The mutation eliminated the carboxy-terminal 80 amino acids. Two other COG1-deficient patients were found with costovertebral dysplasia in cerebrocostomandibular syndrome (CCMS) and in cerebrofaciothoracic dysplasia and spondylocostal dysostosis (Zeevaert et al. 2009b).

Two patients were identified with COG8 mutations. One with a truncating mutation that eliminated 76-carboxy-terminal amino acids had a normal neonatal and infancy, but later developed mental retardation and ataxia from cerebellar atrophy/hypoplasia (Foulquier

et al. 2007). The other patient had a splice site mutation and a deletion that truncated the protein (Kranz et al. 2007). This patient is severely retarded and has a near absence of muscle that resembles malnutrition, but no dysmorphic features. COG5-deficient patient had a splicing mutation that caused exon skipping and severe reduced expression of the COG5 protein, but with mild psychomotor retardation, delayed motor and language development (Paesold-Burda et al. 2009).

COG4 deficiency (Reynders et al. 2009; Richardson et al. 2009) in one patient with a missense mutation and small deletion caused only mild phenotype. Its loss decreased the expression or stability of the other Lobe A subunits, but stability of the complex was still seen in the cytoplasm at lower levels. Another patient with a COG4 defect had a fatal outcome (Ng et al. 2011). A patient has also been identified with lethal mutations in COG6 that caused neurologic disease, seizures, and intracranial bleeding (Lubbehusen et al. 2010).

Fibroblasts from all COG patients show a three to fourfold slower rate of brefeldin A–induced Golgi dissociation, while postwashout Golgi reassembly is normal. This is consistent with COG's role in retrograde transport from Golgi to ER. In all cases except COG4, expression of the normal allele in patients' cells normalizes BFA response (Foulquier 2009; Reynders et al. 2009).

The very broad spectrum of clinical presentations among COG patients may seem puzzling, and although it may be lumped into the "genetic background" explanation, other issues may cloud predictions or interpretations. As with most glycoyslation disorders, the mutated alleles are hypomorphic, not null. Also, patient fibroblasts are convenient, but probably not reflective of the pathology in other cells or tissues. This is especially important because COG mutations frequently involve splice sites that can show variable penetrance. Finally, understanding of COG cell biology is in an early stage, and it is clear that COGs function in intra-Golgi as well as Golgi to ER transport, but it is not known if all the components are required for each role.

Little is known about how the COG complex maintains proper glycosylation, homeostasis, and structural integrity of the Golgi. Recent analysis suggests that the amino-terminal portion of Cogs1–4 are required for assembly of a core complex, whereas the carboxy-terminal domains form "elongated legs" that interact with other glycosylation related proteins. Carboxy-terminal domain of COG1 is required for interaction with the COG 5–8 subunit complex (Lees et al. 2010). Another study shows that small interfering RNA knockdown of lobe A (Cogs1–4) subunits alter Golgi structure, whereas loss of Lobe B (Cogs 5–8) subunits decreased stability of both B4GALT1 and ST6GAL1 (Peanne et al. 2010) used for serum glycoprotein synthesis. COG patients show deficiencies in both galactosylation and sialylation.

Other genetic disorders will likely arise from defects in proteins that directly interact with the COG complex.

VACUOLAR ATPase

Autosomal recessive cutis laxa (ARCL) is associated with a progeroid appearance, lax and wrinkled skin, osteopenia, and mental retardation (Morava et al. 2008). One of the major diagnostic criteria is abnormal elastin fibers (Morava et al. 2009), but the genetic deficiencies of many cutis laxa patients were unknown. Mutations in *ATP6V0A2*, the a2 subunit of the vacuolar H^+-ATPase (V-ATPase) were shown to affect N- and O-glycosylation and alter Golgi trafficking as determined by BFA treatment (Guillard et al. 2009; Kornak et al. 2008). Cells from patients had distended Golgi cisternae with accumulation of abnormal lysosomes and multivesicular bodies. Tropoelastin (TE) accumulated in the Golgi and in large, abnormal intracellular and extracellular aggregates. siRNA knockdown of ATP6V0A2 showed similar phenotype. The delayed secretion and increased intracellular retention of TE in the Golgi and reduced extracellular deposition of mature elastin increased apoptosis of elastogenic cells (Hucthagowder et al. 2009). It is likely that the vacuolar ATPase maintains correct pH in lysosomes, synaptic vesicles,

endosomes, secretory granules, and the Golgi apparatus (Saroussi and Nelson 2009). Another candidate disease gene could be the Golgi pH regulator (*GPHR*) that shows a voltage-dependent anion-channel activity and alters glycosylation (Maeda et al. 2008).

GOLGINS

Golgins bind to the cytoplasmic side of *cis*- and *trans*-Golgi and can act as tethers using their coiled coil domains that project from the cisternae as long thin fibers (Ramirez and Lowe 2009; Sztul and Lupashin 2009; Goud and Gleeson 2010). They interact with small GTPases, and some bind to the cytoskeletal elements. Golgin GMAP210 recruits γ tubulin complexes to form Golgi ribbons and also interacts with Arf1 (Drin et al. 2008; Cardenas et al. 2009). GMAP210 binds to highly curved membranes and is thought to tether tubules or vesicles to the *cis*-Golgi (Drin et al. 2008). Overepression induces Golgi fragmentation and disturbs ER to Golgi trafficking (Ramirez and Lowe 2009). It is widely expressed and may be redundant because deficient mouse embryos have a normal Golgi. This protein anchors the cilia component of IFT20 to the Golgi and these cells from the mutants have shorter primary cilia, suggesting that it plays some role in the sorting of membrane components to the cilia (Follit et al. 2008). This conclusion matched the known data, but did not explain why GMAP210-deficient mice died soon after birth, apparently of heart and complex lung defects (Follit et al. 2009). The answer emerged from a forward genetic screen focused on determining the genes responsible for neonatal lethal skeletal defects in mice (Smits et al. 2010). The target gene *Trip11* encoded GMAP210 and the mouse phenotype resembled that of a human disorder achondrogenesis type Ia (Smits et al. 2010). Subsequent analysis of patients showed mutations in *TRIP11*. The phenotype could not have been predicted from the cell biology, especially because GMAP210 is widely expressed. Chondrocytes from mutant mice show impaired differentiation, distended ER, and altered glycosylation. Many of the large matrix proteins such as type II collagen and aggrecan were correctly localized, but perlecan, a large heparan sulfate proteoglycan accumulated in the ER. This argued that GMAP210 deficiency caused a selective protein trafficking defect in a tissue that carried a heavy demand for glycosylation of proteoglycans. Early death seemed to result from lung compression caused by an underdeveloped skeleton rather than a defect in the primary cilium assembly and localization. There is evidence that golgins can play a specific role in cargo protein selection (Drin et al. 2008; Ungar 2009). This example is important because it points out the difficulty of predicting which proteins, organ systems, or time frames will be affected by mutations in Golgi trafficking proteins.

Gerodermia osteodysplastica is a genetic disorder that causes premature wrinkly skin and osteoporosis, defining it as a progeroid disorder (Al-Gazali et al. 2001). Mapping studies localized the defect to *SCYL1BP1* loss-of-function mutations. The protein is highly expressed in those locations and it increases during osteoblast differentiation (Hennies et al. 2008). Immunofluorescence localized it to the Golgi, and yeast two-hybrid pull down showed it interacted with RAB6, in its GTP-activated state. The protein contained coiled coil domains, localized to the Golgi, and bound RAB6, identifying it as a golgin. These results reinforce the relation of the secretory pathway in selected tissues with age-related changes in connective tissues (Hennies et al. 2008). Additional patients had similar phenotypes and mutations in this gene, but transferrin glycosylation was normal (Al-Dosari and Alkuraya 2009). As seen with *GMAP210* mutations that cause achondrogenesis type Ia, the effects on glycosylation may be protein selective and tissue specific.

SEC PROTEINS

Another example showing unpredictable functional consequences is also seen in two disorders caused by mutations in the family of COPII coat protein complexes, SEC23A and SEC23B (Lang et al. 2006; Bianchi et al. 2009).

Mutations in *SEC23A* cause the cranio-lenticulo-sutural dysplasia syndrome and affect facial development (Boyadjiev et al. 2006). In zebrafish, positional cloning mapped the defect of a malformed craniofacial skeleton, kinked pectoral fins and a short body length to the homolog of *sec23b*. The mutant fish also showed accumulation of ECM components including type II collagen in the ER (Lang et al. 2006). Mice mutated in the transcription factor BBF2H7 that activates Sec23A transcription have a similar phenotype to GMAP210-deficient mice including abnormal chondrogenesis and accumulation of collagen II and cartilage oligomeric matrix protein in the ER. Introduction of Sec23A into chondrocytes normalized the secretion of matrix components (Stagg et al. 2008; Saito et al. 2009).

The mutant form of SEC23A poorly recruits the SEC13-SEC31 complex, inhibiting vesicle formation. Surprisingly, this effect is modulated by the SAR1 GTPase paralog used in the reaction, indicating distinct affinities of the two human SAR1 paralogs for the SEC13–SEC31 complex. Patient cells accumulate numerous tubular cargo-containing ER exit sites devoid of observable membrane coat, likely representing an intermediate step in COPII vesicle formation (Fromme et al. 2007).

In contrast, *SEC23B* mutations cause a completely different glycosylation disorder called congenital dyserythropoietic anemia or HEMPAS (Schwarz et al. 2009). These patients have poor erythropoiesis generating bi- and multinucleated erythroblasts in bone marrow, suggesting abnormal cytokinesis (Denecke and Marquardt 2009). In peripheral red blood cells, proteins and glycolipids are incompletely glycosylated (Fukuda 1990). This leads to a progressive splenomegaly, gallstones, and iron overload potentially with liver cirrhosis or cardiac failure. Knockdown of SEC23B via shRNA mimics the defective cytokinesis, and zebrafish morphants have abnormal enrythrocyte development (Bianchi et al. 2009; Schwarz et al. 2009). Surprisingly analysis of one family showed that heterozygous parents had detectable abnormalities in erythrocyte membrane glycans, but a healthy child was completely normal, thus suggesting the presence of a subclinical haploinsufficiency (Zdebska et al. 2002).

LIPID HOMEOSTASIS AND TRAFFICKING

Lipid and glycolipid trafficking also involve the Golgi together with other compartments in the cell (Lev 2006; Chandran and Machamer 2008; van Meer et al. 2008). Recent proposed explanations for the organization, dynamics, and flux of both proteins and lipids through the Golgi are based primarily on their physical properties, and sites of synthesis/depletion to construct membranes of different compositions, dimensions, and curvatures (Lippincott-Schwartz and Phair 2010). Lipid and protein sorting are interwoven and interdependent in this attractive, but unproven model (Emr et al. 2009; Gong et al. 2010). However, it provides a setting to discuss the possibility that altered glycosylation may be a useful marker or play a role in pathophysiology of the disorders. These include various disorders of cholesterol trafficking such as Niemann–Pick type C (Urano et al. 2008; Lloyd-Evans and Platt 2010). Defects in this protein of still unknown function also affect the esterification of dolichol (Turunen and Schedin-Weiss 2007) and glycosylation of ApoE in mouse models (Chua et al. 2010). Surprisingly, study of lipid storage and trafficking models in *Drosophila*, shows that cholesterol storage alters the trafficking of a glycolipid from the biosynthetic to the degradative endolysosomal pathway and cholesterol depletion eliminates glycolipid recycling. Lactosyl ceramide diverts a neutral cargo (dextran) away from the lysosome (Hortsch et al. 2010).

PERSPECTIVES AND CONCLUSIONS

Current and emerging gene sequencing technology as well as falling costs (Ng et al. 2009; Horn et al. 2010; Roach et al. 2010) will reveal new inherited disorders in the near future. The examples in this article show that Golgi-associated disorders that affect glycosylation will be well represented. Protein homology predictions may not produce accurate predictions of patients' phenotypes (e.g., compare

SEC23A and *SEC23B* or *PIGM* vs. *PIGV*). Forward genetic screens in model organisms (GMAP210 causing lethal achondrogensis) may help identify interacting pathways and protein binding partners. Some of the defective genes will be familiar, others will have unknown functions or be poorly annotated making their physiological function difficult to unravel. In some cases, biochemical confirmation of altered glycosylation can be a quality control indicator that implicates the Golgi in the physiological functions of defective genes (Nilsson et al. 2009). Genetic defects commonly associated with various medical specialties will require increased interaction and cooperation with basic cell biologists and biochemists to explain and translate these genetic errors into physiology and potential treatments.

SUMMARY

The Golgi apparatus sorts, organizes, and traffics intracellular protein and lipid cargos. It glycosylates cargo and maintains factory organization for efficient pickups and deliveries between the ER and final destinations (plasma membrane or endocytic organelles). Impaired performance by mutated Golgi resident proteins creates severe and highly variable pathologies. Emerging developments in patients' genetic analysis should identify new Golgi components/processes, in which glycosylation is a good indicator of Golgi and the patients' health or pathology.

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