FIGURE 39.5. Mechanisms of viral entry into host cells. (a) Influenza virus initiates host cell contact and entry by binding to cell-surface sialic acid receptors through its surface glycoprotein hemagglutinin. After intracellular replication, a cell-surface neuraminidase cleaves sialic acid from the cell membrane allowing viral escape. (b) Herpes simplex virus (HSV) engages host cells first through a low-affinity engagement of heparan sulfate proteoglycans via its surface glycoproteins gB and gC. Subsequently, a higher-affinity binding of viral protein gD to a member of the tumor necrosis factor–nerve growth factor (TNF/NGF) receptor family promotes membrane fusion. (c) Human immunodeficiency virus (HIV) surface glycoprotein gp120 binds sequentially to the CD4 receptor on T cells and then to a coreceptor such as chemokine receptor CCR4. The latter interaction triggers a conformational change in gp120, which exposes gp41, the HIV factor capable of initiating membrane fusion.
FIGURE 1.5. Recommended symbols and conventions for drawing glycan structures. (Top panel) The monosaccharide symbol set from the first edition of Essentials of Glycobiology is modified to avoid using the same shape or color, but with different orientation to represent different sugars. Each monosaccharide class (e.g., hexose) now has the same shape, and isomers are differentiated by color/black/white/shading. The same shading/color is used for different monosaccharides of the same stereochemical designation, e.g., Gal, GalNAc, and GalA. To minimize variations, sialic acids and uronic acids are in the same shape, and only the major uronic and sialic acid types are represented. When the type of sialic acid is uncertain, the abbreviation Sia can be used instead. Only common monosaccharides in vertebrate systems are assigned specific symbols. All other monosaccharides are represented by an open hexagon or defined in the figure legend. Anomeric notation and destination linkages can be indicated without spacing/dashes. Although color is useful, these representations will survive black-and-white printing or photocopying with the colors represented in different shades (the color values in the figure are the RGB triplet color settings)*. Modifications of monosaccharides are indicated by lowercase letters, with numbers indicating linkage positions, if known (e.g., 9Ac for the 9-O-acetyl group, 3S for the 3-O-sulfate group, 6p for a 6-O-phosphate group, 8Me for the 8-O-methyl group, 9Ac for the 9-O-acetyl group, and 9Lt for the 9-O-lactyl group). Ester and ethers are shown attached to the symbol with a number. For N-substituted groups, it is assumed that only one amino group is on the monosaccharide with an already known position (e.g., NS for an N-sulfate group on glucosamine, assumed to be at the 2-position). Typical branched “biantennary” N-glycan with two types of outer termini, depicted at different levels of structural details. (Bottom panel) Some typical glycosaminoglycan (GAG) chains. *Note: To reproduce precise colors in RGB format, use the following triplet values: Galactose stereochemistry: Yellow (255,255,0); Glucose stereochemistry: BLUE (0,0,250); Mannose stereochemistry: GREEN (0,200,50); Fucose: RED (250,0,0); Xylose: ORANGE (250,100,0); Neu5Ac: PURPLE (125,0,125); Neu5Gc: LIGHT BLUE (200,250,250); KDN: GREEN (0,200,50); GlcA: BLUE (0,0,250); IdoA: TAN (150,100,50); GalA: Yellow (255,255,0); ManA: GREEN (0,200,50).

FIGURE 8.4. (Facing page) Processing and maturation of an N-glycan. The mature Dol-P-P-glycan, synthesized as described in Figure 8.3, is transferred to Asn-X-Ser/Thr sequons during proteinsynthesis as proteins are being translocated into the ER. Following transfer of the 14-sugar Glc3 Man9GlcNAc2 glycan to protein, glucosidases in the ER remove the three glucose residues, and ER mannosidase removes a mannos residue. These reactions are intimately associated with the folding of the glycoprotein assisted by the lectins calnexin and calreticulin, and they determine whether the glycoprotein continues to the Golgi or is degraded. Another lectin, termed EDEM (ER degradation-enhancing α-mannosidase I–like protein), binds to mannose residues on misfolded glycoproteins and escorts them via retrotranslocation into the cytoplasm for degradation. The removal of the first glucose (and therefore all glucose) can be blocked by castanospermine, leaving Glc3 Man9GlcNAc2Asn, which may subsequently have terminal mannose residues removed during passage through the Golgi. For most glycoproteins, additional mannose residues are removed in the ciscompartment of the Golgi until Man5GlcNAc2Asn is generated. The mannosidase inhibitor deoxymannojirimycin blocks the removal of these mannose residues, leaving Man5GlcNAc2Asn, which is not further processed. The action of GlcNAcT-I on Man5GlcNAc2Asn in the medial-Golgi initiates the first branch of an N-glycan. This reaction is blocked in the Lec1 CHO mutant in which GlcNAcT-I is inactive, leaving Man5GlcNAc2Asn, which is not further processed. α-Mannosidase II removes two outer mannose residues in a reaction that is blocked by the inhibitor swainsonine. The action of α-mannosidase II generates the substrate for GlcNAcT-II.

FIGURE 8.1. Biosynthesis and interconversion of monosaccharides. The relative contributions of each pathway under physiological conditions are unknown. (Rectangles) Donors; (ovals) monosaccharides; (asterisks) control points; (6PG) 6-phosphogluconate; (PEP) phosphoenolpyruvate; (KDN) 2-keto-3-deoxy-d-glycero-β-galactonononic acid; (Dol) dolichol.
FIGURE 8.4. (continued) The resulting biantennary N-glycan is extended by the addition of fucose, galactose, and sialic acid to generate a complex N-glycan with two branches. The addition of galactose does not occur in the Lec8 CHO mutant, which has an inactive UDP-Gal transporter. In Lec8 mutants, complex N-glycans terminate in N-acetylglucosamine. The addition of sialic acid does not occur in the Lec2 CHO mutant, which has an inactive CMP-sialic acid transporter. In Lec2 mutants, complex N-glycans terminate with galactose. Complex N-glycans can have many more sugars than shown in this figure, including additional residues attached to the core, additional branches, branches extended with poly-N-acetyllactosamine units, and different “capping” structures (see Chapter 13). Also shown is the special case of lysosomal hydrolases that acquire a GlcNAc-1-P at C-6 of mannose residues on oligomannose N-glycans in the cis-Golgi. The N-acetylglu-cosamine is removed in the trans-Golgi by a glycosidase, thereby exposing Man-6-P residues that are recognized by a Man-6-P receptor and routed to an acidified, prelysosomal compartment, as described in Chapter 30. The inhibitors of N-glycan processing are described in Chapter 50, and the CHO mutants blocked in N-glycan synthesis are described in Chapter 46. (Adapted, with permission of the Annual Review of Biochemistry, from R. Kornfeld and S. Kornfeld. 1985. Annu. Rev. Biochem. 54: 631–634.)
There are eight O-GalNAc glycan core structures (Table 9.1), most of which may be further substituted by other sugars. N-Acetylgalactosamine is converted to core 1 (Gal\(\beta_1-3\)GalNAc-) by a core 1 \(\beta_1-3\) galactosyltransferase termed T synthase or C1GalT-1 (Figure 9.3). This activity is present in most cell types. However, to be exported from the endoplasmic reticulum in vertebrates, T synthase requires a specific molecular chaperone called Cosmc. Cosmc is an ER protein that appears to bind specifically to T synthase and ensures its full activity in the Golgi. Lack of core 1 synthesis can be due to either defective T synthase or the absence of functional Cosmc chaperone. The result is high expression of Tn and sialyl-Tn antigens. Immunoglobulin A nephropathy in humans is associated with low expression of Cosmc, and a mutation in the Cosmc gene gives rise to a condition called the Tn syndrome.

**FIGURE 9.3.** Biosynthesis of core 1 and 2 O-GalNAc glycans. Shown is the biosynthesis of some extended core 1 and core 2 O-GalNAc glycans. The linkage of N-acetylgalactosamine to serine or threonine to form the Tn antigen, catalyzed by polypeptide-N-acetylgalactosaminyltransferases (ppGalNAcTs), is the basis for all core structures. Core 1 \(\beta_1-3\) galactosyltransferase (C1GalT-1, T-synthase) synthesizes core 1 (T antigen) and requires a specific molecular chaperone, Cosmc. Core 1 may be substituted with sialic acid or N-acetylgalcosamine (as shown) or by fucose or sulfate (not shown). Substitution of core 1 by a \(\beta_1-3\)-linked N-acetylgalcosamine prevents the synthesis of core 2 by core 2 \(\beta_1-6\) N-acetylgalcosaminyltransferase (C2GnT), an enzyme that is highly specific for unmodified core 1 as a substrate. Red cross marks blocked pathway. In breast cancer cells, C2GnT and \(\alpha_2-3\) sialyltransferase (ST3Gal I) compete for the common core 1 substrate. If sialyltransferase activity is high, chains will be mainly short with sialylated core 1 structures; if C2GnT activity is high, chains will be complex and large. The Gal\(\beta_1-3\) residue of the core 1 O-GalNAc glycan and both N-acetylgalcosamine and galactose branches of the core 2 O-GalNAc glycan may be extended and carry terminal blood group and Lewis antigens (see Table 9.1).
O-GalNAc glycosylation is probably an essential process because all mammalian cell types studied to date express ppGalNAcTs. However, when the ppGalNAcT-1 gene was deleted in mice the animals appeared to be unaffected, possibly due to the fact that another ppGalNAcT replaces the function of ppGalNAcT-1. In the secreted mucins of the respiratory, gastrointestinal, and genitourinary tracts, as well as those of the eyes, the O-GalNAc glycans of mucous glycoproteins are essential for their ability to hydrate and protect the underlying epithelium. Mucins also trap bacteria via specific receptor sites within the O-glycans of the mucin. Some sugar residues or their modifications can mask underlying antigens or receptors. For example, O-acetyl groups on the sialic acid residue of the sialyl-Tn antigen prevent recognition by anti-sialyl-Tn antibodies. Gut bacteria enzymatically remove this blocking group. Bacteria can cleave sulfate with sulfatases or terminal sugars with glycosidases. Because the O-glycans are hydrophilic and usually negatively charged, they promote binding of water and salts and are major contributors to the viscosity and adhesiveness of mucus, which forms a physical barrier between lumen and epithelium. The removal of microbes and particles trapped in mucus is an important physiological process.
The essential events in GPI precursor biosynthesis are, like the core structure, highly conserved. There are, however, variations on the theme, and *T. brucei* and mammalian cell GPI pathways are used here to represent these differences (Figure 11.4). In all cases, GPI biosynthesis involves the transfer of N-acetylglucosamine from UDP-GlcNAc to phosphatidylinositol (PI) to give GlcNAc-PI via an ER-membrane-bound multiprotein complex (Table 11.2, Figure 11.5). This step occurs on the cytoplasmic face of the ER, as does the second step of the pathway, the de-N-acetylation of GlcNAc-PI to GlcN-PI. Notable differences between the *T. brucei* and mammalian GPI-biosynthetic pathways occur from GlcN-PI onward, including (1) the timing and reversibility of inositol acylation, (2) substrate channeling.

There are many examples of transmembrane signaling via the cross-linking of GPI-anchored proteins with antibody and clustering with a second antibody on various cells, particularly leukocytes. Cellular responses include rises in intracellular Ca++, tyrosine phosphorylation, proliferation, cytokine induction, and oxidative burst. These antibody-induced signaling events are clearly dependent on the presence of a GPI anchor and might be due to the coalescence of small lipid rafts. Lipid-raft-resident GPI-binding transmembrane proteins have also been postulated to account for the missing link between the outer and inner leaflets of the plasma membrane bilayer. One candidate for this in leukocytes is the β2-integrin complement receptor type 3. Despite the plethora of GPI-protein cross-linking/signal-transduction examples, it should be noted that there are no receptor/ligand pairs of established physiological relevance that signal in a GPI-dependent way. Thus, GPI-anchored proteins known to be involved in transmembrane signaling, such as the glial-cell-line-derived neurotrophic factor receptor-α (GDNFR-α), need to be associated with transmembrane β coreceptors to transmit their signals. Similarly, GPI-anchored CD14 (the LPS/LPS-binding protein receptor) functions equally well with a GPI anchor or with a spliced transmembrane domain, and the signal-transducing partner for CD14 has been identified as the transmembrane Toll-like receptor-4.

An example of a different type of cross-linking exists in mammals and involves the biosynthesis of inter-α-trypsin inhibitor (ITI), the major urinary proteinase inhibitor. The complex is composed of one or two heavy chains that are linked via the α-carboxyl group on the carboxy-terminal aspartate residue to the C-6 hydroxyl groups of N-acetylgalactosamine residues in a short (12–18 disaccharides) chondroitin sulfate chain attached to the core protein bikunin through a typical proteoglycan linkage region (see Chapter 16). All of the protease inhibitory activity occurs in the bikunin molecule (Figure 12.8). The cross-linking occurs in the Golgi of hepatocytes, but other cells can also carry out the reactions. A complex series of reactions involving proteolytic cleavage of the heavy chains and their esterification with the chondroitin sulfate chains occurs in the Golgi. When these proteins are secreted, they may encounter hyaluronan, which serves as an acceptor in a trans-esterification reaction releasing the chondroitin sulfate–bikunin molecule. These heavy-chain-modified hyaluronan molecules can then assemble into cable-like structures that are thought to have a role in inflammation (see Chapter 15). Details of the specificity for trans-esterification reactions are not known.
FIGURE 12.8. Model for the structure and function of a chondroitin sulfate (CS) glycan that bridges several proteins. The inter-α-trypsin inhibitor (ITI) family of molecules are synthesized in the Golgi of hepatocytes. The inhibitor contains three protein chains, HC1 (heavy chain 1), HC2, and serine protease inhibitor bikunin, all held together via a chondroitin sulfate chain. The CS chain is attached to Ser-10 of bikunin via a standard glycosaminoglycan attachment (see Chapter 16), whereas the HCs are linked through ester bonds between carboxylate groups of their carboxy-terminal aspartic acid residues and the C-6 hydroxyls of internal N-acetylgalactosamine units in the CS chain. The CS-bound HCs are then transferred to C-6 hydroxyls of N-acetylglucosamine residues in HA via a transesterification involving the carboxy-terminal aspartic acids. This leads to the construction of extracellular matrices by the aggregation of SHAP (serum-derived hyaluronan-associated protein)-HA molecules into a “cable-like structure” containing the complexes and the interaction of the matrices with inflammatory cells. (Redrawn, with permission of the American Society for Biochemistry and Molecular Biology, from Zhuo L, Hascall V.C., and Kimata K. 2004. J. Biol. Chem. 279: 38079–38082.)

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Poly-N-acetyllactosamines

As noted above, poly-N-acetyllactosamine biosynthesis is directed by the alternating actions of β1–4 galactosyltransferases and β1–3 N-acetylglucosaminyltransferases (Figure 13.2). Some glycoproteins and glycolipids are preferentially modified to carry poly-N-acetyllactosamine. This implies that the glycosyltransferases responsible for poly-N-acetyllactosamine biosynthesis can discriminate between glycoproteins or glycolipids that appear to present the same terminal GlcNAc and galactose glycan.

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cumulus cells up-regulate Has2 and a link module family hyaladherin encoded by tumor necrosis factor-stimulated gene 6 (TSG-6). The expression of these proteins initiates production of hyaluronan and its organization into an expanding matrix around the cumulus cells. Concurrently, the follicle becomes permeable to serum, which introduces an unusual molecule called inter-α-trypsin inhibitor (ITI), composed of the trypsin inhibitor bikunin and two heavy chains all covalently bound to a chondroitin sulfate chain (see Chapter 12). In a complex process, TSG-6 catalyzes the transfer of heavy chains from chondroitin sulfate onto the newly synthesized hyaluronan. In the absence of either TSG-6 or ITI, the matrix does not form, and the phenotype of mice null for either of these molecules is female infertility. At the time of ovulation, hyaluronan synthesis ceases, and ovulation of the expanded cumulus cell–oocyte complex occurs. Prior to fertilization, individual sperm undergo capacitation enabling them to penetrate and fertilize an ovum. During this process, SPAM1/PH20, a GPI-anchored hyaluronidase, redistributes and accumulates in the sperm head. SPAM1 binds hyaluronan in the cumulus, causing an increase in Ca++ flux and sperm motility. It also helps dissolve the cumulus matrix as the sperm moves through the hyaluronan vestment. A soluble form of SPAM1 is secreted during the acrosome reaction. The release of acrosomal hyaluronidase and proteases renders the sperm capable of fusing with the egg and eventually destroys the entire matrix to allow the fertilized oocyte to implant and develop.

**FIGURE 16.1.** The large cartilage CS proteoglycan (aggrecan) forms an aggregate with hyaluronan and link protein (see Chapter 15). (Redrawn, with permission of Springer Science and Business Media, from Rodén L. 1980, In The biochemistry of glycoproteins and proteoglycans [ed. WJ. Lennarz], p. 291. Plenum Press, New York.)
As mentioned above, KS I proteoglycans maintain the even spacing of type I collagen fibrils in the cornea, allowing the passage of light without scattering. Defects in sulfation (macular corneal dystrophy) or chain formation (keratoconus) cause distortions in fibril spacing.

### TABLE 16.3. Examples of heparan sulfate proteoglycans

<table>
<thead>
<tr>
<th>Proteoglycan</th>
<th>Core protein (kD)</th>
<th>Number of glycosaminoglycan chains</th>
<th>Tissue distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perlecan</td>
<td>400</td>
<td>1–3 HS</td>
<td>secreted; basement membranes; cartilage</td>
</tr>
<tr>
<td>Agrin</td>
<td>200</td>
<td>1–3 HS</td>
<td>secreted; neuromuscular junctions</td>
</tr>
<tr>
<td>Collagen type XVIII</td>
<td>147</td>
<td>2–3 HS</td>
<td>secreted; basement membranes</td>
</tr>
<tr>
<td>Syndecans 1–4</td>
<td>31–45</td>
<td>1–3 HS</td>
<td>membrane bound; epithelial cells and fibroblasts</td>
</tr>
<tr>
<td>Betaglycan</td>
<td>110</td>
<td>1–2 CS</td>
<td>membrane bound; fibroblasts</td>
</tr>
<tr>
<td>Glypicans 1–6</td>
<td>−60</td>
<td>1–3 HS</td>
<td>membrane bound; epithelial cells and fibroblasts</td>
</tr>
<tr>
<td>Serglycin</td>
<td>10–19</td>
<td>10–15 heparin/CS</td>
<td>intracellular granules; mast cells</td>
</tr>
</tbody>
</table>

**BACKGROUND**

Eubacteria and Archaea (grouped together as prokaryotes) produce a variety of glycoconjugates and polysaccharides of enormous structural diversity and complexity. These glycans include many unusual sugars not found in vertebrates, such as Kdo (3-deoxy-D-manno-octulosonic acid), heptoses, and variously modified hexoses, which have important roles in the biology, and sometimes the pathogenicity, of bacterial cells.
Archaea have rigid cell walls with diverse structures. They contain many unusual lipids with repeating isoprenyl groups linked to glycerol and an S layer of glycoproteins in a lattice-like arrangement attached to the membrane. They lack the peptidoglycan found in almost all prokaryotes and instead, in methanogens, contain a pseudomurein layer, which is similar to the peptidoglycan structure. In general, archaeal structures have been studied in less detail than the corresponding bacterial structures.

Bacteria and Archaea produce numerous glycan-binding proteins. These proteins include adhesins that facilitate bacterial colonization, exotoxins that bind to host membrane glycans, and single-sugar-binding proteins involved in metabolism. Bacterial glycan-binding proteins are discussed in detail in Chapter 34. Contrary to prior misconceptions, Eubacteria and Archaea also produce glycoproteins that contain a number of different linkages. As discussed below, some of the pathways appear to be highly conserved between Eubacteria, Archaea, and Eukaryota. However, many differences also exist, which has allowed extensive use of bacteria as expression system for making recombinant glycoproteins (see Chapter 51).

Addition of L-Ala, D-Glu, m-DAP or other bifunctional amino acids, and D-Ala results in the formation of UDP-MurNAc-pentapeptide. The addition of each amino acid requires a specific ATP-dependent amino acid ligase and the final two amino acids (D-Ala-D-Ala) added as a dipeptide unit. Cytoplasmic enzymes catalyze all of these reactions.

**TABLE 20.1. Examples of capsular polysaccharides**

<table>
<thead>
<tr>
<th>Capsule type</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1 (polysialic acid)</td>
<td>α8 α8 α8 α8 α8 α8 α8 α8</td>
</tr>
<tr>
<td>K5 (N-acetylheparosan)</td>
<td>α4 β4 α4 β4 α4 α4</td>
</tr>
<tr>
<td>Group A Streptococcus</td>
<td>β4 β3 β4 β3 β4</td>
</tr>
</tbody>
</table>


Uridine-P-P-GlcNAc + Manα1-(N-glycan)-Lysosomal enzyme

↓ "Phosphotransferase"

Uridine-P + GlcNAcα1-P-6-Manα1-(N-glycan)-Lysosomal enzyme

↓ "Uncovering Enzyme"

GlcNAc + P-6-Manα1-(N-glycan)-Lysosomal enzyme

↓ *Lysosomal Phosphatase*

P + Manα1-(N-glycan)-Lysosomal enzyme
FIGURE 36.2. Model proposed for the quality control of glycoprotein folding. Proteins entering the ER are N-glycosylated by the oligosaccharyltransferase (OST) as they emerge from the translocon (SEC61) (1). Two glucose residues are removed by the sequential action of α-glucosidases GI and GII to generate monoglucosylated species (2) that are recognized by CNX and/or CRT (only CNX is shown) and are associated with ERp57 (3). The complex between the lectins and folding intermediates/misfolded glycoproteins dissociates upon removal of the last glucose by GII, and is reformed by α-glucosyltransferase (UGGT) (4). Once glycoproteins have acquired their native conformations, either free or bound to lectins, GII hydrolyzes the remaining glucose residue and releases the glycoproteins from the lectin anchors (5). These species are not recognized by UGGT and are transported to the Golgi (6). Glycoproteins remaining in misfolded conformations are retrotranslocated to the cytoplasm, where they are deglycosylated and degraded by the proteasome (7). One or more mannose residues may be removed during the whole folding process. (Modified, by permission of Oxford University Press, from Winchester B. 2005. Glycobiology 15: R1–R15.)

Under normal conditions, a portion of the lipid-linked oligosaccharide (LLO) glycans in the ER are hydrolyzed in the lumen rather than being transferred to protein. Free LLO-derived glycans are rapidly deglucosylated and exported from the ER into the cytoplasm (Figure 36.3). Although no specific transporter has been found, the process requires both ATP and Ca^{++} and removal of the glucose residues. Free mannose can block their export, suggesting that the carrier may recognize the nonreducing end of the chain. Small N-linked glycopeptides can arise within the ER as well, presumably by proteolysis. These are transported by a different mechanism that does not depend on deglucosylation and exhibits different cation requirements. After entry into the cytoplasm, PNGase cleaves the glycan, as described for the degradation of glycoproteins retrotranslocated into the cytoplasm.
It is not clear whether accumulating different types of undegraded material in a lysosome leads to the different symptoms characteristic of each disease. There is no evidence that the stored material causes lysosomes to burst and spew their contents into the cytoplasm. Some leakage may occur or the cell may sense an “engorged” lysosome. The pathology likely depends on the cell type and the cellular balance of synthesis and turnover rates. For instance, dermatan sulfate predominates in connective tissue, which might explain the bone, joint, and skin problems in mucopolysaccharidosis (MPS) I, II, VI, and VII. Keratan sulfate is present in cartilage; therefore, MPS IV is largely a skeletal disease. GM2 ganglioside is abundant in neurons but not in other tissues; therefore, gangliosidosis is predominantly a brain disorder. The importance of glycogen for muscle explains the impact of Pompe disease on the heart and diaphragm, leading to rapid lethality in that disease.

The glycosaminoglycans (GAGs), including heparan sulfate (HS), chondroitin sulfate (CS), dermatan sulfate (DS), keratan sulfate (KS), and hyaluronan, are degraded in a highly ordered fashion. The first three are O-xylose-linked to core proteins. KS can be both N- and O-linked depending on the tissue source, whereas hyaluronan is made as a free glycan (see Chapter 15). Some proteoglycans are internalized from the cell surface and the protein portion is degraded. The GAG chains are then partially cleaved by enzymes such as endo-β-glucuronidases or endohexosaminidases that clip at a few specific sites. Endoglycosidase cleavage creates multiple terminal residues that can be degraded by unique or overlapping sets of sulfatases and exoglycosidases. Structural analysis of partially degraded fragments in the lysosomes of cells from patients with genetic defects in these pathways cause mucopolysaccharidoses (MPS). MPS disorders were critical to dissecting the degradation pathways (see Table 41.2). Note that there is a range of clinical severities and manifestations with mutations in the same gene. For instance, MPS I is clinically subdivided into Hurler, Hurler/Scheie, and Scheie syndromes, although the three disorders represent a continuum of the same disease. Hurler is the most severe form of the disease (Table 41.2). Hurler/Scheie patients progress more slowly and die in early adulthood, whereas Scheie patients can survive to middle or old age. The milder forms of this disease do not cause mental retardation.

The ability to screen large libraries against sulfotransferases has been facilitated by the development of high-throughput assays. Most low-throughput assays measure the transfer of \[^35S\] from \[^35S\]PAPS or measure the radiolabel transfer to a carbohydrate substrate bearing a hydrophobic tail that can easily be isolated using a reverse-phase cartridge. Some sulfotransferases will catalyze the reverse reaction in the presence of high concentrations of a sulfated donor. For example, β-arylsulfotransferase IV (β-AST-IV) will catalyze the reverse transfer of the sulfuryl group from \(p\)-nitrophenol sulfate to PAP, generating PAPS and \(p\)-nitrophenolate ion. When coupled to another sulfotransferase of interest, β-AST-IV regenerates PAPS and stoichiometric amounts of the ion, which can be monitored by UV absorbance. The enzyme also will transfer sulfate to water. A library of 35,000 compounds with purine and pyrimidine scaffolds was screened using β-AST-IV and a fluorescence assay that measured desulfation of 4-methylumbelliferone sulfate. Multiple hits were obtained with moderate inhibition, and subsequent structure elaboration of the library resulted in the generation of a very tight binding small molecule inhibitor with a \(K_m\) value five orders of magnitude lower than the natural substrate. In theory, this approach can be exploited for other enzymes for which high-throughput assays can be developed.

**Complex glycan** A glycan containing more than one type of monosaccharide.
Heparan sulfate  A glycosaminoglycan defined by the disaccharide unit (GlcNAcα1-4Glcβ1-4/IdoAα1-4)n, containing N- and O-sulfate esters at various positions, and typically found covalently linked to a proteoglycan core protein.
In contrast to poly-N-acetyllactosamine, which is a relatively common structure, tandem repeats of LacdiNAc and type-1 sequences are uncommon, although poly-N-acetyllactosamine sequences are sometimes terminated with a type-1 unit. The structures and biosynthesis of poly-N-acetyllactosamine are discussed further in Chapter 13.

**From Chapter 46**  
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**TABLE 46.4. Dominant gain-of-function mutants expressing a new activity**

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Biochemical change</th>
<th>Glycosylation phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEC10 (CHO)</td>
<td>GlcNAcT-III expressed</td>
<td>complex N-glycans have the bisecting N-acetylglucosamine residue</td>
</tr>
<tr>
<td>LEC11 (CHO)</td>
<td>α3FucT-VI expressed</td>
<td>fucose on poly-N-acetyllactosamine generates Le^3 SLe^3 and VIM-2 determinants</td>
</tr>
<tr>
<td>LEC12 (CHO)</td>
<td>α3FucT-IX expressed</td>
<td>fucose on poly-N-acetyllactosamine generates Le^3 and VIM-2 determinants</td>
</tr>
<tr>
<td>LEC14 (CHO)</td>
<td>GlcNAcT-VII expressed</td>
<td>N-glycan core has an extra N-acetylgalactosamine on β1-4-linked Man</td>
</tr>
<tr>
<td>LEC18 (CHO)</td>
<td>GlcNAcT-VIII expressed</td>
<td>N-glycan core has an extra N-acetylgalactosamine on β1-4GlcNAc</td>
</tr>
</tbody>
</table>

Note on nomenclature: Uppercase is used for gain-of-function mutants (e.g., LEC10); lowercase is used for loss-of-function mutants (e.g., Lec1).

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arising from cleavage on the reducing side of each HexNAc residue (usually referred to as A-type ions) whose masses define important structural features of N- and O-glycans, including the types of capping sugars and the presence or absence of poly-N-acetyllactosamine sequences. In MS/MS experiments, additional fragment ions are produced by cleavage on either side of susceptible glycosidic linkages.

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**Keratan sulfate**  
A poly-N-acetyllactosamine \( [Galβ1-4GlcNAcβ1-3]_n \) with sulfate esters at C-6 of N-acetylgalactosamine and galactose residues, found as a side chain of a keratan sulfate proteoglycan.

**Mannan**  
Mannose-rich polysaccharide found in certain bacteria, fungi, and plants.

**Mannose-6-phosphate receptors**  
See P-type lectins.