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ASSEMBLY OF ASPARAGINE-LINKED OLIGOSACCHARIDES

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PERSPECTIVES AND SUMMARY

The subject of asparagine-linked oligosaccharide synthesis and processing was last reviewed in this series in 1981 by Hubbard & Ivatt (1). These authors concluded their excellent chapter with the following statement: "Although the main outlines of this pathway are now clear, several areas, including the detailed enzymology of the synthesizing and processing enzymes, the organization of these enzymes in the cell, the regulation of the pathway, and the

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factors that direct the terminal processing reactions are only beginning to be understood."

During the past few years considerable progress has been made in each of these areas. In addition, much new information has accumulated on the occurrence of oligosaccharide processing in the animal and plant kingdoms and on the repertoire of asparagine-linked oligosaccharides that are synthesized in nature. In this review we have attempted to focus on this new information, particularly the factors that serve to control the extent of processing and thereby determine the final oligosaccharide structures that are assembled. We will begin with a brief review of asparagine-linked oligosaccharide structure, including examples of recently deduced structures. Due to space limitations we are not able to review the important advances that have been made in the area of oligosaccharide structural analysis and the determination of oligosaccharide conformation. The interested reader is referred to several recent reviews of these topics (2–5). In addition, the reader may wish to consult other recent reviews on the synthesis of asparagine-linked oligosaccharides (6–9a).

STRUCTURES OF ASPARAGINE-LINKED OLIGOSACCHARIDES

The advances in techniques for oligosaccharide structural analysis, including the use of 360 and 500 MHz ^{1}H-NMR, have made it possible to deduce the complete structure of hundreds of asparagine-linked oligosaccharides from a variety of plant and animal sources. When these structures are examined, it is evident that they fall into three main categories termed high mannose, hybrid, and complex. Typical examples of these oligosaccharides are shown in Figure 1. They all share the common core structure Manα1–3(Manα1–6) Manβ1–4GlcNAcβ1–4 GlcNAc-Asn, contained within the boxed area in Figure 1, but differ in their outer branches. The high mannose-type oligosaccharides typically have two to six additional mannose residues linked to the pentasaccharide core. The hybrid molecules are so named because they have features of both high-mannose and complex-type oligosaccharides. Most hybrid molecules contain a "bisecting" *N*-acetylglucosamine linked β1,4 to the β-linked mannose residue, although some exceptions exist (10–12). The complex-type structure shown in Figure 1 contains two outer branches with the typical sialyl lactosamine sequence and shows two other commonly found substituents, namely a fucose in α1,6 linkage to the innermost *N*-acetylglucosamine residue, and a "bisecting" *N*-acetylglucosamine linked β1,4 to the β-linked mannose residue. This complex-type structure may be modified both by the addition of extra branches on the α mannose residues or by the addition of extra sugar residues that elongate the outer chains. The majority of complex-type oligosaccharides contain two, three, or four outer branches, but units with five

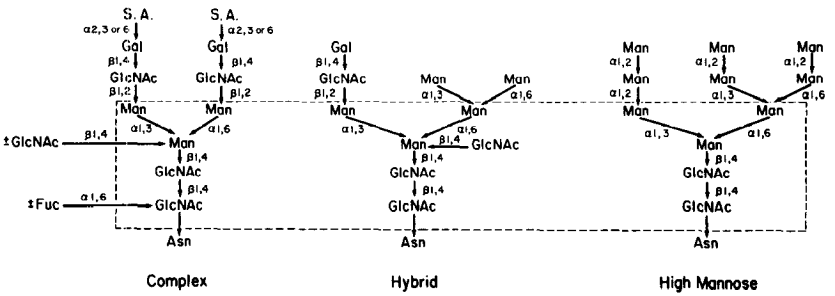


Figure 1 Structures of the major types of asparagine-linked oligosaccharides. The boxed area encloses the pentasaccharide core common to all N-linked structures.

outer branches have been found on avian ovomucoids (13–16). Figure 2 shows the variations in the substituents which have been found attached to outer chain *N*-acetylglucosamine residues of complex oligosaccharides. The basic Galβ1-4GlcNAc or lactosamine sequence may contain a fucose in α1,3 linkage to the GlcNAc, as for example in human lactotransferrin (17, 18), or a sialic acid in α2,6 linkage to galactose, as in human transferrin (19), but it cannot contain both substituents (20, 21). Alternatively the galactose may be substituted with a fucose residue in α1,2 or α1,6 linkage (2) or with another galactose residue in α1,3 linkage (22–24). An unusual polysialosyl sequence (NeuAcα2–8)$_n$ NeuAcα2–3Gal→ has been found in the complex glycopeptides of developing rat brain by Finne (25). The number of sialic acid residues varied from 8 to 12 in glycopeptides having 3 or 4 outer branches. Rothbard et al (26) have shown that the developmentally regulated neural cell adhesion molecule (N-CAM) from chicken brain is a glycoprotein containing 30% sialic acid in the embryonic form and only 10% in the adult form. This sialic acid probably occurs in polysialosyl chains since Finne et al (27) have shown that the analogous mouse brain cell surface protein (BSP-2) contains polysialosyl units of α2,8-linked sialic acid residues which decrease in both amount and chain length during embryonic to adult development. Polysialosyl glycopeptides have also been isolated from a neural tumor cell line (28). Another unusual outer chain sequence, shown in Figure 2, is the β1,3-linked Gal→GlcNAc disaccharide in which the GlcNAc bears a sialic acid substituent. This sequence has another sialic acid attached to the galactose through an α2,4 linkage in bovine serum fibronectin (29) and through an α2,3 linkage in the bovine coagulation factors IX, X, and prothrombin (30–32). It is obvious that a vast number of different oligosaccharide structures can be constructed by varying the number and type of outer chains, but in fact the three outer branches to the left in Figure 2 have been found to occur very frequently and those to the right more rarely.

Another outer branch structure consists of repeating lactosamine disaccharides (Galβ1–4GlcNAcβ1–3)$_n$. First discovered on erythrocyte membrane

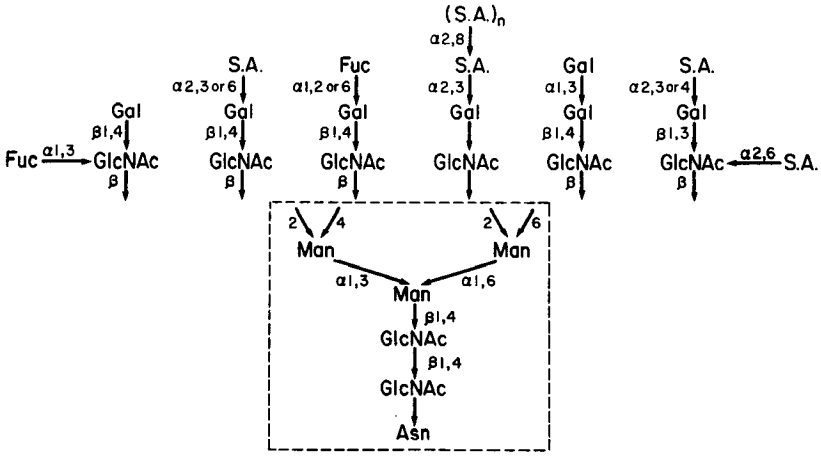


Figure 2 Various outer chain sequences found in complex-type oligosaccharides. The boxed area encloses the core region to which four outer chains may be attached.

glycoproteins (33, 34) and called erythroglycans, these polylactosamine oligosaccharides may be substituted in various ways and have been shown to carry ABH and Ii blood group antigens (33, 35). Fukuda et al. (36, 37) have deduced the complete structure of the biantennary lactosaminoglycans derived from the Band 3 glycoprotein of fetal and adult erythrocytes and shown that the adult form (I) contains chains with multiple branches of the type

$$\text{Gal}\beta1\text{–}4\text{GlcNAc}\beta1\diagdown$$
$$(\text{Gal}\beta1\text{–}4\text{GlcNAc}\beta1\text{–}3)^{6}_{n}\ \text{Gal}\beta1\text{–}4\text{GlcNAc}\beta1\text{–}3$$

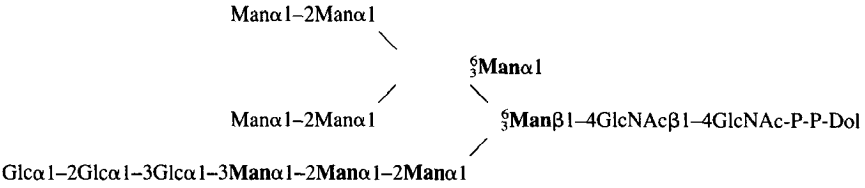
whereas the fetal form (i) has unbranched chains. Spooncer et al (38) have isolated a tetraantennary neutral lactosaminoglycan from human granulocyte glycoproteins in which the *N*-acetylglucosamine residues of the lactosamine chain are substituted with fucose to form the antigenic determinant Galβ1–4(Fucα1–3)GlcNAc expressed on granulocytes (39). This determinant is also expressed on other cell types of human and murine origin, including the murine preimplantation embryo where it has been detected as a stage specific embryonic antigen (SSEA-1) (39–41). Galβ1–4GlcNAcβ1–3, sulfated on C-6 of the GlcNAc and some of the Gal residues, is also the repeating disaccharide of corneal keratan sulfate, the only proteoglycan known to be derived from a complex type of N-linked oligosaccharide precursor (42–47).

All of the asparagine-linked oligosaccharide structures have the common pentasaccharide core structure (see Figure 1) because they all arise from the same biosynthetic precursor lipid-linked oligosaccharide which is transferred to nascent peptide chains and then processed to form these various structures.

ASSEMBLY AND TRANSFER OF THE LIPID-LINKED OLIGOSACCHARIDE

Assembly

The steps in the synthesis of the lipid-linked oligosaccharide precursor



have been reviewed (1, 6, 9). The oligosaccharide is assembled in the endoplasmic reticulum on the lipid carrier dolichol phosphate. The sugars are added in a stepwise fashion with the first seven sugars (two GlcNAc and five Man residues) derived from the nucleotide sugars, UDP-GlcNAc and GDP-Man (**Man** residues in bold type) whereas the next seven sugars (four Man and three Glc residues) are derived from the lipid intermediates, Dol-P-Man and Dol-P-Glc. While it has been established that protein glycosylation occurs in the lumen of the rough endoplasmic reticulum (48–53), the site of lipid-linked oligosaccharide assembly is not fully understood. The problem centers around the fact that the nucleotide sugar donors are unable to enter the lumen of the RER (54, 55). Several studies have attempted to define the membrane orientation of the lipid-linked oligosaccharide intermediates and the enzymes involved in their synthesis. Snider and coworkers (56, 57) used the lectin Con A to probe the orientation of the lipid-linked oligosaccharide precursors in ER-derived vesicles. Their data indicate that the $Man_{3-5}GlcNAc_2$ species face the cytoplasm whereas the $Man_{6-9}GlcNAc_2$ and $Glc_{1-3}Man_9GlcNAc_2$ species face the lumen of the ER. This suggests that the $Man_5GlcNAc_2$ species is assembled on the cytoplasmic side of the ER membrane and then is translocated to the lumenal side where assembly is completed and transfer to protein occurs. Since dolichol derivates do not translocate spontaneously across the lipid bilayer (58), it is likely that the translocation is protein mediated. This pathway fits nicely with the fact that the $Man_5GlcNAc_2$ intermediate is assembled directly from nucleotide sugars. However, this scheme also requires that Dol-P-Man and Dol-P-Glc face the lumen of the ER even though these intermediates are synthesized from cytoplasmic nucleotide sugars by the reaction: Dol-P + XDP-Sugar → Dol-P-Sugar + XDP. Haselbeck & Tanner (59) have proposed a solution to this problem. They have shown that yeast Dol-P-Man synthetase, when incorporated into an artificial membrane, can catalyze the transmembrane movement of Dol-P-Man. In this way the lipid-linked monosaccharides could be synthesized from nucleotide sugars on the cytoplasmic face and then

translocated to the lumenal face where they could serve as donors for the elongation of the $Man_5GlcNAc_2$ intermediate. Many of the enzymes involved in both the early and late stages of lipid-linked oligosaccharide assembly have protease-sensitive sites on the cytoplasmic side of microsomal membranes (55, 60–63), although the $Man_5GlcNAc_2$ translocation mechanism predicts that early enzymes of this pathway would be protease-sensitive but late enzymes, e.g., the glucosyltransferases, would not be. This discrepancy can be explained by proposing that the biosynthetic enzymes are in a transmembrane orientation (55). Also at variance with the model is the observation by Hanover & Lennarz (55, 64) that the intermediate $GlcNAc_2$-P-P-Dol in sealed hen oviduct microsomes is inaccessible to a soluble galactosyltransferase. In contrast, exogenous $GlcNAc_2$-P-P-Dol added to the microsomes was accessible to the galactosyltransferase and did not become inaccessible as a function of time. Possibly the endogenous intermediate is localized within the membrane in a manner which makes it inaccessible to the probe, but still capable of being elongated at the cytoplasmic face by an intramembraneous mannosyltransferase.

Transfer-Oligosaccharide Structural Requirements

Several studies (65–72) have shown that the glucose residues on the lipid-linked oligosaccharide facilitate the in vitro transfer of the oligosaccharide to protein, but the presence of glucose residues is not an absolute requirement for transfer. Robbins and coworkers have shown that yeast mutants defective in various steps in the synthesis of the lipid-linked oligosaccharide transfer nonglucosylated oligosaccharides ranging in size from $Man_9GlcNAc_2$ to $Man_{1-2}GlcNAc_2$ to protein (73, 74). The transfer of $Man_{1-2}GlcNAc_2$ is consistent with previous in vitro studies (71, 72, 75–78). The protozoa *Trypanosoma cruzi* (79–81), *Crithidia fasciculata* (82, 83), and *Leishmania mexicana* (84) synthesize nonglucosylated lipid-linked oligosaccharides which are transferred directly to protein. Interestingly, in *Trypanosoma cruzi*, a transient glucosylation occurs after the oligosaccharide is bound to protein (80, 81). It is not known if the oligosaccharyltranferase of higher organisms will transfer nonglucosylated lipid-linked oligosaccharides. A Con A–resistant cell line that cannot glucosylate lipid-linked oligosaccharides has been isolated, but transfer of this oligosaccharide to protein has not been demonstrated (85).

Hoflack et al (86) have proposed that glucosylation protects the lipid-linked oligosaccharides from degradation. They studied intact rat spleen lymphocytes which incorporate nucleotide sugars into lipid-linked oligosaccharides and found that the nonglucosylated lipid-linked oligosaccharides were selectively degraded by a phosphodiesterase.

While glucose residues facilitate oligosaccharide transfer to protein in some cell types, the number of mannose residues has little effect on protein glycosylation. In protozoa the completed lipid-linked oligosaccharides contain nine

mannoses (79), seven mannoses (82, 83) and six mannoses (84). The green flagellate *Volvox carteri* synthesizes a lipid-linked oligosaccharide with the composition $Glc_1Man_5GlcNAc_2$ which is transferred to protein (87, 88). The oligosaccharyltransferase of higher organisms also appears to be indifferent to the number of mannose residues on the lipid-linked oligosaccharide precursor. Removal of mannose residues from the $Glc_3Man_9GlcNAc_2$ precursor with α-mannosidase does not impair transfer to protein (66, 69). Mutant cell lines that synthesize truncated lipid-linked oligosaccharides with five or seven mannoses transfer these species to protein (89–93). Cells starved of glucose (94–96) or treated with CCCP, an uncoupler of oxidative phosphorylation (97), synthesize lipid-linked $Glc_3Man_5GlcNAc_2$ which is transferred to protein. The effects seen with glucose starvation or energy depletion vary with the cell type and cell density. Spiro et al (98) found that energy deprivation of thyroid slices resulted in depletion of $Glc_3Man_9GlcNAc_2$-P-P-Dol, an accumulation of the $Man_9GlcNAc_2$ lipid-linked species and a concomitant decrease in protein glycosylation. In this system, the $Man_9GlcNAc_2$-P-P-Dol was stable.

Transfer-Role of Peptide Acceptor

Glycosylated asparagines almost always occur in the sequence Asn-X-Ser/Thr where X can be any amino acid except possibly proline and aspartic acid (99, 100). There is one report of glycosylation at an Asn-Ala-Cys sequence (100a). In vitro glycosylation with synthetic peptides has shown that the Asn-X-Ser/Thr sequence is sufficient for oligosaccharide transfer although glycosylation is not efficient unless both the α-amino group of asparagine and the α-carboxyl moiety of the hydroxyl amino acid are blocked (71, 72, 101–105). Increasing the acceptor chain length to a hexa- or octapeptide greatly enhances the rate of oligosaccharide transfer, possibly by allowing the formation of a higher order structure which appears to facilitate the reaction (106, 107).

Studies by Bause & Legler on the role of the hydroxyl amino acids in the glycosylation reaction have provided important insights into the mechanism of the reaction and the requirement of a favorable conformation (108). Using a series of hexapeptides derived from Tyr-Asn-Gly-X-Ser-Val in which X was varied, they found that threonine-, serine- and cysteine-containing derivatives could be glycosylated, but at very different rates (threonine>serine>cysteine) whereas valine and O-methylthreonine analogs were inactive. They concluded that there is an absolute requirement in the glycosylation reaction mechanism for formation of a hydrogen bond in the side chain of the hydroxyl amino acid. A model was proposed in which a hydrogen-bond interaction between the amide of asparagine (the hydrogen-bond donor) and the oxygen of the hydroxyl group of the hydroxyl amino acid (the hydrogen acceptor) increased the nucleophilicity of the amide electron pair, resulting in a higher reactivity toward the glycosyl donor. By analyzing space-filling models, Bause found

that β-turns or loops represent spatial arrangements of the peptide chain that favor the required hydrogen-bonded contacts (109). Proline-containing peptide analogs that did not serve as acceptors were unable to achieve a conformation that allowed the necessary hydrogen bonding. The relevance of these findings to the in vivo situation is supported by statistical studies of naturally occurring glycoproteins which indicate that most *N*-glycosylated asparagines are located in peptide segments that favor the formation of β-turns (110, 111).

Lau et al (112) examined the effect of synthetic peptides on the transfer of oligosaccharide to nascent polypeptides during coupled translation/glycosylation in a reticulocyte lysate, dog pancreas microsome system. They found that peptides that were acceptors acted as competitive inhibitors of nascent chain glycosylation, but did not affect translocation of the nascent chains. Nonacceptor peptides had no effect, indicating that the inhibition was a result of competition for the active site of the oligosaccharyltransferase.

An examination of protein sequences has revealed that only about one third of the potential Asn-X-Ser/Thr sites in proteins are actually glycosylated (113). In addition, while glycosylation in general is highly efficient, the process is incomplete at some Asn-X-Ser/Thr sites (114–116). It would appear that the ability of the tripeptide signal to achieve a favorable conformation in the presence of short-range interactions with neighboring amino acids may have a significant effect on the rate and extent of glycosylation. Such favorable conformations would be needed to provide the correct hydrogen bonding as well as adequate accessibility to the oligosaccharyltransferase. Since glycosylation occurs cotranslationally, the asparagine that is to be glycosylated is part of a growing peptide chain that is in the process of folding. Consequently, the period of time during which glycosylation can occur may be quite brief. Once the protein has folded, potential glycosylation sites are no longer accessible to the oligosaccharyltransferase (117). Another factor in determining the level of glycosylation is the availability of the lipid-linked oligosaccharide donor. Carson et al found that added Dol-P increased the glycosylation of secreted RNase from 12% to 90% in bovine pancreas tissue slices (118). Thus, Dol-P availability may regulate asparagine-linked glycosylation under some circumstances. In other instances, such as the regenerating rat liver (118a), and hormone treated thyroid (118b) and oviduct tissue (118c), the level of oligosaccharyltransferase activity may control the extent of protein glycosylation. The oligosaccharyltransferase has been partially purified by two groups (119, 120).

In summary, the efficient glycosylation of proteins is dependent on a sufficient pool of completely assembled and glucosylated lipid-linked oligosaccharide donor, an adequate activity of oligosaccharyltransferase, and a properly oriented and accessible Asn-X-Ser(Thr) sequence in the acceptor.

OLIGOSACCHARIDE PROCESSING

Sequence of Processing

The enzymatic pathway of oligosaccharide processing and the subcellular location of the various reactions in that sequence are schematically depicted in Figure 3. The precursor $Glc_3Man_9GlcNAc_2$ is transferred from the lipid donor to an asparagine in a nascent polypeptide during its vectorial transport across the membrane of the RER (reaction 1). Processing is initiated by the removal of the terminal glucose residue by a specific α1,2 glucosidase I (reaction 2). The two inner glucose residues are then removed (reaction 3) by a single α1,3 specific glucosidase II. These glucosidases are located in the membranes of the RER as is a specific α-mannosidase which catalyzes the removal of at least one α1,2-linked mannose residue (reaction 4). These processing events have been shown by Atkinson & Lee (121) to occur cotranslationally (i.e., on polysome-bound nascent polypeptides) for the viral membrane glycoprotein (G protein) of vesicular stomatitis virus-infected HeLa cells. Both integral membrane and secreted glycoproteins undergo this same sequence of events in the RER, but with various glycoproteins some or all of the steps, including the glycosylation event, may occur after the polypeptide chain is completely synthesized (122–123a). Resident ER glycoproteins such as HMG CoA reductase (124) and the ribophorins (121) and glycoproteins which exit the ER only after a long lag time (125, 126) may undergo further mannose trimming to give rise to $Man_6GlcNAc_2$ species. Parodi and his coworkers have shown that protein-bound nonglucosylated high mannose units may be transiently reglucosylated by an ER glucosyltransferase (127). This reaction has been demonstrated in mammalian, avian, protozoan, and plant cells but is not detectable in yeast (81, 127, 128). While no function for this reglucosylation has been demonstrated, Parodi has speculated that it could serve to protect protein-linked oligosaccharides from extensive degradation by the ER mannosidase. With the exception of glycoproteins that are permanent residents of the ER membrane, the newly synthesized glycoproteins are next transported to the cis Golgi cisternae by means of vesicles which are believed to bud from the RER and then fuse with the Golgi membrane (129). The rate at which these proteins exit the RER varies widely depending on the specific glycoprotein and the tissue examined (126, 130, 131). This variation in the rate of exit from the ER of different glycoproteins in the same cell suggests that the vesicular transport may involve a receptor-mediated process rather than simple pinching off of bulk phase lumenal contents. 1-Deoxynojirimycin, a specific inhibitor of glucosidases I and II, delays the exit of some, but not all, glycoproteins from the RER (132–134). On this basis it has been postulated that glucose trimming is necessary for efficient movement from the RER to the Golgi, possibly because the deglucosylated oligosaccharides form part of a recognition site for a transport receptor for

certain secretory proteins (132). An alternative explanation for these findings is that glucose removal is necessary for the glycoprotein to mature to a correct functional conformation (135).

When the glycoproteins arrive in the Golgi, they traverse the stack from the

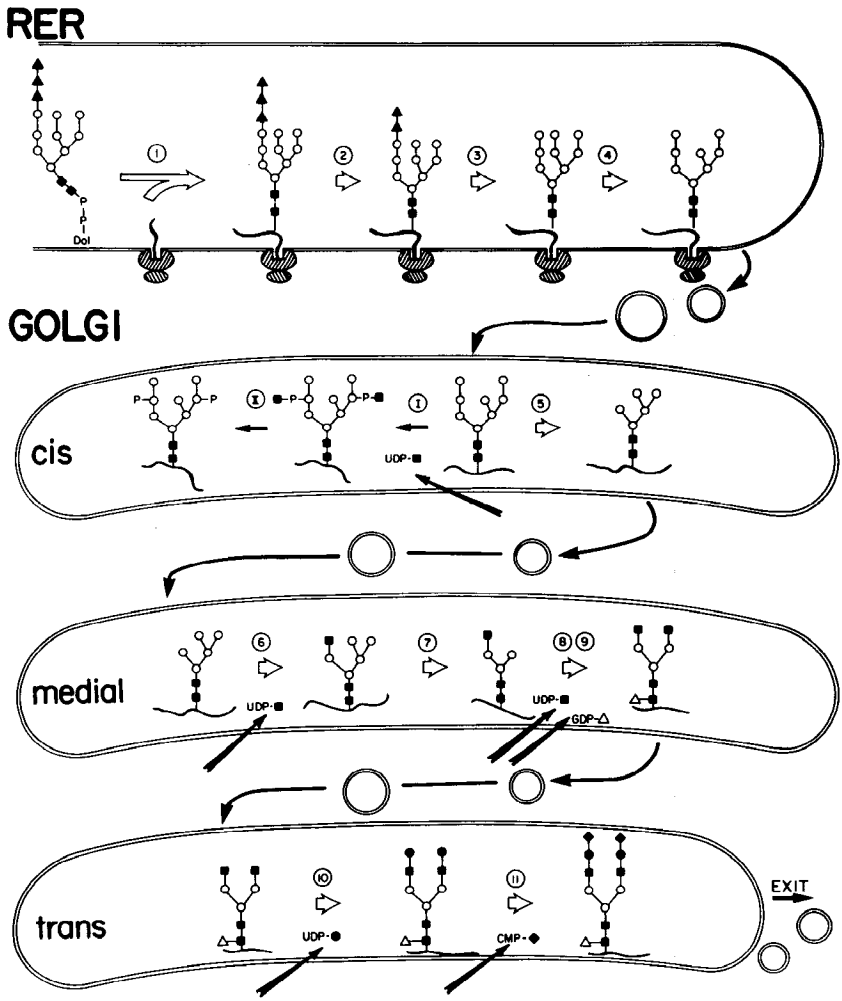


Figure 3 Schematic pathway of oligosaccharide processing on newly synthesized glycoproteins. The reactions are catalyzed by the following enzymes: (1) oligosaccharyltransferase, (2) α-glucosidase I, (3) α-glucosidase II, (4) ER α1,2-mannosidase, (I) *N*-acetylglucosaminylphosphotransferase, (II) *N*-acetylglucosamine-1-phosphodiester α-*N*-acetylglucosaminidase, (5) Golgi α-mannosidase I, (6) *N*-acetylglucosaminyltransferase I, (7) Golgi α-mannosidase II, (8) *N*-acetylglucosaminyltransferase II, (9) fucosyltransferase, (10) galactosyltransferase, (11) sialyltransferase. The symbols represent: ■, *N*-acetylglucosamine; ○, mannose; ▲, glucose; △, fucose; ●, galactose; ◆, sialic acid.

cis through medial to trans cisternae by vesicular transport (136–138). A special subset of glycoproteins, the lysosomal enzymes, undergo a highly specific mannose phosphorylation catalyzed by two enzymes, *N*-acetylglucosaminylphosphotransferase (reaction I) and *N*-acetylglucosamine-1-phosphodiester α-*N*-acetylglucosaminidase (reaction II), which have been localized to a dense Golgi membrane subfraction presumed to correspond to the cis Golgi cisternae. The high mannose oligosaccharides on nonlysosomal glycoproteins can be further trimmed by the Golgi α1,2 mannosidase (Golgi mannosidase I) to yield a $Man_5GlcNAc_2$ structure (reaction 5). This reaction is believed to occur in either the cis or the medial cisternae (137, 138). In the medial cisternae, those oligosaccharide chains destined to become complex-type structures are further processed by addition of a *N*-acetylglucosamine residue catalyzed by *N*-acetylglucosaminyltransferase I (reaction 6), followed by the removal of two mannose residues by Golgi α-mannosidase II (reaction 7) and the subsequent addition of another outer chain *N*-acetylglucosamine residue catalyzed by *N*-acetylglucosaminyltransferase II (reaction 8). At this stage fucosyltransferase may act to transfer a fucose residue to the innermost GlcNAc residue on the oligosaccharide (reaction 9). The final steps of complex oligosaccharide synthesis occur in the trans Golgi cisternae and consist of addition of outer chain galactose and sialic acid residues catalyzed by galactosyl- and sialyl-transferases in reactions 10 and 11. Other terminal sugar additions (Figure 2) must also occur at this late stage. The newly synthesized glycoproteins then exit the Golgi and are transported to their final destination.

The specific localization of the processing enzymes to the RER or the cis, medial, or trans Golgi cisternae provides a mechanism for controlling their sequential action on newly synthesized glycoproteins in both space and time. The final oligosaccharide structure assembled on a glycoprotein is dictated to a large extent by the order in which that glycoprotein encounters the processing glycosidases and glycosyltransferases, and their specificity. These factors are reviewed in more detail below.

Subcellular Localization of Processing Enzymes

A variety of techniques have been employed to localize oligosaccharide processing reactions to specific intracellular organelles. Subcellular fractionation by density gradient centrifugation provides separation of the RER membranes from the Golgi complex membranes and on this basis many of the processing enzymes have been localized to one or the other compartment. Finer mapping of some of the Golgi-associated enzymes to specific regions of the Golgi stack has been achieved more recently. The most definitive method to emerge involves immunocytochemical labeling of ultrathin sections to localize specific proteins within the cell at the electron microscope level. Using colloidal gold particles coated with Protein A as the marker, Roth & Berger (139) showed that galactosyltransferase was localized in only two or three trans

cisternae of the Golgi stacks in thin sections of HeLa cells pretreated with antibody to galactosyltransferase. Slot & Geuze (140), using immunogold labeling of ultrathin cryosections, also observed that galactosyltransferase was confined to the trans-most cisternae in human hepatoma cells. Dunphy & Rothman (141), using horseradish peroxidase coupled to Protein A as the marker to detect bound antibody on thin sections, have localized *N*-acetylglucosaminyltransferase I to the medial Golgi cisternae. Novikoff et al (142), using a similar method, found Golgi α-mannosidase II distributed throughout the Golgi stack and in portions of the ER in rat liver. Other approaches have provided convincing, albeit less definitive, evidence for the intracellular localization of a number of these enzymes. Dunphy et al (143) devised a sucrose density gradient centrifugation method that gave partial separation of Chinese hamster ovary cell membranes containing the early-acting α-mannosidase I from the late-acting galactosyl- and sialyltransferases, and proposed that the enzymes were in separate compartments of the Golgi. Dunphy & Rothman (144) extended this study to show that *N*-acetylglucosaminyltransferases I and II and α-mannosidase II cofractionate with α-mannosidase I. Using a modification of these workers' sucrose gradient technique to fractionate the membranes of cultured mouse lymphoma cells, Goldberg & Kornfeld (145) observed that Golgi associated enzyme activities could be partially separated into four regions on the gradient. Going from most to least dense these regions contained (1) *N*-acetylglucosaminylphosphotransferase, (2) *N*-acetylglucosamine-1-phosphodiester α-*N*-acetylglucosaminidase (3) *N*-acetylglucos aminyltransferases I and V, α-mannosidase II, and fucosyltransferase, and (4) galactosyltransferase. Deutscher et al (146) were able to separate rat liver Golgi membranes containing *N*-acetylglucosamine-1-phosphotransferase from those enriched in *N*-acetylglucosamine-1-phosphodiester α-*N*-acetyl glucosaminidase.

Other investigators have mapped the subcellular distribution of glycoprotein oligosaccharides by using lectins that react with specific carbohydrate structures. For example, using the immunogold technique, Griffiths et al (147) found that the galactose binding lectin RCA-I only labeled Golgi cisternae in the middle and on the trans side of the stack in frozen thin sections of virus-infected cells. They concluded that the viral membrane proteins acquire galactose in the trans Golgi cisternae. Using lightly fixed saponin-permeabilized, IgM-secreting myeloma cells, Tartakoff & Vassalli (148) found that peroxidase-conjugated Concanavalin A (Con A), which preferentially binds to high mannose oligosaccharides, stained the nuclear envelope, the RER, and the proximal (or cis) Golgi cisternae but not the cell surface. In contrast, peroxidase-conjugated WGA, which binds to sialic acid and outer chain *N*-acetylglucosamine residues of complex-type oligosaccharides, stained the more distal (or trans) Golgi cisternae and associated vesicles and the cell

surface, but not the nuclear envelope, RER, or cis Golgi cisternae. Although such lectin studies only indirectly localize the processing enzymes by mapping their oligosaccharide products, they are entirely consistent with the idea that these enzymes have a restricted distribution in the Golgi cisternae that fits the sequential processing of glycoproteins moving from the cis to trans face of the stack. Based on studies employing a combination of mapping techniques in conjunction with the use of monensin to inhibit glycoprotein transport through the Golgi, Griffiths et al (149) concluded that the Golgi stack can be divided into three functionally distinct compartments, the cis, medial, and trans. Rothman and his coworkers have provided evidence for this Golgi compartmentation using a different approach. They followed the transport of the VSV G protein from one Golgi compartment to another by monitoring its acquisition of outer chain sugar residues. The initial in vitro studies (150, 151) employed Golgi membranes from VSV-infected mutant cells lacking *N*-acetylglucosaminyl transferase I, mixed with Golgi membranes from uninfected wild-type cells and showed that the G protein acquired outer chain *N*-acetylglucosamine presumably due to transfer of the G protein between Golgi subcompartments. In subsequent in vivo studies (137) the same result occurred when pulse-labeled VSV-infected mutant cells were fused with wild-type cells. In similar experiments (138) the G protein from VSV infected cells deficient in galactosylation or in sialylation were shown to acquire galactose or sialic acid residues after cell fusion with uninfected cells having that capacity. These intercompartment transfers were very efficient. Importantly, the transport of a cohort of labeled G protein from one compartment to another in fused cells only occurred during a brief interval after the pulse. If fusion was delayed so that transport had already occurred to the next compartment in the host cell, G protein could not acquire the later sugars in the Golgi of the fusion partner. The authors concluded that transfer is a dissociative process most probably mediated by vesicles budding from the rims of the donor Golgi cisterna and moving to and fusing with the target cisterna. These experiments further support the idea that there are three functionally distinct Golgi compartments, the cis, medial, and trans, as shown in Figure 3.

Processing in Lower Organisms

Plant cells synthesize lipid-linked $Glc_3Man_9GlcNAc_2$ which is transferred to protein (69, 152, 153). In soybean cells, the protein-bound oligosaccharide is processed by the removal of the glucose residues and up to four mannose residues (154). In the case of soybean agglutinin only glucose is removed to form the $Man_9GlcNAc_2$ oligosaccharide found on the mature agglutinin (155). Vitale & Chrispeels have studied the biosynthesis of phytohemagglutinin (PHA) in cotyledons of the common bean (*Phaseolus vulgaris* L.) (156). The mature protein contains two Asn-linked oligosaccharides, one with the

composition $Man_{8-9}GlcNAc_2$ and the other with the composition $Xyl_{0.5}Fuc_{0.6}Man_{3.8}GlcNAc_2$. This latter oligosaccharide is derived from a high mannose unit which becomes resistant to endo H after the protein moves from the ER to the Golgi, indicating that the late stage processing events occur in this organelle. The oligosaccharide also acquires one or more outer GlcNAc residues which are subsequently removed when the PHA arrives at its destination in the protein body, a lysosome-like organelle. Pineapple stem bromelein also has an N-linked oligosaccharide-containing xylose, fucose, mannose, and *N*-acetylglucosamine which presumably is formed by processing of a high mannose precursor (157).

In yeast, the precursor $Glc_3Man_9GlcNAc_2$ is trimmed to $Man_8GlcNAc_2$ by α-glucosidases I and II (158) and an α1,2 mannosidase localized in the ER (152, 159–161a). In the Golgi body the $Man_8GlcNAc_2$ species is then elongated by the action of a series of mannosyltranferases to form the large mannan oligosaccharides found on yeast mannoproteins. These structures usually contain 50–150 mannose residues and some of them have Man-P-Man sequences (162, 163). Mosquito cells trim the $Glc_3Man_9GlcNAc_2$ precursor all the way down to a $Man_3GlcNAc_2$ structure (164), but are unable to form complex-type oligosaccharides (164, 165). *Trypanosoma cruzi* cells trim the $Glc_3Man_9GlcNAc_2$ precursor to a mixture of high mannose species, the smallest of which is $Man_6GlcNAc_2$ (81).

Specificity of Processing Enzymes

EARLY ACTING ENZYMES It is apparent that many thousands of different oligosaccharide structures could be assembled from the combined and sequential action of the processing enzymes. Yet in fact the synthesis of only a limited number of structures is observed. This is due to the rigid substrate specificity of these enzymes. Since the earlier reviews (1, 7, 8) include detailed discussions of prior studies on the specificity of the various processing enzymes, this account will emphasize the more recent findings.

Glucosidase I has been purified from calf liver (166) while glucosidase II has been purified from rat liver and kidney (167, 168). Evidence that glucosidase II removes both α1,3 glucose residues from $Glc_2Man_9GlcNAc_2$ has come from studies with the purified enzyme and from the finding that extracts of a glucosidase II–deficient mouse lymphoma cell line are unable to remove glucose from either $Glc_1Man_9GlcNAc_2$ or $Glc_2Man_9GlcNAc_2$ (169). Burns & Touster (167) showed that glucosidase II action on the artificial substrate *p*-nitrophenyl-α-glucoside was activated by 2-deoxyglucose and mannose whereas those compounds inhibited its action on $Glc_{1-2}Man_9GlcNAc$. Based on their observation and the earlier findings of others (170–172) that glucosidase II splits $Glc_{1-2}Man_9GlcNAc$ much better than $Glc_{1-2}Man_7GlcNAc$ or

$Glc_{1-2}Man_4GlcNAc$, they suggested that glucosidase II has, in addition to its catalytic site, a binding site for one or more α1,2 mannose residues. Such a site would provide the basis for very high affinity binding to the physiological substrate. The finding that a mutant cell line that synthesizes a truncated lipid-linked oligosaccharide with the structure $Glc_3Man_5GlcNAc_2$ is able to process this oligosaccharide to typical complex-type species demonstrates that glucosidase II can act on such structures in vivo (173).

The other processing enzyme in the RER is an α-mannosidase which removes α1,2 mannose residues from $Man_9GlcNAc$ and less well from $Man_{6-8}GlcNAc$ (174). This enzyme has been solubilized from rat liver ER and differs from the two Golgi processing α-mannosidases in its substrate specificity and its failure to bind to Con A-Sepharose. However, its properties are virtually identical to the "cytosolic" α-mannosidase (175), suggesting that the latter enzyme may be derived by proteolytic cleavage of the ER α-mannosidase. Yeast cell extracts contain a very specific α1,2 mannosidase that has similarities to mammalian cell ER α1,2 mannosidase (159). The yeast enzyme removes a single mannose residue from the middle branch of $Man_9GlcNAc$ to produce the $Man_8GlcNAc$ isomer shown in Figure 3, which is the precursor to the elongated polymannose chains of yeast mannoprotein (163). High field proton NMR studies, which indicated that the α1,6-linked branch has a different conformation in this $Man_8GlcNAc$ than in $Man_9GlcNAc$, led Byrd et al (159) to propose that the altered conformation could facilitate the subsequent addition of mannose to the α1,6 branch. Interestingly, rat liver Golgi α-mannosidase I, although capable of removing all four α1,2 mannose residues from $Man_9GlcNAc$ in vitro (176, 177), works better on the $Man_8GlcNAc$ shown in Figure 3 and its pattern of mannose removal from $Man_9GlcNAc$ generates $Man_8GlcNAc$, $Man_7GlcNAc$, and $Man_6GlcNAc$ isomers that retain the α1,2 mannose residue on the middle branch (176).

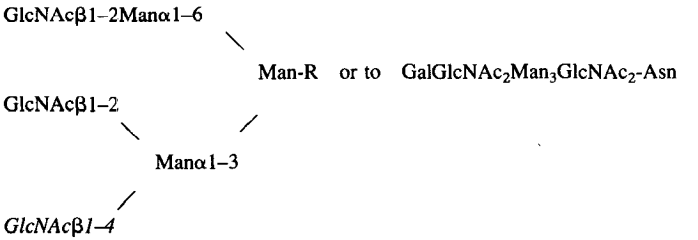
The particular isomers of the Man_8-, Man_7-, and $Man_6GlcNAc_2$ oligosaccharides found on mature glycoproteins is a reflection of the pattern of mannose removal they underwent during processing. That pattern, being determined by the specificities of the α-mannosidases responsible for the trimming, should reflect the extent of mannose removal that occurred in each processing compartment. In general, the smallest high mannose oligosaccharide found on mature glycoproteins is the $Man_5GlcNAc_2$ from which all α1,2 mannose residues have been removed. This oligosaccharide is the preferred substrate in vitro for *N*-acetylglucosaminyltransferase I purified from bovine colostrum (178) and rabbit liver (179) but Manα1-3(Manα1-6)Manβ1-4 $GlcNAc_2$-Asn will accept as will the trisaccharide Manα1-3Manβ1-4GlcNAc at much higher concentrations (180). The first committed step in complex oligosaccharide synthesis is catalyzed by Golgi α-mannosidase II which removes the terminal α1,3 and α1,6 linked mannose residues from $GlcNAcMan_5$

$GlcNAc_2$-Asn (177, 181–183). This enzyme has a very strict substrate specificity and cannot remove those same mannose residues from $Man_5GlcNAc$ or from $GlcNAc(GlcNAc)Man_5GlcNAc_2$-Asn in which a "bisecting" GlcNAc residue has been attached to the core β-mannose residue. This latter reaction is catalyzed by *N*-acetylglucosaminyltransferase III and represents a committment to hybrid oligosaccharide synthesis. Harpaz & Schachter (182) suggested that the relative abundance of α mannosidase II and *N*-acetylglucosaminyltransferase III in a given tissue could determine whether it synthesized complex or hybrid oligosaccharides. In a latter study (184) it was shown that membranes from hen oviduct, which produce ovalbumin containing hybrid oligosaccharides, catalyzed the in vitro conversion of $GlcNAcMan_5GlcNAc_2$-Asn to hybrid oligosaccharides containing the "bisecting" GlcNAc residue. The plant alkaloid swainsonine, which blocks the synthesis of complex oligosaccharides in vivo (185–189) causing accumulation of $GlcNAcMan_5GlcNAc_2$, inhibits purified rat liver Golgi α-mannosidase II in vitro (183).

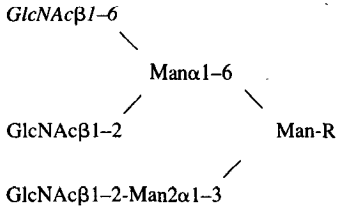
CHAIN ELONGATING ENZYMES The product of α mannosidase II action, $GlcNAcMan_3GlcNAc_2$-Asn, serves as the substrate for *N*-acetylglucosaminyltransferase II catalyzed addition of an *N*-acetylglucosamine residue in β1,2 linkage to the terminal Manα1,6 residue (178, 190, 191). This transferase cannot act on $Man_3GlcNAc$ or $Man_5GlcNAc$. Its product, $GlcNAc_2$-$Man_3GlcNAc_2$-Asn, is the precursor to complex oligosaccharides having two, three, or four outer branches. If *N*-acetylglucosaminyltransferase III acts on the product to attach a "bisecting" GlcNAc residue to the β mannose then the oligosaccharide cannot be further branched (192). Campbell & Stanley (193) have described a lectin resistant CHO cell line that has induced expression of *N*-acetylglucosaminyltransferase III, an enzyme activity undetectable in the parental or a revertant cell line. A high proportion of the oligosaccharide chains on VSV G protein synthesized by the mutant were biantennary complex-type structures containing a "bisecting" GlcNAc residue, whereas no such structures were found on G protein synthesized in parental cells.

In the absence of prior action by *N*-acetylglucosaminyltransferase III on $GlcNAc_2Man_3GlcNAc_2$-Asn, other *N*-acetylglucosaminyltransferases can act to add *N*-acetylglucosamine in β1,4 linkage to the α1,3 mannose (transferase IV) or in β1,6 linkage to the α1,6 mannose (transferase V) to produce structures with three or four outer branch points. Schachter et al (7) have reviewed the substrate specificities known for a number of these *N*-acetylglucosaminyltransferases and point out that they often compete for a common substrate and the order in which specific GlcNAc residues are attached can determine the subsequent route of synthesis. This occurs because transfer of a GlcNAc to a particular linkage in an oligosaccharide can convert it from being a good substrate for a subsequent transferase to being a poor substrate and vice

versa. This situation also obtains for the action of the fucosyl and galactosyltransferases. *N*-acetylglucosaminyltransferase IV from hen oviduct (194) can transfer GlcNAc to $GlcNAc_2Man_3GlcNAc_2$-Asn in vitro to give

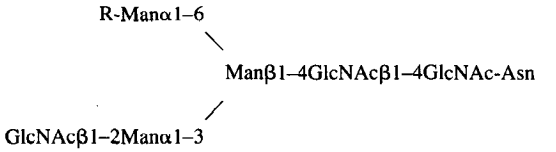


when a Gal residue is on the GlcNAcβ1–2Manα1–6 arm but not when the Gal residue is on the GlcNAcβ1–2Manα1–3 arm. *N*-acetylglucosaminyltransferase V from mouse lymphoma cells (195) acts in vitro on $GlcNAc_2Man_3GlcNAc_2$-Asn to give



and cannot act on $Gal_2GlcNAc_2Man_3GlcNAc_2$-Asn. Since galactosyltransferase is localized to a later Golgi compartment than the *N*-acetylglucosaminyltransferases, galactosylated oligosaccharides are probably not encountered by *N*-acetylglucosaminyltransferases in vivo.

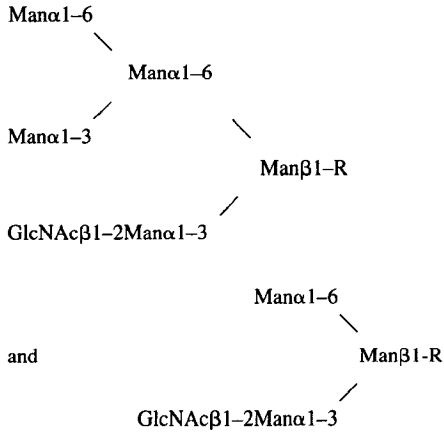
The fucosyltransferase that transfers fucose in α1,6 linkage to the *N*-acetylglucosamine residue attached to asparagine acts on oligosaccharides with the structure



where R is H, GlcNAcβ1–2, or Manα1–6(Manα1–3), but not on substrates where the β Man carries a "bisecting" *N*-acetylglucosamine residue or when GlcNAcβ1–2 on both arms is substituted with galactose (196). The requirement of the fucosyltransferase for the GlcNAcβ1–2Manα1–3 sequence explains why most high mannose oligosaccharides do not have fucose linked to

the core *N*-acetylglucosamine residue. The two exceptions that have been reported are high mannose oligosaccharides on β-glucuronidase (197) and cathepsin D (198).

Schachter et al (7) has noted that a number of processing enzymes appear to interact with the GlcNAcβ1–2Manα1–3 sequence which is present in both

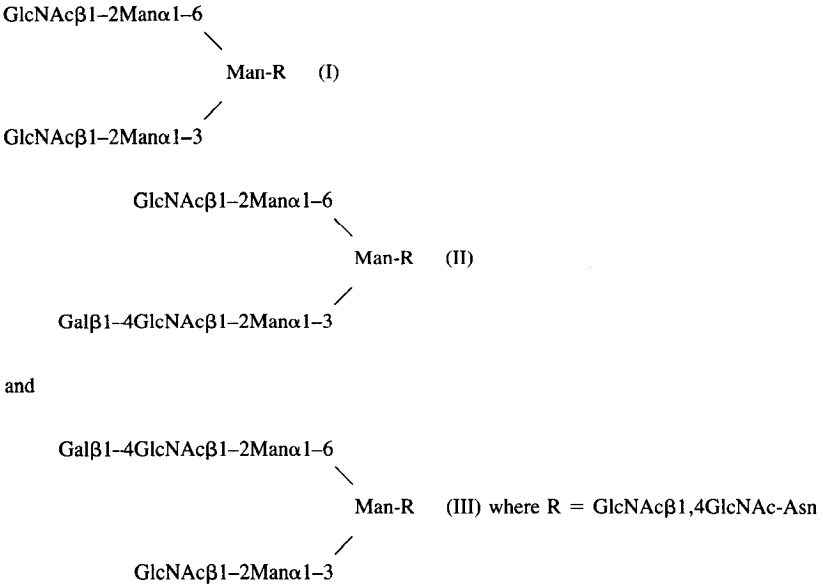


(where R = GlcNAcβ1–4GlcNAc-Asn) although their catalytic attack occurs on very different parts of the structures. These enzymes include those that can act on both substrates (*N*-acetylglucosaminyltranferases III and IV and the α1,6 fucosyltransferase); an enzyme that acts only on $GlcNAcMan_5GlcNAc_2$-Asn (α-mannosidase II); and an enzyme that acts only on the $GlcNAcMan_3GlcNAc_2$-Asn (*N*-acetylglucosaminyltransferase II). However, introduction of a "bisecting" GlcNAc residue into either structure by *N*-acetylglucosaminyltransferase III converts that structure to a nonacceptor for the other enzymes. An explanation for these observations comes from the three-dimensional conformation of these oligosaccharide structures determined by Brisson & Carver (199). The Manα1–3 arm in these N-linked oligosaccharides maintains a fixed orientation in relation to the core Manβ1–4GlcNAcβ1–4GlcNAc segment even when the "bisecting" GlcNAc residue is attached to C-4 of the Manβ1–4 residue. But the added GlcNAc residue blocks access to one face of the GlcNAcβ1–2Manα1–3 segment, with which the other enzymes must presumably interact.

The galactosyltransferase that transfers galactose residues in β1,4 linkage to terminal *N*-acetylglucosamine residues on N-linked oligosaccharides has been purified to homogeneity from a variety of tissues and secretions and the extensive earlier studies on this transferase have been reviewed by Beyer et al (8). Rao & Mendicino (200) first observed that this enzyme acted best on a biantennary complex oligosaccharide with two terminal *N*-acetylglucosamine

residues (K_m 0.25mM), less well on the monogalactosylated derivative (K_m = 2mM) and very poorly on a substrate with a S.A.→Gal on one branch (K_m 10mM).

More recently two groups have established that β1,4 galactosyltransferase displays branch specificity in its action. Paquet et al (201) using purified rat liver Golgi galactosyltransferase with the substrates



found that their apparent K_m's as acceptors were 0.13, 0.43, and 6.28 mM respectively. By monitoring the products formed by galactosyltransferase acting on 0.18 mM structure (I) these workers showed that structure (II) was formed rapidly, structure (III) more slowly, and the digalactosylated product gradually accumulated. The rate constants derived from this study showed that galactose transfer to the GlcNAcβ1–2Manα1–3 branch occurred about 5 times faster than transfer to the GlcNAcβ1–2Manα1–6 branch. Using a double-radio-labeling technique, Blanken et al (202) have shown that the galactosyltransferases from bovine colostrum and calf thymus also preferentially transfer galactose to the 1,3 branch of an acceptor oligosaccharide with structure (I) with a preference ratio of about 4:1 over transfer to the 1,6 branch. These specificity studies were carried out in vitro with glycopeptide or oligosaccharide substrates. However, in vivo the biantennary complex oligosaccharide of human and bovine IgG is monogalactosylated on the Manα1–6 arm (203, 204). This seeming paradox suggests that the relative accessibility of the Manα1–3 arm and the Manα1–6 arm to galactosyltransferase is altered when the oligosaccharide is covalently attached to a protein with which it can

interact. In fact, X-ray crystallographic studies of the F_c fragment of human IgG have shown that the oligosaccharide is disposed in a fixed conformation stabilized by carbohydrate-protein interactions (205, 206). Similar observations have been made on crystals of the F_c fragment of rabbit IgG (207).

LATE ACTING ENZYMES The β-galactoside α2–6 sialyltransferase that is responsible for sialylating N-linked oligosaccharides has been purified from bovine colstrum and its substrate specificity extensively studied as reviewed by Beyer et al (8). Recently van den Eijnden et al (208) showed that this enzyme displays "branch specificity", preferring to sialylate the galactose on the Galβ1–4GlcNAcβ1–2Manα1–3 arm of a biantennary glycopeptide. In the triantennary glycopeptide both branches arising from Manα1–3 were sialylated whereas the Galβ1–4GlcNAcβ1–2Manαβ1–6 branch was not. Interestingly, the core GlcNAc residue to which the β-mannose residue is attached in biantennary oligosaccharides is essential for this branch specificity (209). Reduction of this residue to *N*-acetylglucosaminitol led to decreased branch specificity as well as decreased sialic acid transfer and its removal led to random attachment of sialic acid to the galactose residues in both branches. The authors proposed that interaction of the sialyltransferase with this *N*-acetylglucosamine residue in acceptor oligosaccharides positions the enzyme on the substrate in such a way that transfer to galactose on the 1–3 branch is favored.

Paulson et al (20) and Beyer et al (21) have studied the in vitro glycosylation of asialotransferrin, which contains biantennary complex oligosaccharides, using purified β-galactoside α2,6 sialyltransferase and *N*-acetylglucosaminide α1,3 fucosyltransferase. The latter enzyme adds fucose to C-3 of the *N*-acetylglucosamine residue in the sequence Galβ1–4GlcNAcβ1–2Man and the product of its action Galβ1–4(Fucα1–3)GlcNAcβ1–2Man will not serve as a substrate for the α2,6 sialyltransferase. Similarly, prior sialylation of the asialotransferrin prevented subsequent fucosylation of the *N*-acetylglucosamine residues. The mutually exclusive action of these two transferases on the same oligosaccharide substrate accounts for the fact that the sequence S.A.α2–6Galβ1–4(Fucα1–3) GlcNAc- has not been found on glycoprotein oligosaccharides. An analogous situation appears to account for the observations of Finne et al (210, 211) that a variant mouse melanoma cell line selected for resistance to the sialic acid binding lectin wheat germ agglutinin has increased sensitivity to the fucose binding lectin from *Lotus tetragonolobus* and a 70-fold increase in *N*-acetylglucosaminide α1,3 fucosyltransferase activity compared to the parent melanoma cell line. The oligosaccharides synthesized by the parent cells contained α2,3-linked sialic acid which was reduced in the variant cells concomitant with the appearance of high amounts of Fucα1–3GlcNAc. Thus prior action by the 1,3 fucosyltransferase may also hinder the action of α2,3 sialyltransferase on the same

oligosaccharide and vice versa, although structures containing both substituents have been reported, e.g., in rat brain by Krusius & Finne (212). Campbell & Stanley (213) have also described two mutant Chinese hamster ovary cell lines that are resistant to wheat germ agglutinin and express *N*-acetylglucosaminide α1,3 fucosyltransferase activity not present in the parental cell line. The mutant cells also express the stage-specific embryonic antigen SSEA-1, Galβ1–4(Fucα1–3)GlcNAc, (40, 41) on their surfaces and the parental cells do not.

The α1–3 fucosyltransferase activity that is found in tissues from many species (including human) appears to be a different enzyme than the human Lewis blood group specified α1–3/4 fucosyltransferase which forms the Le^a antigenic structure Galβ1–3(Fucα1–4)GlcNAc (214, 215). This latter enzyme, isolated from human milk by Prieels et al (216), was shown to catalyze fucose transfer to *N*-acetylglucosamine in α1–3 linkage using Galβ1–4GlcNAc as acceptor and in α1–4 linkage using Galβ1–3GlcNAc as acceptor. The two activities occurred in a constant ratio throughout a 500,000-fold purification indicating that they are catalyzed by a single enzyme. This enzyme is thus an exception to the dictum: one linkage—one enzyme.

The α2,3 sialyltransferase that sialylates Galβ1–3(4)GlcNAc sequences in N-linked oligosaccharides has been purified to homogeneity from rat liver by Weinstein et al (217, 218) and its substrate specificity for oligosaccharides and glycoproteins compared with that of the Galβ1–4GlcNAc α2–6 sialyltransferase purified to homogeneity from the same source. The best acceptors for the α2,3 sialyltransferase contained the sequence Galβ1–3GlcNAc-R which was inactive with the α2,6 transferase. The product, SAα2–3Galβ1–3 GlcNAc-R, has only been reported to occur in glycoproteins containing the disialylated structure SAα2–3Galβ1–3(SAα2–6)GlcNAc-(30–32). The α2,3 sialyl transferase also acted on acceptors with the sequence Galβ1–4GlcNAc-R to form SAα2–3Galβ1–4GlcNAc-R. These acceptors were even more active with the α2,6 sialyltransferase which formed SAα2–6Galβ1–4GlcNAc-R. This is in accord with the fact that the *N*-linked oligosaccharides of many glycoproteins contain one or both of these sialylated sequences in their outer branches. Both enzymes showed higher affinity for glycoprotein acceptors than for the analogous oligosaccharide acceptors. Asialo α-1 acid glycoprotein could be almost quantitatively sialylated by either enzyme even though in its isolated form it contains sialic acid in α2,6 linkage. These investigators, on the basis of transfer experiments using mixtures of the α2,3 and α2,6 enzymes, suggest that the relative proportions of these enzymes in a tissue will not totally account for the relative proportion of α2,3 vs α2,6 sialylation on glycoproteins and that the two enzymes probably have complementary but overlapping branch specificity. Interestingly, the α2,3 sialyltransferase could not act on the fucosylated pentasaccharide Galβ1–3(Fucα1–4)GlcNAcβ1–3Galβ1–4Glc. The isomeric structure containing Galβ1–4(Fucα1–3)GlcNAc-R

was not tested as an acceptor but its α2,3-sialylated form has been found in oligosaccharides (212).

Paulson et al (219) have identified an *N*-acetylglucosaminide α2,6 sialyltransferase in rat liver Golgi which, acting with α2,3 sialyltransferase, can form the disialylated sequence SAα2–3Galβ1–3(SAα2–6)GlcNAc found in bovine prothrombin (32). SAα2–3Galβ1–3GlcNAc was the preferred substrate of the GlcNAc α2,6 sialyltransferase. Using desialyzed acceptors (either oligosaccharide or glycoprotein) and both α2,3 sialyltransferase and GlcNAc α2,6 sialyltransferase it was shown that the former acted first and the latter second to form the disialylated product. However the α2,3 sialyltransferase can act on the milk oligosaccharide Galβ1–3 (SAα2–6)GlcNAcβ1–3Galβ1–3Glc (218).

The repeating disaccharides (Galβ1–4GlcNAcβ1–3) of polylactosamine chains are thought to be assembled by the alternating action of β1,4 galactosyltransferase and β1,3 *N*-acetylglucosaminyltransferase, and the branches formed by the action of a β1,6 *N*-acetylglucosaminyltransferase. Three groups (220–222) have reported that normal human serum contains a β1,3 *N*-acetylglucosaminyltransferase activity that acts on lactose to form GlcNAcβ1–3Galβ1–4Glc. Each group found that Galβ1–4GlcNAc was a better acceptor than lactose while Yates & Watkins (221) and Piller & Cartron (222) found that Galβ1–3GlcNAc was a poor acceptor, but a variety of structures with terminal Galβ1–4GlcNAc- sequences were good acceptors. Interestingly the latter group found that the branched tetrasaccharide Galβ1–4GlcNAcβ1–3(GlcNAcβ1–6)Gal had poor acceptor activity but the branched pentasaccharide Galβ1–4GlcNAcβ1–3(Galβ1–4GlcNAcβ1–6)Gal was very active. Piller et al have identified the β1,6 *N*-acetylglucosaminyltransferase using GlcNAcβ1–3Galβ1–4Glcβ-OMe as substrate (223). This enzyme has no activity with acceptors having terminal Gal residues. These results indicate that branching of polylactosamine structures only occurs during elongation of the chains and that at branching points two GlcNAc residues are incorporated in a strictly ordered manner into terminal nonreducing galactose. The addition of the Gal residues is also ordered, with the GlcNAc-linked β1–6 being substituted first (224). Using a different approach van den Eijnden et al (225) tested asialo α-1 acid glycoprotein labeled in its terminal galactose residues as an acceptor for *N*-acetylglucosaminyltransferases in crude homogenates of Novikoff ascites tumor cells. They observed that *N*-acetyl–glucosamine was transferred in both β1,3 and β1,6 linkage to terminal galactose residues as revealed by methylation analysis of the product. However, they did not find evidence for branch formation on these terminal galactoses. The serum β1,3 *N*-acetylglucosaminyltransferase activity (221, 222) was more active on asialo α-1 acid glycoprotein than on asialo fetuin which fits with the fact that the oligosaccharides of the former glycoprotein have been shown to have the repeating lactosamine sequence on some of their

GlcNAcβ1–6 Manα1–6 arms whereas fetuin oligosaccharides do not have the repeating sequence. Structural analysis of mouse lymphoma cells (226) and BHK (227) glycoproteins have shown that the repeating lactosamine sequence is present on a high proportion of the tetra and triantennary N-linked oligosaccharides, but rarely on the biantennary oligosaccharides. A mutant cell line deficient in α mannoside β1,6 *N*-acetylglucosaminyltransferase (*N*-acetylglucosaminyltransferase V) has a greatly reduced content of repeating lactosamine sequences and those present are found predominantly on triantennary chains (226). Taken together these observations suggest that the β galactoside β1,3 *N*-acetylglucosaminyltransferase may have a preference for elongating oligosaccharide chains linked to C-6 of the Manα1-6 residue.

Some of the polylactosamine chains in the mouse lymphoma oligosaccharides referred to above were terminated by an additional Galα1–3 residue (226). This was also observed by Eckhardt & Goldstein (22) in the case of the polylactosamine chains on tetraantennary oligosaccharides in Ehrlich ascites tumor cells. Blake & Goldstein (228) obtained a partially purified α1,3 galactosyltransferase activity from these cells and showed that it catalyzed transfer of galactose from UDP-Gal to lactosamine to form Galα1–3Galβ1–4GlcNAc. The transferase preferred the Galβ1–4GlcNAc-R sequence but also acted on Galβ1–3GlcNAc-R. The enzyme was distinguished from the blood group B α-galactosyltransferase by its lack of reaction with Fucα1–2Galβ1–4GlcNAc-R, the preferred substrate for that transferase. Van Halbeek et al (229) and van den Eijnden et al (230) have studied an α-galactosyltransferase activity in calf thymus that transfers galactose in α1,3 linkage to Galβ1–4GlcNAc and to the Galβ1–4GlcNAc-termini in asialo α-1 acid glycoprotein. This enzyme is different from other galactosyltranferases in calf thymus that act on GalNAc-R in ovine submaxillary asialo mucin and on GlcNAc. During the analysis of the products of the α1,3 galactosyltransferase, it was noted that the added Gal residue was cleaved by some β galactosidases due to their contamination with α galactosidase activity, often barely detectable against *p*-NO_2-phenyl-α-galactoside (230). This prompted the authors to suggest that the Galβ1–3Galβ1–4 GlcNAc- sequences previously reported to occur on calf thymus membrane glycoproteins (231, 232), in which the terminal linkage had been assigned as β on the basis of its susceptibility to cleavage by β galactosidase, should be reevaluated.

OTHER POSTTRANSLATIONAL MODIFICATIONS

Asparagine-linked oligosaccharides may undergo further posttranslational modifications, including phosphorylation of mannose residues, sulfation of mannose and *N*-acetylhexosamine residues, and O-acetylation of sialic acid residues. Lysosomal hydrolases acquire phosphomannosyl residues which serve as the essential component of a recognition marker which allows binding

to a specific receptor (the Man 6-P receptor) in the Golgi and subsequent translocation to lysosomes (233). This recognition marker is generated by the sequential action of two Golgi enzymes (Figure 3). First, *N*-acetylglucosaminylphosphotransferase transfers *N*-acetylglucosamine 1-phosphate from UDP-GlcNAc to selected mannose residues of high mannose oligosaccharides and then *N*-acetylglucosamine 1-phosphodiester α-*N*-acetylglucosaminidase removes the *N*-acetylglucosamine residue to expose the phosphomannosyl group (234). The phosphates may be linked to five different mannose residues on the oligosaccharide and individual molecules may contain one or two phosphates (234). While oligosaccharides with phosphomonoesters are the physiologic ligands for binding to the receptor (11, 235–237), lysosomal enzymes containing several oligosaccharides with phosphodiesters have a weak interaction with the receptor (238). Partially purified *N*-acetylglucosaminylphosphotransferase phosphorylates lysosomal enzymes at least 100-fold better than nonlysosomal glycoproteins which contain identical high mannose–type oligosaccharides (239, 240). Isolated high mannose oligosaccharides and glycopeptides are extremely poor substrates for the enzyme, indicating that the high affinity interaction between the transferase and lysosomal enzymes is mediated primarily by protein-protein interactions. This has been shown directly by demonstrating that deglycosylated lysosomal enzymes are potent inhibitors of the phosphorylation of intact lysosomal enzymes (241). Based on these data it has been proposed that the *N*-acetylglucosaminylphosphotransferase recognizes a protein domain that is common to all lysosomal enzymes, but is absent in nonlysosomal glycoproteins (239–241); however, the identity of this common protein domain is unknown. Fibroblasts from patients with the lysosomal enzyme storage diseases, I-cell disease (mucolipidosis II), and pseudo-Hurler polydystrophy (mucolipidosis III) are severely deficient in this enzyme activity (233, 234). Their inability to generate phosphomannosyl residues prevents the receptor-mediated targeting of newly synthesized acid hydrolases to lysosomes, and consequently the enzymes are secreted into the extracellular milieu. Cell fusion studies indicate that there are three complementation groups among patients with mucolipidosis III (241). Fibroblast extracts of all three groups have very low *N*-acetylglucosaminylphosphotransferase activity toward lysosomal enzyme acceptors, but in one group there is nearly normal activity toward the substrate analog, α-methylmannoside (242, 243). To explain this result, it was proposed that the *N*-acetylglucosaminylphosphotransferase contains a binding site, distinct from the catalytic site, which is involved in the specific recognition of lysosomal enzymes, and that this site is selectively altered by the mutation (243). High mannose units with Man-6-P are also present on lysosomal enzymes of the slime mold *Dictyostelium discoideum* (244, 245), but almost all of these residues are in the form of an acid-stable diester which has been identified as methylphosphomannose (246). The pathway by which this diester is synthesized is unknown.

Another recognition system involving Man-6-P has been detected in the embryonic chick-neural retina where a family of glycoproteins bind to the cell-surface baseplate protein, called ligatin (247). These glycoproteins contain high mannose oligosaccharides with Man-6-P residues in diester linkage to the 1-carbon of terminal glucose residues (248). The binding to ligatin is mediated by the diester rather than the monoester as in the case of the Man-6-P receptor. A UDP-glucose: glycoprotein glucose 1-phosphotransferase has been detected in homogenates of embryonic chicken neural retina (249). Thus this diester is synthesized in a fashion analogous to the transfer of GlcNAc-P to mannose residues of lysosomal enzymes. Presumably in this system the glucose is not removed since the specificity of the ligatin is for the phosphodiester (248). Hen oviduct contains an enzyme which transfers Gal-1-P from UDP-Gal to UDP-GlcNAc to form UDP-GlcNAc-6-P-Gal (250). The latter compound had previously been isolated from hen oviduct, but its physiologic function is not known. The enzyme was also detected in the liver, ovary, and uterus of the laying hen.

A variety of secretory and membrane glycoproteins have been shown to contain sulfate residues on their N-linked oligosaccharides (12, 245, 251–260). In calf thyroid plasma membrane glycoproteins, the asparagine-linked GlcNAc is 6-O-sulfated and the outer GlcNAc residues are 4-O-sulfated (256). The glycoproteins of paramyxovirus SV5 also contain 6-O-sulfate residues on their innermost GlcNAc residues (255). A small fraction (3%) of ovalbumin contains an unusual hybrid-type species in which one of the outer mannose residues is 4-O-sulfated (12). Several pituitary glycoproteins, including bovine lutropin (LH) and thyroid stimulating hormone (TSH), contain, on their α-subunits, biantennary complex-type oligosaccharides with 4-O-sulfated GlcNAc and GalNAc residues at the two nonreducing termini (257–259). By contrast, placental hCG, which shares the common α subunit, lacks SO_4 and has terminal sialic acid residues indicating that the sulfation reaction may be tissue specific (261, 262). The sulfation of the pituitary glycoprotein hormones also differs from most of the other examples in that the oligosaccharides have a full residue of SO_4 per hexosamine whereas in other glycoproteins the substitution is incomplete (253, 255, 256). The high mannose units of the *D. discoideum* lysosomal enzymes contain SO_4 in addition to methylphosphate residues (245). The SO_4, which appears to be present on both the *N*-acetylglucosamine and mannose residues, has been identified as the "common" antigen of these proteins (263).

CONTROL OF OLIGOSACCHARIDE PROCESSING

An analysis of oligosaccharide structures on the same protein from different species and even different tissues reveals that major variations frequently exist (24, 29, 32, 264–270). It is evident from the preceding discussion that a key

factor in determining the synthesis of particular N-linked oligosaccharides is the level of expression of the various glycosyltransferases. Differences in the relative activity of these enzymes among species and tissues can account for many of the variations in oligosaccharide structures that occur. But beyond this another level of control must be operative in individual cells. Although most cells have the capacity to process the common protein-bound oligosaccharide precursor to a great number of structures ranging from simple high mannose units to extended complex-type units and potentially could produce such an array at any particular asparagine that is glycosylated, this type of gross heterogeneity is not common. Rather, individual glycosylation sites tend to have characteristic oligosaccharides, and when heterogeneity is encountered, it usually consists of a family of closely related oligosaccharides which may differ from oligosaccharides at another site on the same protein (271). The factors which control this level of oligosaccharide processing are beginning to be understood. One determinant of oligosaccharide processing is the location of the protein in the cell. Thus resident ER glycoproteins, not having been exposed to the Golgi processing enzymes, would be expected to have only high mannose units. In fact, HMG-CoA reductase has $Man_8GlcNAc_2$ and $Man_6GlcNAc_2$ units (124). Oligosaccharide processing of proteins that exit the ER is controlled by a number of factors, including the conformation of the polypeptide backbone. This was demonstrated by analyzing the types of oligosaccharides present on closely related viral glycoproteins isolated from the same host cells. Since the virus utilizes the host cell processing enzymes, any difference in oligosaccharide structure should reflect differences in the viral glycoproteins. Hunt et al (10) found that the G protein of the Indiana serotype of vesicular stomatitis virus (VSV) contains sialylated complex-type oligosaccharides at both its glycosylation sites when grown in baby hamster kidney cells. In contrast, the Hazelhurst subtype of the New Jersey serotype of VSV contained a similar unit at one site, but a mixture of hybrid and high mannose (predominantly $Man_{5-6}GlcNAc_2$) species at the other site. Similar differences in oligosaccharide processing were observed when closely related murine leukemia viruses were grown in a number of hosts under various growth conditions (272). The simplest explanation for these findings is that differences in the conformation of these closely related viral glycoproteins influences the extent of processing. Further evidence that protein structure influences oligosaccharide processing has been obtained by incorporating the amino acid analogs β-hydroxyleucine (leucine analog) and 4-thioisoleucine (isoleucine analog) into the immunoglobulin light chain synthesized by MOPC-46B cells (273). The incorporation of these analogs prevented the normal processing of the high mannose precursor to a complex-type unit without significantly decreasing the secretion of the protein.

Some insight into one mechanism by which polypeptide structure influences oligosaccharide processing has come from studies using endo H to probe the

relative accessibility of high mannose units on native and denatured glycoproteins. Since this enzyme cleaves the chitobiose unit of the inner core, it can be used to probe the exposure of an oligosaccharide. Three different studies have shown that the extent of oligosaccharide processing correlates with the susceptibility to endo H cleavage. Hsieh et al grew Sindbis virus in a clone of Chinese hamster ovary cells that is deficient in *N*-acetylglucosaminyltransferase I (274). In this cell line the formation of complex-type units is blocked at the $Man_5GlcNAc_2$ stage so that all the N-linked oligosaccharides remain endo H-sensitive. For both Sindbis glycoproteins, endo H preferentially cleaved the oligosaccharides located at the sites that normally contain complex-type units. The selectivity of the endo H cleavage was lost when viruses were digested with pronase or incubated with detergent. Similarly, treatment of yeast carboxypeptidase Y and invertase with endo H released the highly processed large, phosphorylated oligosaccharides ($Man_{11-18}GlcNAc$) whereas the smaller, less processed species ($Man_{8-12}GlcNAc$ with no phosphate) were inaccessible to endo H until the proteins were denatured (275). Finally, treatment of human spleen β-glucuronidase with endo H released the most processed oligosaccharides (the phosphorylated high mannose species and $Man_{5-7}GlcNAc$) whereas the $Man_9GlcNAc_2$ units were only released after the protein was denatured (276). These findings indicate that physical accessibility of the oligosaccharide to processing enzymes can control its processing. The interaction of protein subunits may also influence oligosaccharide processing by this same mechanism. For example, the association of the two heavy chains of IgG places the two oligosaccharide units in direct contact with each other, leading to carbohydrate-carbohydrate interactions as well as protein-carbohydrate interactions (207). It has been postulated that the specific pairing of these oligosaccharides dictates the extent of terminal glycosylation.

There is also evidence that host-dependent factors influence the extent of oligosaccharide processing. Hsieh et al (277) examined the Asn-linked oligosaccharides at the individual glycosylation sites of the two Sindbis virus glycoproteins in virus grown in three different host cells. They found that one of the two glycosylation sites in E1 had exclusively complex-type oligosaccharides regardless of the host cell type. However the other site had a high mannose unit on virus grown in chick embryo fibroblasts, a complex-type unit in virus from baby hamster kidney cells, and both complex and high mannose-type units in virus from Chinese hamster ovary cells. A significant amount of the high mannose units were processed to the $Man_5GlcNAc_2$ species, suggesting that the next processing step, the addition of GlcNAc by *N*-acetylglucosaminyltransferase I, occurs more slowly at some glycosylation sites than at others. Similar host-dependent variation in oligosaccharide processing of viral glycoproteins has been noted by others (278–281). Hsieh et al pointed out that these differences in processing among cell types could be due to intrinsic differences in the cellular processing enzymes, differences in the duration

of intracellular transit of glycoproteins, or differences in the physical accessibility of oligosaccharides during processing (277). Williams & Lennarz have obtained evidence that indicates that the substrate specificities of the processing enzymes can differ between tissues and/or species (282). They found that rat liver Golgi membranes could process the high mannose units of native bovine RNase B (mainly $Man_5GlcNAc_2$) to complex-type units whereas bovine pancreas Golgi membranes failed to do so unless the RNase B was denatured. Presumably one or more of the processing enzymes of the bovine pancreas (presumably *N*-acetylglucosaminyltransferase I and/or α-mannosidase II) are constrained by some aspect of the native conformation of RNase B that is not inhibitory to the same processing enzymes of the rat liver Golgi. These findings indicate that the control of processing involves more sophisticated mechanisms than simple exposure of oligosaccharide chains.

It may be that interaction of protein signals with certain glycosyltransferases which mediate the terminal steps in oligosaccharide assembly determines which of the many possible structures the mature oligosaccharide will have. This would be analogous to the specific recognition of a protein determinant on lysosomal enzymes by *N*-acetylglucosaminylphosphotransferase. Alternatively, differences in the interaction of the oligosaccharide with the underlying peptide may determine the nature of the final structure. In this case accessibility rather than a specific signal would be the critical factor.

Pollack & Atkinson noted that the location of glycosylation sites in the polypeptide chain correlates with processing (283). Their survey of glycoproteins revealed that glycosylation sites in the first 100 amino acid residues were enriched in complex-type units whereas high mannose units predominated at sites at amino acid residue 200 or higher. The mechanism whereby the location of an oligosaccharide along the linear polypeptide chain influences processing is unclear since, in most instances, the polypeptide will have folded by the time it enters the Golgi.

Literature Cited

1. Hubbard, S. C., Ivatt, R. J. 1981. *Ann. Rev. Biochem.* 50:555–83
2. Kobata, A. 1984. In *Biology of Carbohydrates*, ed. V. Ginsburg, P. W. Robbins, 2:87–161. New York: Wiley-Interscience
3. Vliegenthart, J. F. G., Dorland, L., van Halbeek, H. 1983. *Adv. Carbohydr. Chem. Biochem.* 41:209–374
4. Carver, J. P., Brisson, J. R. 1984. See Ref. 2, pp. 289–331
5. Montreuil, J. 1980. See Ref. 3, 37:157–223
6. Snider, M. D. 1984. See Ref. 2, pp. 163–98
7. Schachter, H., Narasimhan, S., Gleeson, P., Vella, G. 1983. *Can. J. Biochem. Cell Biol.* 61:1049–66
8. Beyer, T. A., Sadler, J. E., Rearick, J. I., Paulson, J. C., Hill, R. L. 1981. *Adv. Enzymol. Relat. Areas Mol. Biol.* 52:23–175
9. Staneloni, R. J., Leloir, L. F. 1982. *Crit. Rev. Biochem.* 12:289–326
9a. Presper, K., Heath, E. C. 1983. In *The Enzymes*, ed. P. D. Boyer, 16:449. Academic
10. Hunt, L. A., Davidson, S. K., Golemboski, D. B. 1983. *Arch. Biochem. Biophys.* 226:347–56

11. Varki, A., Kornfeld, S. 1983. *J. Biol. Chem.* 258:2808–18
12. Yamashita, K., Veda, I., Kobata, A. 1983. *J. Biol. Chem.* 258:14144–47
13. Paz-Parente, J., Wieruszeski, J. M., Strecker, G., Montreuil, J., Fournet, B., et. al. 1982. *J. Biol. Chem.* 257:13173–76
14. Yamashita, K., Kammerling, J. P., Kobata, A. 1983. *J. Biol. Chem.* 258: 3099–106
15. Paz-Parente, J., Strecker, G., Leroy, Y., Montreuil, J., Fournet, B., et. al. 1983. *FEBS Lett.* 152:145–52
16. Francois-Gerard, C., Pierce-Cretel, A., Andre, A., Dorland, L., van Halbeek, H., et. al. 1983. In *Glycoconjugates, Proc. 7th Int. Symp. Glycoconjugates,* ed. M. A. Chester, D. Heinegard, A. Lundblad, S. Svensson, pp. 169–70. Sweden: Lund-Ronneby
17. Spik, G., Strecker, G., Fournet, B., Bouquelet, S., Montreuil, J., et. al. 1982. *Eur. J. Biochem.* 121:413–19
18. Matsumoto, A., Yoshima, J., Takasaki, S., Kobata, A. 1982. *J. Biochem.* 91: 143–55
19. Dorland, L., Haverkamp, J., Schut, B., Vliegenthart, J. F. G., Spik, G., et. al. 1977. *FEBS Lett.* 77:15–20
20. Paulson, J. C., Prieels, J.-P., Glasgow, L. R., Hill, R. L. 1978. *J. Biol. Chem.* 253:5617–24
21. Beyer, T. A., Rearick, J. I., Paulson, J. C., Prieels, J.-P., Sadler, J. E., Hill, R. L. 1979. *J. Biol. Chem.* 254:12531–41
22. Eckhardt, A. E., Goldstein, I. J. 1983. *Biochemistry* 22:5290–97
23. Dorland, L., van Halbeek, H., Vliegenthart, J. F. G. 1984. *Biochem. Biophys. Res. Commun.* 122:859–66
24. Spiro, R. G., Bhoyroo, V. D. 1984. *J. Biol. Chem.* 259:9858–66
25. Finne, J. 1982. *J. Biol. Chem.* 257: 11966–70
26. Rothbard, J. B., Brackenbury, R., Cunningham, B. A., Edelman, G. M. 1982. *J. Biol. Chem.* 257:11064–69
27. Finne, J., Finne, U., Deagostini-Bazin, H., Goridis, C. 1983. *Biochem. Biophys. Res. Commun.* 112:482–87
28. Margolis, R. K., Margolis, R. V. 1983. *Biochem. Biophys. Res. Commun.* 116: 889–94
29. Takasaki, S., Yamashita, K., Suzuki, K., Iwanaga, S., Kobata, A. 1979. *J. Biol. Chem.* 254:8548–53
30. Mizuochi, T., Taniguchi, T., Fujikawa, K., Titani, K., Kobata, A. 1983. *J. Biol. Chem.* 258:6020–24
31. Mizuochi, T., Yamashita, K., Fujikawa, K., Titani, K., Kobata, A. 1980. *J. Biol. Chem.* 255:3526–31
32. Mizuochi, T., Yamashita, K., Fujikawa, K., Kisiel, W., Kobata, A. 1979. *J. Biol. Chem.* 254:6419–25
33. Krusius, T., Finne, J., Rauvala, H. 1978. *Eur. J. Biochem.* 92:289–300
34. Jarnefelt, J., Rush, J., Li, Y.-T., Laine, R. A. 1978. *J. Biol. Chem.* 253:8006–9
35. Childs, R. A., Feizi, T., Fukuda, M., Hakomori, S. I. 1978. *Biochem. J.* 173:333–36
36. Fukuda, M., Dell, A., Fukuda, M. N. 1984. *J. Biol. Chem.* 259:4782–91
37. Fukuda, M., Dell, A., Oates, J. E., Fukuda, M. N. 1984. *J. Biol. Chem.* 259:8260–73
38. Spooncer, E., Fukuda, M., Klock, J. C., Oates, J. E., Dell, A. 1984. *J. Biol. Chem.* 259:4792–801
39. Huang, L. C., Civin, C. I., Magnani, J. L., Shaper, J. H., Ginsburg, V. 1983. *Blood* 61:1020–23
40. Gooi, H. C., Feizi, T., Kapadia, A., Knowles, B. B., Solter, D., Evans, M. J. 1981. *Nature* 292:156–58
41. Kannagi, R., Nudelman, E., Levery, S. B., Hakomori, S. 1982. *J. Biol. Chem.* 257:14865–74
42. Nilsson, B., Nakazawa, K., Hassell, J. R., Newsome, D. A., Hascall, V. C. 1983. *J. Biol. Chem.* 258:6056–63
43. Nakazawa, K., Newsome, D. A., Nilsson, B., Hascall, V. C., Hassell, J. R. 1983. *J. Biol. Chem.* 258:6051–55
44. Hassell, J. R., Newsome, D. A., Hascall, V. C. 1979. *J. Biol. Chem.* 254:12346–54
45. Roden, L. 1980. In *The Biochemistry of Glycoproteins and Proteoglycans.* ed. W. J. Lennarz, pp. 267–371. New York: Plenum
46. Keller, R., Stein, T., Stuhlsatz, H. W., Greiling, H., Ohst, E., Muller, E., Scharf, H.-D. 1981. *Hoppe-Seyler's Z. Physiol. Chem.* 362:327–36
47. Stein, T., Keller, R., Stuhlsatz, H. W., Greiling, H., Ohst, E., Muller, E., Scharf, H.-D. 1982. *Hoppe-Seyler's Z. Physiol. Chem.* 363:825–33
48. Bergman, L. W., Kuehl, W. M. 1978. *Biochemistry* 17:5174–80
49. Roberts, J. L., Phillips, M., Rosa, P. A., Herbert, E. 1978. *Biochemistry* 17:3609–18
50. Ronnett, G. O., Lane, M. D. 1981. *J. Biol. Chem.* 256:4704–7
51. Katz, F. W., Rothman, J. E., Lingappa, V. R., Blobel, G., Lodish, H. F. 1977. *Proc. Natl. Acad. Sci. USA* 74:3278–82
52. Lingappa, V. R., Lingappa, J. R., Prasad, R., Ebner, K. E., Blobel, G. 1978. *Proc. Natl. Acad. Sci. USA* 75:2338–42
53. Hanover, J. A., Lennarz, W. J. 1980. *J. Biol. Chem.* 255:3600–4

54. Carey, D. J., Sommers, L. W., Hirschberg, C. B. 1980. *Cell* 19:597–605
55. Hanover, J. A., Lennarz, W. J. 1982. *J. Biol. Chem.* 257:2787–94
56. Snider, M. D., Rogers, O. C. 1984. *Cell* 36:753–61
57. Snider, M. D., Robbins, P. W. 1982. *J. Biol. Chem.* 257:6796–801
58. McCloskey, M. A., Troy, F. A. 1980. *Biochemistry* 19:2061–66
59. Haselbeck, A., Tanner, W. 1982. *Proc. Natl. Acad. Sci. USA* 79:1520–24
60. Snider, M. D., Sultzman, L. A., Robbins, P. W. 1980. *Cell* 21:385–92
61. Nilsson, O. S., DeTomas, M. E., Peterson, E., Bergman, A., Dallner, G., Hemming, F. W. 1978. *Eur. J. Biochem.* 89:619–28
62. Bergman, A., Dallner, G. 1978. *Biochem. Biophys. Acta* 512:123–35
63. Eggens, I., Dallner, G. 1980. *FEBS Lett.* 122:247–50
64. Hanover, J. A., Lennarz, W. J. 1979. *J. Biol. Chem.* 254:9237–46
65. Turco, S. J., Stetson, B., Robbins, P. W. 1977. *Proc. Natl. Acad. Sci. USA* 74:4411–14
66. Spiro, M. J., Spiro, R. G., Bhoyroo, V. D. 1979. *J. Biol. Chem.* 254:7668–74
67. Staneloni, R. J., Ugalde, R. A., Leloir, L. F. 1980. *Eur. J. Biochem.* 105:275–78
68. Murphy, L. A., Spiro, R. G. 1981. *J. Biol. Chem.* 256:7487–94
69. Staneloni, R. J., Tolmasky, M. E., Petriella, C., Leloir, L. F. 1981. *Plant Physiol.* 68:1175–79
70. Trimble, R. B., Byrd, J. C., Maley, F. 1980. *J. Biol. Chem.* 255:11892–95
71. Sharma, C. B., Lehle, L., Tanner, W. 1981. *Eur. J. Biochem.* 116:101–8
72. Lehle, L., Bause, E. 1984. *Biochem. Biophys. Acta* 799:246–51
73. Huffaker, T. C., Robbins, P. W. 1983. *Proc. Natl. Acad. Sci. USA* 80:7466–70
74. Runge, K. W., Huffaker, T. C., Robbins, P. W. 1984. *J. Biol. Chem.* 259:412–17
75. Chen, W. W., Lennarz, W. J. 1977. *J. Biol. Chem.* 252:3473–79
76. Harford, J. B., Waechter, C. J. 1979. *Arch. Biochem. Biophys.* 197:424–35
77. Nakayama, K., Araki, Y., Ito, E. 1976. *FEBS Lett.* 72:287–90
78. Hoflack, B., Debeire, P., Cacan, R., Montreuil, J., Verbert, A. 1982. *Eur. J. Biochem.* 124:527–31
79. Parodi, A. J., Quesada-Allue, L. A. 1982. *J. Biol. Chem.* 257:7637–40
80. Parodi, A. J., Cazzulo, J. J. 1982. *J. Biol. Chem.* 257:7641–45
81. Parodi, A. J., Lederkremer, G. Z., Mendelzon, D. H. 1983. *J. Biol. Chem.* 258:5589–95
82. Parodi, A. J., Quesada-Allue, L. A., Cazzulo, J. J. 1981. *Proc. Natl. Acad. Sci. USA* 78:6201–5
83. Katial, A., Prakash, C., Vijay, I. K. 1984. *Eur. J. Biochem.* 141:521–26
84. Parodi, A., Martin-Barrientos, J. 1984. *Biochem. Biophys. Res. Commun.* 118:1–7
85. Krag, S. S. 1979. *J. Biol. Chem.* 254:9167–77
86. Hoflack, B., Cacan, R., Verbert, A. 1981. *Eur. J. Biochem.* 117:285–90
87. Bause, E., Muller, T., Jaenicke, L. 1983. *Arch. Biochem. Biophys.* 220:200–7
88. Muller, T., Bause, E., Jaenicke, L. 1984. *Eur. J. Biochem.* 138:153–59
89. Trowbridge, I. S., Hyman, R. 1979. *Cell* 17:503–8
90. Chapman, A., Trowbridge, I. S., Hyman, R., Kornfeld, S. 1979. *Cell* 17:509–15
91. Stoll, J., Robbins, A. R., Krag, S. S. 1982. *Proc. Natl. Acad. Sci. USA* 79:2296–300
92. Chapman, A., Li, E., Kornfeld, S. 1979. *J. Biol. Chem.* 254:10243–49
93. Hunt, L. A. 1980. *Cell* 21:407–15
94. Gershman, H., Robbins, P. W. 1981. *J. Biol. Chem.* 256:7774–80
95. Rearick, J. I., Chapman, A., Kornfeld, S. 1981. *J. Biol. Chem.* 256:6255–61
96. Turco, S. J. 1980. *Arch. Biochem. Biophys.* 205:330–39
97. Datema, R., Schwarz, R. T. 1981. *J. Biol. Chem.* 256:11191–98
98. Spiro, R. G., Spiro, M. J., Bhoyroo, V. D. 1983. *J. Biol. Chem.* 258:9469–76
99. Marshall, R. D. 1972. *Ann. Rev. Biochem.* 41:673–702
100. Marshall, R. D. 1974. *Biochem. Soc. Symp.* 40:17–26
100a. Stenflo, J., Fernlund, P. 1982. *J. Biol. Chem.* 257:12180–90
101. Struck, D. K., Lennarz, W. J., Brew, K. 1978. *J. Biol. Chem.* 253:5786–94
102. Hart, G. W., Brew, K., Grant, G. A., Bradshaw, R. A., Lennarz, W. J. 1979. *J. Biol. Chem.* 254:9747–53
103. Bause, E., Lehle, L. 1979. *Eur. J. Biochem.* 101:531–40
104. Ronin, C., Granier, C., Caseti, C., Bouchilloux, S., van Rietschoten, J. 1981. *Eur. J. Biochem.* 118:159–64
105. Welply, J. K., Shenbagamurthi, P., Lennarz, W. J., Naider, F. 1983. *J. Biol. Chem.* 258:11856–63
106. Aubert, J. P., Helbecque, N., Loucheux-Lefebvre, M. H. 1981. *Arch. Biochem. Biophys.* 208:20–29

107. Ronin, C., Aubert, J. P. 1982. *Biochem. Biophys. Res. Commun.* 105:909–15
108. Bause, E., Legler, G. 1981. *Biochem. J.* 195:639–44
109. Bause, E. 1983. *Biochem. J.* 209:331–36
110. Aubert, J. P., Biserte, G., Loucheux-Lefebvre, M. H. 1976. *Arch. Biochem. Biophys.* 175:410–18
111. Beeley, J. G. 1977. *Biochem. Biophys. Res. Commun.* 76:1051–55
112. Lau, J. T. Y., Welply, J. K., Shenbagamurthi, P., Naider, F., Lennarz, W. J. 1983. *J. Biol. Chem.* 258:15255–60
113. Kronquist, K. E., Lennarz, W. J. 1978. *J. Supramol. Struct.* 8:51–65
114. Plummer, T. H., Hirs, C. H. W. 1964. *J. Biol. Chem.* 239:2530–38
115. Anderson, D. R., Samaraweera, P., Grimes, W. J. 1983. *Biochem. Biophys. Res. Commun.* 116:771–76
116. McCune, J., Fu, S., Kunkel, H., Blobel, G. 1981. *Proc. Natl. Acad. Sci. USA* 78:5127–31
117. Pless, D. D., Lennarz, W. J. 1977. *Proc. Natl. Acad. Sci. USA* 74:134–38
118. Carson, D. D., Earles, B. J., Lennarz, W. J. 1981. *J. Biol. Chem.* 256:11552–57
118a. Oda-Tamai, S., Kato, S., Hara, S., Akamatsu, N. 1985. *J. Biol. Chem.* 260:57–63
118b. Franc, J.-L., Hovsepian, S., Fayet, G., Bouchilloux, S. 1984. *Biochem. Biophys. Res. Commun.* 118:910–15
118c. Singh, B. N., Lucas, J. J. 1981. *J. Biol. Chem.* 256:12018–22
119. Aubert, J. P., Chiroutre, M., Kerckaert, J. P., Helbecque, N., Loucheux-Lefebvre, M. H. 1982. *Biochem. Biophys. Res. Commun.* 104:1550–59
120. Das, R. C., Heath, E. C. 1980. *Proc. Natl. Acad. Sci. USA* 77:3811–15
121. Atkinson, P. H., Lee, J. T. 1984. *J. Cell Biol.* 98:2245–49
122. Hubbard, S. C., Robbins, P. W. 1979. *J. Biol. Chem.* 254:4568–76
123. Bergman, L. W., Kuehl, W. M. 1982. In *The Glycoconjugates*, ed. M. I. Horowitz, III:81–98. New York: Academic
123a. Compton, T., Courtney, R. 1984. *J. Virology* 52:630–37
124. Liscum, L., Cummings, R. D., Anderson, R. G. W., DeMartino, G. N., Goldstein, J. L., Brown, M. S. 1983. *Proc. Natl. Acad. Sci. USA* 80:7165–69
125. Hickman, S., Theodorakis, J. L., Greco, J. M., Brown, P. H. 1984. *J. Cell Biol.* 98:407–16
126. Gabel, C., Kornfeld, S. 1984. *J. Cell Biol.* 99:296–305
127. Parodi, A. J., Mendelzon, D. H., Lederkremer, G. Z., Martin-Barrientos, J. 1984. *J. Biol. Chem.* 259:6351–57
128. Parodi, A. J., Mendelzon, D. H., Lederkremer, G. Z. 1983. *J. Biol. Chem.* 258:8260–65
129. Jamieson, J. D., Palade, G. E. 1968. *J. Cell Biol.* 39:589–603
130. Lodish, H. F., Kong, N., Snider, M., Strous, G. J. A. M. 1983. *Nature* 304:80–83
131. Fries, E., Gustafsson, L., Peterson, P. A. 1984. *EMBO J.* 3:147–52
132. Lodish, H. F., Kong, N. 1984. *J. Cell Biol.* 98:1720–29
133. Gross, V., Andus, T., Tran-Thi, T.-A., Schwarz, R. T., Decker, K., Heinrich, P. C. 1983. *J. Biol. Chem.* 258:203–9
134. Lemansky, P., Gieselmann, V., Hasilik, A., von Figura, K. 1984. *J. Biol. Chem.* 259:10129–35
135. Schlesinger, S., Malfer, C., Schlesinger, M. J. 1984. *J. Biol. Chem.* 259:7597–601
136. Bergmann, J. E., Singer, S. J. 1983. *J. Cell Biol.* 97:1777–87
137. Rothman, J. E., Urbani, L. J., Brands, R. 1984. *J. Cell Biol.* 99:248–59
138. Rothman, J. E., Miller, R. L., Urbani, L. J. 1984. *J. Cell Biol.* 99:260–71
139. Roth, J., Berger, E. G. 1982. *J. Cell Biol.* 93:223–29
140. Slot, J. W., Geuze, H. J. 1983. *J. Histochem. Cytochem.* 31:1049–56
141. Dunphy, W. G., Rothman, J. E. 1985. *Cell.* 40:463–72
142. Novikoff, P. M., Tulsiani, D. R. P., Touster, O., Yam, A., Novikoff, A. B. 1983. *Proc. Natl. Acad. Sci. USA* 80:4364–68
143. Dunphy, W. G., Fries, E., Urbani, L. J., Rothman, J. E. 1981. *Proc. Natl. Acad. Sci. USA* 78:7453–57
144. Dunphy, W. G., Rothman, J. E. 1983. *J. Cell Biol.* 97:270–75
145. Goldberg, D., Kornfeld, S. 1983. *J. Biol. Chem.* 258:3159–65
146. Deutscher, S. L., Creek, K. E., Merion, M., Hirschberg, C. B. 1983. *Proc. Natl. Acad. Sci. USA* 80:3938–42
147. Griffiths, G., Brands, R., Burke, B., Louvard, D., Warren, G. 1982. *J. Cell Biol.* 95:781–92
148. Tartakoff, A. M., Vassalli, P. 1983. *J. Cell Biol.* 97:1243–48
149. Griffiths, G., Quinn, P., Warren, G. 1983. *J. Cell Biol.* 96:835–50
150. Fries, E., Rothman, J. E. 1981. *J. Cell Biol.* 90:697–704
151. Fries, E., Rothman, J. E. 1980. *Proc. Natl. Acad. Sci. USA* 77:3870–74
152. Lehle, L. 1981. *FEBS Lett.* 123:63–66
153. Hori, H., James, D. W. Jr., Elbein, A. D. 1982. *Arch. Biochem. Biophys.* 215:12–21

154. Hori, H., Elbein, A. D. 1983. *Arch. Biochem. Biophys.* 220:415–25
155. Dorland, L., van Halbeek, H., Vliegenthart, J. F. G., Lis, H., Sharon, N. 1981. *J. Biol. Chem.* 256:7708–11
156. Vitale, A., Chrispeels, M. J. 1984. *J. Cell Biol.* 99:133–40
157. Ishihara, H., Takahashi, N., Oguir, S., Tejima, S. 1979. *J. Biol. Chem.* 254:10715–19
158. Kilker, R. D. Jr., Saunier, B., Tkacz, J. S., Herscovics, A. 1981. *J. Biol. Chem.* 256:5299–303
159. Byrd, J. C., Tarentino, A. L., Maley, F., Atkinson, P. H., Trimble, R. B. 1982. *J. Biol. Chem.* 257:14657–66
160. Parodi, A. J. 1981. *Arch. Biochem. Biophys.* 210:372–82
161. Esmon, B., Esmon, P. C., Schekman, R. 1984. *J. Biol. Chem.* 259:10322–27
161a. Tsai, P.-K., Ballou, L., Esmon, B., Schekman, R., Ballou, C. E. 1984. *Proc. Natl. Acad. Sci. USA* 81:6340–43
162. Ballou, C. E. 1980. In *Fungal Polysaccharides, ACS Symp. Ser.*, ed. P. A. Sandford, D. Matsuda, 126:1–14. Washington: Am. Chem. Soc., 284 pp.
163. Tsai, P. K., Frevert, J., Ballou, C. E. 1984. *J. Biol. Chem.* 259:3805–11
164. Hsieh, P., Robbins, P. W. 1984. *J. Biol. Chem.* 259:2375–82
165. Butters, T. D., Hughes, R. C. 1981. *Biochem. Biophys. Acta* 640:655–71
166. Hettkamp, H., Legler, G., Bause, E. 1984. *Eur. J. Biochem.* 142:85–90
167. Burns, D. M., Touster, O. 1982. *J. Biol. Chem.* 257:9991–10,000
168. Brada, D., Dubach, U. 1984. *Eur. J. Biochem.* 141:149–56
169. Reitman, M. L., Trowbridge, I. S., Kornfeld, S. 1982. *J. Biol. Chem.* 257: 10357–63
170. Grinna, L. S., Robbins, P. W. 1980. *J. Biol. Chem.* 255:2255–58
171. Michael, J. M., Kornfeld, S. 1980. *Arch. Biochem. Biophys.* 199:249–58
172. Spiro, R. G., Spiro, M. J., Bhoyroo, V. D. 1979. *J. Biol. Chem.* 254:7659–67
173. Kornfeld, S., Gregory, W., Chapman, A. 1979. *J. Biol. Chem.* 254:11649–54
174. Bischoff, J., Kornfeld, R. 1983. *J. Biol. Chem.* 258:7907–10
175. Shoup, V. A., Touster, O. 1976. *J. Biol. Chem.* 251:3845–52
176. Tabas, I., Kornfeld, S. 1979. *J. Biol. Chem.* 254:11655–63
177. Tulsiani, D. R. P., Hubbard, S. C., Robbins, P. W., Touster, O. 1982. *J. Biol. Chem.* 257:3660–68
178. Harpaz, N., Schachter, H. 1980. *J. Biol. Chem.* 255:4885–93
179. Oppenheimer, C. L., Hill, R. L. 1981. *J. Biol. Chem.* 256:799–804
180. Vella, G. J., Paulsen, H., Schachter, H. 1984. *Can. J. Biochem. Cell Biol.* 62:409–17
181. Tabas, I., Kornfeld, S. 1978. *J. Biol. Chem.* 253:7779–86
182. Harpaz, N., Schachter, H. 1980. *J. Biol. Chem.* 255:4894–902
183. Tulsiani, D. R. P., Touster, O. 1983. *J. Biol. Chem.* 258:7578–85
184. Allen, S. D., Tsai, D., Schachter, H. 1984. *J. Biol. Chem.* 259:6984–90
185. Elbein, A. D., Solf, R., Dorling, P. R., Vosbeck, K. 1981. *Proc. Natl. Acad. Sci. USA* 78:7393–97
186. Elbein, A. D., Dorling, P. R., Vosbeck, K., Horisberger, M. 1982. *J. Biol. Chem.* 257:1573–76
187. Gross, V., Tran-Thi, T.-A., Vosbeck, K., Heinrich, P. C. 1983. *J. Biol. Chem.* 258:4032–36
188. Kang, M. S., Elbein, A. D. 1983. *J. Virol.* 46:60–69
189. Tulsiani, D. R. P., Harris, T. M., Touster, O. 1982. *J. Biol. Chem.* 257:7936–39
190. Oppenheimer, C. L., Eckhardt, A. E., Hill, R. L. 1981. *J. Biol. Chem.* 256:11477–82
191. Mendicino, J., Chandrasekaran, E. V., Anumula, K. R., Davila, M. 1981. *Biochemistry* 20:967–76
192. Narasimhan, S. 1982. *J. Biol. Chem.* 257:10235–42
193. Campbell, C., Stanley, P. 1984. *J. Biol. Chem.* 259:13370–78
194. Gleeson, P., Schachter, H. 1983. *J. Biol. Chem.* 258:6162–73
195. Cummings, R. D., Trowbridge, I. S., Kornfeld, S. 1982. *J. Biol. Chem.* 257:13421–27
196. Longmore, G. D., Schachter, H. 1982. *Carbohydr. Res.* 100:365–92
197. Howard, D. R., Natowicz, M., Baenziger, J. U. 1982. *J. Biol. Chem.* 257: 10861–68
198. Takahashi, T., Schmidt, P. G., Tang, J. 1983. *J. Biol. Chem.* 258:2819–30
199. Brisson, J. R., Carver, J. P. 1983. *Can. J. Biochem. Cell Biol.* 61:1067–78
200. Rao, A. K., Mendicino, J. 1978. *Biochemistry* 17:5632–38
201. Paquet, M. R., Narasimhan, S., Schachter, H., Moscarello, M. A. 1984. *J. Biol. Chem.* 259:4716–21
202. Blanken, W. M., van Vliet, A., van den Eijnden, D. H. 1984. *J. Biol. Chem.* 259:15131–35
203. Kornfeld, R., Kornfeld, S. 1980. In *The Biochemistry of Glycoproteins and Proteoglycans.*, ed. W. J. Lennarz, pp. 1–34. New York: Plenum
204. Tai, T., Ito, S., Yamashita, K., Mura-

matsu, T., Kobata, A. 1975. *Biochem. Biophys. Res. Commun.* 65:968–74
205. Deisenhofer, J. 1981. *Biochemistry* 20: 2361–70
206. Deisenhofer, J., Colman, P. M., Epp, O., Huber, R. 1976. *Hoppe-Seyler's Z. Physiol. Chem.* 357:1421–34
207. Sutton, B. J., Phillips, D. C. 1983. *Biochem. Soc. Trans.* 11:130–32
208. van den Eijnden, D. H., Joziasse, D. H., Dorland, L., van Halbeek, H., Vliegenthart, J. F. G., Schmid, K. 1980. *Biochem. Biophys. Res. Commun.* 92: 839–45
209. Joziasse, D. H., Schiphorst, W. E. C. M., van den Eijnden, D. H., van Kuik, J. A., van Halbeek, H., Vliegenthart, J. F. G. 1985. *J. Biol. Chem.* 260:714–19
210. Finne, J., Tao, T. W., Burger, M. M. 1980. *Cancer Res.* 40:2580–87
211. Finne, J., Burger, M. M., Prieels, J.-P. 1982. *J. Cell Biol.* 92:277–82
212. Krusius, T., Finne, J. 1978. *Eur. J. Biochem.* 84:395–403
213. Campbell, C., Stanley, P. 1983. *Cell* 35:303–9
214. Johnson, P. H., Yates, A. D., Watkins, W. M. 1981. *Biochem. Biophys. Res. Commun.* 100:1611–18
215. Johnson, P. H., Watkins, W. M. 1982. *Biochem. Soc. Trans.* 10:445–46
216. Prieels, J.-P., Monnom, D., Dolmans, M., Beyer, T. A., Hill, R. L. 1981. *J. Biol. Chem.* 256:10456–63
217. Weinstein, J., de Souza-e-Silva, U., Paulson, J. C. 1982. *J. Biol. Chem.* 257:13835–44
218. Weinstein, J., de Souza-e-Silva, U., Paulson, J. C. 1982. *J. Biol. Chem.* 257:13845–53
219. Paulson, J. C., Weinstein, J., de Souza-e-Silva, U. 1984. *Eur. J. Biochem.* 140:523–30
220. Zielenski, J., Koscielak, J. 1983. *FEBS Lett.* 158:164–68
221. Yates, A. D., Watkins, W. M. 1983. *Carbohydr. Res.* 120:251–68
222. Piller, F., Cartron, J.-P. 1983. *J. Biol. Chem.* 258:12293–99
223. Piller, F., Cartron, J.-P., Maranduba, A., Veyrieres, A., Leroy, Y., Fournet, B. 1984. *J. Biol. Chem.* 259:13385–90
224. Blanken, W. M., Hooghwinkel, G. J. M., van den Eijnden, D. H. 1982. *Eur. J. Biochem.* 127:547–52
225. van den Eijnden, D. H., Winterwerp, H., Smeeman, P., Schiphorst, W. E. C. M. 1983. *J. Biol. Chem.* 258:3435–37
226. Cummings, R. D., Kornfeld, S. 1984. *J. Biol. Chem.* 259:6253–60
227. Yamashita, K., Ohkura, T., Tachibana, Y., Takasaki, S., Kobata, A. 1984. *J. Biol. Chem.* 259:10834–40
228. Blake, D. A., Goldstein, I. J. 1981. *J. Biol. Chem.* 256:5387–93
229. van Halbeek, H., Vliegenthart, J. F. G., Winterwerp, H., Blanken, W. M., van den Eijnden, D. H. 1983. *Biochem. Biophys. Res. Commun.* 110:124–31
230. van den Eijnden, D. H., Blanken, W. M., Winterwerp, H., Schiphorst, W. E. C. M. 1983. *Eur. J. Biochem.* 134:523–30
231. Kornfeld, R. 1978. *Biochemistry* 17: 1415–23
232. Yoshima, H., Takasaki, S., Kobata, A., 1980. *J. Biol. Chem.* 255:10793–804
233. Creek, K. E., Sly, W. S. 1984. In *Lysosomes in Biology and Pathology*, ed. J. T. Dingle, R. T. Dean, W. S. Sly, pp. 63–82. New York: Elsevier
234. Goldberg, D., Gabel, C., Kornfeld, S. 1984. See Ref. 233, pp. 45–62
235. Gabel, C. A., Goldberg, D. E., Kornfeld, S. 1982. *J. Cell Biol.* 95:536–42
236. Fischer, H. D., Creek, K. E., Sly, W. S. 1982. *J. Biol. Chem.* 257:9938–43
237. Natowicz, M., Hallett, D. W., Frier, C., Chi, M., Schlesinger, P. H., Baenziger, J. U. 1983. *J. Cell Biol.* 96:915–19
238. Talkad, V., Sly, W. S. 1983. *J. Biol. Chem.* 258:7345–51
239. Reitman, M. L., Kornfeld, S. 1981. *J. Biol. Chem.* 256:11977–80
240. Waheed, A., Hasilik, A., von Figura, K. 1982. *J. Biol. Chem.* 257:12322–31
241. Lang, L., Reitman, M., Tang, J., Roberts, R. M., Kornfeld, S. 1984. *J. Biol. Chem.* 259:14663–71
242. Mueller, O. T., Honey, N. K., Little, L. E., Miller, A. L., Shows, T. B. 1983. *J. Clin. Invest.* 72:1016–23
243. Varki, A. P., Reitman, M. L., Kornfeld, S. 1981. *Proc. Natl. Acad. Sci. USA* 78:7773–77
244. Freeze, H. H., Miller, A. L., Kaplan, A. 1980. *J. Biol. Chem.* 255:11081–84
245. Freeze, H. H., Yeh, R., Miller, A. L., Kornfeld, S. 1983. *J. Biol. Chem.* 258:14874–79
246. Gabel, C. A., Costello, C. E., Reinhold, V. N., Kurz, L., Kornfeld, S. 1984. *J. Biol. Chem.* 259:13762–69
247. Jakoi, E. R., Marchase, R. B. 1979. *J. Cell Biol.* 80:642–50
248. Marchase, R. B., Koro, L. A., Kelly, C. M., McClay, D. R. 1982. *Cell* 28:813–20
249. Koro, L. A., Marchase, R. B. 1982. *Cell* 31:739–48
250. Nakanishi, Y., Otsu, K., Suzuki, S. 1983. *FEBS Lett.* 151:15–18
251. Heifetz, A., Kinsey, W. H., Lennarz, W. J. 1980. *J. Biol. Chem.* 255:4528–34
252. Heifetz, A., Watson, C., Johnson, A.

R., Roberts, M. K. 1982. *J. Biol. Chem.* 257:13581–86
253. Cummings, R. D., Kornfeld, S., Schneider, W. J., Hobgood, K. K., Tolleshaug, H., et. al. 1983. *J. Biol. Chem.* 258:15261–73
254. Nakamura, K., Compans, R. W. 1978. *Virology* 84:303–19
255. Prehm, P., Scheid, A., Choppin, P. W. 1979. *J. Biol. Chem.* 254:9669–77
256. Edge, A. S., Spiro, R. G. 1984. *J. Biol. Chem.* 259:4710–13
257. Parsons, T. F., Pierce, J. G. 1980. *Proc. Natl. Acad. Sci. USA* 77:7089–93
258. Bedi, G. S., French, W. C., Bahl, O. P. 1982. *J. Biol. Chem.* 257:4345–55
259. Anumula, K. R., Bahl, O. P. 1983. *Arch. Biochem. Biophys.* 220:645–51
260. Hsu, C. H., Kingsbury, D. W. 1982. *J. Biol. Chem.* 257:9035–38
261. Mizuochi, T., Kobata, A. 1980. *Biochem. Biophys. Res. Commun.* 97: 772–78
262. Kessler, M. J., Reddy, M. S., Shah, R. H., Bahl, O. P. 1979. *J. Biol. Chem.* 254:7901–8
263. Freeze, H. H., Mierendorf, R. C., Wunderlich, R., Dimond, R. L. 1984. *J. Biol. Chem.* 259:10641–43
264. Yamashita, K., Hitoi, A., Tateishi, N., Higashi, T., Sakamoto, Y., Kobata, A. 1983. *Arch. Biochem. Biophys.* 225: 993–96
265. Mizuochi, T., Fujii, J., Kisiel, W., Kobata, A. 1981. *J. Biochem.* 90:1023–31
266. Yoshima, H., Matsumoto, A., Mizuochi, T., Kawasaki, T., Kobata, A. 1981. *J. Biol. Chem.* 256:8476–84
267. Mizuochi, T., Nishimura, R., Derappe, C., Taniguchi, T., Hamamoto, T., Tamotsu, H., et. al. 1983. *J. Biol. Chem.* 258:14126–29
268. Takasaki, S., Yamashita, K., Suzuki, K., Kobata, A. 1980. *J. Biochem.* 88:1587–94
269. Fukuda, M., Levery, S. B., Hakomori, S. 1982. *J. Biol. Chem.* 257:6856–60
270. Zhu, B. C-R., Fisher, S. F., Pande, H., Calaycay, J., Shively, J. E., Laine, R. H. 1984. *J. Biol. Chem.* 259:3962–70
271. Anderson, D. R., Grimes, W. J. 1982. *J. Biol. Chem.* 257:14858–64
272. Rosner, M. R., Grinna, L. S., Robbins, P. W. 1980. *Proc. Natl. Acad. Sci. USA* 77:67–71
273. Green, M. 1982. *J. Biol. Chem.* 257:9039–42
274. Hsieh, P., Rosner, M. R., Robbins, P. W. 1983. *J. Biol. Chem.* 258:2555–61
275. Trimble, R. B., Maley, F., Chu, F. K. 1983. *J. Biol. Chem.* 258:2562–67
276. Natowicz, M., Baenziger, J. U., Sly, W. S. 1982. *J. Biol. Chem.* 257:4412–20
277. Hsieh, P., Rosner, M. R., Robbins, P. W. 1983. *J. Biol. Chem.* 258:2548–54
278. Keegstra, K., Sefton, B., Burke, D. J. 1975. *J. Virol.* 16:613–20
279. Burke, D. J., Keegstra, K. 1976. *J. Virol.* 20:676–86
280. Burke, D. J., Keegstra, K. 1979. *J. Virol.* 29:546–54
281. Hunt, L. A. 1981. *Virology* 113:534–43
282. Williams, D. B., Lennarz, W. J. 1984. *J. Biol. Chem.* 259:5105–14
283. Pollack, L., Atkinson, P. H. 1983. *J. Cell Biol.* 97:293–300