CMLS, Cell. Mol. Life Sci. 55 (1999) 368–422
1420-682X/99/030368-55 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Review

Eukaryotic glycosylation: whim of nature or multipurpose tool?*

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Received 2 October 1998; received after revision 17 November 1998; accepted 17 November 1998

Abstract. Protein and lipid glycosylation is a ubiquitous phenomenon. The task of cataloguing the great structural variety of the glycan part has demanded considerable efforts over decades. This patient endeavor was imperative to discern the inherent rules of glycosylation which cannot affirm assumptions on a purely coincidental nature of this type of protein and lipid modification. These results together with theoretical considerations uncover a salient property of oligosaccharides. In comparison with amino acids and nucleotides, monosaccharides excel in their potential to serve as units of hardware for storing biological information. Thus, the view that glycan chains exclusively affect physicochemical properties of the conjugates is indubitably flawed. This original concept has been decisively jolted by the discovery of endogenous receptors (lectins) for distinct glycan epitopes which are as characteristic as a fingerprint or a signature for a certain protein (class) or cell type. Recent evidence documents that these binding proteins are even endowed with the capacity to select distinct low-energy conformers of the often rather flexible oligosaccharides, granting entry to a new level of regulation of ligand affinity by shifting conformer equilibria. The assessment of the details of this recognition by X-ray crystallography, nuclear magnetic resonance spectroscopy, microcalorimetry and custom-made derivatives is supposed to justify a guarded optimism in satisfying the need for innovative drug design in antiadhesion therapy, for example against viral or bacterial infections and unwanted inflammation. This review presents a survey of the structural aspects of glycosylation and of evidence to poignantly endorse the notion that carrier-attached glycan chains can partake in biological information transfer at the level of cell compartments, cells and organs.

Key words. Cell adhesion; drug design; glycan metabolism; glycolipids; glycoproteins; glycosaminoglycans; lectins; recognition.

Introduction

Proteins are ubiquitous compounds in all living organisms with many well-established biological functions. They play important roles, for example as biocatalysts, transport and targeting devices, in establishing cell-type specificity and in various aspects of cell sociology. Posttranslational modification of proteins adds a further level of regulation to their activity and can thus enlarge their capacity to take part in regulatory events. The reversibility of the introduction of small inorganic groups into amino acid side chains such as phosphate allows proteins to be used as molecular switches. Evidently, the capacity of the peptide backbone is not restricted to serving as acceptor for a phosphate group. Another rather common type of posttranslational

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modification is protein glycosylation. A potential indication of a biochemical role of the carrier-immobilized sugar units is furnished by the observation that a second abundant class of biomolecules shares the property of proteins to be decorated with oligosaccharides. Ceramide-based lipids as major constituents of biomembranes can carry attached glycan chains, and the phosphatide phosphatidylinositol with its lipid anchor part and hydrophilic inositol head group is likewise engaged as a means of glycan attachment. Glycoproteins and glycolipids are referred to as glycoconjugates and members of the group of complex carbohydrates. When anchored in cell membranes, their sugar parts are directed into the outer environment and thus constitute the glycocalyx. While the glycan chains of lipids rarely extend beyond a dozen monosaccharide units and therefore remain in close vicinity to the cell surface, those of glycoproteins can protrude further from the cell membrane due to either long glycan chains or linkage to an amino acid at a remote position within the peptide chain.

In the early years of research on these compounds, emphasis had to be laid on the development of analytical tools to allow structure determination as a prerequisite for any taking stock of functional aspects. Now a set of sophisticated and further continuously improved methods is available, consequently making it easier to put the initially puzzling complexity of saccharide sequences on record [1–6]. Only after having pursued structural analysis, which revealed a wide range of glycan structures, could attention be turned to the enzymatic routes of biosynthesis and to the delineation of a putative physiological meaning, these lines of reasoning reflected, for example, in reviews by Roseman [7] and Winterburn and Phelbs [8]. This historical development of the different phases of research on complex carbohydrates is nicely described by a series of authoritative reviews [9–18]. Currently, the enormous strides taken in the area of sugar-mediated recognition suggest that glycan constituents of proteins and lipids establish an alphabetical system, adding to the well-accepted coding importance of nucleic acids and peptide backbones [19, 20]. Citing from the article of Winterburn and Phelbs [8], 'carbohydrates are ideal for generating compact units with explicit informational properties since the permutations on linkages are larger than can be achieved by amino acids, and, uniquely in biological polymers, branching is possible'. Therefore, 'the significance of the glycosyl residues is to impart a discrete recognitional role on the protein' (or any other carrier backbone). This notion has meanwhile continuously received enough support to keep it in the eye of a readership from different areas of life sciences [1, 21–26]. In order to familiarize the reader with this concept, we will summarize the major features of glycan structures, analysis, conformation and biosynthesis, and outline the current concept of the biological role of these fascinating molecules.

The sugar code

The term 'complex carbohydrates' should not solely be thought to connote a technically demanding task of cataloguing structures. Principally, oligomers of any class of biomolecules can establish a coding system for information storage, resembling words in a language. In this sense, structural complexity entails the intriguing capacity to serve as versatile hardware for biological information transfer. The capacities for this duty are not equal for the biochemical coding systems. Amino acids and nucleotides are linked nearly exclusively in

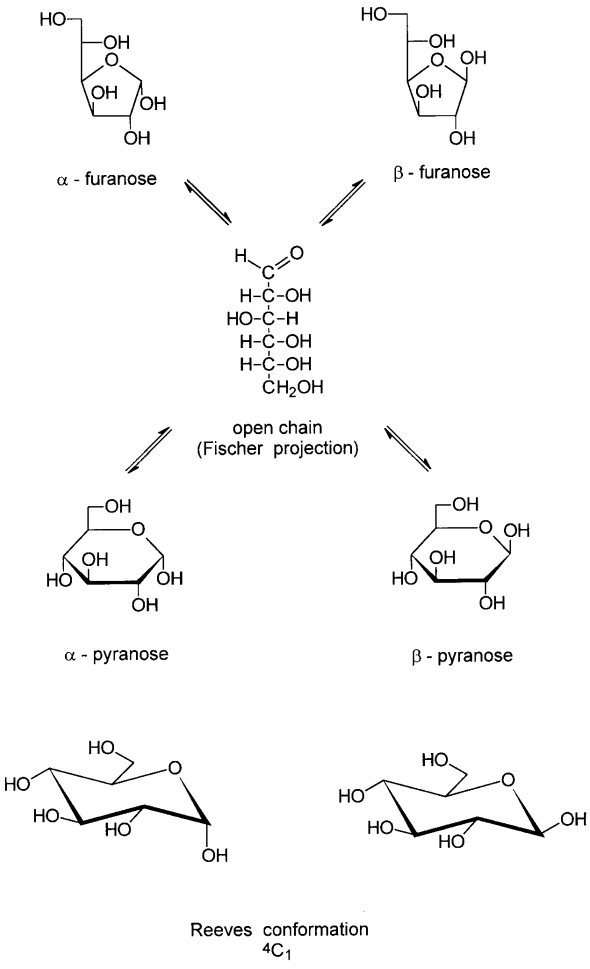

Figure 1. Mutarotation of D-glucose with the different representations of D-glucose as Fischer projection, Haworth projection and Reeves $^4C_1$ chair conformation.

linear chains via peptide or phosphodiester bonds. Only the primary sequence of the monomeric building units in the chains allows for variability in these two cases. This situation changes drastically when oligosaccharides are considered. It is pertinent to recall aspects of the basic biochemistry of sugars to appreciate these extraordinary properties of this type of oligomer, which can reach an unprecedented level of storage capacity.

Textbook biochemistry teaches mutarotation of monosaccharides such as D-glucose primarily from the perspective of a measurable physical parameter (optical rotation). Since linear forms of, for example, any hexose are normally present only in minute amounts, hemiacetals or -ketals are invariably found. The mutarotation of a hexose yields four different ring structures for each monomeric unit, as depicted in figure 1 for D-glucose (Glc). Viewed from the perspective of evaluation of hardware abilities, this result is of relevance. Similarly far-reaching is the fact that each monosaccharide presents a variety of hydroxyl groups. Formation of a bond between two monosaccharide units by condensation can engage diverse possibilities, a daunting task for the organic chemist. When the anomeric center of a monosaccharide participates in the glycosidic bond, each anomer can theoretically react with one of the five, chemically nearly equivalent hydroxyl groups of another hexopyranose unit. Figure 2 shows that permutations on the basis of only these two factors (variations of linkage point and the two anomeric positions) will afford 16 theoretically possible hexopyranose structures with all dimers omitted, in which the two anomeric centers are linked to each other. Sequence permutation doubles this number. In this calculation, the potential for alteration of the ring size (pyranose/furanose, see fig. 1) has not even been incorporated. Using two amino acids or nucleotides, only two different structures can be generated.

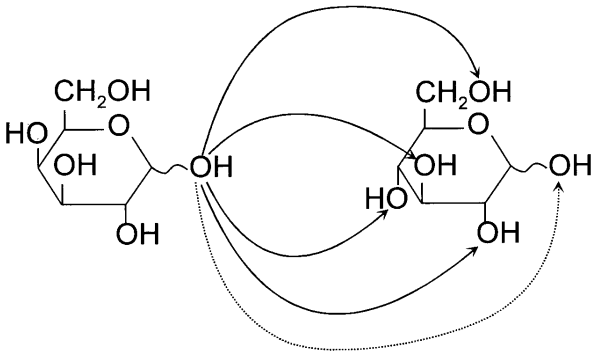

Figure 2. Possible linkage points for a disaccharide consisting of Gal and Glc. The different structures that can be formed result from variations in (i) anomeric configuration (α or β), (ii) ring size, that is furanose or pyranose, with only pyranose depicted in the figure (see fig. 1), (iii) linkage points as indicated by the arrows, with the dotted line for the formation of nonreducing disaccharides involving both anomeric centers, and (iv) the sequence of the two monomers. Excluding linkage via the two anomeric centers, each C1 hydroxyl group can be conjugated to any of the four available hydroxyl groups at C2, C3, C4 and C6 in α- or β-configuration, yielding 8 isomers for each of the two anomeric centers.

If we allow the formation of any linear or branched trisaccharide from a pool of 20 different monosaccharides, we end up with 9×10^6 possibilities, whereas only 8000 tripeptides can be formed from the 20 amino acids [19]. For a hexamer this gap widens to more than seven orders of magnitude. This enormous coding capacity of oligosaccharides renders them highly suitable hardware for information storage. However, it is essential to add that the theoretical number of combinations has by far not been unearthed in nature, probably because the equally high number of corresponding glycosyltransferases is not available in the organism [5, 27–29]. A compensatory diversification is found instead by addition of 'small substituents' such as phosphate, acetate or sulfate [19, 26, 30–38]. As will be illustrated later in several places, single or multiple modifications with such substituents can even occur on one monosaccharide residue, affecting its character as code letter. For example, the 4-sulfation of N-acetyl-D-galactosamine (GalNAc) can be likened to the formation of an umlaut in German language. If now multiple saccharide chains, each containing a set of possible structural variations, are attached to one protein backbone as is the case for many glycoproteins, one can grasp the immediate relevance of these calculations and findings for the theoretical content of information stored in the glycoconjugate.

Posttranslational modification by glycosylation can even lead to the creation of discrete subsets of glycoproteins (glycoforms, according to Rademacher et al. [39]). They share an identical polypeptide sequence but differ in the glycan composition. As will exemplarily be outlined in the section on glycans and disease, monitoring the relative proportions of serum transferrin glycoforms has diagnostic value. It is indicative of clinically defined inherited syndromes or of impairment of liver function by distinct causes. In line with the given reasoning, it is tempting to speculate that this potential is not wasted as a costly whim of nature but rather establishes a multipurpose tool that is taken advantage of in diverse circumstances. Before the functional aspect can be discussed, it is essential to provide an insight into the structural organization of protein and lipid glycosylation.

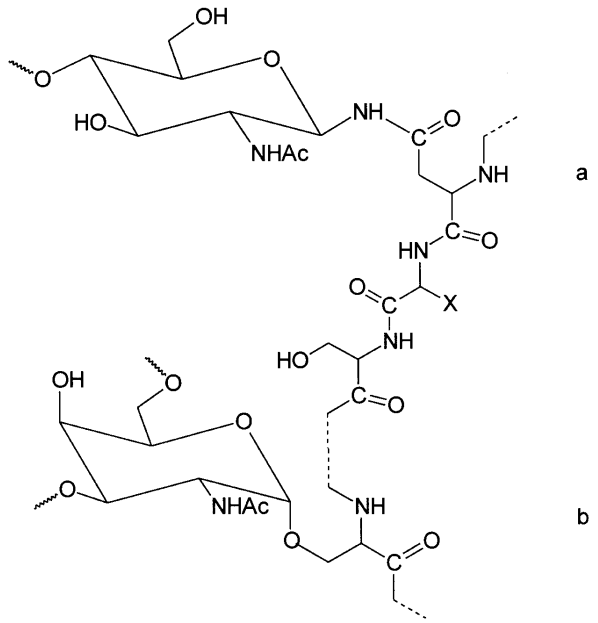

Figure 3. Linkage points for the covalent attachment of common N- and O-glycans to protein. The GlcNAcβ1-Asn-*X*-Ser sequon is characteristic for most N-glycans. The carbohydrate chain is extended at the C-4 of GlcNAc as indicated by the wavy line (*a*); for sites of core fucosylation, see figure 15. The GalNAcα1-Ser structure of mucin-type O-linked glycoproteins can be extended by addition of further saccharides to C-3 and C-6, leading to the introduction of branches (*b*).

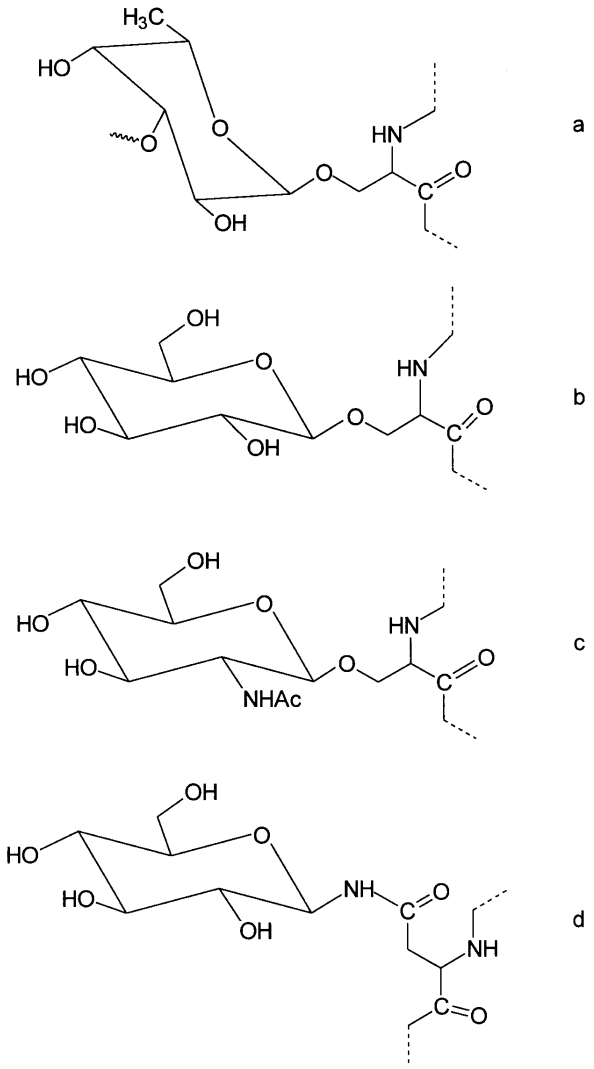

Figure 5. Less common Ser glycosylations are found in the (*a*) -3Fucα1-Ser linkage and (*b*) Glcβ1-Ser linkage in EGF-like domains. Another possible mode of O-glycosylation as well as one of glucose attachment to protein are presented by the linkages (*c*) GlcNAcβ1-Ser and (*d*) Glcβ1-Asn. Type (*c*) is predominantly found in intracellular proteins in the nucleus and cytoplasm.

Glycan attachment to proteins

In glycoproteins, the glycan chains are nearly always covalently linked to suitable functional groups of amino acid side chains within a protein. The elucidation of the structures of the linkage region between carbohydrate and protein was initiated with the description of GlcNAcβ1-Asn for N-glycans and GalNAcα1-Ser or Thr for O-glycans (fig. 3) [1, 10, 25, 26, 40]. Ensuing research considerably extended our knowledge in this area [15, 28, 29, 41, 42]. The current status of delineation of the rules that govern the selection of suitable glycosylation sites in the peptide sequence has enabled explanation of the nonrandom nature of this glycan attachment. It has been recognized that for N-glycosidic linkage a peptide sequence of Asn-X-Ser/Thr (a sequon) is required, in which the variable amino acid X seems to be important for the efficiency of recognition of the resulting sequon [43–45]. Additionally, the amino acid following a sequon (the Y-position in Asn-X-Ser/Thr-

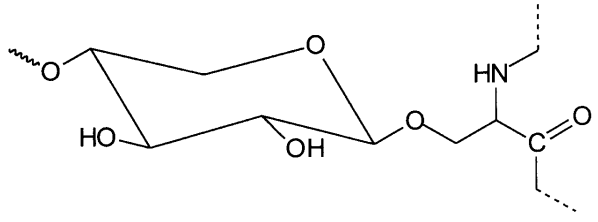

Figure 4. Structure of the -4Xylβ1-Ser linkage of glycosaminoglycans.

Y) has a notable impact on glycosylation efficiency, especially for Asn-X-Ser sequons, Pro, Glu and Trp causing the most negative effect [46, 47]. For human and bovine plasma proteins C, human von Willebrand factor and human CD69 an unusual Asn-X-Cys sequon has been identified for N-glycan attachment [48, 49]. The definition of the sequon allows potential glycosylation sites in any protein to be deduced on the basis of amino acid sequence data. No equivalent peptide sequence requirement has been described so far for classical mucin-type O-glycosylation. The initiation of chain growth by several transferases with varying degrees of stringency of substrate recognition has at least suggested general rules and an algorithm of predictive value [50, 51]. A substantial functional redundancy of these transferases was inferred by mutagenic recombination of one gene with a deletion in the catalytic region, which did not impair O-glycan synthesis [52]. The lack of an O-glycosylation sequon does not mean that the reaction of the uridine diphosphate (UDP)-GalNAc:polypeptide N-acetylgalactosaminyltransferases can take place without sequence requirements. The spatially accessible acceptor amino acid appears to be surrounded preferentially by Ser, Thr, Pro, Ala and Gly residues [28, 29, 50, 53]. Ser/Thr residues not only serve as O-glycan acceptors but also as adaptors for another class of glycans. The third type of complex carbohydrates are the glycosaminoglycans using the glycan-protein linkage 4Xylβ1-Ser/Thr (fig. 4) [54]. Usually, a trisaccharide 4GlcAβ1-3Galβ1-3Galβ1- is attached to the 4-position of Xyl before the typical building blocks of the individual glycosaminoglycan are added [55].

In addition to allowing GalNAc and Xyl attachment, the hydroxyl groups of Ser/Thr are sites for the incorporation of other glycan modifications. Despite their comparatively low frequency they should not be overlooked in this context. On the contrary, they illustrate the existence of cytoplasmic glycosylation pathways independent of these classical routes, each case an economic argument against a merely superfluous expression of the engaged glycosyltransferases. Epidermal growth factor (EGF)-like domains of several glycoproteins including blood coagulation factors VII, IX and XII, plasminogen activators and protein Z are the targets of a sequon-dependent O-fucosylation and O-glucosylation [56, 57]. The enzymatic introduction of α-L-fucose (α-L-Fuc); (fig. 5a) can initiate extension to Glcβ1-3Fucα1-Ser/Thr or to a sialylated N-acetyllactosamine sequence at the 3-position [58, 59]. The sequon dependence of fucosylation in the EGF-like domains of tissue-type plasminogen activator (t-PA) and urokinase was reinforced by the absence of this modification in the human chimeric plasminogen activator K_2tu-PA lacking this sequence element [56, 60]. The consensus sequence for the reaction of this fucosyltransferase is Cys-X-X-Gly-Gly-Ser/Thr-Cys-, which can also be found in other types of proteins besides EGF module-bearing proteins [56, 59]. Once added, protein-bound L-fucose has been intimated to be important for mediating binding of the fucosylated protein to human HepG2 hepatoma cells, because fucose-free EGF-like domains lack this capacity [61]. Since binding and degradation of t-PA is not inhibited by 50 mM L-fucose in the case of human and rat hepatoma cells [62], this suggestion requires further experimental support. Another possibility concerns the

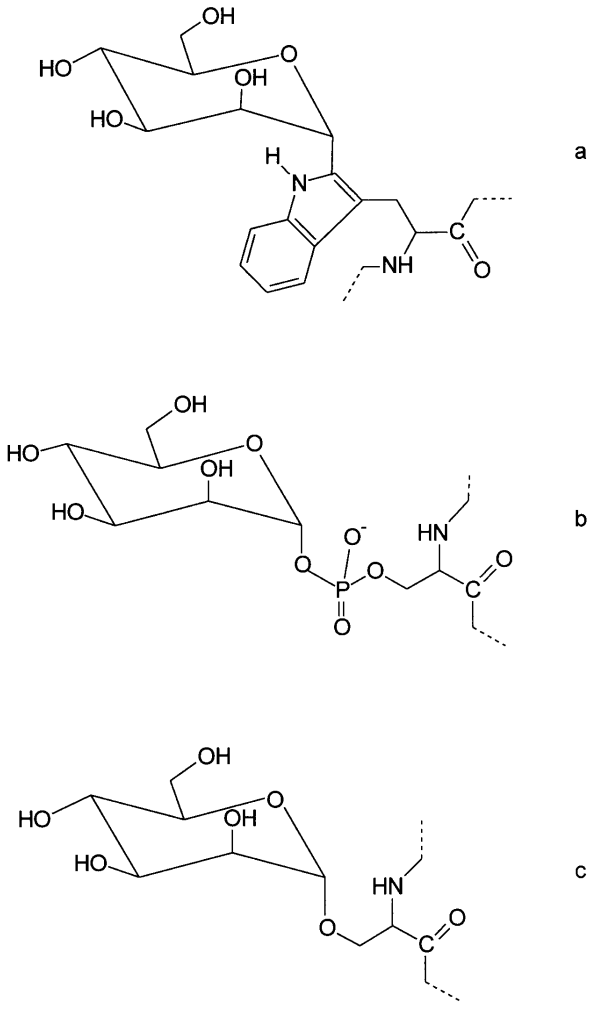

Figure 6. C–C linkage of Man and Trp in human RNase (ribonuclease) U_s (*a*). This is currently the only example known without N- or O-glycosidic linkage between saccharide and protein. Neither biological implications nor enzymatic ways for biosynthesis are known so far. Structure of the Manα1-P-Ser linkage as an example of phosphoglycosylation (*b*). Instead of D-mannose, Fuc, GlcNAc and Xyl may also be found. Manα1-Ser linkage as common glycosylation motif in yeast (*c*).

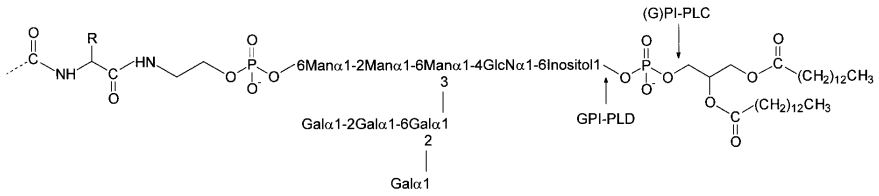

Figure 7. GPI anchor structure. All members of this family of molecules are embedded in the cell membrane via the fatty acids (often C14:0) of the phospholipid part which can comprise ceramide, mono- or diacylglycerol or alkyl/acylglycol. Starting with D-glucosamine, an oligosaccharide that varies structurally among the different glycoproteins is covalently attached to this membrane-embedded anchor part. Incorporation of substituents (saccharides, phosphoethanolamine) at each central D-mannose unit is a major source of structural variability. Via a phosphodiester bridge, ethanolamine is introduced to the α1-2-linked mannose residue of the core glycan. Transamidation follows cleavage of the peptide backbone 10–12 residues away from the hydrophobic C-terminus, which generates the new C-terminus. A protein is attached by its ω-site (preferentially Asp, Asn, Gly, Ala, Ser or Cys), yielding its glypiation. Potential cleavage sites for (G)PI-PLC and GPI-PLD are indicated. Introduction of a modification at the inositol residue will hamper PI-PLC action. (G)PI-PLC/D: (glycosyl) phosphatidylinositol phospholipases C/D.

positive influence of fucosylation at Thr18 on the growth factor activity of the EGF-like domain in human urokinase-type plasminogen activator [63, 64]. As indicated above, the EGF module for example in t-PA does not only harbor the O-fucosylation. It also carries one O-linked glucose unit (fig. 5b) in the consensus sequence Cys-X-Ser-*X*-Pro-Cys of the EGF domain [56, 57]. The glucose moiety may further be substituted at the 3-position by Xylα1-3Xylα1-, as found in blood coagulation factors VII and IX, protein Z, t-PA and bovine thrombospondin [56, 65].

In contrast to the rather restricted appearance of these two modifications [56], a β-linked GlcNAc (fig. 5c) can be attached to an extended array of proteins [66–70]. So far, no elongation of this type of modification has been reported. It is confined to a single monosaccharide linked to the protein, and is present on a steadily growing list of intracellular proteins, for example cytoskeletal constituents such as erythrocyte band 4.1 protein, neurofilaments, cytokeratins 8, 13 and 18, transcription factor Sp1 or c-myc, estrogen receptor and p53 [70, 71]. A reciprocal relationship to phosphorylation is assumed for the reversible formation of GlcNAcβ1-Ser/Thr, as such glycosylation sites may also be targets of protein kinase(s) action. Functional implications also include a role in transcriptional initiation by RNA polymerase II and in cytoskeletal assembly and organization [70–72]. GlcNAcylation is also possible for hydroxyproline in slime molds, where a Gal/Fuc-containing pentasaccharide appears to be the nonimmunogenic product of a hitherto unrecognized cytoplasmic O-glycosylation pathway [73]. The lack of immunogenicity is reason to suspect occurrence of similar structures in mammals. Hydroxyl groups of amino acid side chains can further accommodate conjugation with α-Gal (Ser in plant glycoproteins such as the potato lectin), with α-Glc (Tyr in glycogenin, the primer for glycogen biosynthesis), β-Gal (Hyl in collagen) which can be extended to a disaccharide by α1,2-linked Glc (surprisingly, the presence of this otherwise uncommon disaccharide O-linked to Thr is also known from the surface layer glycoprotein of halobacteria [74, 75]) and α-Man (Ser/Thr in α-dystroglycan which can be extended to the Siaα2-3Galβ1-4GlcNAcβ1-2Man sequence and in yeast glycoproteins) [17, 29, 41, 76–78]. The O-mannosylation in mammals has originally been described for derivatives of a GlcNAcβ1-3Man core structure in the chondroitin sulfate proteoglycan of rat brain [79, 80]. A consensus sequence for yeast and mammalian O-mannosylation has not yet been delineated, if it is possible at all. Yeast cells employ dolichol-phosphate-β-Man as donor for the first transfer to the protein acceptor in the endoplasmic reticulum by the dolichol-phosphate-Man:protein mannosyltransferase [77, 81].

In this context, an even more exceptional analogy to members of a different urkingdom can be drawn for a

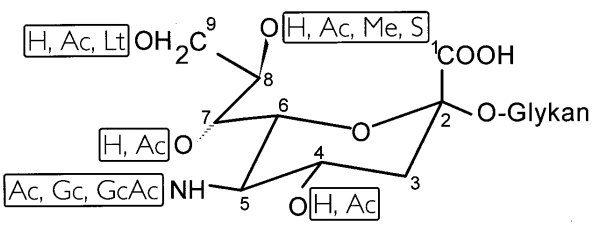

Figure 8. Substitution pattern at neuraminic acid. The whole group of possible derivatives is summarized under the name sialic acids.

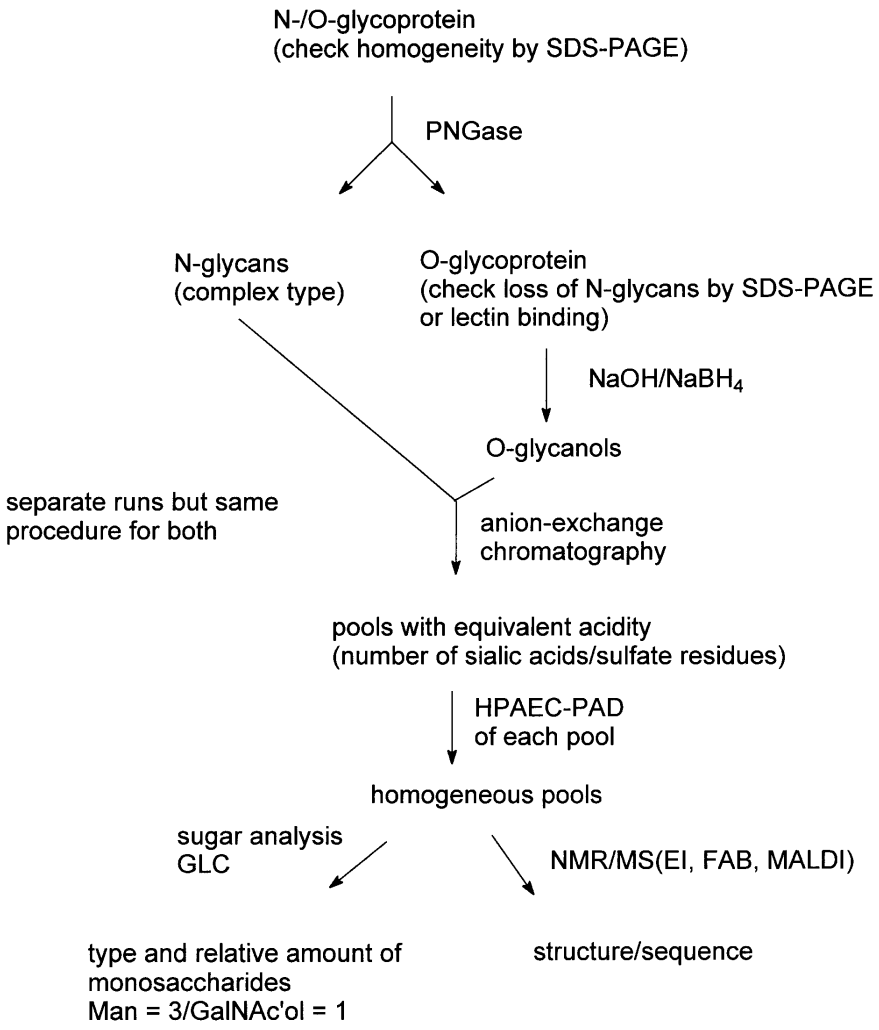

Figure 9. Flow diagram of a typical analytical strategy for the structural elucidation of N-/O-glycans. The given procedures represent commonly used steps. Depending on the type of glycans and the equipment available, other methods may also be used, as given in the text, for example separation of glycans with capillary zone electrophoresis as well as methylation analysis of the glycan followed by modified mass spectrometrical analyses.

second type of glucosylation besides that of EGF domains. Glcβ1-Asn (fig. 5d) is described with a stoichiometry of at least one modified site per molecule to occur in the B2 chain of mammalian laminin, a major basement membrane glycoprotein [82]. This linkage unit had been discovered before in surface glycoproteins of extreme halophilic archaebacteria [83]. In *Halobacterium halobium*, asparaginylglucose of an Asn-X-Thr(Ser) sequence is extended by a short chain of two or three β1-4-linked D-glucuronic acid (GlcA) residues sulfated in the 3-position [74]. As will be described for animal glycosaminoglycans, a partial substitution of GlcA by L-iduronic acid (IdoA) is detectable [84]. In moderate halophiles such as *Haloferax volcanii*, a chain of 9 or 10 β1-4-linked Glc moieties replaces the highly charged glycosaminoglycan-like chain attached to asparaginylglucose [85]. A plant-specific modification of L-hydroxyproline-rich glycoproteins such as Ser-Hyp_4-containing extensins, repetitive proline-rich proteins, arabinogalactan-proteins or solanaceous lectins is arabinosylation, which appears to follow the rule of Hyp contiguity [86, 87]. In retrospect, all the multiple documented modes of O-glycosylation can confound nonspecialists. The distinction between the abundant mucin-type form starting with α-GalNAc and less frequent non-mucin-type structures has therefore taken root in the literature.

In the examples given the acceptor group on the protein is either an amide N atom or in a more promiscuous fashion a hydroxyl O atom in the side chains of Ser/Thr/Tyr/Hyl/Hyp. Although these two conjugation modes indubitably are the most abundant and prominent reactions in protein glycosylation, the enzymology of sugar incorporation has found at least two other means of glycan attachment. The recent description of C-mannosylation of tryptophan (fig. 6a) in the case of ribonuclease 2 from cells of different mammals [88] prompts further investigation into the mechanism of this reaction. Its product is not found in recombinant ribonuclease 2 expressed in plant protoplasts, insect cells or *Escherichia coli*. In addition to the C-glycosylation, an indirect attachment of saccharide to protein is described with phosphoglycosylation [89]. Its occurrence explains why it would be incorrect to exclusively assume a direct connection between the glycan and the protein during attachment. For example, α-Man is linked to Ser via a phosphodiester bridge in this instance (fig. 6b). Oligosaccharides linked to protein in this way differ from the common N- and O-glycans by

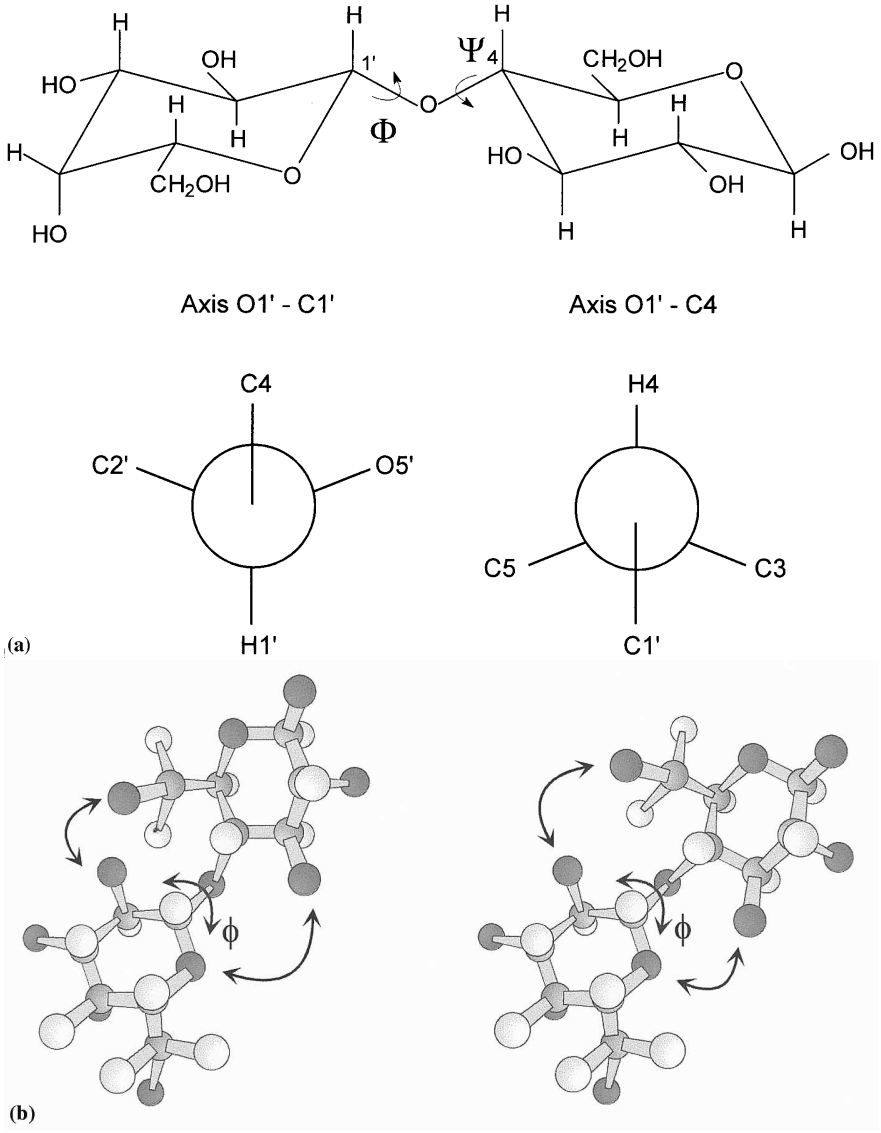

Figure 10. Torsion angles Φ and Ψ for the glycosidic linkage of lactose (see fig. 2: β1-4-linkage) with the corresponding Newman projections (*a*). The impact of an alteration of only one torsional angle (Φ; about 25°) on the relative positioning of two selected pairs of OH-groups in the two monosaccharide moieties is indicated by arrows (*b*).

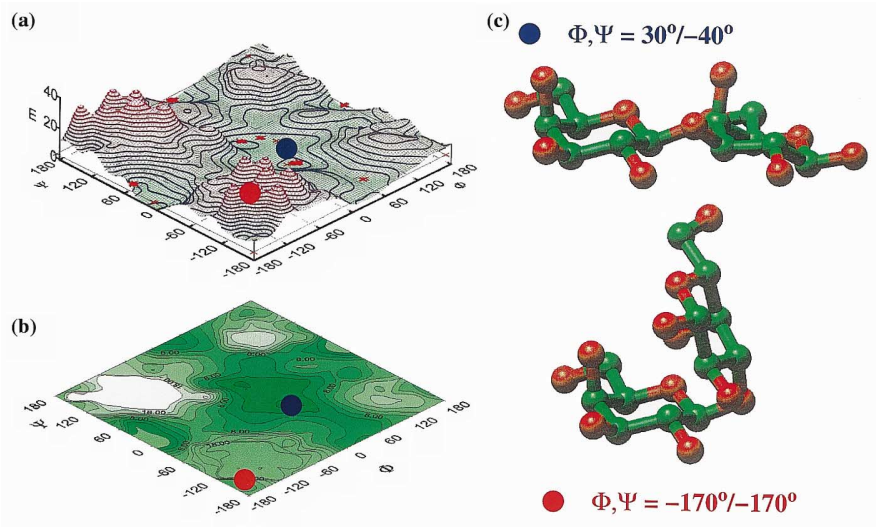

Figure 11. Illustration of the conformational energy contour maps for the Φ/Ψ-torsion angles of the disaccharide Galβ1-3Gal in 3D- (*a*) and 2D-representation (*b*). In addition to the relaxed map the crosses indicate low-energy positions based on the conformational clustering analysis. Calculations according to this protocol remove the Φ/Ψ-grid constraint from the molecule. To provide an insight into the geometry of a low-energy (blue dot) and a high-energy (red dot) conformation, their representations are also given (*c*). Kindly provided by Dr. C.-W. von der Lieth, Heidelberg.

their behavior towards various methods used for release of glycans from protein [89–91]. Since these techniques will be outlined in the next section in the context of glycan analysis, it is sufficient to note here that this phosphoglycosyl linkage is resistant to PNGase (N-glycanase or peptide-N^4-[N-acetyl-β-D-glucosaminyl]-asparagine amidase) treatment, which effects de-N-glycosylation in vivo and in vitro [92, 93]. To remove the glycan, its sensitivity towards mild acid hydrolysis resulting in a phosphorylated L-serine residue, and a released glycan chain is exploited. Alkaline treatment will cleave phosphoglycans if a monosaccharide residue is attached to the phosphate group, but phosphodiester-linked oligosaccharides have proven rather resistant to liberation under similar conditions [89, 94]. For chain extension on the D-mannose residues, several possibilities exist which can even link a protein to a phospholipid. As already briefly indicated with respect to yeast and mammalian O-mannosylation, D-mannose appears to be a very versatile linkage point for protein modification, the structure of α-attachment of Man to Ser in yeast [76, 77, 81, 95–97] being documented in figure 6c. Besides D-mannose, also α-GlcNAc, Xyl or β-Fuc can be linked to L-phosphoserine/threonine in slime molds and unicellular parasites [94, 98, 99]. Phosphoglycosylation with GlcNAc or Fuc has been identified so far in cysteine proteinases or spore coat proteins SP75 and SP96 from *Dictyostelium discoideum* [98–100] and with Xyl in phosphoglycoproteins of *Trypanosoma cruzi* [94].

A phosphate group can additionally be moved between two monosaccharides, as seen for *R*-Manα1-PO_4-6Glc(or Gal)-R in the proteophosphoglycan of *Leishmania mexicana* amastigotes [91]. Such a phosphodiester linkage is not totally uncommon. High mannose-type glycans of acid lysosomal hydrolases acquire it transiently during glycan processing on the C6-hydroxyl group of a D-mannose residue (preferentially terminal α1-2-linked mannose units but also penultimate ones) by the action of a UDP-N-acetylglucosamine:lysosomal enzyme N-acetylglucosamine-1-phosphotransferase [31, 32]. The name of the enzyme plainly lays down that the synthesis of the crucial sugar modification critically depends on features of the target protein for proper selection of the objects of glycosyltransferase activity [101]. A deficiency in this enzyme accounts for a severe storage disease, illuminating the impact of the D-mannose-6-phosphate signal generated by a second enzymatic activity for targeting, that is α-N-acetylglucosaminyl phosphodiester glycosidase (see sections on N-glycans as well as on glycans and disease for further details).

Furthermore, O-mannosylated rat glycoproteins are the targets for a UDP-glucose: glycoprotein glucose-1-phosphotransferase, which generates a Glcα1-p-6-Man linkage cleavable by a α-glucose-1-phospate phosphodiesterase [102, 103]. The functional role of this phosphodiester linkage is less unraveled than that of the

intermediate en route to the D-mannose-6-phosphate routing signal. This is also the case for another putative phosphodiester. Release of radioactive GalNAc-containing glycans by mild acid treatment from rat liver glycoproteins and acceptor properties of N-cadherin for the activity of a chicken neural retina cell surface N-acetylgalactosaminyl phosphotransferase argue in favor of the presence of phosphodiester-linked αGalNAc in further mammalian glycoconjugates [104, 105]. Turning back to the protozoan proteophosphoglycans, both the presently ill-defined functional aspect and the potential to develop antiparasite drugs that interfere with the biosynthesis will spur further work. The provocative suggestion that this constellation of the various types of phosphoglycosylation might be a whim of nature can yet only be refuted by providing clear-cut evidence of biological relevance.

Functional reasoning for the phosphodiester is rather evident, when the phosphate group extends from a D-mannose residue to ethanolamine. A bridging role can be played by the glycan and its attached phosphatidylinositol, joining a protein's C-terminus to a

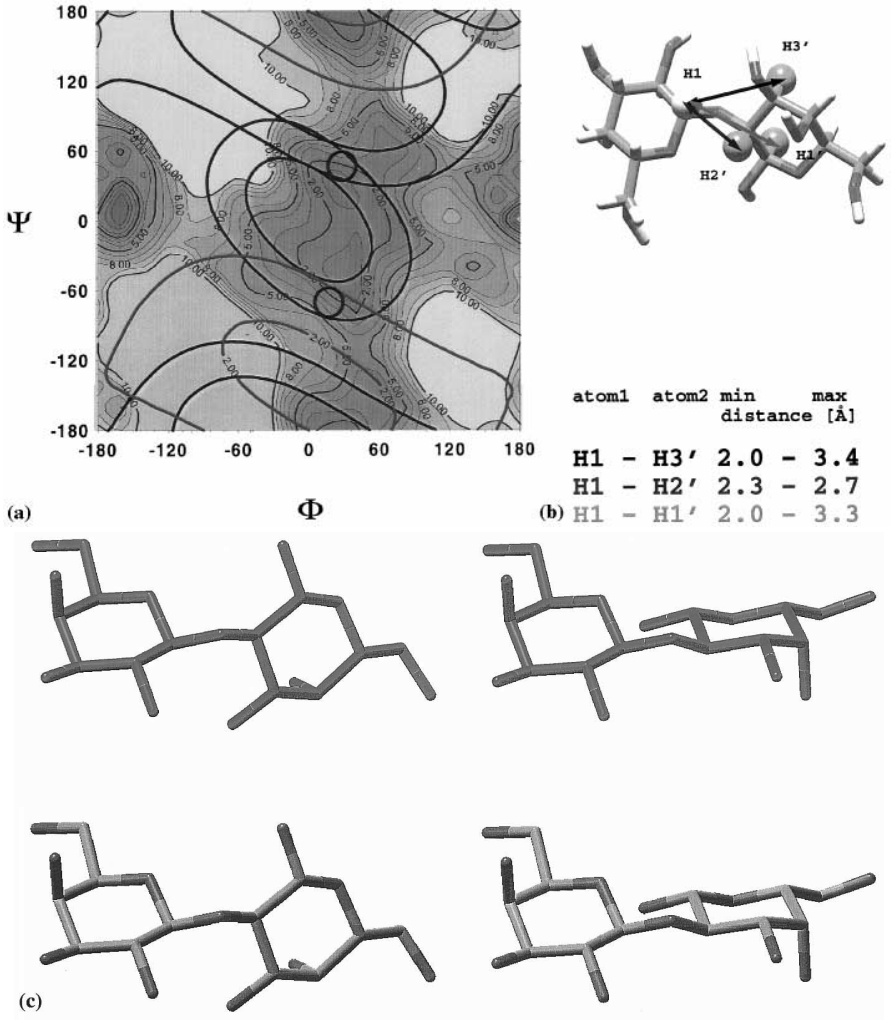

Figure 12. Illustration of the distance-mapping approach for Galβ1-2Gal with three NMR-derived constraints. The three pairs of distance lines, calculated from the NMR data and given in (*a*), have no common area of overlap in the Φ,Ψ,E-plot. The characteristics of each pair are based on the signal intensities of an interresidual contact of the anomeric proton H1 with H1′, H2′ or H3′, respectively, of the β1-2-linked sugar unit (*b*). Overlap between two sets of distance lines encircles sections of the two low-energy territories in the central valley of the Φ,Ψ,E-plot (*a*). This result of time- and ensemble-averaged NMR data argues in favor of two rapidly interconverting, energetically privileged conformations, presented to view in (*c*). Kindly provided by Dr. H.-C. Siebert, Munich, and Dr. C.-W. von der Lieth, Heidelberg.

membrane lipid, graphically demonstrated by the group of glycosyl phosphatidylinositol (GPl) anchors (fig. 7). They were first discovered in *Trypanosoma* [106]. In these structures the membrane anchor is formed by two fatty acids from a phospholipid often with defined chain lengths for the individual species, for example C14:0 for the variable surface glycoprotein (VSG) of *Trypanosoma brucei*. Diacylglycerol, 1-alkyl-2-acylglycerol and monoacylglycerol have been identified as hydrophobic membrane-entering domains [107, 108]. Moreover, diacylglycerol ceramide can serve as lipid part. Lipooligosaccharides containing ceramide do not belong to the group of GPI anchors and should be called glycosyl inositolphosphoceramides [107].

GPI anchors not only harbor differences in the lipid part. Via D-glucosamineα1-6inositol one phosphate group carries a saccharide chain consisting of D-galactose, D-mannose and N-acetyl-D-galactosamine in variable species-specific arrangements. The basic unit is further extended via a phosphodiester involving a D-mannose residue, as seen in phosphoglycosylation, and ethanolamine. Its amino group forms an amide bond to the C-terminus of a (glyco)protein, completing the molecular bridge between the lipid anchor and a variety of (glyco)proteins [107, 109, 110]. In acetylcholinesterase of human erythrocytes the structural theme is further varied by esterification of the inositol residue with a fatty acid, even augmenting the contact to the membrane. The glycan part of certain GPI anchors and related structures also presents a noteworthy structural feature. It attests to the possibility of furanose ring formation of D-galactose (see fig. 1). Actually, the occurrence of galactofuranose has been demonstrated in glycosyl inositolphosphoceramide and -phospholipids of *T. cruzi* and *Leishmania* species [111].

Since the anchor structures expose specific cleavage sites for (glycosyl) phosphatidylinositol phospholipases C [(G)PI-PLC] and glycosyl phosphatidylinositol phospholipases D (GPI-PLD), it is possible to convert the (glyco)protein part from membrane-bound to soluble form [108, 112, 113]. This selective release of certain membrane-associated proteins by a phospholipase was substantial evidence to describe this linkage type and to guide structural analysis [114, 115]. Whereas (G)PI-PLC cleaves the linkage between phosphate and glycerol, GPI-PLD acts on the ester bond between phosphate and inositol (fig. 7). The metabolites of these reactions or other degradative steps produce a number of second messengers such as diacylglycerol, phosphatidic acid or inositolphosphate glycan [112, 116–119]. Besides the structural role that spatially associates a (glyco)protein to a membrane, GPI anchors are thus also reservoirs for signaling mediators upon stimulation of degradation.

Especially the examples of rare modifications illustrate the enormous technical progress in detecting and delineating new linker structures for glycans that occur often only in minor amounts. To think oneself capable of mastering the ensuing task of structure determination of the glycans is the result of an admirable increase in the power of the analytical tools available. The spectrum of natural ways of conjugating a glycan to a protein requires careful consideration of each step of the analytical procedures starting with the separation of the glycan from the carrier. Phosphoglycosylation, for example, may have escaped discovery for a long time because it is an acid-sensitive linkage and is thus hydrolyzed under conditions that are not typical for glycan–protein bond cleavage. Following their release from the protein part, the glycan chains are amenable to structural analysis.

Glycan analysis

The enormous potential for variation in glycan structures calls for analytical tools to unambiguously identify the nature and sequence of monosaccharides, anomeric configuration, linkage position as well as the nature and positioning of substituents on individual residues. Sophisticated strategies employing diverse approaches are required for complete structural elucidation [2–4, 6, 120].

Glycan analysis of glycoproteins may start with degradation of the protein part to a small peptide around the glycan linkage region. Hydrolytic cleavage of the peptide bonds is achieved by relatively unspecific proteases [3, 4]. To avoid formation of a mixture of small peptide fragments that may interfere with the subsequent carbohydrate analysis and therefore have to be separated from the glycopeptide, the already indicated release of the saccharide from the complete protein part is often preferred. This removal is performed chemically with sodium or potassium hydroxide/sodium borohydride or tritiated sodium borohydride yielding [^{3}H]-labeled oligosaccharide alditols in the case of O-glycans [1, 121, 122]. For the liberation of N-glycans, hydrazine is used, which destroys the polypeptide backbone completely [123]. In cases where it is desirable to retain partially or entirely deglycosylated protein for further analysis of its properties, trifluoromethanesulfonic acid (TFMSA) is a recommendable choice [124–126]. Since these methods are neither absolutely specific nor necessarily complete and may cause unwanted side reactions within the glycan chain such as loss of O- and N-acyl groups or peptide core degradation, the conditions have to be tested systematically to find a suitable way to remove all glycan chains with minimal harmful effects on the oligosaccharide chain. These problems are often addressed by enzymes requiring less drastic conditions for cleavage, although protein denaturation to ensure accessibility is still beneficial.

Endoglycosidases release complete or nearly complete glycan chains from the linkage area [127–129]. Several N-glycanases from different sources are available for action on N-glycans [93, 129, 130]. Similar to chemical deglycosylation, the enzymatic release of glycan chains may nonetheless be incomplete. Steric hindrance, which hampers accessibility of the N-glycosidic bond to the enzyme's reaction center, is usually overcome by protein denaturation. In the case of N-deglycosylation of native cell surfaces, the use of detergents has to be minimized or better avoided if the cells are to be taken for further studies. Commercially available enzymes exhibit fine specificities that allow a further discrimination between the various forms of N-glycans, for example high-mannose or complex types [93, 131, 132]. Enzymatic O-glycan removal is facilitated by at least two enzymes. Their activities towards O-glycans show a narrow specificity. Therefore, these enzymes cannot be expected to perform liberation of all O-linked saccharides [133–135]. An enzymatic means to demonstrate presence of O-glycans is the fragmentation of the glycoproteins by O-sialoglycoprotein endopeptidase [130].

Following release, the saccharide chains must be separated. Depending on the inherent complexity of the glycan mixture various techniques can be applied. Often, separation based on the number of acidic groups (sulfate or sialic acid) within one chain is performed as a first step. The availability of anion-exchange resins with high capacity and good resolution power also for high-performance liquid chromatography (HPLC) renders this step relatively easy. The choice of methods used for further separation of the fractions obtained depends on the characteristics of the components in the actual mixture. In many instances HPLC on a variety of resin types can be applied [136–139], for example anion-exchange, amino-phase, or reversed-phase columns as the case may be for individual saccharides or their derivatives to be separated. Relatively new and powerful methods are anion-exchange chromatography at high pH, mostly in combination with pulsed amperometric detection (HPAEC-PAD) [140–142], and capillary zone electrophoresis (CZE) which may be coupled, for example, to photometric or mass spectrometric detection systems [143–145]. Capillary electrophoresis with ultraviolet (UV) detection at 190 nm has been reported to be about 4000 times more sensitive than measurements by HPAEC-PAD [146]. At this stage, a characteristic fingerprint of neutral and mono- up to tetrasialylated N-glycans was obtained [147], pointing to the general applicability of this method.

To analyze the sugar composition of a glycan, methanolysis of the purified oligomers to monosaccharides is often performed by treatment with methanolic HCl yielding methyl glycosides of the monosaccharides or by aqueous hydrolysis with, for example, trifluoroacetic acid followed by reduction of the monosaccharides formed with sodium borohydride to the corresponding alditols. A set of methods is available for the following determination of the nature of the individual sugars [3, 4, 6, 148], for example gas-liquid chromatography (GLC) analysis of either the methyl glycosides after trimethylsilylation to yield the per-O-trimethylsilyl ether derivatives or of the acetylated alditols [4, 149, 150]. HPAEC-PAD or HPLC after derivatization with, for example, 2-aminopyridine or 2-aminobenzamide are also exploited for monosaccharide analysis [4, 6, 138, 151–153]. When the hydrolysis of an oligosaccharide can deliberately be limited with site-specific tools, these techniques are the basis for establishing an approach to sequencing. By sequentially employing specific exoglycosidases such as sialidase, β-galactosidase, hexosaminidase and α-/β-mannosidases, the sequence and anomeric configuration of the monomeric sugars within a typical N-glycan can be determined [2, 154–157]. Analyses of the liberated monosaccharides and the remaining oligosaccharides after each step of enzyme treatment are run in this procedure. This technique has further been refined up to the stage that kits are now commercially available [158].

At this point in chemical and enzymatic analysis the types of monosaccharides, sequence and anomeric configuration are clarified but in most cases not the position of each glycosidic linkage, a major source of sequence variability (fig. 2). Chemically, this problem is solved by methylation analysis, a rather old and ingenious technique [159]. It has been significantly improved over the years [160] by reducing possible contamination from the reagents, by modifying the procedure to ascertain complete methylation and by scaling down the amount of saccharide needed for modification. It is now even possible to analyze 100 ng of an oligosaccharide by this method in conjunction with gas-liquid chromatography/mass spectrometry (GLC-MS) analysis with selected ion monitoring of the permethylated, liberated and derivatized monosaccharides [6, 160]. A comparison of the old protocols for methylation of carbohydrates with up-to-date technology is probably one of the most telling examples for continuous improvement of already well-established methods.

For monosaccharide analysis there is still one major problem, the analysis of sialic acids. In contrast to other monosaccharides, this sugar is often already unwittingly removed from a glycan chain under relatively mild conditions. At the same time, it is easily degraded by acid or alkaline treatment. Thus, under conditions necessary to clip hexoses and (N-acetyl-)hexosamines from a glycan, sialic acids are almost completely destroyed. Moreover, the presence of a number of small substituents such as acetyl and lactyl groups (fig. 8) causes serious problems. These ester linkages are labile under

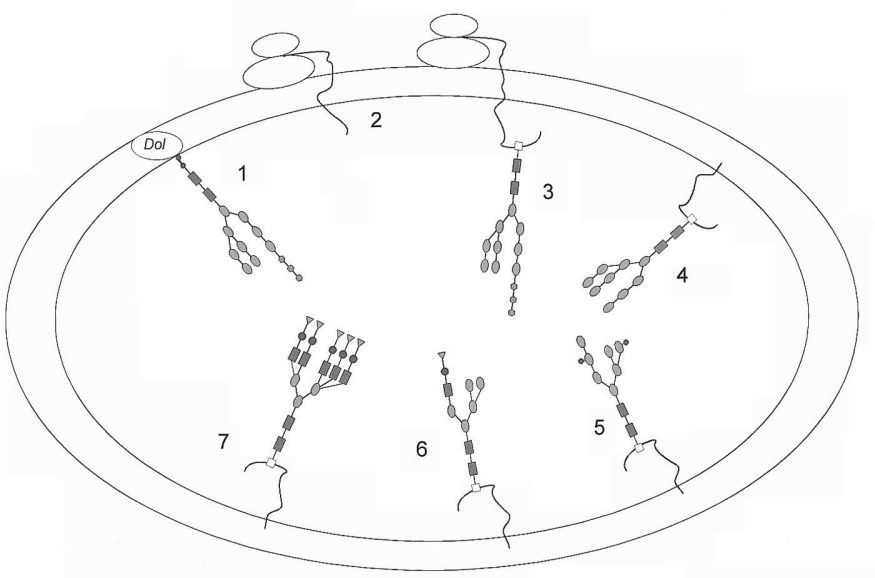

Figure 13. Illustration of the initial reactants and common products of processing of N-glycans in the endoplasmic reticulum. Starting with the oligosaccharyltransferase-mediated attachment (3) of the preassembled dolichol-linked precursor (1) to the nascent protein (2), several principal processing options for main types of products are possible, that is generation of high-mannose (4), hybrid (6) and bi- to pentaantennary (7) complex N-glycans. 6-Phosphorylation of D-mannose residues (5) decorates the protein for transport to the lysosomes by engaging P-type lectins (see table 1).

an array of conditions [30, 33, 34, 161–163]. The ester at the 4, 7, 8 or 9-hydroxyl positions can then be lost. In this context of sugar analysis the product of a peculiar monosaccharide modification reminiscent of Lys/Pro derivative formation deserves comment. In free sialic acids the amino function at C-5 is always acylated, in glycoconjugate-bound form in almost all cases. Acetylation to N-acetylneuraminic acid (Neu5Ac) does not occur at the stage of the amino-C9-sugar. It is already present on the biosynthetic precursor N-acetylmannosamine-6-phosphate, which is condensed with phosphoenolpyruvate to form 9-O-phosphorylated Neu5Ac [34, 164]. This phosphate group is finally displaced from Neu5Ac by a specific phosphatase. Unlike any N-acetyl group of other letters of the carbohydrate alphabet used in eukaryotes, this part of Neu5Ac is the target for hydroxylation to produce N-glycolylneuraminic acid (Neu5Gc). After activation to cytidine monophosphate (CMP)-β-Neu5Ac, monooxygenation can take place to supply CMP-β-Neu5Gc as the starting point for the synthesis of all Neu5Gc derivatives [165, 166]. The glycolyl OH function of Neu5Gc can even be acetylated, as found in rat thrombocytes [167], or can carry another sialic acid residue in glycoproteins from the starfish *Asterias rubens* [168]. Although Neu5Gc presence is widespread in mammals, normal human tissues do not contain this type of sialic acid [34, 164, 169]. The reason for this lack in biosynthetic capacity is the loss of one exon coding for the N-terminal sequence from the gene of the human monooxygenase when compared with the genomic organization of the mouse homologue [170, 171]. This species-specific restriction notwithstanding, the sialic acid backbone is definitely the scaffold for the introduction of the largest number of modifications in the realm of carbohydrate biochemistry. Although the linkage establishment for the principal sugar unit is confined to α-2–3/6/8-bond formation [34, 172], manipulation at the functional groups graphically and positively impinges on the extent of potential coding units, as theoretically discussed in the section on the sugar code.

By derivatization of one or several of the OH functions of Neu5Ac or Neu5Gc at C-4, 7, 8 and 9, about 30 naturally occurring derivatives are formed carrying acetyl, lactyl, methyl, sulfate or phosphate as small substituents [30, 33, 34, 161–163, 168, 169, 173–176], with acetylation as the by far most abundant form of substitution. Another 10 modified forms are found in nature as derivatives of the 2,3-unsaturated sialic acid 2,3-dehydro-N-acetyl-D-neuraminic acid (Neu5Ac2en) [34, 169, 177]. Interestingly, Neu5Ac2en and its N-glycolyl derivative are very effective inhibitors of sialidases [178, 179]. This sugar has been used as lead structure for computer-aided drug design of a potent inhibitor of

influenza virus sialidases to fight the flu [180, 181]. It has also proven pertinent in inferring the biological role in growth and differentiation control of regulatable cell surface ganglioside sialidase activity of cultured human neuroblastoma cells [182–185]. It goes without saying that the presented structural complexity surely calls for an adequate panel of analytical techniques to accurately map the presence of any sialic acid. The methods used for sialic acid identification are colorimetry, thin-layer chromatography (TLC), HPLC, GLC-MS, different MS techniques and NMR. The enormous problems in sialic acid analysis, including loss and migration of acetyl groups even under physiological conditions and strategies that should solve them to avoid artifacts, have been detailed in several reviews [33, 34, 162, 163, 186]. Besides improving the handling of these delicate substances, solid insights into their enzymatic production have been gained. After many years of research the proteins essential for 9-O-acetylation of sialic acids appear to have been identified [187, 188]. If they conferred no functionally relevant properties, then the constitutive presence of a viral esterase, an enzyme that destroys sialic acid 9-O-acetyl groups [34, 189], to initiate a futile cycle would be without consequences. Obviously, the indispensable nature of 9-O-acetyl groups is underscored by a report on their destruction in situ in transgenic mice, which leads to arrest of development at the two-cell stage [190]. The addition of this 'small substituent' is therefore not a random event but most probably a site-specific modification regulating the ligand properties of the sugar unit [34, 38, 191, 192]. Together with site-specific routing signals (e.g. Man-6-p or SO_4-4GalNAc, to be further discussed in the section on N-glycans) and docking points (e.g. 3-O-sulfation of the central modified GlcN unit in the serpin-binding site of heparin to be detailed in the section on proteoglycans), this result makes clear how important 'small substituents' are even on the level of the organism.

Instead of processing by monosaccharide analysis, whole purified oligosaccharide chains can often directly enter structural investigation. The various mass spectrometric techniques are excellently suited to precisely determine molecular weights, branching or substitution patterns of glycans, when a battery of standards is available. Employing electrospray ionization or matrix-assisted laser desorption with, for example, 2,5-dihydroxybenzoic acid as matrix, even pseudomolecular ions have become readily accessible for analysis without prior derivatization. The major drawback of this technique is that structural information is obtained in terms of hexose, N-acetylhexosamine and so on; discrimination between epimers such as D-glucose and D-galactose is not possible. With MS-MS techniques, analysis of rather complex mixtures is also feasible. In the course of such measurements, a mass spectrometric peak resulting from the ionized 'parent molecule' is further fragmented, and the resulting 'sequence ions' originating from this individual peak are recorded in the spectrum (daughter-ion scan). In addition, it is possible to screen for the original fragment from which a certain peak is formed (parent-ion scan). In this way, interpretation of a mass spectrum does not only depend on the occurrence of a given fragment that may also be derived from a contaminant but also on a set of interdependent ions, which increases the reliability. Mass spectrometry is also very often performed after initial runs with separation methods such as GLC, HPLC or CZE [193–201]. Homogeneous glycans can then be subjected to the most powerful analytical tool for glycan analysis. ^{1}H-NMR analysis invariably demands highly purified substances. With this method it is not only possible to identify individual monosaccharides, in almost all cases including type and position of substituents on these residues, but also to deduce anomeric configurations, linkage points and sequence of the residues [202]. This analytical step concludes the processing, as summarized in a scheme for a strategy of glycan analysis (fig. 9).

This figure illustrates that a complete run obviously asks for access to expensive equipment and precious methodological know-how. If the experimental question does not require a complete analysis, versatile and less demanding one-step methods would be ideal to detect the presence and type of glycosylation and of defined sequences. A rapid and efficient means of documenting the presence of glycans with certain structural features in a mixture is the monitoring with epitope-specific probes, that is antibodies or lectins. They are conveniently purified by the versatile technique of affinity chromatography on resins with immobilized carbohydrates or glycoconjugates [203–208]. Either solid-phase assays or (serial) affinity chromatography with well-characterized marker proteins (lectins or monoclonal

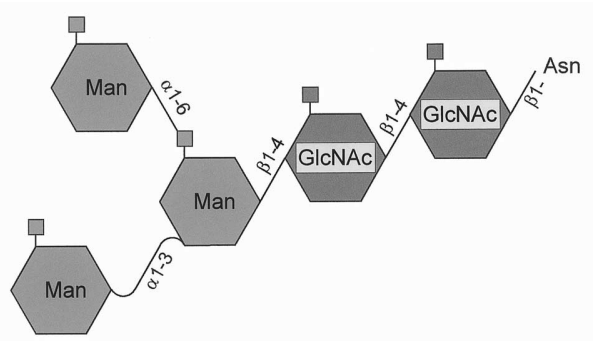

Figure 14. Core structure of N-glycans, that is $Man_3GlcNAc_2$-Asn (see fig. 13; common part of structures 4–7).

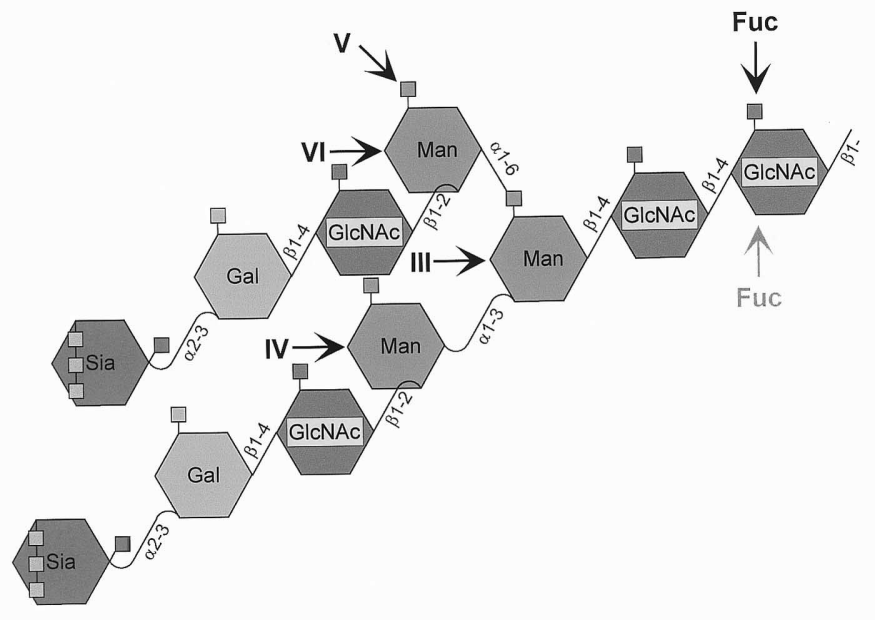

Figure 15. Mature biantennary N-glycan. In the process of maturation this N-glycan can accommodate several substituents, which initiate the formation of other complex N-glycans, for example a pentaantennary product given as structure 7 in figure 13. Further GlcNAc residues may be attached to the core at the positions indicated by the arrows to enable addition of antennae (see structure 7 in fig. 13 for scheme). The roman numbers refer to the temporal sequence, in which the corresponding GlcNAc transferases have to act to assemble tri- to pentaantennary structures. An exception is the transferase III inserting a so-called bisecting GlcNAc, which cannot serve as acceptor for further chain elongation. Fucosylation at the peptide linkage GlcNAc (see fig. 3) occurs at the 3- and 6-position depending on the phylum. The terminal sialic acid residues may also be linked α2-6 instead of α2-3 as indicated. Finally, the different antennae may not be complete or may contain varying lengths of N-acetyllactosamine repeats and distinct modifications such as LacdiNAc-SO_4. The LacdiNAc terminus can alternatively be modified by α2-6 sialylation or α1-3 fucosylation.

antibodies) may be pertinent to prove the presence of a certain glycan type or sugar epitope in one step [208–215]. The tools will not only act on isolated compounds but can also localize carbohydrate epitopes in cell preparations and tissue sections [208, 216–220]. Beyond the solution of a technical problem, this application also points to a role of this recognition process in the cell biological context. It also argues in favor of the validity of the assumption of an already mentioned 'discrete recognitional role' for glycans, when the tools and the analyzed samples both originate, for example, from mammals.

Glycan conformation

Having solved the problems of sequence analysis, including correct assignment of anomeric configurations, linkage points and positioning of substituents, the description of the structural characteristics of oligosaccharides can move to the next level, that is the three-dimensional (3D) depiction of the conformation. To address this issue properly, a combined approach has proven valuable. Already at this stage it should be mentioned that the thorough and reliable description of glycan (ligand) conformation in solution will have a far-reaching impact for drug design [221, 222]. By letting the thumbs of each hand touch, the reader can easily form an instructive model of the flexibility of a disaccharide. By then starting to rotate each hand separately, the major source of inherent flexibility about the dihedral angles is readily visualized (fig. 10a). Naturally, not all possible conformations defined by the two angles Φ and Ψ will be equivalent in terms of their energy levels. As illustrated for a change in only one torsion angle for lactose (fig. 10b), the distances of pendant groups are affected markedly by this alteration. To calculate the correlation between the geometric and energetic factors, a force field has to be established. Considering each energetic contribution such as bond deformations, valence angle distortions or nonbonded interactions, topographic maps can be drawn linking energy levels to sets of conformations [223–231]. They can be presented in 3D- or 2D-formats, as shown in figure 11a,b. To familiarize the reader with the view of the molecule's shape at different energy levels, the con-

formations of two positions from high- and low-energy levels are shown in figure 11c. It is remarkable that not only one low-energy position in the map can result from these calculations. As seen in figure 11 for Galβ1-3Gal and in figure 12 for Galβ1-2Gal, several area sections are energetically preferred regions [20, 232]. To underscore that this result is not exceptional, it is worth pointing out that conformers of β-lactoside derivatives in the low-energy region occupy approximately 5% of the total potential energy surface [233]. Evidently, it is possible that different area sections are on similar energy levels, for example in two parts of the central valley (figs 11, 12). The histoblood group A tetrasaccharide, too, appears to lack rigidity and may switch conformational parameters between two energetically rather equal positions [234]. In contrast, Le^x and Le^y epitopes are essentially rigid molecules, highlighting the conclusion that no general a priori statements are justified.

In order to change the conformation and move to a new position in the Φ,Ψ,E-maps, energy barriers must be crossed with the amount of motional energy available to the atoms. By running molecular-dynamics simulations, the question can be answered whether the different energetically preferred regions in the entire Φ/Ψ-space will actually be populated. These calculations gain access to 'snapshots' of the molecule, which will not complete stretch vibrations of a C–H-bond between two computational recordings [229]. Consequently, the time interval between two computational recordings will be in the order of 1 fs. These calculations for Galβ1-2Gal, whose topographical Φ/Ψ-map is shown in figure 12a, reveal a rapid oscillation in the central low-energy valley with only a rare crossing of energy barriers to one side minimum [20, 235]. The computer-assisted calculations thus allow us to discern an important aspect of oligosaccharide behavior, that is the inherent mobility. This factor will certainly not be without impact on the chances for success of crystallization. Indeed, it can be the cause of frustration for crystallographers. Despite the logical development of the algorithms and these truly unpleasant indications of their validity, these calculations inevitably are in need of further experimental confirmation.

The experimental data basis originates from NMR spectroscopy using the nuclear Overhauser effect (NOE, a through-space magnetization transfer with r^{-6}-dependence) as ruler for atomic distances up to a limit of 3.5 Å for oligosaccharides [224, 236–238]. One crucial difference between the calculations and the NMR measurements concerns the different time scales [237, 239–241]. Within a period of milliseconds during NMR monitoring, the molecules can frequently change conformations, yielding time and ensemble averaging [237, 239–241]. At any rate, each NOE contact provides a distance constraint for the molecule. In the topographical maps this constraint is incorporated as pair of lines which delimit the allowed Φ/Ψ-section [242]. For Galβ1-2Gal, three contacts are measurable (fig. 12a,b) [20, 232]. Since no conformation can be seen upon examination of the Φ,Ψ,E-map which can satisfy the three pairs of experimental distance constraints (fig. 12a), such a conformation is referred to as 'virtual' [237, 243]. However, two areas of overlap for two pairs of contour lines exist (fig. 12a), and their positioning makes it reasonable to assume that two conformations in the two low-energy sections of the central global valley (fig. 12c) fulfill the NMR-derived criteria [20, 232, 235]. This interpretation reconciles theoretical and experimental data. In cases where signal overlap causes severe problems, additional spectral dispersion is offered by heteronuclear experimets with isotope (^{13}C)-enriched oligosaccharides [244, 245]. To extend such measurements to the level of glycan chains, for example for glycan intermediates during intracellular processing and cell surface-presented oligosaccharides [192, 246, 247], it is essential to have sequence information of cellular glycoconjugates available.

Table 1. Main families of animal lectins.

Family	Structural motif	Carbohydrate ligand	Modular arrangement
C-type	conserved CRD	variable (mannose, galactose, fucose, heparin tetrasaccharide)	yes
I-type	immunoglobulin-like CRD	variable ($Man_6GlcNAc_2$, HNK-1 epitope, hyaluronic acid, α2,3/α2,6-sialyllactose)	yes
Galectins (formerly *S*-type)	conserved CRD	Galβ1,3(4)GlcNAc core structures with species- and galectin type-dependent differences in affinity for extensions to blood group A, B or H epitopes; internal stretches of poly(N-acetyllactosamine) chains	variable
Pentraxins	pentameric subunit arrangement	4,6-cyclic acetal of β-galactose, galactose, sulfated and phosphorylated monosaccharides	yes
P-type	conserved CRD	mannose-6-phosphate-containing glycoproteins	yes

CRD, carbohydrate recognition domain; from [298] with modification.

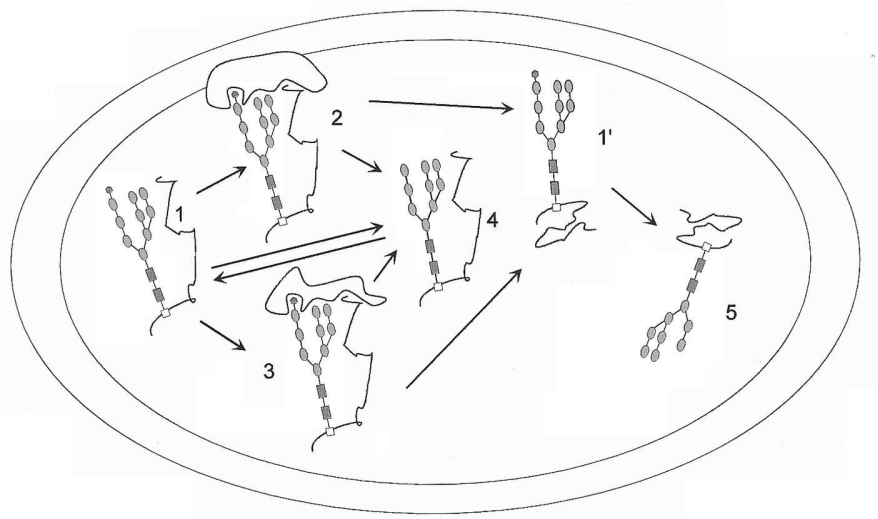

Figure 16. Illustration of a role of the monoglucosylated glycan intermediate(s) in quality control. The molecular chaperones calnexin (type I membrane protein) and its soluble homologue calreticulin bind this sugar structure (2, 3) and can affect the folding process positively, leading to a mature product (1′). Cleavage by an endo-α-mannosidase at this stage (5) or by sequential action of α-glucosidase II and the ER α-mannosidase removing the α1-2-linked mannose moiety not from the α1-3 arm but from the α1-3 branch of the α1-6 arm yielding the classical isomeric $Man_8GlcNAc_2$ structure precludes further retention of the glycoprotein in this compartment and re-entry into quality control. This is otherwise possible via the concerted actions of α-glucosidase II and UDP-glucose:glycoprotein glucosyltransferase (1,4).

N-Glycans

Glycoproteins of this type usually contain the GlcNAcβ1-Asn linkage (fig. 3) discussed above. The sugar chains mainly contain GlcNAc, Man, Gal, Fuc and sialic acids. An essential feature of these glycans is their preassembly on a lipid-based anchor in the endoplasmic reticulum. This glycan chain composed of nine Man, two GlcNAc and three Glc residues and attached to dolicholdiphosphate is transferred en bloc onto the amide function of an appropriate Asn residue in a sequon within a nascent protein chain by an oligosaccharyltransferase (fig. 13) [28, 29, 248–253]. Glycosylation and folding of a nascent protein chain can compete in vivo, which explains the lack of utilization of sequons which are rapidly rendered inaccessible [254, 255]. Following the transfer of the preassembled glycan to the protein, this chain is processed immediately. Nonetheless, the cotranslational addition of a glycan can instigate shifts in the folding pathway of the nascent protein chain [251, 256]. In a defined order individual sugars are released from this glycan. Remarkably, computer-assisted calculations of the mono-, di- and triglucosylated $Man_9GlcNAc_2$-chains reveal full accessibility of the glucose moieties to any putative receptor protein [246]. This process is the prerequisite for enzymatic remodeling of the sugar chain, producing one of the three common subtypes of N-glycans, that is high-mannose, hybrid and complex-type chains [1, 25, 75, 257–259]. The topological arrangement of glycosyltransferases and the levels of substrates are decisive and fine-tunable factors which control the glycosylation potential of the Golgi apparatus [260–262]. These factors introducing natural variability are accounted for by rigorous batch controls of the products of recombinant gene expression in mammalian cells by techniques, which have been outlined in the section on glycan analysis, to ensure uniform pharmaceutical quality [263–265]. As depicted in figure 13, the main types of N-glycans can be generated by enzymatic extension of the processed precursor. They have the core structure $Man_3GlcNAc_2$ in common (fig. 14). In this structure only the five monosaccharides closest to the GlcNAcβ1-Asn linkage region of the original high-mannose type ($Man_9GlcNAc_2$) are still present. Especially in the case of the complex type, the outer part of the antennae can present unique termini, giving distinct glycoproteins a characteristic signature. The crucial step in complex-type glycan biosynthesis is the attachment of GlcNAc at the α1-3-linked D-mannose residue even before the α1-6 arm has been completely processed. Further introduction of GlcNAc molecules paves the way to multiantennary structures. In figure 15 an example of a resulting biantennary N-glycan is depicted together with the positions, where elongation of additional antennae is initiated. The Glc-

NAc transferases required for extending the number of chains act in a certain sequence. For the example given, the GlcNAc linked to the α1-3-bound Man is the first to be added, followed by one at the O-2 of the Manα1-6 residue. Further GlcNAc residues can be added by the specific transferases IV to VI in this sequence and at the positions given in figure 15, yielding a pentaantennary structure [28, 29, 248]. Since sequential GlcNAc transfer is a prerequisite for the synthesis of complete complex chains, elongation at these residues must not take place and Man substitution will be incomplete if only Gal is incorporated too early.

A control point committing glycan synthesis to hybrid or complex N-glycans is provided by the action of GlcNAc transferase III. Whereas GlcNAc transferase I acts as a GO signal, the modification of the inner β-linked mannose moiety prior to α-mannosidase II processing (incorporation of bisecting GlcNAc) prevents the actions of this α3/6-trimming enzyme, GlcNAc transferases II, IV and V and core α1-6-fucosyltransferase [28]. The product of GlcNAc transferase III at this stage can thus be considered as a STOP signal. On the other hand, foregoing processing by α-mannosidase II, which trims the α1-6-linked antenna, guides the glycan in the direction of complex-type chains, often terminating with a sialic acid in branches. If sialic acid is added, it will be in α2-3 or α2-6 linkage, as already indicated in the preceding chapter. A peculiar sialylation can enzymatically follow at an α2,3-linked sialic acid moiety of an N-glycan of a neural cell adhesion molecule (N-CAM). Surprisingly, this α2-8-linked polysialic acid with a chain length of 8 to above 100 is structurally identical to a component of the surface coat of Gram-negative bacteria [266–268].

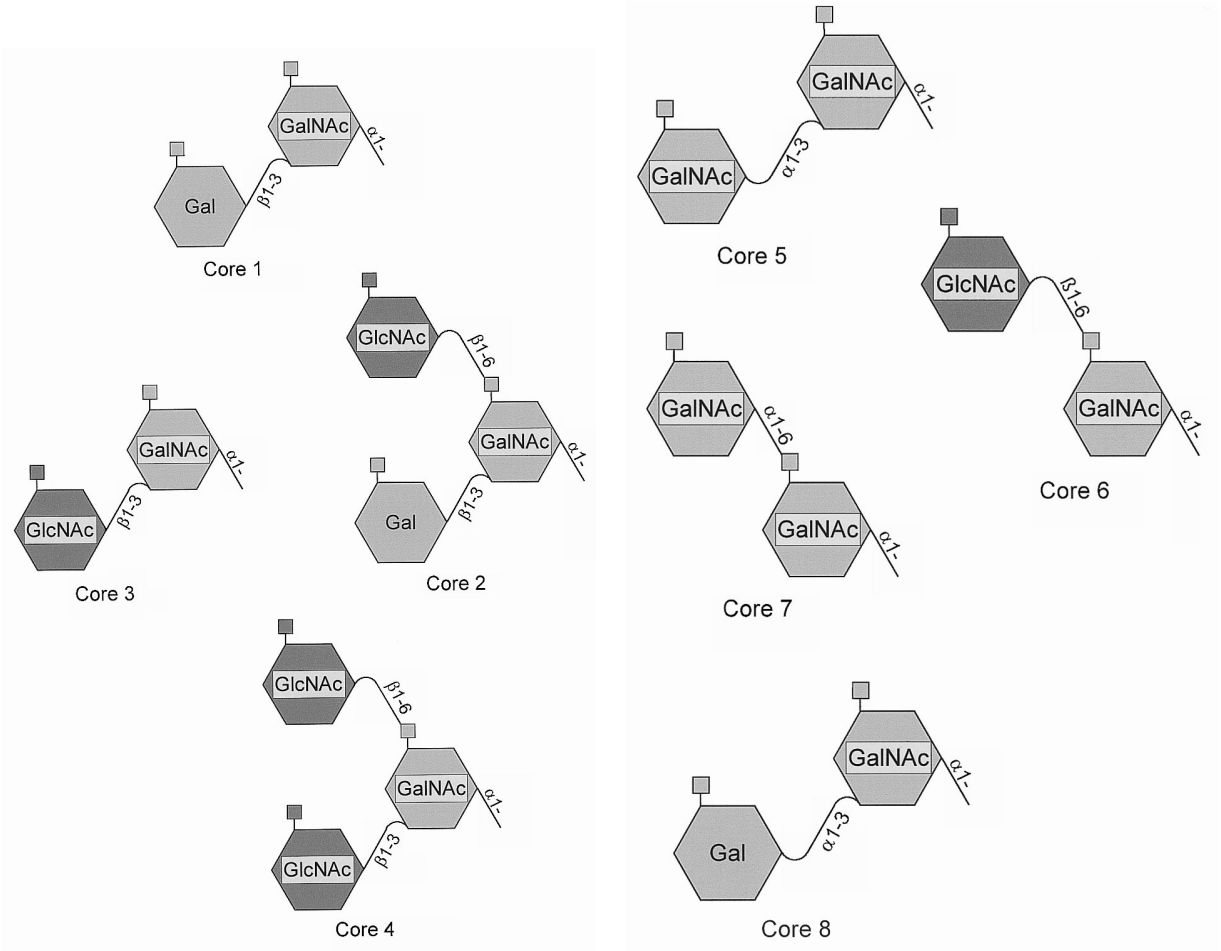

Figure 17. Structures of the eight known core types of mucin-type O-glycans starting with the common GalNAcα1-Ser/Thr linkage to protein (see fig. 3).

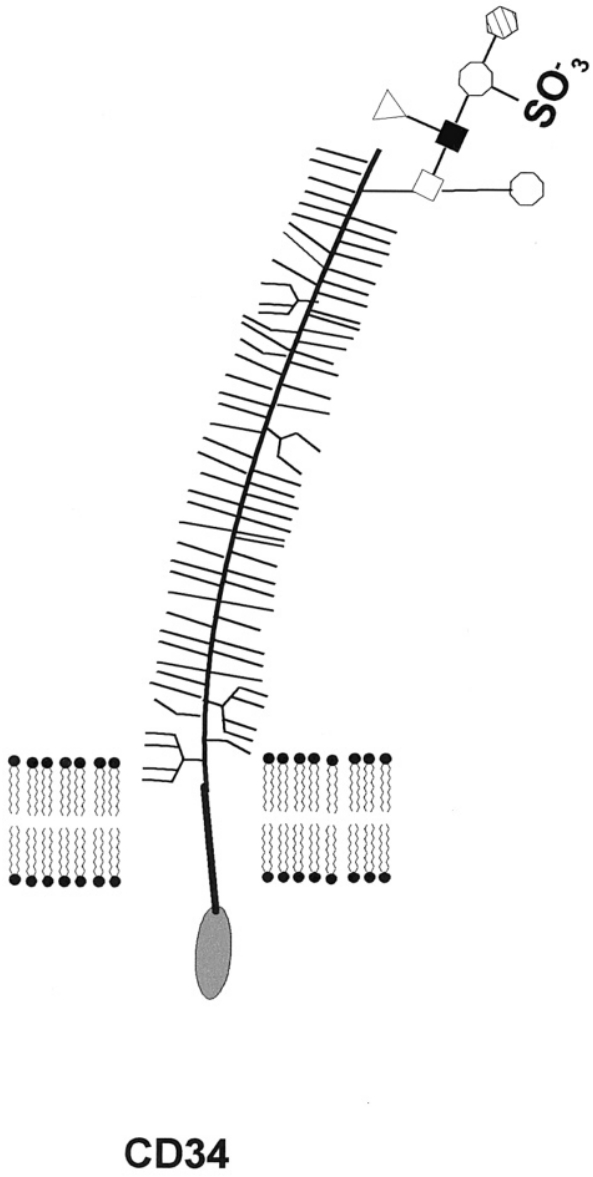

Figure 18. Illustration of the appearence of a mucin-type glycoprotein, that is CD34 expressed on hematopoietic precursor cells and vascular endothelium. The terminal part of the glycoform expressed by high endothelial venules which is accessible for interactions most readily contains a structure serving as docking point for L-selectin (see fig. 31), that is a sulfated Le^x-epitope. Kindly provided by Priv.-Doz. Dr. H. Kaltner, Munich.

The two types of ubiquitous enzymatic sialylation may even occur on different antennae in one glycan. Remarkably, chemoenzymatic synthesis has mastered the problem of delivering these glycan chains and introducing custom-made neoglycoproteins with, for example, an undecasaccharide to cell biological and histopathological testing [269–272]. Coupling of purified glycans from natural sources is thus no longer the only approach to recruit complex oligosaccharides as tools for receptor search [273–276], whose concepts and successes will be presented in the section on glycan recognition. When no further processing will reduce the size of the $Man_5GlcNAc_2$ chain, it is committed to the generation of high-mannose-type glycans, which are common products of yeasts but are also present in animal glycoproteins such as ovalbumin. Interestingly, monitoring their expression has unraveled dynamic remodeling even after reaching the cell membrane. For high-mannose-type N-glycans on the plasma membrane of rat hepatocytes and hepatoma cell lines, remodeling of the glycan chains at the cell surface or during endocytosis was reported. Postbiosynthetic modification includes removal of up to three mannose residues by stepwise demannosylation [277]. Trimming already processed glycoproteins by reglycosylation has also been suggested for complex-type N-glycans on the basis of the significantly shorter turnover rates of terminal sugars (sialic acid, D-galactose, L-fucose) when compared with the protein [278]. Thus, reglycosylation may serve as a repair mechanism of these complex compounds which had encountered glycosidases [279, 280]. This process is different from the appearance of free oligomannosides in the cytoplasm after their formation by hydrolysis as a bypass of the dolichol cycle [281].

Besides elaboration of the outer sections of the antennae, the immediate core residue is also the target of a modification. L-Fucose can be added to the GlcNAc residue linked to Asn. In mammals it is bound to the 6-position, in plants at the 3-position [40, 75, 87, 282]. In insect cells difucosylation is observed [283]. This modification requires two different enzymes and follows a strict order in adding first the α1-6-linked fucose and then the α1-3-linked residue [284] (fig. 15). The ensuing immunogenicity of the α1-3-modified structure is one reason for concern to employ insect or plant cells for the production of therapeutic glycoproteins [285–287]. This aspect will be further discussed at the end of this chapter.

Along the route that N-glycans move within the cell the corresponding enzymes needed to complete the synthesis of these complex molecules are located. In the endoplasmic reticulum, the en bloc transfer from the dolichol precursor to the protein takes place followed by the first steps of modification, before any trimmed N-glycan moves to the cis-Golgi. Within the Golgi network further modification to the complex-type N-glycans takes place, and sialylation as the final modification occurs by the corresponding sialyltransferases in the trans-Golgi [288–292]. The complexity of the enzymatic reactions and the topological order of the enzymes involved along the route of a glycoprotein's

biosynthesis determine the display of glycans on the cell surface and the sorting of N-glycosylated proteins. One example is provided by the preferential labeling of lysosomal enzymes with mannose-6-phosphate in a two-step reaction [31, 32, 293, 294]. The transient presence of a phosphodiester linkage after the first step has already been noted in the chapter on glycan attachment to proteins when talking about phosphoglycosylation. As pointed out in figure 13 and its legend, this signal is the equivalent of a postal code taking advantage of specific receptors (P-type lectins; see table 1 and the section on animal lectins for further information) for prompt delivery to the final destination. Without it, misrouting of the hydrolases destined to end up in lysosomes will occur, and a severe lysosomal storage disease with dense inclusion cells (I cells) develops, as further explained in the section on glycans and disease. The analogy between defined (uncommon or unique) sugar compounds and postal codes is tempting. Indeed, analysis of intracellular transport of glycoproteins contradicts the view of the cargo system as a singular fortuitous event. In the routing of N-glycoproteins between the endoplasmic reticulum and the cis-Golgi and further post-Golgi trafficking, several lectins (calnexin, calreticulin, ERGIC53, VIP36, comitin) are involved [295–299]. Evidence in the case of ERGIC53 has accumulated that this lectin plays a role in efficient intracellular transport of a certain subset of glycoproteins [300, 301]. Having already emphasized the spatial accessibility of the only transiently present glucose units on each processed N-glycan, it is intriguing that the putative receptors have been defined, that is calnexin and calreticulin [296, 302, 303]. The presence of one glucose residue is needed for recognition by the molecular chaperones calnexin and calreticulin, which are engaged in aiding correct folding of various proteins as an early quality control during biosynthesis (fig. 16). If the recognition signal constituted by the monoglucosylated glycan ($GlcMan_9GlcNAc_2$) is prematurely clipped off by a glucosidase II, incompletely folded glycoproteins can still be recognized by UDP-Glc: glycoprotein glucosyltransferase and relabeled for retention in the endoplasmic reticulum [304, 305]. As with UDP-N-acetylglucosamine:lysosomal enzyme N-acetylglucosamine-1-phosphotransferase and UDP-N-acetylgalactosamine:glycoprotein hormone N-acetylgalactosaminyltransferase, a recognition site on the target glycoprotein is indispensable for this glycosyltransferase to regenerate monoglucosylated N-glycans [101]. Although the monoglucosylated form serves as an active but not essential tag in this control system, with several backup mechanisms to compensate any loss in

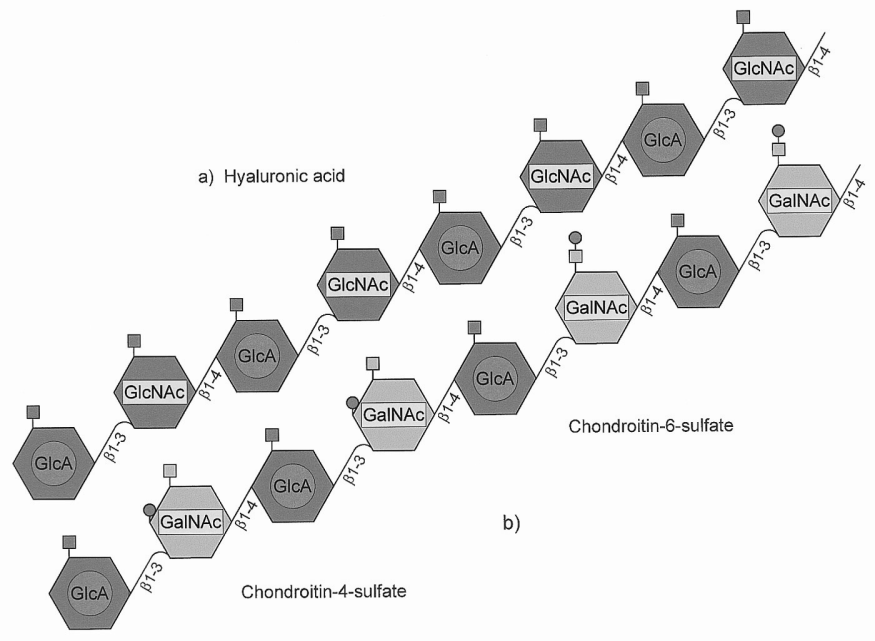

Figure 19. Structures of hyaluronic acid built of the repeating disaccharide unit -4GlcAβ1-3GlcNAcβ1- (*a*) and the typical units of chondroitin-4-sulfate (*b*, left) and chondroitin-6-sulfate (*b*, right). The structure of this glycosaminoglycan differs from hyaluronic acid by substitution of GlcNAc by GalNAc, with further modification of this amino sugar via sulfation in the 4- or 6-position. Usually, blocks with both types of sulfation occur within one chain.

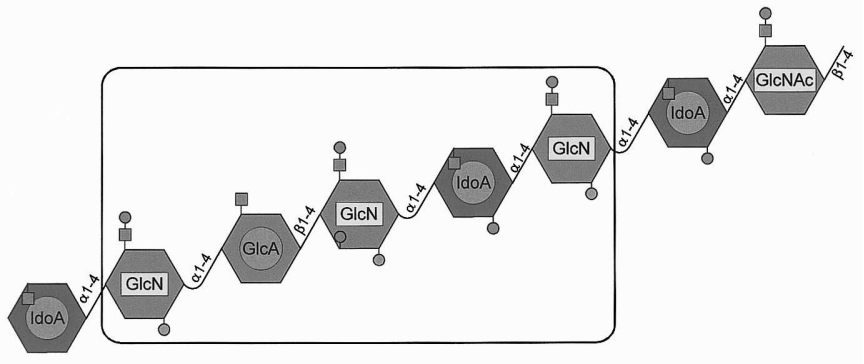

Figure 20. Example of a structural element of heparin. As described in the text, a number of modifications with regard to chondroitin sulfate have to be introduced to form heparin. It is the glycosaminoglycan with the highest extent of structural variation within one chain. The antithrombin-binding pentasaccharide sequence of heparin harbors special structural features especially with the 3-sulfation of the central GlcN moiety. One functionally operative structure is represented within the frame.

one section, this enzyme is apparently not universally expressed in eukaryotes, *Saccharomyces cerevisiae* being a documented exception lacking this activity [306, 307]. The rhetorical question of the purpose of the glycan part [302] and on the universal occurrence of N-glycans in eukaryotes can thus be answered with reference to the significance of the monoglucosylated intermediate in protein folding and of more mature forms in intracellular routing. Once this precursor has served its function, it is disposable, and remodeling of glycans can ensue. This process tailors them for other functions at the cell surface or in circulation. Remarkably, calreticulin's functional spectrum is also not confined to a role in this compartment. Reports on its activity or that of a very closely related molecule as mediator of integrin responses, inhibitor of steroid hormone-regulated gene expression, as cell surface lectin and as C1q-binding protein encourage further work on this structurally highly conserved and ancient molecule [308–310].

During the routing of the N-glycoprotein, irreversible removal of the recognition epitope can proceed via the action of an endo-α-mannosidase, which sets the Glcα1-3Man disaccharide free in one enzymatic step relative to the consecutive actions of glucosidase II and α1-2-mannosidase [311]. Since a phylogenetic survey has determined a late evolutionary appearance, this enzyme is a fairly recent addition to the N-glycan-processing machinery in contrast to the trimming exoglycosidases [312]. Both systems erase the information for calnexin/calreticulin binding from the glycosignature, obliterating a retention code. To emphasize the role of a distinct glycosylation motif as a postal code, the introduction of SO_4-4GalNAcβ1-4GlcNAc (sulfated LacdiNAc) into the termini of selected glycan antennae initiated by the N-acetylgalactosaminyltransferase mentioned in the preceding paragraph is a further instructive example [37, 101]. The essentially rapid clearance of the glycoprotein lutropin is accounted for by signal-mediated endothelial cell homing to the liver [37, 313]. The measured target specificity sets a shining precedent for work on drug delivery or radioimaging employing neoglycoproteins, synthetic matrices or liposomes as smart carriers [314–329]. This strategy exploits paradigmatically the natural capacity of cells like hepatocytes or macrophages to carry out lectin-dependent endocytosis [330–336].

N-Glycosylation is not restricted to mammalian cells. It is found in all eukaryotes, albeit with specific glycan motifs. In yeast, the common structure $Man_3GlcNAc_2Asn$ (fig. 14) is extended by five or more Man residues with the possibility of frequent branching [77]. This distinct pattern of glycan presentation is ideal for preparing molecules of innate immunity to weed out infectious yeast cells (see section on animal lectins). Plant N-glycans are characterized by a D-xylose residue β-linked to the 2-position of the branching Man residue [40, 282]. The biantennary structure may contain an α1-6-linked L-fucose moiety at the GlcNAc residue(s) in the antennae and can be elongated up to the Gal residues [87]. Sialylation is not found in plants, with the exception of one example described in the literature [337]. Insect glycoproteins share the possibility of α1-3 core fucosylation with plants but do not exhibit the Xylβ1-2Man-modified cores [283]. Instead of complex-type chains, fucosylated paucimannosidic (truncated) N-glycans are characteristic insect products [283, 286]. The α1-3-fucosylation of the innermost GlcNAc residue is suspected to form an immunoglobulin E (IgE)-reactive determinant that causes broad allergenic cross-reactivity among various allergens from insects and plants

[287, 338]. In contrast to the biosynthesis of the N-glycans with its initiation by a preassembled precursor, other types of glycosylation can proceed entirely in a stepwise manner, as shown at first for mucin-type O-glycans in the next section.

Mucin-type O-glycans

As already indicated, O-glycans differ from N-glycans in their attachment point to the protein (fig. 3). In comparison to the multiantennary structures decorating the N-type glycoprotein parts, O-glycans are rather compact structures. Already at the GalNAc residue attached to the protein branching can occur [28, 29, 42, 57, 339–341]. Eight different core structures are presently known for extension beyond the GalNAcα1-Ser/Thr linkages, as shown in figure 17. Introduction of, for example, sialylation and other modifications further increases the diversity of O-glycans. Therefore, the total number of known O-glycan structures is considered to be much higher than that of N-glycans. To give a compelling example, human lung mucin alone contains more than 100 different O-glycans [342, 343]. It is speculated that the high diversity in O-glycan structures of, for example, bronchial mucins is responsible for trapping inhaled microorganisms and thus protects the underlying epithelia [343, 344]. The structural variations are not made feasible by an extension of the pool of monosaccharides. A common feature of all of these structures is the presence of the limited number of different monosaccharide residues, namely GalNAc, Gal, GlcNAc, Fuc and sialic acids. Sulfation is an additional, frequently detected modification of mucin-type O-glycans. From comparison with the constituents of N-linked saccharides, it is clear that Man is a typical monosaccharide for N-glycans. Owing to the discovery of the presence of GalNAc in N-glycans from invertebrates and vertebrates [345–349] already spoken of with respect to the pivotal 4′-sulfated derivative at the termini of glycan antennae of pituitary glycoprotein hormones [37], this sugar should no longer be considered as typical for O-glycans.

Biosynthesis of O-glycosidically linked saccharide chains [29, 42, 339, 340] is completely different from that of N-glycans. Instead of transfer of a preassembled carbohydrate precursor individual nucleotide-activated monosaccharides are added by site-specific glycosyltransferases in a stepwise manner. This process is highly organized in such a way that at certain branching points of biosynthesis the addition of the next sugar determines the route for further elongation, as detailed in informative reviews on this aspect [28, 42, 339, 341]. Many of these transferases have meanwhile been char-

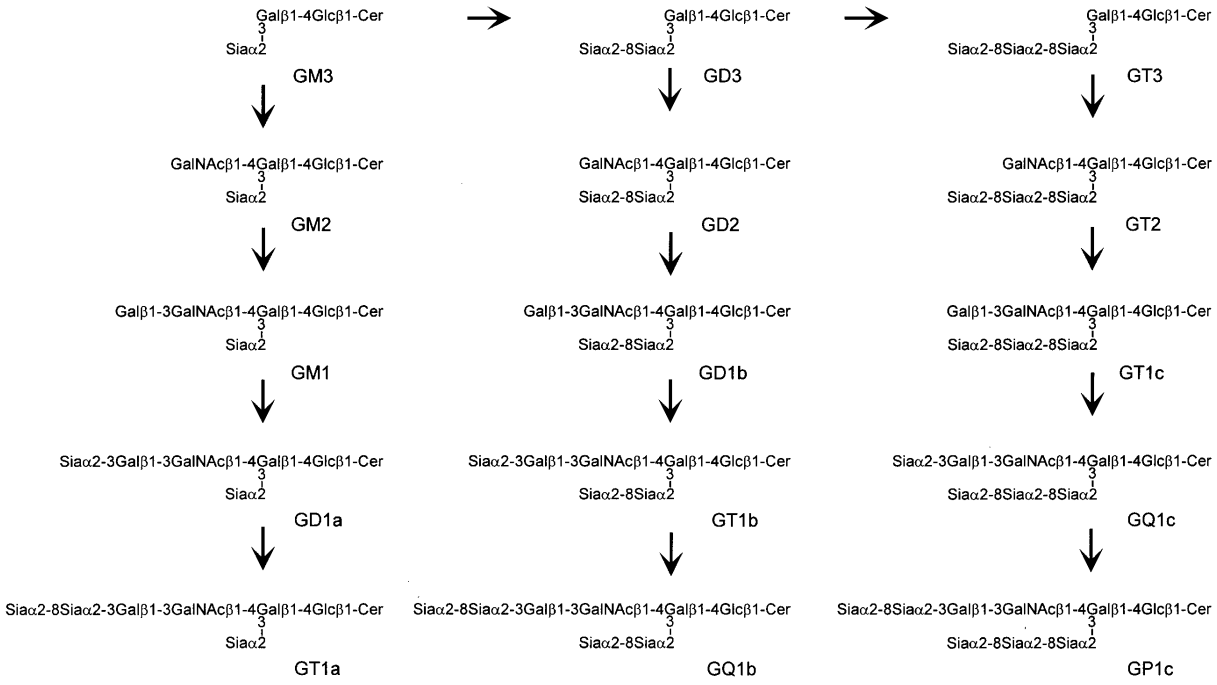

Figure 21. Biosynthetic pathway for gangliosides of the ganglio series a, b and c.

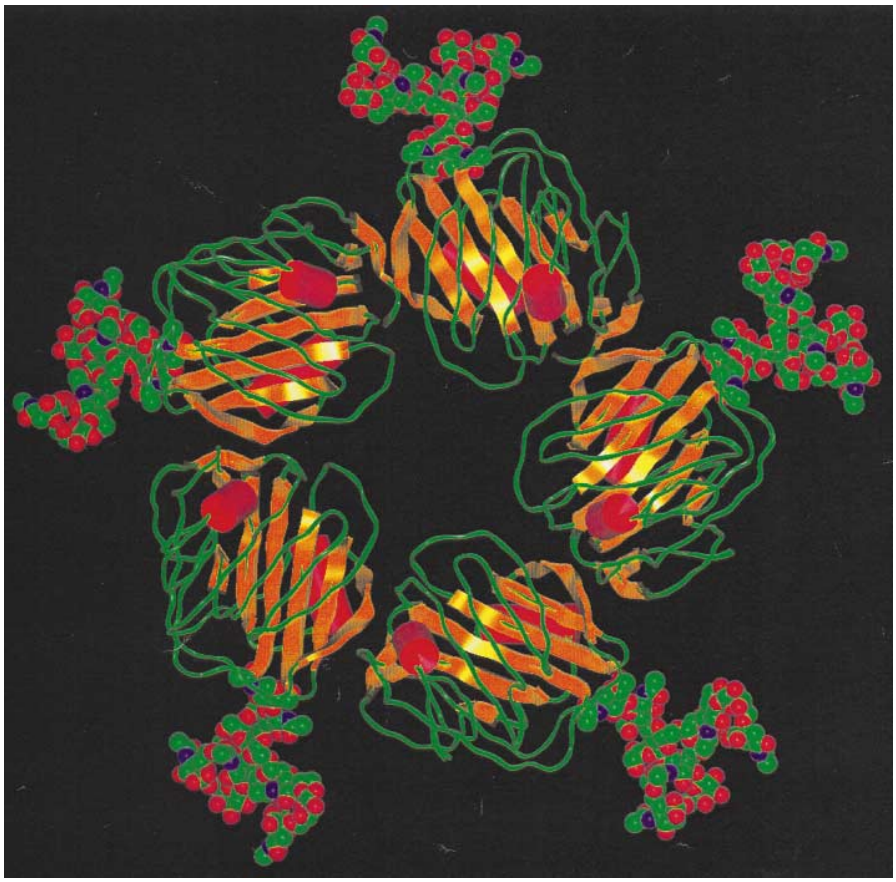

Figure 22. Illustration of the secondary structure of the pentraxin serum amyloid P component with its N-linked glycan on Asn32 attached to each monomer (entry 1SAC into the Brookhaven Protein Data Bank). Kindly provided by Dr. E. Tajkhorshid, Heidelberg, and Dr. C.-W. von der Lieth, Heidelberg.

acterized and cloned, as already mentioned for the first type of enzyme initiating O-glycan synthesis in the first paragraph of the section on glycan attachment to protein, thus providing tools for refined chemoenzymatic synthesis [29, 270, 350, 351].

It is perhaps the lack of stringent structural requirements around the linkage region that allows multiple O-glycosylation on Ser/Thr residues in close vicinity. Mucins usually contain a large number of short O-glycan chains constituting areas with a high saccharide density (fig. 18). This structure can be compared to the bristles of a brush [352], reminiscent of the arrangement of clusters of glycosaminoglycan chains in proteoglycans [55]. Elongation by poly(N-acetyllactosamine) repeats and versatile branching can extend the length of individual O-glycans. These highly glycosylated regions with a carbohydrate content of up to 80% furnish a dense local clustering of negative charge by the constituent sialic acids and sulfate. Similar to the glycosaminoglycan hyaluronic acid (see next section) mucins in solution cause high viscosity and on surfaces a barrier that cannot easily be crossed. They also offer protection from proteolytic attack. On the other hand, the glycan structures are not only passive barriers but implicated in molecular interactions as well [340, 353–356]. Since they serve as recognition signals and adhesion points for other molecules and cells, initiating cell contacts in inflammation and infection, they are role models for the design of antiadhesion drugs [309, 343, 357–362]. As an analogy has just been drawn to the structural appearance of proteoglycan sugar chains, their synthesis will be presented in the next section.

Glycosaminoglycans and proteoglycans

Proteoglycans are conjugates of at least one covalently bound glycosaminoglycan chain with perplexing poten-

tial for inter- and intrachain heterogeneity attached to a protein core. In addition to this special type of glycan incorporation via the linkage unit shown in figure 4, the protein part, itself belonging to different families as discussed in the second part of this section, can often carry N- and O-glycans. Although issues of nomenclature are rarely inspiring, it is not too difficult to condense the essentials of glycosaminoglycan structure in order to provide the reader a conceptual grasp of this section of glycosciences.

The glycosaminoglycans form a distinct group of complex carbohydrates. Generally, they are defined as unbranched (linear) acidic polysaccharides formed from repeating disaccharide units. They are composed of a uronic acid (D-glucuronic acid or L-iduronic acid) or D-galactose and an amino sugar (D-glucosamine or D-galactosamine) [55, 363–366]. Monosaccharides in the chain can richly be modified by 'small substituents', enhancing the potential for variability enormously. The amino sugars are in most cases N-acetylated, sometimes N-sulfated, yielding an intrachain heterogeneity most notable in the case of heparin and heparan sulfate [367–372]. The term 'heparin' is conscripted to refer to the presence of a high proportion (generally $> 80\%$) of N-sulfation of the GlcN residues. As a clue for the organization of the enzymatic assembly line, this factor entails abundant O-sulfation and a high IdoA:GlcA ratio. Since current methods do not attain a definitive sequence assignment of intact microheterogeneous glycosaminoglycan chains, manageable fragments are produced by nitrous acid or heparin lyase treatment. The polydisperse mixture of di- and oligosaccharides can then be fractionated with sophisticated techniques such as HPLC or CZE, as already outlined in the section on glycan analysis. Unambiguous structure determination mainly relies upon NMR and mass spectrometrical analysis [373]. Besides the presence of N-sulfation, sulfate substituents can also decorate OH groups of the amino sugars and/or uronic acids.

Characterization and cloning of the sulfotransferases responsible for the individual reactions on proteoglycans but also on N- and O-glycans of glycoproteins and on glycolipids has advanced considerably [36]. Sulfotransferase-dependent substrate modification has already been shown in the last section to endow the pituitary glycoprotein hormones with a routing signal. It is therefore not surprising that a similar interpretation with the positioning of these groups as sensors for adaptive processes is advocated, especially for heparan sulfates [374].

The lowest degree of modification among the group of glycosaminoglycan chains is recorded for hyaluronic acid (fig. 19a), with D-glucuronic acid and N-acetyl-D-glucosamine as building blocks of the repeating disaccharide unit [375–377]. It is the only member of this group which is not covalently linked to protein and which is free of sulfate groups. All other types are integral parts of proteoglycans with the sulfate positioning deserving special attention. In chondroitin sulfates, D-glucuronic acid is found together with N-acetyl-D-galactosamine, which is further substituted by sulfate at positions 4 or 6, yielding chondroitin-4-sulfate or chondroitin-6-sulfate (fig. 19b). Within one chain there can be sequence sections with 4- together with others displaying 6-sulfation, designated as copolymer. A similar structural feature also occurs in dermatan

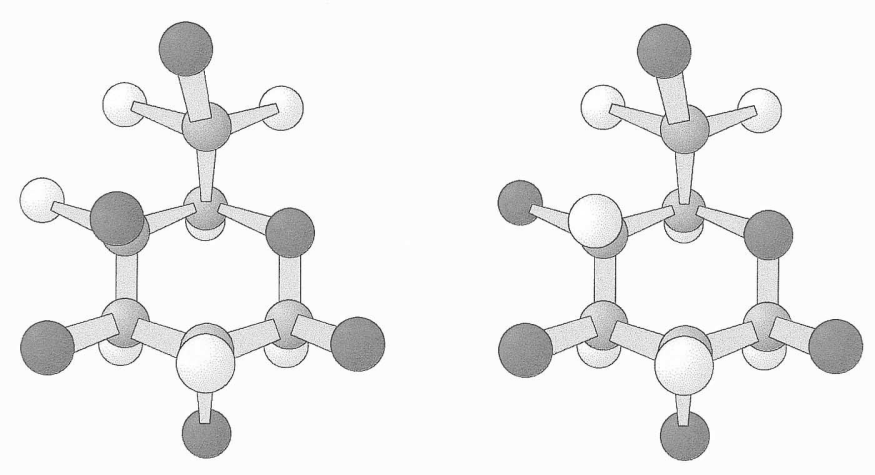

Figure 23. Stick and ball conformation of D-galactose (left) and D-glucose (right).

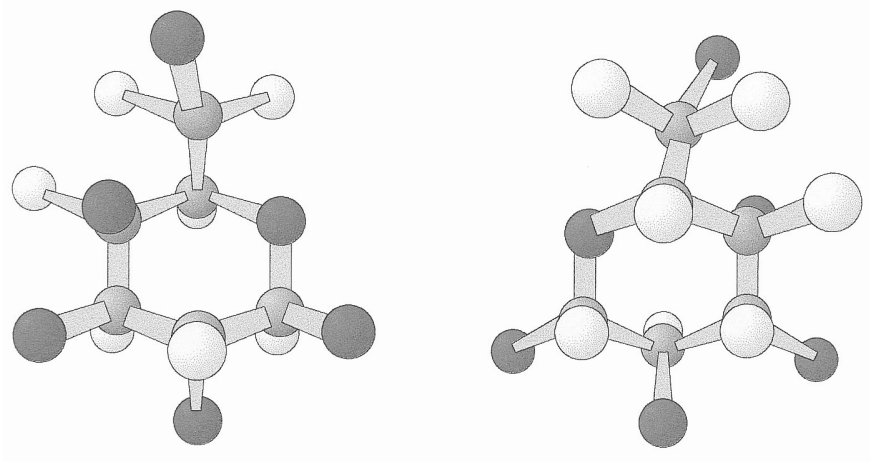

Figure 24. Polar A and unpolar B sides of D-galactose as stick and ball model.

sulfate, in which a part of the uronic acid residues of chondroitin sulfate is enzymatically epimerized to L-iduronic acid that additionally may carry O-sulfate groups.

Owing to its clinical application as anticoagulant heparin is the most popular proteoglycan. Its sugar chains contain either D-glucuronic acid or L-iduronic acid together with D-glucosamine as the basic disaccharide [363, 367]. An insight into its structure is given in figure 20. It illustrates intrachain heterogeneity of monosaccharides due to differential modification by sulfate and the extent of C5-epimer formation of the uronic acid. The glycosidic linkage points of the uronic acids to the amino sugars remain unaltered; the change from α to β is a consequence of the stereochemical alteration (from D to L). Due to the interconversion of IdoA conformers ($^1C_4$, $^2S_0$) essentially through rotation around the C2–C3 bond, the shape of the polysaccharide is amenable to energetically inexpensive modulation, establishing an extra degree of intrachain freedom of mobility [378]. Sequence heterogeneity in heparin and heparan sulfates extends to at least 10 different -GlcN- HexA and 17 different -HexA- GlcN disaccharide units with definitely nonrandom occurrence despite mechanistic constraints during synthesis [369]. Theoretically, 48 different disaccharides, including the presence of rarely found GlcN, are possible [379]. Similar to N- and O-glycans, not all devisable structures are actually biological. Nonetheless, the actually measured variability is still amazing. Again, the exquisitely well-ordered structure of the product depends on a high degree of topological organization of the biosynthetic machinery in the Golgi apparatus and the *trans*-Golgi network. Fundamentally, two catalytic activities are sufficient to fulfill the job of chain elongation. Prior to this process the preparation of the core must be accomplished. It starts with the transfer of Xyl onto Ser within the peptide chain (fig. 4). The subsequent addition of two Gal residues is catalyzed by two different β-galactosyltransferases because the substrates are Xylβ1-Ser and Galβ1-4Xyl, respectively. With the attachment of GlcA to Gal in β1-3-linkage, the common core structure for further elongation is completed [380]. This -4GlcAβ1-3Galβ1-3Galβ1-4Xylβ1-Ser linkage is identical for chondroitin sulfate, dermatan sulfate, heparin and heparan sulfate. A deficiency of galactosyltransferase I in a 4-year-old Danish male was associated to an Ehlers-Danlos-type of connective tissue disorder with impaired decorin (a secreted proteoglycan with normally one dermatan sulfate chain) [381]. The clinical relevance of this biochemical defect in glycan modification merits devoting a separate section to the general aspect, merging clinical medicine and glycosciences.

That settled, we can continue to dig into the workings of glycosaminoglycan construction. The chain elongation makes progress by stepwise operation of only two enzyme activities. UDP-N-acetyl-D-glucosamine or -D-galactosamine and UDP-D-glucuronic acid (UDP-D-galactose) are the activated forms that enable alternate transfer of the monosaccharides onto the growing chain. A GlcNAc or GalNAc transferase center, depending on the type of N-acetylhexosamine to be incorporated, and glucuronosyl(galactosyl)transferase activity accomplish this task. Elegant work on heparin/heparan sulfate biosynthesis spearheading this aspect of work on glycosaminoglycans has revealed that two activities reside in a single 70-kDa protein [382]. All modifications which are characteristic for the individual

glycosaminoglycan are thereafter introduced in a defined sequence during ongoing chain elongation [370, 383, 384]. In the case of heparin, a series of five enzymatic activities is coordinated: N-deacetylase followed by N-sulfotransferase and then O-sulfotransferase and GlcA C5-epimerase [370, 385]. The first two activities are harbored in one protein for which mouse tissues express two different genes [386]. For the two latter enzymes N-sulfation is required for substrate recognition. Finally, a specific 3-O-sulfotransferase introduces an infrequent modification. This feature is crucial to turn the sequence stretch into an antithrombin-binding unit. In addition to this special sulfation three additional sulfate groups at monosaccharide unit I (O-sulfation), III and V (N-sulfations) complete the changes of the framework for high-affinity binding [369, 387–389]. Actually, these modifications must already be present to impart substrate properties for the 3-O-sulfotransferase to the pentasaccharide. The product of the shaping of the high-affinity serpin ligand is shown in figure 20. Circulating heparan sulfate can mimic the potent anticoagulant heparin and thus contribute to a fatal clinical outcome by serious bleeding in individual patients [390]. Neutralization of the anticoagulant activity by protamine sulfate or platelet factor 4 is mandatory in such a case. The exception already noted owing to the presence of an uronic acid is seen for keratan sulfate chains. In this case Gal alternates with GlcNAc. Sulfate incorporation at C-6 of Gal and GlcNAc maintains the acidic appearance of this glycosaminoglycan, a chick corneal form being linked to its core protein via Asn [391]. Moreover, O-linked attachment is possible for this chain-type in aggrecan [392].

Not only the sequences of the glycosaminoglycan chains and the intra- and interchain heterogeneity furnish sites for regulation. Reactivity with binding partners in their vicinity and/or attachment to protein cores with glycoform formation are the material for a series of captivating stories on the versatility of glycosaminoglycan functions [55, 366, 371, 384, 392–395]. In this respect, the discussion is simplified for hyaluronan, because the long (up to 10 μm) chains are not attached to a protein core. Their physicochemical properties, including swelling with high viscosity of solutions in water and inherent elasticity, are ideal for a lubricant in joints and on surfaces sliding along each other. In addition, this

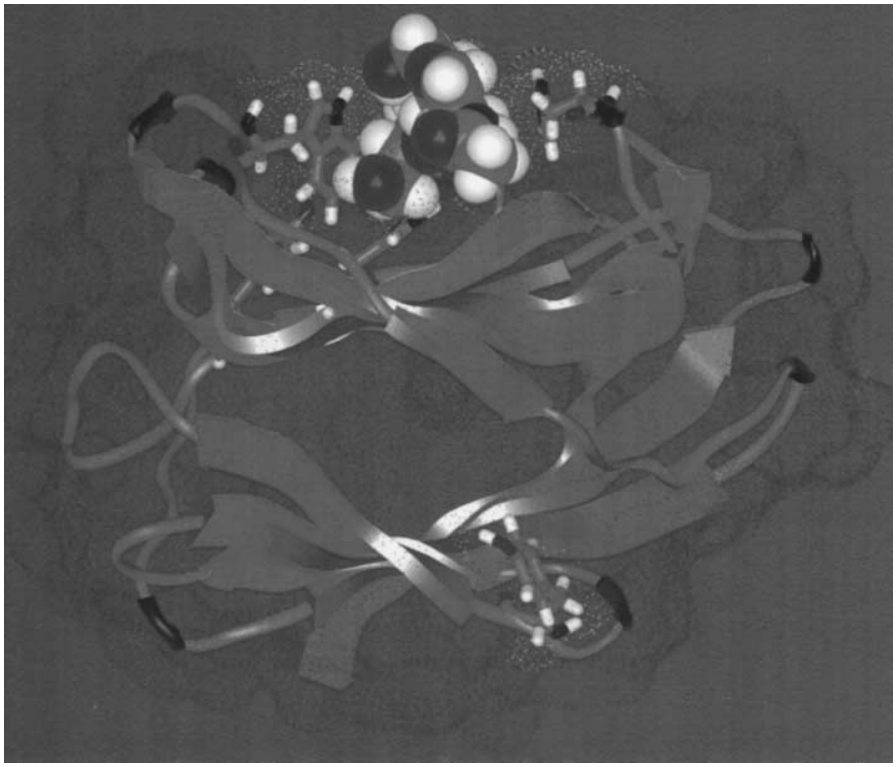

Figure 25. Illustration of the peptide backbone of one monomer of bovine galectin-1 (see table 1; entry 1SLT into the Brookhaven Protein Data Bank) with emphasis on the close spatial contact between Trp68 and the ligand. The solvent-accessible surface is highlighted. Kindly provided by Dr. C.-W. von der Lieth, Heidelberg.

polymer can be likened to a highway for cell migration and the scaffolding for a molecular network in the extracellular space. It is thus not surprising that a variety of hyaluronan receptors have been put on record, among them CD44, a receptor for hyaluronic acid-mediated motility (RHAMM), cartilage link protein as well as aggrecan and the other members of the modular proteoglycan subfamily, versican, brevican and neurocan [376, 395–399]. Already very early in development, the entry of migrating mesenchymal cells into the blastocoel exploits glycosaminoglycan to set the stage for the multilayered body plan, as underscored by the harm done by hyaluronidase injections. Similarly, cell movements from the dorsal neural tube appear to make headway on hyaluronic acid.

Concerning elasticity, proteoglycans with the sulfated types of glycosaminoglycan chains can contribute to regulating this physicochemical property. A variety of subfamilies of protein cores without a unifying sequence motif can be enlisted as carriers of the chains. From the point of view of molecular networking, the extracellular hyaluronic acid-binding proteoglycans are evidently endowed with bridging properties for various partners due to their imaginative modular design [366, 395, 400]. The irreplaceable character of aggrecan is convincingly proven by the detrimental effects of a mutation in an animal model [401]. Together with the correlation between decorin deficiency and development of a progeroid syndrome, these model studies help to embrace the concept of the vital relevance of the proteoglycans. The structural trait shared by aggrecan, versican, neurocan and brevican is a tridomain structure. The central region harboring the glycosaminoglycan chains is flanked by N-terminal hyaluronan-binding sequence stretches and a C-terminal section with EFG-like domains, a C-type lectin domain and a complement-binding consensus repeat. Clustering of these three modules is known from the mosaic-like structure of selectins and *Limulus* factor C [298], which will be discussed in the chapter on animal lectins (see also fig. 31 for an illustration of the modular architecture of selectins). Chimeric structural design is also characteristic of the other subgroup of extracellular proteoglycans. They lack capacity for binding to hyaluronic acid, but have acquired other networking abilities. Perlecan, agrin and testican present an array of modules which can include laminin domain G-, III- or IV-like, low-density lipoprotein (LDL) receptor-like or secreted protein acidic and rich in cysteine (SPARC)-like sequences or a Cys-Trp-Cys-Val (CWCV) domain [395]. A prototype molecule of this group is perlecan with its matrix protein (for example laminin and collagen type IV)- and cell-binding properties [366, 402]. The staggering growth of our knowledge on proteoglycan cores is further attested by the fact that secreted pericellular proteoglycans even comprise another distinct category. Their members, decorin among them, display a central domain composed of leucine-rich repeats flanked by two cysteine-rich regions with a total molecular weight of 36–42 kDa [366, 403]. These SLRPs (small leucine-rich proteoglycans) also participate in matrix assembly and confer cell adhesive properties extending to the regulation of cell proliferation [366, 395, 404].

The assumed impact on matrix assembly is readily apparent for certain transmembrane proteoglycans, whose name is derived from the Greek verb *syndein* (to bind together). Syndecans and other transmembrane proteoglycans such as CD44 or thrombomodulin can connect the extracellular environment to the actin filaments of the cytoskeletal organization [366, 392, 405–410]. Anchoring the core does not exclusively depend on a transmembrane domain. As with other proteins a core protein can be associated to a membrane by the GPI anchor, introduced in figure 7. Tissue- and stage-specific expression and work on the *dally* (division abnormally delayed) locus in *Drosophila melanogaster* implicate the glypicans (currently five vertebrate forms are known; mutations in the glypican-3 gene are connected with the Simpson-Golabi-Behmel syndrome characterized by visceral and skeletal abnormalities and pre- and postnatal overgrowth) in regulation of growth control and patterning during development [366, 411].

Besides the modules in the diverse protein cores the glycosaminoglycan chains will contribute noticeably to the interactive potential complementing their rather passive physicochemical properties. Monitoring of specific binding of probes constituted by labeled glycosaminoglycans to cells in tissue sections is one approach to detect this aspect of proteoglycan function [412]. This receptor-directed activity does not only encompass the famous textbook example of serpin binding by the heparin pentasaccharide shown in figure 20. Also, a variety of proteins including laminin, thrombospondin, fibronectin, cell adhesion molecules such as N-CAM, lectins and virulence factors are proven binding partners for the sugar part of proteoglycans [298, 371, 372, 413–421]. This property is likewise relevant for cytokines and growth factors. By attachment to a glycosaminoglycan chain they are apparently either safely stored for a period and released upon stimulation of protease or heparitinase activities or acquire a functionally effective state in a ternary complex with their protein receptor [371, 422–431]. In striking analogy to oligosaccharides released from plant cell walls in a defense reaction, glycosaminoglyan components can also serve as signaling molecules after enzymatic generation from the chain [432]. From a merely passive role on the basis of their charge density, our judgment of glycosaminoglycans has steadily made a remarkable transition towards the appreciation of their 'recogni-

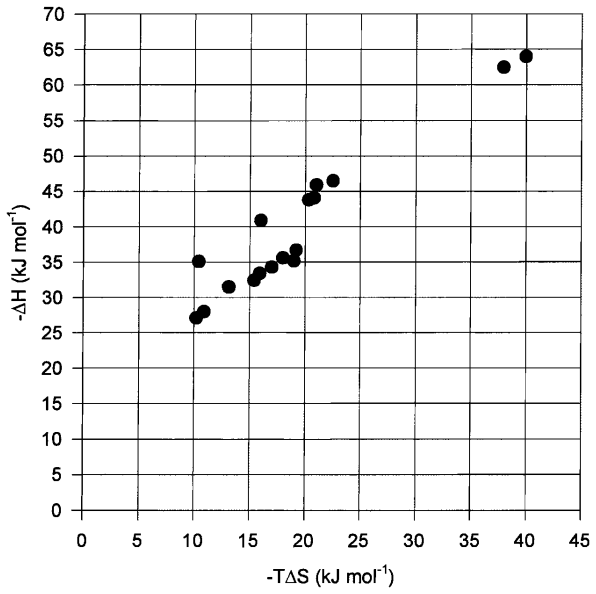

Figure 26. Enthalpy-entropy compensation plot for the thermodynamic data of binding of mono- and disaccharides to the galactoside-specific mistletoe lectin.

tional' capacity with impact on biosignaling. The pattern of maturation of scientific reception is similar in the case of glycolipids.

Glycosphingolipids

In contrast to glycoproteins and proteoglycans, glycolipids are not high molecular weight compounds on the basis of their structure, with only about 1150 Da for, for example, sialyllactosylceramide (GM3; fig. 21), a widely occurring glycolipid. They contain as noncarbohydrate part a lipophilic adapter by which they become a part of the outer leaflet of the lipid bilayer. While the ceramide portion can display a certain degree of heterogeneity due to varying chain length, unsaturation, hydroxylation and branching, classification of a glycolipid calls for homogeneity in its carbohydrate part. Sialic acid-containing glycosphingolipids are called gangliosides, and their biosynthetic relations are shown in figure 21. The membrane anchoring of the glycolipids and the comparatively short chain length preclude extensive reaching of glycolipids into the extracellular space. Hence, the saccharides are rather in close vicinity to the membrane surface. Owing to their lipid part most glycolipids are poorly soluble in water. In an aqueous phase they usually form micelles, with the polar sugars facing outward. Isolation of glycolipids from biological materials therefore requires organic solvents or detergents.

As seen for the mucin-type O-glycan chains, there are numerous structural variations within this group, although they are likewise built with only few different monosaccharides, that is Glc, Gal, GalNAc, GlcNAc, Fuc and Sia [433, 434]. Man has been reported to be a constituent of invertebrate glycolipids, leading to the arthro- and mollu-oligosaccharide series [435]. As reflected in figure 21 for gangliosides, the biosynthetic pathway of glycosphingolipids follows the strategy used for O-glycans or glycosaminoglycans. Individual monosaccharides are added in a stepwise manner from nucleotide-activated precursors by specific glycosyltransferases [436–440]. As accentuated in figure 21, the order of the individual biosynthetic steps is well elucidated. Final stages of ganglioside synthesis take place in the *trans*-Golgi network. Already in the *cis*-Golgi synthetic routes are committed to certain product lines. The acceptor sugar of major importance is the Gal moiety of GM3. As soon as a GalNAc moiety is transferred to the 4′-position of this D-galactose unit, no further α2-8-sialylation of the GM3 sialic acid can occur but only chain elongation via GalNAc. A similar behavior evocative of the guiding role of the introduction of a bisecting GlcNAc residue in N-glycan synthesis or of N-sulfation in heparin/heparan sulfate synthesis is found in the case of GD3, channeling sialylation. Thus, the number of sialic acid residues attached to the D-galactose unit of GM3 is the basis for distinguishing a, b and c series gangliosides. The continuous elaboration of purification protocols and glycan analysis techniques, the latter already outlined in this review, fueled a stunning increase in the number of gangliosides. From 1978 to 1989 the number of characterized species jumped from 28 to 79 [441]. The same is true for the different classes of neutral glycosphingolipids (i.e. ganglio- and isoganglio-, globo- and isoglobo-, lacto- and neolacto-, muco- and gala series), sulfatoglycosphingolipids and fucoglycosphingolipids [438, 442]. By 1989 267 identified glycosphingolipids had been registered [441]. Especially cells with aberrant glycosylation after malignant transformation are a rich source for newly detected variants [439, 443, 444].

Once these glycoconjugates have reached their final destination primarily on the cell surface, the sugar chain can be subject to enzymatic remodeling similar to the N-glycans of glycoproteins. We have already referred to the presence of a cell surface ganglioside sialidase in the section on glycan analysis. This enzyme activity elevates the levels of presentation of the ganglioside GM1 and of lactosylceramide with consequences on cell proliferation [182, 184, 185, 445]. Since this regrouping in glycolipid subpopulations is associated with growth control and differentiation in cultured human neuroblastoma cells, as, for example, shown with the already mentioned sialidase inhibitor Neu5Ac2en, the biological rel-

evance of the display alterations offers an insight to functional traits. The common degradation of glycosphingolipids proceeds after endocytosis in lysosomes by exoglycosidases without accessory proteins. However, once the sugar chain has been shortened below a length of five units, the remaining distance to the intracellular membrane surface is no longer sufficient to guarantee that the degrading enzymes can find a solid grip on their substrate. For hexosaminidases acting on GM2 (for structure, see fig. 21) it has actually been shown that for steric reasons cleavage of glycosidic linkages cannot take place [446, 447]. Therefore, an additional protein, a so-called activator protein, is essential, which works as a 'molecular handle' or 'kind of detergent' in interacting with an appropriate glycolipid and lifting it measurably out of the membrane, thus rendering the sugar chain accessible to the hydrolase [447]. If the action of this protein is impaired, degradation is abrogated [446, 448]. Mutations in activator proteins such as the GM2 activator are the cause of clinical symptoms of lysosomal storage diseases, in this case nearly indistinguishable from the classical Tay-Sachs disease, where the mutation resides in the α-chain of the β-hexosaminidase A gene [448]. This aspect will be discussed further at the end of this section. Accumulation of glycolipids due to defective degradation can be alleviated by therapeutically routing enzymes to the badly afflicted cell types such as macrophages, an approach termed enzyme replacement therapy, with the enzyme's trimmed complex-type N-glycans as a routing signal to the C-type lectins of the target cells [298, 449]. Instead of exoglycosidase treatment of the naturally occurring β-glucocerebrosidase or expression in GlcNAc-transferase I-deficient mammalian cells, insect cells may also be a source of mannose-presenting enzymes, as explained in the section on N-glycans [450, 451]. This example again emphasizes the role of glycans in cell-targeted delivery. If the routing signal itself is not correctly synthesized, for example a mannose-6-phosphate group in lysosomal acid hydrolases, clinical symptoms can be directly assigned to a lack of functionality in the glycan chains, as reported in the context of phosphoglycosylation and N-glycan processing and discussed more in detail in the next section.

Owing to their strategic positioning in the outer leaflet of the mammalian cell membrane, the head group of glycolipids can be accessible for protein/carbohydrate-carbohydrate interactions, which will be explained in the section on glycan recognition. The most famous example is the highly specific binding of cholera toxin to ganglioside GM1, an irresistible argument against a merely arbitrary role of sugars as headgroups in glycosphingolipids [442]. Growth control by sialidase-dependent ganglioside modification in neuroblastoma cells has already been cited as a further example, most probably involving protein-carbohydrate interactions with an endogenous lectin [185]. This report underscores the well-appreciated potential of this class of glycoconjugates to partake in biosignaling extending to the level of kinases or transcription factors [437–439, 446, 452–455]. Besides reacting with their sugar headgroups, glycosphingolipids can be converted to bioreactive compounds such as ceramide or sphingosine-1-phosphate or N,N-dimethylsphingosine, which are signals for protein kinases among others [453, 456]. Evidently, glycolipids are very versatile modulators of a host of reactivities at different stages of their life cycle. In this context the putative formation of glycolipid microdomains with unique properties in ligand presentation is a hot topic of debate [457, 458]. A further analogy to, for example, mucin-type O-glycans (see figs. 17 and 18) can be drawn, as the glycan part of glycolipids can likewise impersonate docking points for microbes, an attractive target for antiadhesion therapy [358, 442, 459]. The underlying structural similarity of parts of glycans from both classes of complex carbohydrates can mean that aspects of their functions can be shared by lipid- and protein-linked determinants [460]. Similarly intriguing, the appearance of glycosphingolipid-based stage-specific embryonic antigens suggests possible explanations of molecular events which are programmed by this signal [461, 462]. Since the prefix *sphingo* derives from the Greek myth of the sphinx, the magnitude of the functional riddle posed by the molecules is adequately signified.

A recently emerging approach to discovering the functions of glycolipids is to generate knockout mice with deficiencies in a distinct glycosyltransferase, as reviewed recently by Hathaway and Shur [463]. While only subtle defects in neuronal functions (for example neuronal conduction velocity) were measurable in mice lacking complex gangliosides due to disruption of GM2/GD2 synthase, galactocerebroside and its sulfatides have been shown to maintain the insulator function of the membrane bilayer of oligodendrocytes and Schwann cells and to target axons by oligodendrocyte processes [464–467]. The same strategy, that is to disrupt genes for selected enzymes at the stem cell level, has been employed to study glycolipid degradation. Of remarkable clinical interest is the production of animal models for heritable neurodegenerative disorders caused by incomplete hydrolysis of the glycan part with excessive accumulation of gangliosides [448, 468]. However, the study of the variants of GM2 gangliosidoses reveals that an identical genetic defect in mice and humans will not necessarily cause the same symptoms. Despite deficiency in the GM2-degrading β-hexosaminidase A, the 'Tay-Sachs' mice are phenotypically normal. The explanation of this finding is attributable to a mouse sialidase. It is able to convert GM2 to a substrate of the

remaining intact mouse β-hexosaminidase, whose action produces lactosylceramide [469]. In this case, species differences in enzyme specificity preclude obtaining the desired animal model.

The crucial role of an enzyme defect for the apparent consequences on the cellular level can be unequivocally demonstrated by gene import into cultured fibroblasts. The deliberate deficit correction ensues significant clearance of accumulated substrates, as demonstrated with transgenic 'Fabry' mice expressing a human α-galactosidase [470, 471]. Clearance of accumulated material will also be beneficial for alleviating defects in lysosomal degradation of other glycoconjugates. Given the strict order of enzymatic steps in such a pathway, glycan analysis of the abundant material clarifies the nature of the deficiency on the analytically accessible level, which concerns glycosphingolipids [439, 472–474], glycogen [475–477], glycosaminoglycans [478, 479] and glycoproteins [480, 481]. Notably, the compilation of the predominant products of diverse variants with inherited defects in N-glycan processing makes it possible to schematically represent the chain of events in degradation, comparable to the example given for GM2 gangliosidoses [481–483]. Data from rigorous testing with site-specific glycosylation and processing inhibitors can easily be reconciled with these results, proving the chain of events independently [484–489]. The further analysis of the responsible enzymes from the patients will define the site of mutation and its actual impact on the protein's features [490–494]. This research can also provide clues to a more complicated regulation than originally thought when one degradation step is impaired with disparate consequences. While, for example, in mucolipidosis I sialylated N- and O-glycans are excessively found due to a lack of sialidase activity, only corresponding O-glycans are detected in Kanzaki disease, although the same enzyme is faulty [495, 496], necessitating clarification of the precise nature of the biochemical defects.

In our context, supportive evidence for a clear-cut answer to the provocative question in the title would be inarguable if it were possible to present clinical examples for an indubitable correlation of aberrant glycosylation and clinical symptoms. Beyond the importance for basic science, the diagnosis can pave the way for an effective rational therapy at least on the level of the symptoms. Such a case has already been discussed for autosomal recessive Morbus Gaucher by the targeted enzyme (i.e. β-glucocerebrosidase) replacement [449, 451, 497].

Glycans and disease

A disease is considered as an aberration from the normal status. If the molecular cause of this experiment of nature can be defined, and no further additional defects complicate the interpretation, the reduced efficiency of the organism in one or several aspects highlights the necessity for strict regulatory events and a functionality of the respective target of the deviation. The fact that glycans should not be randomly added to any protein is graphically proven by a mechanism for pathogenesis of severe hemophilia A. Abnormal N-glycosylation, introduced as a consequence of mutations in, for example, positions 572 or 1772 of the factor VIII-coding DNA sequence, which create new sequons for N-glycosylation, blocks the procoagulant activity of factor VIII [498]. Decreased functionality by this mechanism has also been observed for antithrombin Rouen-III. A mutation from Ile to Asn at position 7 established a new sequon within the heparin-binding domain [499]. Once added, all glycan chains are subject to the events of processing. They appear to be derailed in certain aspects in various diseases [500–503]. Acquired dysfibrinogenemia seen in patients with hepatitis or primary hepatoma involves a delayed rate and extent of fibrin polymerization. A correction of this defect and a normalization of the thrombin time was brought about in vitro by an enzymatic decrease in the hypersialylation of the four biantennary N-glycans [500]. Various degrees of liver dysfunction are associated with the elevated retention of α_1-trypsin inhibitor in the hepatocyte as a consequence of glycosylation abnormalities linked to mutations in the peptide backbone, for example Glu342Lys [500]. The substantial heterogeneity of glycan structures of other acute-phase glycoproteins in response to pathophysiological conditions intimates that glycan presentation is at least of diagnostic relevance [501]. Monitoring of this aspect for α_1-acid glycoprotein has even encouraged the notion that distinct glycoforms harboring the sialyl Lewis^x-epitope may have antiinflammatory potency to restrict an excessive selectin-mediated leukocyte extravasation into the inflamed tissue section [504, 505]. The importance of the fucosylated sugar determinant will be discussed further in this chapter with respect to an inherited deficiency in producing GDP-Fuc de novo, the essential precursor of fucosylation.

In addition to affecting remodeling, impairment of liver functions can translate into the production of carbohydrate-deficient glycoproteins up to the level of a lack of glycosylation. Such a deficiency for the serum glycoprotein transferrin has debatable value as a diagnostic indicator for example for chronic alcohol abuse [506–508]. In this case the patient himself can be blamed for the self-inflicted symptoms on the level of the serum marker. Hypoglycosylation with a characteristic pattern of distribution of transferrin glycoforms is also indicative of a family of presently four subtypes of inherited disorders whose initial description dates from 1980 [359, 503, 509–514]. From the panel of analytical tools isoelectric focusing or anion exchange chromatography are instrumental in delineating the relative proportions

of isoforms, for example tetra- and disialotransferrin [515], in the serum of patients with the multisystemic problems. Electrospray mass spectrometry has verified the assumed loss of one or two N-glycans [359, 511]. Currently, the molecular defects causing a characteristic pattern of hypoglycosylation in the carbohydrate-deficient glycoprotein syndromes (CDGSs) are defined for type Ia, Ib and II. Clinical abnormalities in type Ia include severe neurologic defects (for example brainstem atrophy, cerebellar hypoplasia and alternating esotropia), dysmorphic features, developmental delay, hypogonadism, lipodystrophy and blood coagulation defects. The severity of the symptoms can vary. The responsible genetic abnormality often lies in the gene for phospomannomutase, the enzyme which converts D-mannose-6-phosphate to D-mannose-1-phosphate [513, 516, 517]. This deficiency causes a disturbance of GDP-D-mannose production, representing an example of impairment in the supply of the activated form of this hexose for N-glycan precursor assembly as $Glc_3Man_9GlcNAc_2$-P-P-Dol (fig. 13) and also for biosynthesis of GPI anchors and GDP-L-fucose. In assays with skin fibroblasts from CDGS type 1 patients, D-glucose deprivation or D-mannose supplementation exerted a corrective influence on the size of the lipid- or protein-linked glycans [518, 519]. A variant with a rather mild symptomatology is based on impaired glucosylation of this lipid-linked precursor, which constitutes a suboptimal substrate for transfer to the nascent protein [520]. Phosphomannomutase activity is normal, leaving open the answer to the question on the primary defect.

Besides this most common form and the newly defined variant, the subtype Ib has recently been categorized as a primarily gastrointestinal disorder with protein-losing enteropathy and no indication for psychomotor or mental retardation [521, 522]. While diagnostically indistinguishable from common type Ia on the basis of transferrin analysis, enzyme assays revealed a single-site defect for phosphomannose isomerase. This enzyme converts D-fructose-6-phosphate into D-mannose-6-phosphate. Thus, the precursor for GDP-D-mannose can still be synthesized by a hexokinase, provided the mannose supply is sufficient. Dietary sources and salvage pathways indeed deliver the material for direct D-mannose use, which can make a significant contribution to N-glycan biosynthesis [523]. This reasoning has prompted the design of a rational therapeutic approach with diet supplementation. It worked well in the treated patient [522]. The success of this approach also provides hints to explain dissimilar clinical presentations. Since mannose uptake and/or reuse can be different among cell types, the molecular defect in this enzyme can well be compensated to varying extents by D-mannose-6-phosphate generation, bypassing the shortage in isomerase functionality.

Abnormalities in serum transferrin analysis upon isoelectric focusing were also conducive to pinpoint the type II CDGS, with considerably elevated disialo levels and dramatically reduced tetrasialo values. Peripheral neuropathy could not be diagnosed, in contrast to delayed myelination and global cortical atrophy. The defect in this rare disease was traced to N-acetylglucosaminyltransferase II [513, 517, 524, 525]. As can be deduced from figure 15, the α1-6-linked Man arm of the N-glycan core will not be extended to complex-type chains upon losing this enzyme's activity. Differences in the clinical presentation relative to type I patients are also seen for type III and type IV patients with characteristic changes in the serum transferrin pattern following isoelectric focusing [513, 526]. Although the biochemical nature of the defects in these two inherited diseases has not yet been defined, the fact that abnormal glycosylation can be linked to a variety of clinical phenotypes is further legitimate incentive to teach the basics of glycan structure to medical students to keep them abreast of this development.

It is essential not to disregard that a certain symptom may even depend on two entirely different defects in glycan synthesis. For example, hypergonadotropic hypogonadism is not only associated with CDGS type I but also with galactose-1-phosphate uridyltransferase deficiency [513, 527]. A deficiency in N-acetylglucosaminyltransferase II will not only be responsible for the development of CDGS type II. Expression of a mutant enzyme can lead to mild anemia, hepatomegaly, cirrhosis and hemosiderosis of the liver, classified as congenital dyserythropoietic anemia type II or hereditary erythroblastic multinuclearity with positive acidified-serum test (HEMPAS) [359, 513, 528]. The conversion of the common glycan precursor (see fig. 13) to complex-type N-glycans is also reduced with impaired α-(3/6)-mannosidase II activity. This processing enzyme acts after N-acetylglucosaminyltransferase I on the two residual extensions of the central α1-6-linked mannose prior to N-acetylglucosaminyltransferase II (fig. 15). Again, two different biochemical defects are responsible for the symptoms of one clinically defined syndrome [503, 528]. A clue to understanding the development of the dyserythropoietic anemia in the course of the disease caused by the α-mannosidase II aberration was obtained by knockout mice mutants [529]. In the null mice, nonerythroid cell types avert severe problems via an alternate pathway. The theoretical concept of invoking the presence of a separate α3/6-processing enzyme, termed α-mannosidase III, for removal of the two mannose residues from the α1-6 arm despite a lack

of prior GlcNAc-extension by N-acetylglucosaminyltransferase I was experimentally proven. Both pathways end up in the same substrate for N-acetylglucosaminyltransferase II. The detection of this compensatory (possibly exemplary) backup mechanism can be interpreted as a sign of the vital importance of maintaining correct processing of N-glycans. This view is corroborated by independent experience with (i) glycosylation inhibitors such as swainsonine from plants of the genus *Swainsona*, which cause a disease known as locoism [530], or tunicamycin whose deadly effects due to blocking N-glycosylation at the first step of the dolichol cycle, that is the formation of Dol-P-P-GlcNAc, are counterbalanced by gene amplification [531] as well as (ii) with deliberately engineered loss-of-function mutants after altering a glycosyltransferase gene in the mouse genome [463, 532, 533]. Knockout mice with gene disruption in N-acetylglucosaminyltransferase I, which effectively reduces glycosylation to a 'yeast-or insect-like' status, are invariably subject to embryonic lethality, although mutant cells in vitro suffer no overt consequences [463, 532, 533]. Growth retardation and semilethality were apparent in mice with engineered defects in a β1-4-galactosyltransferase gene [534, 535]. In contrast, absence of the bisecting GlcNAc residue (fig. 15) caused no behavioral or neuromuscular alterations, accessory processes such as cell homing requiring further attention [536].

Genetic defects with clinical impact are not only linked to enzymes of N-glycan processing. A variety of other diseases, including variable hemolytic anemia by increased GalNAcα1-Ser presentation due to reduced O-glycan chain extension, progeroid syndrome with connective tissue abnormalities due to reduced core unit assembly of glycosaminoglycan chains, osteochondrodysplasias and macular corneal dystrophy due to (assumed) undersulfation of certain glycosaminoglycan chains (defect in sulfate transport, 3′-phosphoadenosine-5′-phosphosulfate (PAPS) synthesis or sulfotransferases) and paroxysmal nocturnal hemoglobinuria due to defective assembly of the GPI anchor precursor and ensuing failure of GPI anchoring, prove that the other types of glycans are also relevant for upholding a normal phenotype [26, 503, 513, 537]. As a further resource for valuable insights, the conditional lethality of mutant yeast cells (*gpi1*) with a defect in the synthesis of N-acetylglucosaminyl phosphatidylinositol, the first intermediate in GPI synthesis (see fig. 7 for GPI anchor structure), underlines the importance of GPI anchoring and its potential exploitation by devising antifungal drugs that interfere with GPI synthesis [538], as similarly suggested with respect to parasitic phosphoglycosylation in the section on glycan attachment to proteins. But these are not the only lessons we can learn from the analysis of the biomedical consequences of natural changes in glycosylation. Tracing the pathway between mutation and phenotype has delineated ligand properties of carbohydrate structures in two clinically relevant cases. As already noted in the context of processing of high-mannose-type N-glycans (fig. 13), a D-mannose-6-phosphate signal is attached to acid hydrolases via a two-step stamping process, outlined in the context of presentation of phosphoglycosylation. As a direct result of the elucidation of the molecular basis of the I (inclusion)-cell disease (mucolipidosis II) and its milder variant (Pseudo-Hurler polydystrophy), the P-type lectin-mediated targeting of marker-bearing glycoproteins to the lysosomes was discovered [31, 32]. The reduced activity of the UDP-N-acetylglucosamine: lysosomal enzyme N-acetylglucosaminyl-1-phosphotransferase abrogates proper enzyme targeting. As a consequence misrouting of D-mannose-6-phosphate-lacking hydrolases into the secretory pathway mimics a catabolic enzyme defect of a severe lysosomal storage disease. The expedience of this knowledge is also documented by experimental attempts of enzyme replacement therapy in murine mucopolysaccharidosis VII with D-mannose-6-phosphate-bearing β-glucuronidase obtained in vitro from receptor-deficient L-cells which secrete most of the desired signal-containing enzyme [539, 540].

Severe consequences with mental and growth retardation and recurrent infections are also apparent in another inherited disease, autosomal recessive leukocyte adhesion deficiency (LAD) type II syndrome [541]. This disease has already been referred to in the first part of this section with respect to the potentially antiinflammatory properties of glycoforms of α_1-acid glycoprotein. Its molecular cause is attributed to glycan synthesis in contrast to the type I syndrome. The type I syndrome has been traced back in biochemical terms to mutations in the β_2 integrin (CD18) gene. Either no product, a defective form unable to associate with the partner α chains to build the three heterodimeric integrins LFA-1, CR3 and p150,95 (CD11a,b,c CD18) or dysfunctional β_2 integrins are the hallmarks of the genetic deviation [542, 543]. Under all conditions, recurrent and/or chronic infections despite persistent leukocytosis in the circulation point to a conspicuous inability of leukocytes to migrate into the inflamed tissue part. An inherited defect in production of the activated fucose donor, GDP-Fuc, accounts for the underlying defect in the type II syndrome [541]. Explicitly, the de novo pathway at the stage of regulation of GDP-D-mannose-4,6-dehydratase activity by a yet unidentified regulatory protein is likely to be affected [544, 545]. The requirement for fucosylated ligands on

leukocytes was elegantly underscored by knockout mice for the α1-3-fucosyltransferase VII. Their immunophenotype resembled that of LAD type II syndrome patients [546]. The dependence on fucose incorporation into cell surface glycans for proper activity as addressins is different for leukocytes and nonlymphoid endothelial cells, only the former relying on correct fucosylation for their trafficking behavior [544]. The ongoing debate about the actual nature of the ligand structures in this process notwithstanding [547–549], it is safe to conclude that fucosylated and sialylated/sulfated carbohydrate epitopes are implicated in the initiation of leukocyte-endothelium contacts during inflammation at least on the level of leukocyte glycans. The findings in I-cell disease and LAD type II syndrome collectively and strongly argue in favor of a special case of operative glycan recognition, that is the role as a ligand for an endogenous lectin. In this sense, our discussion on glycan-related diseases has guided us to appreciate that natural tampering with enzymes of the glycosylation machinery engenders a variety of syndromes. Moreover, the documented medical relevance prompts us to look more deeply into the aspects of glycan recognition and glycan receptors (lectins).

Glycan recognition

As attested by a causal relation between a defect in glycosylation and symptoms of a clinically manifest disease, the glycan part of glycoconjugates, already coined as bioinformation hardware, is clearly able to modulate biomolecular features of carrier biomolecules. For proteins, physicochemical factors include solubility and charge variations, passive steric hindrance guiding folding, oligomer assembly, proteolysis and immunogenicity, and active intramolecular interactions stabilizing protein conformations and affecting enzyme and receptor activities [25, 26, 75, 249, 512, 550–552]. Especially the core constituents such as the N,N′-diacetylchitobiosyl or attached fucose moieties can be tightly associated to the protein backbone as if they are an integral part of the carrier [553–555]. This intimate contact is able to modulate and/or restrict the mobility of glycan chains, for example the α1-6-branch of the Asn78-linked biantennary complex-type undecasaccharide of the α-subunit of human chorionic gonadotropin (for illustration of the structure, see fig. 15) [556]. Although the terminal parts of a glycan chain appear to be extended in solution, as shown for the Asn32-attached glycan of human serum amyloid P component in figure 22, the nature of the immediate surroundings constituted by the peptide backbone and side chains can impart differential presentation to the environment and accessibility of the oligosaccharides. The interdependence of conformational preferences between individual torsion angles of glycosidic linkages and between protein (or other groups in the immediate vicinity) and glycan parts will be crucial for the actual selection of conformational minima, which necessitates looking more deeply into the concept of environment-dependent shaping of the potentially bioactive glycan [192, 247, 557–559]. Assessment of the relaxation behavior in NMR analysis or avidity of lectin binding is conducive to delineating such effects [271, 556, 560–562]. Recalling the description of the remarkable flexibility of free oligosaccharides in the section on glycan conformation, the restriction of the inherent mobility of the sugar part of glycoconjugates up to freezing conformations into bioactive or bioinert constellations will have implications for the ligand properties of the corresponding glycans.

The term ‘ligand properties’ can inadvertently imply binding exclusively to a protein. However, intermolecular contacts can be made to a suitably positioned glycan (homotypic and heterotypic carbohydrate-carbohydrate interaction) as well, warranting a comment. Clustering of individual low-affinity sites in carbohydrate-carbohydrate recognition adds up to yield stable cell aggregates involving aggregation factors (polyanionic glycans) in sponges and neutral or sialylated glycolipids in higher eukaryotes [453, 455, 563, 564]. Intermolecular protein-carbohydrate interactions have already been substantiated at several places in this article, for example concerning intracellular N-glycan-presenting glycoprotein routing. This molecular interaction is governed by common principles of recognition involving cooperative or bidentate hydrogen bonds, dispersion forces and

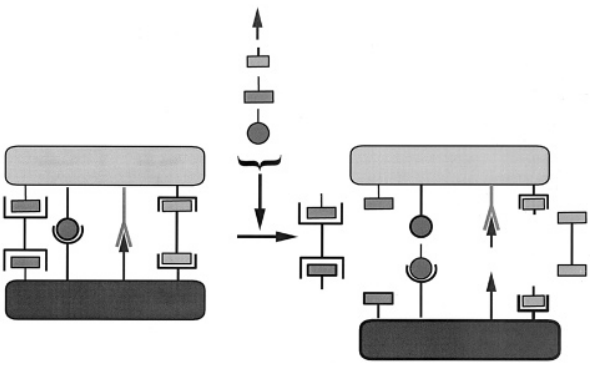

Figure 27. Illustration of the concept for lectin-based antiadhesion therapy. Therapeutic inhibitors (glycostructures or glycomimetics) block the intercellular interaction established by lectin-ligand contact including cellular association by bridging of epitopes. Kindly provided by Dr. H.-C. Siebert, Munich, and Dr. C.-W. von der Lieth, Heidelberg.

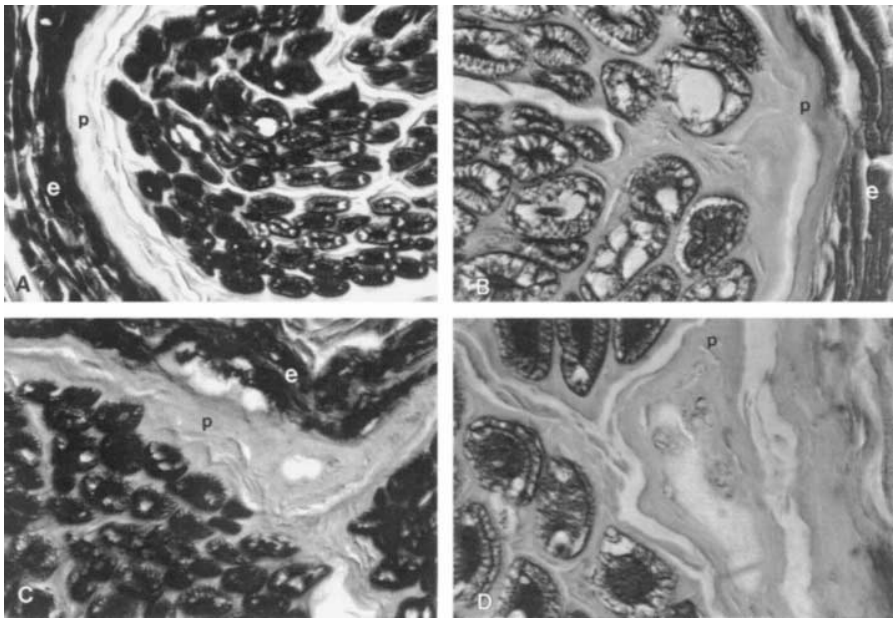

Figure 28. Localization of specific binding sites for β-D-xyloside (A), α-D-mannoside (B), β-D-galactoside (C) and N-acetyl-β-D-glucosaminide (D) in the epineurium (e) of fixed sections of human accessory nerve. Perineurium (p) and endoneurium failed to present staining, obtained by the common DAB reaction and metal ion intensification; magnification $\times$ 422 (A), $\times$ 845 (B), $\times$ 528 (C) and $\times$ 1056 (D).

hydrophobic stacking [222, 298, 565–572]. While the abundance of hydroxyl groups naturally intimates the relevance of donor/acceptor hydrogen bonds, the actual involvement of stacking and dispersion forces requires an explanation which necessarily falls back on the inspection of the monosaccharide structure given in the section on the sugar code.

The three basic hexoses for N- and O-glycans are assumed to be selected for incorporation owing to their relative levels of thermodynamic stability [573]. Unfavorable 1,3-diaxial interactions of bulky groups are minimized in glucose and its 2- and 4-epimers. For the consideration of interactions not only the relative positioning of the hydroxyl groups is important. As compellingly illustrated in figure 23, an epimerization also affects polarity distribution. Moreover, the upper and lower sides of a hexose unit can be disparate with respect to this property. Explicitly, the two surfaces of D-galactose present undeniably different characters in terms of polarity (fig. 24). The B-face with its aliphatic protons is thus likely to stack against aromatic side chains, as seen in the crystal structure of bovine galectin-1 (fig. 25). Since this aromatic amino acid is thereby shielded from solvent exposure by the carbohydrate ligand, some of its protons will no longer be accessible for nuclear polarization by a laser photoactivated dye. Monitoring spin polarization of the aromatic side chains by NMR spectroscopy has thus been instrumental in verifying this structural role of the only tryptophan residue in galectin-1 in solution [241]. Also, plant lectins such as hevein domain-containing proteins with affinity for N-acetyl-D-glucosamine harbor aromatic residues in their combining sites for a similar purpose, as shown in solution by NMR spectroscopy and microcalorimetry [574–577].

This example teaches that a variety of enthalpic forces add up to result in the measurable enthalpy of the entire binding process. The enthalpic gain is inevitably linked to changes in ligand flexibility. When a disaccharide such as Galβ1-2Gal with its rapid transition between two low-energy conformers is accommodated into a binding site, the necessity of an ensuing entropic penalty is readily apparent. To document the validity of the concept of an assumed conformer selection in solution, NMR techniques are the method of choice, especially the monitoring of interresidual ligand signals based on a through-space magnetization transfer, termed transferred nuclear Overhauser effects [20, 222, 228, 240, 557, 578–580]. This technique was instrumental in proving differential conformer selection by plant and animal galactoside-specific lectins [232, 235]. While the free ligand population is distributed between the two

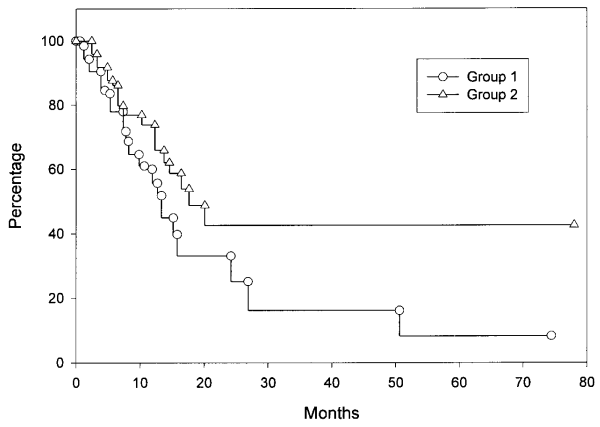

Figure 29. Illustration of the survival of lung cancer patients (in months) after surgery, given as percentage of the total number of patients, at each time point. Category formation is based on the capacity of tumor cells to specifically bind the histoblood group H (type 1) trisaccharide (group 1: no binding, group 2: binding), presented as part of a histochemically inert and labeled carrier, that is substituted poly(2-hydroxyethyl acrylamide).

low-energy positions in the central valley of the ϕ, ψ-plot, documented in figure 12a, its association to a plant or animal lectin accounts for shifting the dynamic equilibrium to one of the two conformers. A single saccharide sequence can thus be viewed as a 'bunch of keys' [239], which can open more than one lock. E. Fischer's famous 'lock and key' analogy for enzyme activities [581] is thus extended, placing emphasis on recognition as a shape, not a sequence problem [557]. Another documented case for this entropically unfavorable process even reveals its operation in homologous

Figure 30. Illustration of the apparently strategic positioning of the two ligand-binding sites on homodimeric bovine galectin-1 (entry 1SLT into Brookhaven Protein Data Bank) to form a versatile cross-linking reagent (see also fig. 27). Kindly provided by Dr. C.-W. von der Lieth, Heidelberg.

locks (proteins), that is the selectins [582] (for further information on selectins, see next section). Evidently, the strategic design of sterically hindered derivatives (glycans or their mimetics) could be a safe section of the tour of the minefield towards reaching the envisaged destination in rational drug design, that is the perfect inhibitor. However, the complexity of the entire binding process warns against ignoring other, potentially decisive factors. In addition to the actual interactions between receptor and ligand, which extend beyond hydrogen bonding, the systematic testing of monodeoxy ligand derivatives, which are valuable for chemical mapping of the binding site [583], has clearly underscored the importance of other terms such as solvent and protein effects in the case of trimannoside binding to the model lectin concanavalin A [584]. Another argument in favor of this line of reasoning is the observation that extended glycan structures appear to be bound with different energetic mechanisms despite conservation of binding sites by *Diocleinae* lectins [585, 586].

A fundamental principle of the binding process is enthalpy-entropy compensation, balancing out for an enthalpic stabilization of approximately 5 kcal/mol [222, 228, 587–589]. Solvent reorganization and freezing of degrees of motional freedom by strong enthalpic interactions will certainly play a salient role in explaining this general property of weak intermolecular interactions, illustrated for mono- and disaccharide binding to a plant lectin as model in figure 26. However, it is essentially premature to assign a molecular cause to the properties determined by microcalorimetry. An example from recent research illustrates this prickly issue. Despite similar extents of restriction of ligand mobility of β1-2(3)-linked digalactosides, the enthalpic gain and the entropic penalty can vary significantly, although still obeying the mutual compensation [235, 590]. Again, the meticulous analysis of protein and solvent features is understood to be imperative to delineating crucial parameters for entirely reconciling thermodynamic and structural studies. It is not preposterous to predict that this approach garnered from several areas will have merit for the development of high-affinity agents with testable perspective in lectin-mediated drug delivery or antiadhesion therapy in, for example, inflammation [222, 324, 357, 591–597]. A scheme for successful performance of a carbohydrate inhibitor or a mimetic thereof is presented in figure 27.

Coupling of these high-affinity binding partners to a biologically inert matrix without impairing their ligand properties is also a major step towards generating a versatile class of lectin-detecting tools, that is neoglycoconjugates [317, 320, 327, 598–604]. Their application in solid-phase assays, in cell biological test systems and in tissue sections fosters the detection of sugar-binding molecules which retain activity under experimental con-

Table 2. Functions of animal lectins.

Activity	Example of lectin
Ligand-selective molecular chaperones in endoplasmic reticulum	calnexin, calreticulin
Intracellular routing of glycoproteins and vesicles	ERGIC-53, VIP-36, P-type lectins, comitin
Intracellular transport and extracellular assembly	nonintegrin 67-kDa elastin/laminin-binding protein
Cell type-specific endocytosis	hepatic asialoglycoprotein receptor, macrophage C-type lectins, hepatic endothelial cell receptor for GalNAc-4-SO_4-bearing glycoproteins
Recognition of foreign glycans (β1,3-glucans, LPS)	CR3 (CD11b/CD18), *Limulus* coagulation factors C and G
Recognition of foreign or aberrant glycosignatures on cells (incl. endocytosis or initiation of opsonization or complement activation)	collectins, pentraxins (CRP, limulin), C-type macrophage receptors, L-ficolin
Targeting of enzymatic activity in multimodular proteins	acrosin
Bridging of molecules	homodimeric and tandem-repeat galectins, cytokines (for example IL-2:IL-2R and CD3 of TCR), cerebellar soluble lectin
Effector release (H_2O_2, cytokines etc.)	galectins, selectins, CD23
Cell growth control and apoptosis	galectins, C-type lectins, amphoterin-like protein, cerebellar soluble lectin
Cell routing	selectins, I-type lectins, galectins
Cell-cell interactions	selectins and other C-type lectins, galectins, I-type lectins
Cell-matrix interactions	galectins, heparin- and hyaluronic acid-binding lectins
Matrix network assembly	proteoglycan core proteins (C-type CRD), galectins, nonintegrin 67-kDa elastin/laminin-binding protein

See [298, 669–671].

ditions [272, 320, 602, 603, 605–607]. Besides commercial offers for labeling kits, the natural activity of enzymes can be exploited by covalent conjugation to visualize a binding event of the sugar part in simplified protocols [606, 608–610]. Concerning ligand synthesis, the already mentioned combination of chemical and enzymatic techniques (see section on N-glycans) has matured considerably and is competitive to the purification of oligosaccharides from natural sources. This progress extends the range of testable ligands and their complexity considerably [29], as recently shown for bi-antennary N-glycans [271]. An especially charming aspect of this work aside from its scientific merit is the inherent genuine beauty of histochemical slides. As exemplarily documented in figure 28 for carbohydrate-reactive sites in the epineurium of a human accessory nerve [611, 612], specific glycoligand-dependent binding can readily be assessed. Thus, the monitoring of accessible binding sites on cells and in tissues by glycohistochemistry [218, 219, 606, 613, 614] can guide further efforts to purify the observed activity. In histopathology, this class of markers leads to results equivalent to the application of antilectin antibodies [220, 615–622]. Glycohistochemistry with neoglycoconjugates is, for example, helpful in muscle fiber typing in fixed sections and tumor diagnosis in different classes such as brain, breast, colon, lung or prostate malignancies [326, 616, 617, 623–631]. In addition to diagnostic questions, the binding of distinct sugar epitopes such as A- and H-histoblood group determinants, as shown in figure 29 for the H-epitope, is of predictive importance for prognostic evaluations in lung cancer [632, 633]. The pursuit of this analysis can also contribute to clarifying the riddle of the relevance of blood group presence in tumor pathology [634–637]. Since endogenous lectins have been implicated in growth control and induction of apoptosis [298], this glycohistochemical information is wedded at least phenomenologically to the concept of glycan function.

Indeed, the structural analysis of glycan-lectin interactions and their functional implications are a hallmark of glycan relevance. Research on animal lectins, whose path through the decades has been traced elsewhere [638–640], has matured to the stage of classification of

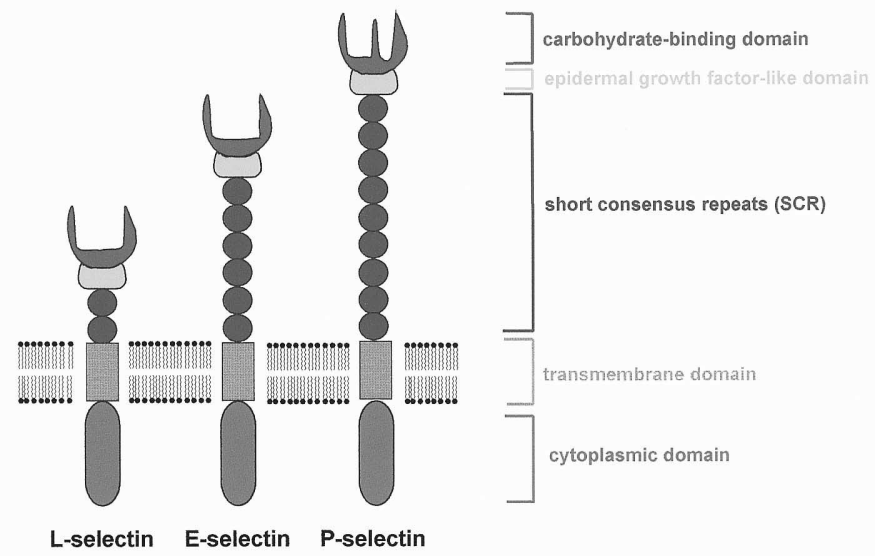

Figure 31. Illustration of the modular architecture of the members of the group of selectins, a subfamily of C-type animal lectins (see table 1). Kindly provided by Priv.-Doz. Dr. H. Kaltner, Munich.

main families and elucidation of various functions to be reviewed in the next section.

Animal lectins: current classification and function

Structural characterization of invariant sequence motifs and distinct folding patterns has paved the way for a well-accepted classification scheme (table 1). The question of the design of carbohydrate-binding sites is presently answered, for example, by noting the compactness of β-strand organization, seen in the jelly-roll topology with its antiparallel β-sandwich arrangement (fig. 30), the stacked β-sheets of the Ig-superfamily members or the flattened β-barrel arrangement of a P-type lectin with similarity to avidin [298, 568, 569, 571, 641–645]. Compilations of the frequency of lectin occurrence with respect to their target sugars have unveiled a preponderance for receptors specific for D-galactose and its derivatives such as sialyllactose [573, 646]. This phenomenological evidence points to a correlation between the spatial accessibility of these determinants at the outer part of sugar antennae and their operativity as lectin ligands. A similar conclusion had been drawn on the basis of the transient presentation of a glucose unit during N-glycan processing and the presence of calnexin and calreticulin (fig. 16). These two lectins lead off the compilation of functions, given in table 2.

The suggestion of a noncoincidental positioning is reinforced when the modular constitution of several lectins implicated in cell adhesion is scrutinized [298, 361, 647–650]. The C-type subgroup of the selectins which has been mentioned in preceding sections as targets for anti-inflammatory drug design expose their lectin domain well beyond the glycocalyx by spacer modules (fig. 31). Thus, leukocytes and platelets in rapid circulation can sense the presence of docking sites on inflamed endothelium leading to initial contact formation [298, 361, 362, 651–653]. As seen for hyaluronic acid-binding extracellular proteoglycans (see section on glycosamino-

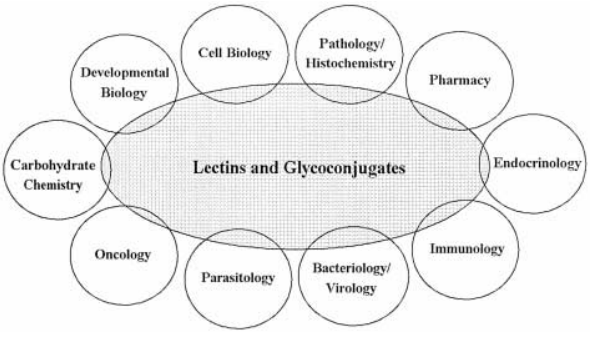

Figure 32. Illustration of the relevance of lectins and carbohydrates for various disciplines in basic and applied natural and clinical sciences.

glycans and proteoglycans), we again meet EGF-like and complement-binding consensus-repeat-like domains in the vicinity of the C-type lectin fold. A similar principle holds true for the sialoadhesin group of I-type lectins employing C2-set immunoglobulin-like tandem repeats, and most galectins can readily bridge two accessible glycans by the strategic occurrence of their binding pockets at opposing sides of homodimeric and tandem-repeat proteins, as shown in figure 30 [298, 654]. This binding, effectively forming cross-linked complexes [655–658], may not only establish or strengthen cell clusters. It is most probably a trigger for the role of galectins in cell growth control and apoptosis [119, 185, 298, 654, 659], the available evidence justifying an entry into table 2.

Evidently, positioning of the binding sites is essential for the consequences which follow molecular association. This correlation is not only encountered in the case of cell surface contacts on 'self' cells. Since bacteria and yeast cells differ markedly in the presentation of glycans, for example harboring a high density of mannans on infectious yeasts like *Candida albicans* (see section on N-glycans), it is not surprising when accepting the concept of a role of sugars as recognition units that components of the innate immune system take advantage of this glycosignature to identify invaders [298, 660–663]. Either α-helical coiled-coil-mediated noncovalent association of lectin-containing subunits ('bundle-of-tulips' arrangement) or tandem-repeat presentation of receptor sites in one contiguous polypeptide chain enable the construction of sensors to track down a 'nonself' pattern (table 2). Subsequent complement activation or opsonization lead from initial contact to destruction of the invading microorganism. Interestingly, the generation of the coagulin gel which will engulf Gram-negative bacteria in the hemolymph of the horseshoe crab to render them targetable by antimicrobial substances (anti-bacterial lipopolysaccharide (LPS) factor or tachyplesins) is initiated by two β-glucan sensors [664, 665]. The spatial vicinity of this unit to an activatable proteolytic center is a prerequisite to transform the signal on the presence of a foreign glycosignature into the eventually protective reaction cascade (table 2). Similar to the already mentioned relevance of endocytosis receptors for routing determinants such as GalNAc-4-SO_4 on the terminal part of N-glycan antennae of a limited set of glycoproteins [37], the occurrence of pattern-recognizing lectins in host defense attests how meaningful messages of the glycocode can be deciphered to the benefit of the organism. As likewise emphasized, therapeutic routing has also been beneficially exploited in enzyme replacement therapy involving the C-type tandem-repeat mannose receptor of macrophages [298, 335, 449, 451]. These examples and further information given in table 2 make easily understandable why current research activities on lectins and glycoconjugates touch on various disciplines from the realms of basic and applied science (fig. 32).

Concluding remarks

Having started this review with a provocative question, we have furnished the reader with a wealth of information to rebuff the notion that glycosylation is a superfluous, nonfunctional event in protein and lipid processing. Experience with recombinant expression of glycoproteins has helped teach instructive lessons in not underestimating the role of glycans in protein stability and therapeutic performance [263–265, 666, 667]. Albeit still laborious, glycan analysis continues to nourish the concept of lasting value that the sugar code has an unsurpassed and actually operative capacity to store biological information. It is reassuring that the envisioned 'discrete recognitional role' [8] has turned out to be prophetic. It is a great asset for this view that endogenous receptors (lectins) for distinct glycoepitopes have been purified. Their classification based on folding patterns and the architecture of carbohydrate-binding sites and the delineation of functions by molecular rendezvous between lectins and their sugar partners are making rapid progress, encouraging scanning of the horizon for potentially remunerative clinical applications which do not reek of science fiction. It is thus presently fair to conclude that it is not clear how to contradict the positive answer to the initial question in principle, although we still face the challenge of clarifying further, yet undiscovered aspects of glycan structure and functionality. We are reminded of an apt allegory, given by J. Montreuil in 1975 [668] quoting A. Gottschalk, who compared the realm of glycoconjugate research to a great unexplored continent. Montreuil concluded in 1995 that 'we have crossed the shores of the great unexplored continent announced by A. Gottschalk and, filled with wonder, we are discovering its fascinating secrets. We have entered the golden age of glycoconjugates' [16]. Yes, indeed!

Acknowledgments. We are indebted to Priv.-Doz. Dr. H. Kaltner, Dr. H.-C. Siebert and Dr. C.-W. von der Lieth for providing artwork, as explicitly given in the corresponding figure legends, to R. Ohl for excellent processing of the manuscript and to the Deutsche Forschungsgemeinschaft, the Dr.-Mildred-Scheel-Stiftung für Krebsforschung, the Fonds der Chemischen Industrie, the INTAS-program of the EC, the Verein zur Förderung des biologisch-technologischen Fortschritts in der Medizin e.V., and the Volkswagenstiftung for financial support. A sincere apology is directed to our colleagues whose relevant publications could not completely be included in the reference section due to space limitations.

1 Berger E. G., Buddecke E., Kamerling J. P., Kobata A., Paulson J. C. and Vliegenthart J. F. G. (1982) Structure, biosynthesis and function of glycoprotein glycans. Experientia **38:** 1129–1162
2 Dwek R. A., Edge C. J., Harvey D. J. and Wormald M. R. (1993) Analysis of glycoprotein-associated oligosaccharides. Annu. Rev. Biochem. **62:** 65–100
3 Hounsell E. F. (ed.) (1993) Glycoprotein Analysis in Biomedicine, Humana Press, Totowa, NJ
4 Hounsell E. F. (1997) Methods for glycoconjugate analysis. In: Glycosciences: Status and Perspectives, pp. 15–29, Gabius H.-J. and Gabius S. (eds), Chapman and Hall, London
5 Vliegenthart J. F. G. (1997) Structural glycobiology: how to gain insight into the structure-function relationship. Pure Appl. Chem. **69:** 1875–1878
6 Geyer H. and Geyer R. (1998) Strategies for glycoconjugate analysis. Acta Anat. **161:** 18–35
7 Roseman S. (1970) The synthesis of complex carbohydrates by multiglycosyltransferase systems and their potential function in intercellular adhesion. Chem. Phys. Lipids **5:** 270–297
8 Winterburn P. J. and Phelps C. F. (1972) The significance of glycosylated proteins. Nature **236:** 147–151
9 Montreuil J. (1984) Spatial structures of glycan chains of glycoproteins in relation to metabolism and function. Survey of a decade of research. Pure Appl. Chem. **56:** 859–877
10 Cook G. M. W. (1986) Cell surface carbohydrates: molecules in search of a function? J. Cell Sci. Suppl. **4:** 45–70
11 Neuberger A. (1988) Early work on the glycoprotein egg albumin. Trends Biochem. Sci. **13:** 398–399
12 Faillard H. (1989) The early history of sialic acids. Trends Biochem. Sci. **14:** 237–241
13 Rodén L. (1989) Highlights in the history of heparin. In: Heparin. Chemical and Biological Properties, Clinical Applications, pp. 1–24, Lane D. A. and Lindahl U. (eds), CRC Press, Orlando
14 Yanagishita M. (1993) A brief history of proteoglycans. Experientia **49:** 366–368
15 Cook G. M. W. (1995) Glycobiology of the cell surface: the emergence of sugars as an important feature of the cell periphery. Glycobiology **5:** 449–461
16 Montreuil J. (1995) The history of glycoprotein research, a personal view. In: Glycoproteins, pp. 1–12, Montreuil J., Vliegenthart J. F. G. and Schachter H. (eds), Elsevier Science, Amsterdam
17 Sharon N. (1997) Albert Neuberger (1908–96): founder of modern glycoprotein research. Glycoconjugate J. **14:** 155–158
18 Sharon N. (1998) Glycoproteins now and then: a personal account. Acta Anat. **161:** 7–17
19 Laine R. A. (1997) The information-storing potential of the sugar code. In: Glycosciences: Status and Perspectives, pp. 1–14, Gabius H.-J. and Gabius S. (eds), Chapman and Hall, London
20 von der Lieth C.-W., Siebert H.-C., Kozár T., Burchert M., Frank M., Gilleron M. et al. (1998) Lectin ligands: new insights into their conformations and their dynamic behavior and the discovery of conformer selection by lectins. Acta Anat. **161:** 91–109
21 Sharon N. (1984) Glycoproteins. Trends Biochem. Sci. **9:** 198–202
22 Olden K., Bernard B. A., Humphries M. J., Yeo T.-K., Yeo K.-T., White S. L. et al. (1985) Function of glycoprotein glycans. Trends Biochem. Sci **10:** 78–82
23 Paulson J. C. (1989) Glycoproteins: what are the sugar chains for? Trends Biochem. Sci. **14:** 272–276
24 Elbein A. D. (1991) The role of N-linked oligosaccharides in glycoprotein function. Trends Biotechnol. **9:** 346–352
25 Kobata A. (1992) Structures and functions of the sugar chains of glycoproteins. Eur. J. Biochem. **209:** 483–501
26 Varki A. (1993) Biological roles of oligosaccharides: all of the theories are correct. Glycobiology **3:** 97–130
27 Whitfield D. M. and Douglas S. P. (1996) Glycosylation reactions: present status, future directions. Glycoconj. J. **13:** 5–17
28 Brockhausen I., Schachter H. (1997) Glycosyltransferases involved in N- and O-glycan biosynthesis. In: Glycosciences: Status and Perspectives, pp. 79–113, Gabius H.-J. and Gabius S. (eds), Chapman and Hall, London
29 Sears P. and Wong C.-H. (1998) Enzyme action in glycoprotein synthesis. Cell. Mol. Life Sci. **54:** 223–252
30 Reuter G., Pfeil R., Stoll S., Schauer R., Kamerling J. P., Versluis C. et al. (1983) Identification of new sialic acids derived from glycoprotein of bovine submandibular gland. Eur. J. Biochem. **134:** 139–143
31 Kornfeld S. (1986) Trafficking of lysosomal enzymes in normal and disease states. J. Clin. Invest. **77:** 1–6
32 von Figura K. and Hasilik A. (1986) Lysosomal enzymes and their receptors. Annu. Rev. Biochem. **55:** 167–193
33 Varki A. (1992) Diversity in sialic acids. Glycobiology **2:** 25–40
34 Reuter G. and Gabius H.-J. (1996) Sialic acids. Structure-analysis-metabolism-occurrence-recognition. Biol. Chem. Hoppe-Seyler **377:** 325–342
35 Varki A. (1996) 'Unusual' modifications and variations of vertebrate oligosaccharides: are we missing the flowers for the trees? Glycobiology **6:** 707–710
36 Brockhausen I. and Kuhns W. (1997) Role and metabolism of glycoconjugate sulfation. Trends Glycosci. Glycotechnol. **9:** 379–398
37 Hooper L. V., Manzella S. M. and Baenziger J. U. (1997) The biology of sulfated oligosaccharides. In: Glycosciences: Status and Perspectives, pp. 261–276, Gabius H.-J. and Gabius S. (eds), Chapman and Hall, London
38 Varki A. (1997) Sialic acids as ligands in recognition phenomena. FASEB J. **11:** 248–255
39 Rademacher T. W., Parekh R. B. and Dwek R. A. (1988) Glycobiology. Annu. Rev. Biochem. **57:** 785–838
40 Gleeson P. A. (1988) Complex carbohydrates of plants and animals: a comparison. Curr. Top. Microbiol. Immunol. **139:** 1–34
41 Hayes B. K. and Hart G. W. (1994) Novel forms of protein glycosylation. Curr. Opin. Struct. Biol. **4:** 692–696
42 Brockhausen I. (1997) Biosynthesis and functions of O-glycans and regulation of mucin antigen expression in cancer. Biochem. Soc. Trans. **25:** 871–874
43 Marshall R. D. (1972) Glycoproteins. Annu. Rev. Biochem. **41:** 478–502
44 Shakin-Eshleman S. H., Spitalnik S. L. and Kasturi L. (1996) The amino acid at the X position of an Asn-X-Ser sequon is an important determinant of N-linked core-glycosylation efficiency. J. Biol. Chem. **271:** 6363–6366
45 Kasturi L., Chen H. and Shakin-Eshleman S. H. (1997) Regulation of N-linked core glycosylation: use of site-directed mutagenesis approach to identify Asn-Xaa-Ser/Thr sequons that are poor oligosaccharide acceptors. Biochem. J. **323:** 415–419
46 Gavel Y. and von Heijne G. (1990) Sequence differences between glycosylated and non-glycosylated Asn-X-Thr/Ser acceptor sites: implications for protein engineering. Protein Eng. **3:** 433–442
47 Mellquist J. L., Kasturi L., Spitalnik S. L. and Shakin-Eshleman S. H. (1998) The amino acid following an Asn-X-Ser/Thr sequon is an important determinant of N-linked core glycosylation efficiency. Biochemistry **37:** 6833–6837
48 Bause E. and Legler G. (1981) The role of the hydroxy amino acid in the triplet sequence Asn-Xaa-Thr(Ser) for the N-glycosylation step during glycoprotein biosynthesis. Biochem. J. **195:** 639–644
49 Vance B. A., Wu W., Ribaudo R. K., Segal D. M. and Kearse K. P. (1997) Multiple dimeric forms of human CD69 result from differential addition of N-glycans to typical (Asn-X-Ser/Thr) and atypical (Asn-X-Cys) glycosylation motifs. J. Biol. Chem. **272:** 23117–23122

50 Clausen H. and Bennett E. P. (1996) A family of UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferases controls the initiation of mucin-type O-linked glycosylation. Glycobiology **6:** 635–646

51 Hansen J. E., Lund O. and Brunak S. (1997) O-GLYCBASE version 2.0: a revised database of O-glycosylated proteins. Nucleic Acids Res. **25:** 278–282

52 Hennet T., Hagen F. K., Tabak L. A. and Marth J. D. (1995) T-cell-specific deletion of a polypeptide N-acetylgalactosaminyltransferase gene by site-directed mutagenesis. Proc. Natl. Acad. Sci. USA **92:** 12070–12074

53 Elhammer A. P., Poorman R. A., Brown E., Maggiora L. L., Hoogerheide J. G. and Kezdy F. J. (1993) The specificity of UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase as inferred from a database of in vivo substrates and from the in vitro glycosylation of proteins and peptides. J. Biol. Chem. **268:** 10029–10038

54 Schwartz N. B. (1995) Xylosylation: the first step in synthesis of proteoglycan. Trends Glycosci. Glycotechnol. **7:** 429–445

55 Caplan A. I. (1984) Cartilage. Sci. Am. **251:** 82–90

56 Harris R. J. and Spellman M. W. (1993) O-Linked fucose and other posttranslational modifications unique to EGF modules. Glycobiology **3:** 219–224

57 van den Steen P., Rudd P. M., Dwek R. A. and Opdenakker G. (1998) Concepts and principles of O-linked glycosylation. Crit. Rev. Biochem. Mol. Biol. **33:** 151–208

58 Wang Y., Lee G. F., Kelley R. F. and Spellman R. W. (1996) Identification of a GDP-L-fucose:polypeptide fucosyltransferase and enzymatic addition of O-linked fucose to EGF domains. Glycobiology **6:** 837–842

59 Moloney D. J., Lin A. I. and Haltiwanger R. S. (1997) The O-linked fucose glycosylation pathway. Evidence for protein-specific elongation of O-linked fucose in Chinese hamster ovary cells. J. Biol. Chem. **272:** 19046–19050

60 Bergwerff A. A., van Oostrum J., Asselbergs F. A. M., Burgi R., Hokke C. H., Kamerling J. P. et al. (1993) Primary structure of N-linked carbohydrate chains of human chimeric plasminogen activator K_2tuPA expressed in Chinese hamster ovary cells. Eur. J. Biochem. **212:** 639–656

61 Hajjar K. and Reynolds C. (1994) Fucose mediated binding and degradation of tissue-type plasminogen activator by HepG2 cells. J. Clin. Invest. **93:** 703–710

62 Camani C. and Kruithof E. K. O. (1995) The role of the finger and growth factor domains in the clearance of tissue-type plasminogen activator by hepatocytes. J. Biol. Chem. **270:** 26053–26056

63 Rabbani S. A., Mazar A. P., Bernier S. M., Haq M., Bolivar I., Henkin H. et al. (1992) Structural requirements for the growth factor activity of the amino-terminal domain of urokinase. J. Biol. Chem. **267:** 14151–14156

64 Schnaper H. W., Barnathan E. S., Mazar A., Maheshwari S., Ellis S., Cortez S. L. et al. (1995) Plasminogen activators augment endothelial cell organization in vitro by two distinct pathways. J. Cell. Physiol. **165:** 107–118

65 Bjoern S., Foster D. C., Thim L., Wiberg F. C., Christensen M., Komiyama Y. et al. (1991) Human plasma and recombinant factor VII. Characterization of O-glycosylations at serine residues 52 and 60 and effects of site-directed mutagenesis of serine 52 to alanine. J. Biol. Chem. **266:** 11051–11057

66 Torres C. R. and Hart G. W. (1984) Topography and polypeptide distribution of terminal N-acetylglucosamine residues on the surface of intact lymphocytes. Evidence for O-linked GlcNAc. J. Biol. Chem. **259:** 3308–3317

67 Holt G. D., Haltiwanger R. S., Torres C. R. and Hart G. W. (1987) Erythrocytes contain cytoplasmic glycoproteins. O-linked GlcNAc on band 4.1. J. Biol. Chem. **262:** 14847–14850

68 Hart G. W., Haltiwanger R. S., Holt G. D. and Kelly W. G. (1989) Glycosylation in the nucleus and cytoplasm. Annu. Rev. Biochem. **58:** 841–874

69 Roquemore E. P., Chou T.-Y. and Hart G. W. (1994) Detection of O-linked N-acetylglucosamine (O-GlcNAc) on cytoplasmic and nuclear proteins. Methods Enzymol. **230:** 443–460

70 Hart G. W. (1997) Dynamic O-linked glycosylation of nuclear and cytoskeletal proteins. Annu. Rev. Biochem. **66:** 315–335

71 Haltiwanger R. S., Busby S., Grove K., Li S., Mason D., Medina L. et al. (1997) O-Glycosylation of nuclear and cytoplasmic proteins: regulation analogous to phosphorylation? Biochem. Biophys. Res. Commun. **231:** 237–242

72 Hart G. W., Kreppel L. K., Comer F. I., Arnold C. S., Snow D. M., Ye Z. Y. et al. (1996) O-GlcNAcylation of key nuclear and cytoskeletal proteins: reciprocity with O-phosphorylation and putative roles in protein multimerization. Glycobiology **6:** 711–716

73 Teng-umnuay P., Morris H. R., Dell A., Panico M., Paxton T. and West C. M. (1998) The cytoplasmic F-box binding protein SKP1 contains a novel pentasaccharide linked to hydroxyproline in *Dictyostelium*. J. Biol. Chem. **273:** 18242–18249

74 Sumper M., Wieland F. T. (1995) Bacterial glycoproteins. In: Glycoproteins, pp. 455–473, Montreuil J., Vliegenthart J. F. G. and Schachter H. (eds), Elsevier, Amsterdam.

75 Sharon N. and Lis H. (1997) Glycoproteins: structure and function. In: Glycosciences: Status and Perspectives, pp. 133–162, Gabius H.-J. and Gabius S. (eds), Chapman and Hall, London

76 Herscovics A. and Orlean P. (1993) Glycoprotein biosynthesis in yeast. FASEB J. **7:** 540–550

77 Lehle L. and Tanner W. (1995) Protein glycosylation in yeast. In: Glycoproteins, pp. 475–509, Montreuil J., Vliegenthart J. F. G., Schachter H. (eds), Elsevier, Amsterdam

78 Chiba A., Matsumura K., Yamada H., Inazu T., Shimizu T., Kusunoki S. et al. (1997) Structures of sialylated O-linked oligosaccharides of bovine peripheral nerve α-dystroglycan. The role of a novel O-mannosyl-type oligosaccharide in the binding of α-dystroglycan with laminin. J. Biol. Chem. **272:** 2156–2162

79 Finne J., Krusius T., Margolis R. K. and Margolis R. U. (1979) Novel mannitol-containing oligosaccharides obtained by mild alkaline borohydride treatment of a chondroitin sulfate proteoglycan from brain. J. Biol. Chem. **245:** 10295–10300

80 Krusius T., Finne J., Margolis R. K. and Margolis R. U. (1987) Structural studies on sialylated und sulphated O-glycosidic mannose-linked oligosaccharides in the chondroitin sulfate proteoglycan of brain. Biochem. J. **245:** 229–234

81 Gentzsch M. and Tanner W. (1997) Protein-O-glycosylation in yeast: protein-specific mannosyltransferases. Glycobiology **7:** 481–486

82 Schreiner R., Schnabel E. and Wieland F. (1994) Novel N-glycosylation in eukaryotes: laminin contains the linkage unit β-glucosylasparagine. J. Cell Biol. **124:** 1071–1081

83 Wieland F., Heitzer R. and Schäfer W. (1983) Asparaginylglucose: novel type of carbohydrate linkage. Proc. Natl. Acad. Sci. USA **80:** 5470–5474

84 Wieland F., Lechner I. and Sumper M. (1986) Iduronic acid: constituent of sulfated dolichyl phosphate oligosaccharides in halobacteria. FEBS Lett. **195:** 77–81

85 Mengele R. and Sumper M. (1992) Drastic differences in the glycosylation of related S-layer glycoproteins from moderate and extreme halophiles. J. Biol. Chem. **267:** 8182–8185

86 Kieliszewski M. J., O'Neill M., Leykam J. and Orlando R. (1995) Tandem mass spectrometry and structural elucidation of glycopeptides from a hydroxyproline-rich plant cell wall glycoprotein indicate that contiguous hydroxyproline residues are the major sites of hydroxyproline O-arabinosylation. J. Biol. Chem. **270:** 2541–2549

87 Sturm A. (1995) N-Glycosylation of plant proteins. In: Glycoproteins, pp. 521–541, Montreuil J., Vliegenthart J. F. G., Schachter H. (eds), Elsevier, Amsterdam

88 Krieg J., Gläsner W., Vicentini A., Doucey M.-A., Löffler A., Hess D. et al. (1997) C-Mannosylation of human RNase 2 is an intracellular process performed by a variety of cultured cells. J. Biol. Chem. **272:** 26687–26692

89 Haynes P. A. (1998) Phosphoglycosylation: a new structural class of glycosylation? Glycobiology **8:** 1–5
90 Ilg T., Overath P., Ferguson M. A. J., Rutherford T., Campbell D. G. and McConville M. J. (1994) O- and N-glycosylation of the *Leishmania mexicana*-secreted acid phosphatase. J. Biol. Chem. **269:** 24073–24081
91 Ilg T., Craik D., Currie G., Multhaupt G. and Bacic A. (1998) Stage-specific proteophosphoglycan from *Leishmania mexicana* amastigotes. J. Biol. Chem. **273:** 13509–13523
92 Suzuki T., Kitajima K., Inoue S. and Inoue Y. (1995) N-Glycosylation/deglycosylation as a mechanism for the post-translational modification/remodification of proteins. Glycoconj. J. **12:** 183–193
93 Suzuki T., Kitajima K., Inoue S. and Inoue Y. (1997) Occurrence and potential functions of N-glycanases. In: Glycosciences: Status and Perspectives, pp. 121–131, Gabius H.-J. and Gabius S. (eds), Chapman and Hall, London
94 Haynes P. A., Ferguson M. A. J. and Cross G. A. M. (1996) Structural characterization of novel cell surface glycoconjugates of *Trypanosoma cruzi*. Glycobiology **6:** 869–878
95 Strahl-Bolsinger S. and Tanner W. (1991) Protein O-glycosylation in *Saccharomyces cerevisiae*. Purification and characterization of the dolichyl-phosphate-D-mannose-protein O-D-mannosyltransferase. Eur. J. Biochem. **196:** 185–190
96 Marchase R. B., Bounelis P., Brumley L. M., Dey N., Browne B., Auger D. et al. (1993) Phosphoglucomutase in *Saccharomyces cerevisiae* is a cytoplasmic glycoprotein and the acceptor for a Glc-phosphotransferase. J. Biol. Chem. **268:** 8341–8349
97 Gentzsch M., Strahl-Bolsinger S. and Tanner W. (1995) A new Dol-P-Man:protein O-D-mannosyltransferase activity from *Saccharomyces cerevisiae*. Glycobiology **5:** 77–82
98 Gustafson G. L. and Milner L. A. (1980) Occurrence of N-acetylglucosamine1-phosphate in proteinase I from *Dictyostelium discoideum*. J. Biol. Chem. **255:** 7208–7210
99 Mehta D. P., Ichikawa M., Salimath P. V., Etchison J. R., Haak R., Manzi A. et al. (1996) A lysosomal cysteine proteinase from *Dictyostelium discoideum* contains N-acetylglucosamine-1-phosphate bound to serine but not mannose-6-phosphate on N-linked oligosaccharides. J. Biol. Chem. **271:** 10897–10903
100 Srikrishna G., Wang L. and Freeze H. H. (1998) Fucoseβ1-P-Ser is a new type of glycosylation: using antibodies to identify a novel structure in *Dictyostelium discoideum* and study multiple types of fucosylation during growth and development. Glycobiology **8:** 799–811
101 Baenziger J. U. (1994) Protein-specific glycosyltransferases: how and why they do it! FASEB J. **8:** 1019–1025
102 Srisomsap C., Richardson K. L., Jay J. C. and Marchase R. B. (1989) An α-glucose-1-phosphate phosphodiesterase is present in rat liver cytosol. J. Biol. Chem. **264:** 20540–20546
103 Veyna N. A., Jay J. C., Srisomsap C., Bounelis P. and Marchase R. B. (1994) The addition of glucose-1-phosphate to the cytoplasmic glycoprotein phosphoglucomutase is modulated by intracellular calcium in PC12 cells and rat cortical synaptosomes. J. Neurochem. **62:** 456–464
104 Balsamo J. and Lilien J. (1990) N-Cadherin is stably associated with and is an acceptor for a cell surface N-acetylgalactosaminylphosphotransferase. J. Biol. Chem. **265:** 2923–2928
105 Hayes B. K. and Varki A. (1993) The biosynthesis of oligosaccharides in intact Golgi preparations from rat liver. Analysis of N-linked and O-linked glycans labeled by UDP-[6-^{3}H]-N-acetylgalactosamine. J. Biol. Chem. **268:** 16170–16178
106 Ferguson M. A. J., Haldar K. and Cross G. A. M. (1985) *Trypanosoma brucei* variant surface glycoprotein has a *sn*-1,2-dimyristyl glycerol membrane anchor at its COOH terminus. J. Biol. Chem. **260:** 4963–4968
107 Englung P. T. (1993) The structure and biosynthesis of glycosyl phosphatidylinositol protein anchors. Annu. Rev. Biochem. **62:** 121–138
108 Eckert V., Gerold P. and Schwarz R. T. (1997) GPI-anchors: structure and function. In: Glycosciences: Status and Perspectives, pp. 223–243, Gabius H.-J. and Gabius S. (eds), Chapman and Hall, London
109 Ferguson M. A. J. (1994) What can GPI do for you? Parasitol. Today **10:** 48–52
110 Ferguson M. A. J. (1997) The surface glycoconjugates of trypanosomatid parasites. Phil. Trans. R. Soc. Lond. B **352:** 1295–1302
111 de Lederkremer R. M. and Colli W. (1995) Galactofuranose-containing glycoconjugates in trypanosomatids. Glycobiology **5:** 547–552
112 Spiegel S., Foster D. and Kolesnick R. (1996) Signal transduction through lipid second messengers. Curr. Opin. Cell Biol. **8:** 159–167
113 Singer W. D., Brown H. A. and Sternweis P. C. (1997) Regulation of eukaryotic phosphatidylinositol-specific phospholipase C and phospholipase D. Annu. Rev. Biochem. **66:** 475–509
114 Low M. G. (1987) Biochemistry of the glycosylphosphatidylinositol membrane protein anchors. Biochem. J. **244:** 1–13
115 Ferguson M. A. (1993) GPI membrane anchors: isolation and analysis. In: Glycobiology, pp. 349–384, Fukuda M. and Kobata A. (eds), IRL Press, Oxford
116 Robinson P. J. (1991) Signal transduction by GPI-anchored membrane proteins. Cell. Biol. Int. Rep. **15:** 761–767
117 Thomas L. J., de Gasperi R., Sugiyama E., Chang H.-M., Beck P. J., Orlean P. et al. (1991) Functional analysis of T-cell mutants defective in the biosynthesis of glycosylphosphatidylinositol anchor. Relative importance of glycosylphosphatidylinositol anchor versus N-linked glycosylation in T-cell activation. J. Biol. Chem. **266:** 23175–23184
118 Jones D. R. and Varela-Nieto I. (1998) The role of glycosylphosphatidylinositol in signal transduction. Int. J. Biochem. Cell Biol. **30:** 313–326
119 Villalobo A. and Gabius H.-J. (1998) Signaling pathways for transduction of the initial message of the glycocode into cellular responses. Acta Anat. **161:** 110–129
120 Jackson P. and Gallagher J. T. (eds) (1997) A Laboratory Guide to Glycoconjugate Analysis, BioMethods, vol. 9, Birkhäuser, Basel
121 Duk M., Ugorski M. and Lisowska E. (1997) β-Elimination of O-glycans from glycoproteins transferred to Immobilon P membranes: method and some application. Anal. Biochem. **253:** 98–102
122 Morelle W., Guyetant R. and Strecker G. (1998) Structural analysis of oligosaccharide-alditols released by reductive β-elimination from oviductal mucins of *Rana dalmatina*. Carbohydr. Res. **306:** 435–443
123 Patel T. P. and Parekh R. B. (1994) Release of oligosaccharides from glycoproteins by hydrazinolysis. Methods Enzymol. **230:** 57–66
124 Edge A. S. B., Faltynek C. R., Hof L., Reichert L. E. Jr. and Weber P. (1981) Deglycosylation of glycoproteins by trifluoromethanesulfonic acid. Anal. Biochem. **118:** 131–137
125 Sojar H. T. and Bahl O. P. (1987) Chemical deglycosylation of glycoproteins. Methods Enzymol. **138:** 341–350
126 Horvath E., Edwards A. M., Bell J. C. and Braun P. E. (1989) Chemical deglycosylation on a micro-scale of membrane glycoproteins with retention of phosphoryl-protein linkages. J. Neurosci. Res. **24:** 398–401
127 Kobata A. (1989) Use of endo- and exoglycosidases for structural studies of glycoconjugates. Anal. Biochem. **150:** 1–14
128 Thotakura N. R. and Bahl O. P. (1987) Enzymatic deglycosylation of glycoproteins. Methods Enzymol. **138:** 350–359
129 Maley F., Trimble R. B., Tarentino A. L. and Plummer T. H. Jr. (1989) Characterization of glycoproteins and their associated oligosaccharides through the use of endoglycosidases. Anal. Biochem. **180:** 195–204
130 Mellors A. and Sutherland D. R. (1994) Tools to cleave glycoproteins. Trends Biotechnol. **12:** 15–18

131 Tarentino A. L., Trimble R. B. and Plummer T. H. Jr. (1989) Enzymatic approaches for studying the structure, synthesis and processing of glycoproteins. Methods Cell Biol. **32:** 111–139
132 Tarentino A. L. and Plummer T. H. Jr. (1994) Enzymatic deglycosylation of asparagine-linked glycans: purification, properties and specificity of oligosaccharide-cleaving enzymes from *Flavobacterium meningosepticum*. Methods Enzymol. **230:** 44–57
133 Ishii-Karakasa I., Iwase H., Hotta K., Tanaka Y. and Omura S. (1992) Partial purification and characterization of an endo-α-N-acetylgalactosaminidase from the culture medium of *Streptomyces sp*. OH-11242. Biochem. J. **288:** 475–482
134 Jacob G. S. and Scudder P. (1994) Glycosidases in structural analysis. Methods Enzymol. **230:** 280–299
135 Ishii-Karakasa I., Iwase H. and Hotta K. (1997) Structural determination of O-linked sialyl oligosaccharides liberated from fetuin with endo-α-N-acetylgalactosaminidase-S by HPLC analysis and 600-MHz ^{1}H-NMR spectroscopy. Eur. J. Biochem. **247:** 709–715
136 Lee Y. C. (1990) High-performance anion-exchange chromatography for carbohydrate analysis. Anal. Biochem. **189:** 151–162
137 Baenziger J. U. (1994) High-performance liquid chromatography of oligosaccharides. Methods Enzymol. **230:** 237–249
138 Hase S. (1994) High-performance liquid chromatography of pyridylaminated saccharides. Methods Enzymol. **230:** 225–237
139 Davies M. J., Hounsell E. F. (1998) HPLC and HPAEC of oligosaccharides and glycopeptides. In: Glycoanalysis Protocols, Methods in Molecular Biology, 2nd ed., vol. 76, pp. 79–100, Housell E. F. (ed.), Humana Press, Totowa
140 Hardy M. R. and Townsend R. R. (1994) High-pH anion-exchange chromatography of glycoprotein-derived carbohydrates. Methods Enzymol. **230:** 208–225
141 Rohrer J. S. (1995) Separation of asparagine linked oligosaccharides by high pH anion exchange chromatography with pulsed amperometric detection: empirical relationships between oligosaccharide structure and chromatographic retention. Glycobiology **5:** 359–360
142 Townsend R. R., Lipniunas P. H., Bigge C., Ventom A. and Parekh R. B. (1996) Multimode high performance liquid chromatography of fluorescently labeled oligosaccharides from glycoproteins. Anal. Biochem. **239:** 200–207
143 Taverna M., Baillet A., Biou D., Schlüter M., Werner R. and Ferrier D. (1992) Analysis of carbohydrate-mediated heterogeneity and characterization of N-linked oligosaccharides of glycoproteins by high performance capillary electrophoresis. Electrophoresis **13:** 359–366
144 Hermentin P., Doenges R., Witzel R., Hokke C. H., Vliegenthart J. F. G., Kamerling J. P. et al. (1994) A strategy for the mapping of N-glycans by high-performance capillary electrophoresis. Anal. Biochem. **221:** 29–41
145 Linhardt R. J. (1994) Capillary electrophoresis of oligosaccharides. Methods Enzymol. **230:** 265–280
146 Hermentin P., Witzel R., Dönges R., Bauer R., Haupt H., Patel T. et al. (1992) The mapping by high-pH anion-exchange chromatography with pulsed amperometric detection and capillary electrophoresis of the carbohydrate moieties of human plasma α_1-acid glycoprotein. Anal. Biochem. **206:** 419–429
147 Hermentin P., Witzel R., Kanzy E.-J., Diderrich G., Hoffmann D., Metzner H. et al. (1996) The hypothetical N-glycan charge: a number that characterizes protein glycosylation. Glycobiology **6:** 217–230
148 Weitzhandler M., Rohrer J., Thayer J. R., Avdalovic N. (1998) HPAEC-PAD analysis of monosaccharides released by exoglycosidase digestion using the CaroPac MA1 column. In: Glycoanalysis Protocols, Methods in Molecular Biology, 2nd ed., vol. 76, pp. 71–78, Housell E. F. (ed.), Humana Press, Totowa
149 Kamerling J. P., Gerwig G. J., Vliegenthart J. F. G. and Clamp J. R. (1975) Characterization by gas-liquid chromatography-mass spectrometry and proton-magnetic resonance spectroscopy of pertrimethylsilyl methyl glycosides obtained in methanolysis of glycoproteins and glycopeptides. Biochem. J. **151:** 491–495
150 Merkle R. K. and Poppe I. (1994) Carbohydrate composition analysis of glycoconjugates by gas-liquid chromatography/mass spectrometry. Methods Enzymol. **230:** 1–15
151 Bigge J. C., Patel T. P., Bruce J. A., Goulding P. N., Charles S. M. and Parekh R. B. (1995) Nonselective and efficient fluorescent labeling of glycans using 2-aminobenzamide and anthranilic acid. Anal. Biochem. **230:** 229–238
152 Morelle W., Lemoine J. and Strecker G. (1998) Structural analysis of O-linked oligosaccharide-alditols by electrospray-tandem mass spectrometry after mild periodate oxidation and derivatization with 2-aminopyridine. Anal. Biochem. **259:** 16–27
153 Natsuka S. and Hase S. (1998) Analysis of N- and O-glycans by pyridylamination. In: Glycoanalysis Protocols, Methods in Molecular Biology, 2nd ed., vol. 76, pp. 101–113, Housell E. F. (ed.), Humana Press, Totowa
154 Welply J. K. (1989) Sequencing methods for carbohydrates and their biological applications. Trends Biotechnol. **7:** 5–10
155 Guttman A. and Ulfelder K. A. (1997) Exoglycosidase matrix-mediated sequencing of a complex glycan pool by capillary electrophoresis. J. Chromatogr. A **781:** 547–554
156 Rudd P. M., Guile J. R., Küster B., Harvey D. J., Opdenakker G. and Dwek R. A. (1997) Oligosaccharide sequencing technology. Nature **388:** 205–207
157 Prime S., Merry T. (1998) Exoglycosidase sequencing of N-linked glycans by the reagent array analysis method (RAAM). In: Glycoanalysis Protocols, Methods in Molecular Biology, 2nd ed., vol. 76, pp. 53–69, Housell E. F. (ed.), Humana Press, Totowa
158 Gooley A. A. and Williams K. L. (1997) How to find, identify and quantitate the sugars on proteins. Nature **385:** 557–559
159 Hakomori S. (1964) A rapid methylation of glycolipid and polysaccharide catalyzed by methylsulfinyl carbanion in dimethyl sulfoxide. J. Biochem. **55:** 205–208
160 Geyer R. and Geyer H. (1994) Saccharide linkage analysis using methylation and other techniques. Methods Enzymol. **230:** 86–108
161 Varki A. and Diaz S. (1984) The release and purification of sialic acids from glycoconjugates: methods to minimize the loss and migration of O-acetyl groups. Anal. Biochem. **137:** 236–247
162 Reuter G. and Schauer R. (1994) Determination of sialic acids. Methods Enzymol. **230:** 168–199
163 Reuter G. and Schauer R. (1994) Enzymic methods of sialic acid analysis. Methods Carbohydr. Chem. **10:** 29–39
164 Reutter W., Stäsche R., Stehling P., Baum O. (1997) The biology of sialic acids: insights into their structure, metabolism and function in particular during viral infection. In: Glycosciences: Status and Perspectives, pp. 245–259, Gabius H.-J. and Gabius S. (eds), Chapman and Hall, London
165 Shaw L., Schneckenburger P., Schlenzka W., Carlsen J., Christiansen K., Jürgensen D. et al. (1994) CMP-N-acetylneuraminic acid hydroxylase from mouse liver and pig submandibular glands. Interaction with membrane-bound and soluble cytochrome b_5-dependent electron transport chains. Eur. J. Biochem. **219:** 1001–1011
166 Schlenzka W., Shaw L., Kelm S., Schmidt C. L., Bill E., Trautwein A. X. et al. (1996) CMP-N-acetylneuraminic acid hydroxylase: the first cytosolic Rieske iron-sulphur protein to be described in *Eukarya*. FEBS Lett. **385:** 197–200
167 Kluge A., Reuter G., Lee H., Ruch-Heeger B. and Schauer R. (1992) Interaction of rat peritoneal macrophages with homologous sialidase-treated thrombocytes in vitro: biochemical and morphological studies. Detection of N-(O-acetyl)glycoloylneuraminic acid. Eur. J. Cell Biol. **59:** 12–20

168 Bergwerff A. A., Hulleman S. H. D., Kamerling J. P., Vliegenthart J. F. G., Shaw L., Reuter G. et al. (1992) Nature and biosynthesis of sialic acids in the starfish *Asterias rubens*. Identification of sialo-oligomers and detection of S-adenosyl-L-methionine-N-acetylneuraminate 8-O-methyltransferase and CMP-N-acetylneuraminate monooxygenase activities. Biochimie **74:** 25–37
169 Schauer R., Kelm S., Reuter G., Roggentin P. and Shaw L. (1995) Biochemistry and role of sialic acids. In: Biology of Sialic Acids, pp. 7–67, Rosenberg A. (ed.), Plenum Press, New York
170 Irie A., Koyama S., Kozutsumi Y., Kawasaki T. and Suzuki A. (1998) The molecular basis for the absence of N-glycolylneuraminic acid in humans. J. Biol. Chem. **273:** 15866–15871
171 Irie A. and Suzuki A. (1998) CMP-N-acetylneuraminic acid hydroxylase is exclusively inactive in humans. Biochem. Biophys. Res. Commun. **248:** 330–333
172 Kolter T. and Sandhoff K. (1997) Sialic acids – why always α-linked? Glycobiology **7:** vii–x
173 Manzi A. E., Dell A., Azadi P. and Varki A. (1990) Studies of naturally occurring modifications of sialic acids by fast-atom bombardment-mass spectrometry. Analysis of positional isomers by periodate cleavage. J. Biol. Chem. **265:** 8094–8107
174 Kleineidam R. G., Hofmann O., Reuter G. and Schauer R. (1993) Indications for the enzymatic synthesis of 9-O-lactoyl-N-acetylneuraminic acid in equine liver. Glycoconjugate J. **10:** 116–119
175 Klein A., Diaz S., Ferreira I., Lamblin G., Roussel P. and Manzi A. E. (1997) New sialic acids from biological sources identified by a comprehensive and sensitive approach: liquid chromatography electrospray ionization mass spectrometry (LC-ESI-MS) of SIA quinoxalinones. Glycobiology **7:** 421–432
176 Kelm A., Shaw L., Schauer R. and Reuter G. (1998) The biosynthesis of 8-O-methylated sialic acids in the starfish *Asterias rubens*. Isolation and characterisation of S-adenosyl-L-methionine:sialate-8-O-methyltransferase. Eur. J. Biochem. **251:** 874–884
177 Pilatte Y., Bignon J. and Lambré C. R. (1993) Sialic acids as important molecules in the regulation of the immune system: pathophysiological implications of sialidases in immunity. Glycobiology **3:** 201–217
178 Meindl P., Bodo G., Palese P., Schulman J. and Tuppy H. (1974) Inhibition of neuraminidase activity by derivatives of 2-deoxy-2,3-dehydro-N-acetylneuraminic acid. Virology **58:** 457–463
179 Nöhle U., Shukla A. K., Schröder C., Reuter G., Schauer R., Kamerling J. P. et al. (1985) Structural parameters and natural occurrence of 2-deoxy-2,3-didehydro-N-glycoloylneuraminic acid. Eur. J. Biochem. **152:** 459–463
180 von Itzstein M., Wu W.-Y., Kok G. B., Pegg M. S., Dayson J. C., Jin B. et al. (1993) Rational design of potent sialidase-based inhibitors of influenza virus replication. Nature **363:** 418–423
181 von Itzstein M. and Colman P. M. (1996) Design and synthesis of carbohydrate-based inhibitors of carbohydrate-protein interactions. Curr. Opin. Struct. Biol. **6:** 703–709
182 Kopitz J., von Reitzenstein C., Mühl C. and Cantz M. (1994) Role of plasma membrane ganglioside sialidase of human neuroblastoma cells in growth and differentiation. Biochem. Biophys. Res. Commun. **199:** 1188–1193
183 Kopitz J., Mühl C., Ehemann V., Lehmann C. and Cantz M. (1997) Effects of cell surface ganglioside sialidase inhibition on growth control and differentiation of human neuroblastoma cells. Eur. J. Cell Biol. **73:** 1–9
184 Kopitz J., Sinz K., Brossmer R. and Cantz M. (1997) Partial characterization and enrichment of membrane-bound sialidase specific for gangliosides from human brain tissue. Eur. J. Biochem. **248:** 527–534
185 Kopitz J., von Reitzenstein C., Burchert M., Cantz M. and Gabius H.-J. (1998) Galectin-1 is a major receptor for ganglioside GM1, a product of the growth-controlling activity of a cell surface ganglioside sialidase, on human neuroblastoma cells in culture. J. Biol. Chem. **273:** 11205–11211
186 Reuter G. and Schauer R. (1986) Comparison of electron and chemical ionization mass spectrometry of sialic acids. Anal. Biochem. **157:** 39–46
187 Kanamori A., Nakayama J., Fukuda M. N., Stallcup W. B., Sasaki K., Fukuda M. et al. (1997) Expression cloning and characterization of a cDNA encoding a novel membrane protein required for the formation of O-acetylated ganglioside: a putative acetyl CoA transporter. Proc. Natl. Acad. Sci. USA **94:** 2897–2902
188 Shi W. X., Chammas R. and Varki A. (1998) Induction of sialic acid 9-O-acetylation by diverse gene products: implications for the expression cloning of sialic acid O-acetyltransferases. Glycobiology **8:** 199–205
189 Herrler G., Reuter G., Rott R., Klenk H.-D. and Schauer R. (1987) N-Acetyl-9-O-acetylneuraminic acid, the receptor determinant for influenza C virus, is a differentiation marker on chicken erythrocytes. Biol. Chem. Hoppe-Seyler **368:** 451–454
190 Varki A., Hooshmand F., Diaz S., Varki N. M. and Hedrick S. M. (1991) Developmental abnormalities in transgenic mice expressing a sialic acid-specific 9-O-acetylesterase. Cell **65:** 65–74
191 Zeng F.-Y. and Gabius H.-J. (1992) Sialic acid-binding proteins: characterization, biological functions and application. Z. Naturforsch. **47c:** 641–653
192 Siebert H.-C., von der Lieth C.-W., Dong X., Reuter G., Schauer R., Gabius H.-J. et al. (1996) Molecular dynamics-derived conformation and intramolecular interaction analysis of the N-acetyl-9-O-acetylneuraminic acid-containing ganglioside GD1a and NMR-based analysis of its binding to a human polyclonal immunoglobulin G fraction with selectivity for O-acetylated sialic acids. Glycobiology **6:** 561–572
193 Dell A., Reason A. J., Khoo K.-H., McDowell R. A. and Morris H. R. (1994) Mass spectrometry of carbohydrate-containing biopolymers. Methods Enzymol. **230:** 108–132
194 Bihoreau N., Ramon C., Lazard M. and Schmitter J. M. (1997) Combination of capillary electrophoresis and matrix-assisted laser desorption mass spectrometry for glycosylation analysis of a human monoclonal anti-Rhesus (D) antibody. J. Chromatogr. B **697:** 123–133
195 Hop C. E. C. A. and Bakhtiar R. (1997) An introduction to electrospray ionization and matrix-assisted laser desorption/ionization mass spectrometry: essential tools in a modern biotechnology environment. Biospectroscopy **3:** 259–280
196 Ju D.-D., Lai C.-C. and Her G.-R. (1997) Analysis of gangliosides by capillary zone electrophoresis and capillary zone electrophoresis-electrospray mass spectrometry. J. Chromatogr. A **779:** 195–203
197 Penn S. G., Cancilla M. T., Green M. K. and Lebrilla C. B. (1997) Direct comparison of matrix-assisted laser desorption/ionisation and electrospray ionisation in the analysis of gangliosides by Fourier transform mass spectrometry. Eur. Mass Spectrom. **3:** 67–79
198 Iwase H., Tanaka A., Hiki Y., Kokubo T., Ishii-Karakasa I., Nishikido J. et al. (1998) Application of matrix-assisted laser desorption ionization time-of-flight mass spectrometry to the analysis of glycopeptide-containing multiple O-linked oligosaccharides. J. Chromatogr. B **709:** 145–149
199 Mitamura K. and Shimada K. (1998) Derivatization in LC/MS. Yakugaku Zasshi J. Pharmaceut. Soc. Japan **118:** 206–215
200 Olling A., Breimer M. E., Peltomaa E., Samuelsson B. E. and Ghardashkhani S. (1998) Electrospray ionization and collision-induced dissociation time-of-flight mass spectrometry of neutral glycosphingolipids. Rapid Commun. Mass Spectrom. **12:** 637–645
201 Rhomberg A. J., Ernst S., Sasisekharan R. and Biemann K. (1998) Mass spectrometric and capillary electrophoretic investigation of the enzymatic degradation of heparin-like glycosaminoglycans. Proc. Natl. Acad. Sci. USA **95:** 4176–4181

202 Vliegenthart J. F. G., Dorland L. and van Halbeek H. (1983) High-resolution ^{1}H-NMR spectroscopy as a tool in structural analysis of carbohydrates related to glycoproteins. Adv. Carbohydr. Chem. Biochem. **41:** 209–374

203 Lis H. and Sharon N. (1981) Affinity chromatography for the purification of lectins. J. Chromatogr. **215:** 361–372

204 Pazur J. H. (1981) Affinity chromatography of macromolecular substances on adsorbents bearing carbohydrate ligands. Adv. Carbohydr. Chem. Biochem. **39:** 405–447

205 Freier T., Fleischmann G. and Rüdiger H. (1985) Affinity chromatography on immobilized hog gastric mucin and ovomucoid. A general method for isolation of lectins. Biol. Chem. Hoppe-Seyler **366:** 1023–1028

206 Gabius H.-J. and Engelhardt R. (1987) Interaction of mannose-binding proteins with different types of immobilized ligands. J. Chromatogr. **391:** 452–456

207 Gabius H.-J. (1990) Influence of type of linkage and spacer on the interaction of β-galactoside-binding proteins with immobilized affinity ligands. Anal. Biochem. **189:** 91–94

208 Gabius H.-J. and Gabius S. (eds) (1993) Lectins and Glycobiology, Springer, Berlin

209 Merkle R. K. and Cummings R. D. (1987) Lectin affinity chromatography of glycopeptides. Methods Enzymol. **138:** 232–259

210 Osawa T. and Tsuji T. (1987) Fractionation and structural assessment of oligosaccharides and glycopeptides by use of immobilized lectins. Annu. Rev. Biochem. **56:** 21–42

211 Green E. D. and Baenziger J. U. (1989) Characterization of oligosaccharides by lectin affinity high-performance liquid chromatography. Trends Biochem. Sci. **14:** 168–172

212 Kobata A. and Endo T. (1992) Immobilized lectin columns: useful tools for the fractionation and structural analysis of oligosaccharides. J. Chromatogr. **597:** 111–122

213 Cummings R. D. (1997) Lectins as tools for glycoconjugate purification and characterization. In: Glycosciences: Status and Perspectives, pp. 191–199, Gabius H.-J. and Gabius S. (eds), Chapman and Hall, London

214 Peumans W. J. and van Damme E. J. M. (1998) Plant lectins: specific tools for the identification, isolation and characterization of O-linked glycans. Crit. Rev. Biochem. Mol. Biol. **33:** 209–258

215 Yamamoto K., Tsuji T., Osawa T. (1998) Analysis of asparagine-linked oligosaccharides by sequential lectin-affinity chromatography. In: Glycoanalysis Protocols, Methods in Molecular Biology, 2nd ed., vol. 76, pp. 35–51, Housell E. F. (ed.), Humana Press, Totowa

216 Schrevel J., Gros D. and Monsigny M. (1981) Cytochemistry of cell glycoconjugates. Progr. Histochem. Cytochem. **14/2:** 1–269

217 Spicer S. S. and Schulte B. A. (1992) Diversity of cell glycoconjugates shown histochemically: a perspective. J. Histochem. Cytochem. **40:** 1–38

218 Danguy A., Akif F., Pajak B. and Gabius H.-J. (1994) Contribution of carbohydrate histochemistry to glycobiology. Histol. Histopathol. **9:** 155–171

219 Danguy A., Camby I., Salmon I. and Kiss R. (1997) Modern glycohistochemistry: a major contribution to morphological investigations. In: Glycosciences: Status and Perspectives, pp. 547–562, Gabius H.-J. and Gabius S. (eds), Chapman and Hall, London

220 Kannan S. and Nair M. K. (1997) Lectins and neoglycoproteins in histopathology. In: Glycosciences: Status and Perspectives, pp. 563–583, Gabius H.-J. and Gabius S. (eds), Chapman and Hall, London

221 Marrone T. J., Briggs J. M. and McCammon J. A. (1997) Structure-based drug design. Annu. Rev. Pharmacol. Toxicol. **37:** 71–90

222 Gabius H.-J. (1998) The how and why of protein-carbohydrate interaction: a primer to the theoretical concept and a guide to application in drug design. Pharm. Res. **15:** 23–30

223 Pérez S., Imberty A. and Carver J. P. (1994) Molecular modeling: an essential component in the structure determination of oligosaccharides and polysaccharides. Adv. Computat. Biol. **1:** 147–202

224 Homans S. W. (1995) 3D structure. 2. Three dimensional structure of oligosaccharides explored by NMR and computer calculations. In: Glycoproteins, pp. 67–86, Montreuil J., Vliegenthart J. F. G. and Schachter H. (eds), Elsevier, Amsterdam.

225 Woods R. J., Dwek R. A., Edge C. J. and Fraser-Reid B. (1995) Molecular mechanical and molecular dynamical simulations of glycoproteins and oligosaccharides. J. Phys. Chem. **99:** 3832–3846

226 Kozár T. and von der Lieth C.-W. (1997) Efficient modeling protocols for oligosaccharides: from vacuum to solvent. Glycoconj. J. **14:** 925–933

227 Rasmussen K. (1997) How to develop force fields: an account of the emergence of potential energy functions for saccharides. J. Mol. Struct. **395–396:** 91–106

228 Siebert H.-C., von der Lieth C.-W., Gilleron M., Reuter G., Wittmann J., Vliegenthart J. F. G. et al. (1997) Carbohydrate-protein interaction. In: Glycosciences: Status and Perspectives, pp. 291–310, Gabius H.-J. and Gabius S. (eds), Chapman and Hall, London

229 von der Lieth C.-W., Kozár T. and Hull W. E. (1997) A (critical) survey of modeling protocols used to explore the conformational space of oligosaccharides. J. Mol. Struct. **395–396:** 225–244

230 Imberty A. and von der Lieth C.-W. (1998) The ligand's personality. Carbohydrates in Europe **23**: 30–35

231 Woods R. J. (1998) Computational carbohydrate chemistry: what theoretical methods can tell us. Glycoconj. J. **15:** 209–216

232 Siebert H.-C., Gilleron M., Kaltner H., von der Lieth C.-W., Kozár T., Bovin N. V. et al. (1996) NMR-based, molecular dynamics – and random walk molecular mechanics-supported study of conformational aspects of a carbohydrate ligand (Galβ1-2Galβ1-R) for an animal galectin in the free and in the bound state. Biochem. Biophys. Res. Commun. **219:** 205–212

233 Fernandez P., Jiménez-Barbero J., Martin-Lomas M., Solís D. and Díaz-Mauriño T. (1994) Involvement of the glucose moiety in the molecular recognition of methyl β-lactoside by ricin: synthesis, conformational analysis and binding studies of different derivatives at the C-3 region. Carbohydr. Res. **256:** 233–244

234 Imberty A., Mikros E., Koca J., Mollicone R., Oriol R. and Pérez S. (1995) Computer simulation of histo-blood group oligosaccharides: energy maps of all constituting disaccharides and potential energy surfaces of 14 ABH and Lewis carbohydrate antigens. Glycoconj. J. **12:** 331–349

235 Gilleron M., Siebert H.-C., Kaltner H., von der Lieth C.-W., Kozár T., Halkes K. M. et al. (1998) Conformer selection and differential restriction of ligand mobility by a plant lectin. Conformational behaviour of Galβ1-3GlcNAcβ1-R, Galβ1-3GalNAcβ1-R and Galβ1-2Galβ1-R′ in the free state and complexed with galactoside-specific mistletoe lectin as revealed by random-walk and conformational clustering molecular-mechanics calculations, molecular-dynamics simulations and nuclear Overhauser experiments. Eur. J. Biochem. **252:** 416–427

236 Homans S. W. (1990) Oligosaccharide conformations: application of NMR and energy calculations. Progr. NMR Spectroscopy **22:** 55–81

237 Carver J. P. (1991) Experimental structure determination of oligosaccharides. Curr. Opin. Struct. Biol. **1:** 716–720

238 Bush C. A. and Cagas P. (1992) Three-dimensional conformations of complex carbohydrates. Adv. Biophys. Chem. **2:** 149–180

239 Hardy B. J. (1997) The glycosidic linkage flexibility and time-scale similarity hypotheses. J. Mol. Struct. **395–396:** 187–200

240 Imberty A. (1997) Oligosaccharide structures: theory versus experiment. Curr. Opin. Struct. Biol. **7:** 617–623

241 Siebert H.-C., Adar R., Arango R., Burchert M., Kaltner H., Kayser G. et al. (1997) Involvement of laser photo CIDNP (chemically induced dynamic nuclear polarization)-

reactive amino acid side chains in ligand binding by galactoside-specific lectins in solution. Similarities in the role of tryptophan/tyrosine residues for ligand binding between a plant agglutinin and mammalian/avian galectins and the detection of an influence of single-site mutagenesis on surface presentation of spatially separated residues. Eur. J. Biochem. **249:** 27–38

242 Poppe L. J., von der Lieth C.-W. and Dabrowski J. (1990) Conformation of the glycolipid globoside head group in various solvents and in the micelle-bound state. J. Am. Chem. Soc. **112:** 7762–7771

243 Jardetzky O. (1980) On the nature of molecular conformations inferred from high-resolution NMR. Biochim. Biophys. Acta **621:** 227–232

244 Kiddle G. R., Harris R. and Homans S. W. (1998) Heteronuclear Overhauser effects in carbohydrates. J. Biomol. NMR **11:** 289–294

245 Milton M. J., Harris R., Probert M. A., Field R. A. and Homans S. W. (1998) New conformational constraints in isotopically (^{13}C) enriched oligosaccharides. Glycobiology **8:** 147–153

246 Petrescu A. J., Butters T. D., Reinkensmeier G., Petrescu S., Platt F. M., Dwek R. A. et al. (1997) The solution NMR structure of glucosylated N-glycans involved in the early stages of glycoprotein biosynthesis and folding. EMBO J. **16:** 4302–4310

247 Qasba P. L., Balaji P. V. and Rao V. S. R. (1997) Conformational analysis of Asn-linked oligosaccharides: implications in biological processes. J. Mol. Struct. **395–396:** 333–360

248 Verbert A. (1995) Biosynthesis of N-linked glycoproteins. In: Glycoproteins, pp. 145–152, Montreuil J., Vliegenthart J. F. G. and Schachter H. (eds), Elsevier, Amsterdam

249 O'Connor S. E. and Imperiali B. (1996) Modulation of protein structure and function by asparagine-linked glycosylation. Chem. Biol. **3:** 803–812

250 Silberstein S. and Gilmore R. (1996) Biochemistry, molecular biology and genetics of the oligosaccharyltransferase. FASEB J. **10:** 849–858

251 Imperiali B. (1997) Protein glycosylation: the clash of the titans. Acc. Chem. Res. **30:** 452–459

252 Cacan R., Duvet S., Kmiecik D., Labiau O., Mir A. M. and Verbert A. (1998) "Glyco-deglyco" processing during the synthesis of N-glycoproteins. Biochimie **80:** 59–68

253 Krag S. S. (1998) The importance of being dolichol. Biochem. Biophys. Res. Commun. **243:** 1–5

254 Camphausen R. T., Yu H.-A., Cumming D. A. (1995) The role of polypeptide in the biosynthesis of protein-linked oligosaccarides. In: Glycoproteins, pp. 391–414, Montreuil J., Vliegenthart J. F. G. and Schachter H. (eds), Elsevier, Amsterdam

255 Holst B., Bruun A. W., Kielland-Brandt M. C. and Winther J. R. (1996) Competition between folding and glycosylation in the endoplasmic reticulum. EMBO J. **15:** 3538–3546

256 Imperiali B. and O'Connor S. E. (1998) The conformational basis of asparagine-linked glycosylation. Pure Appl. Chem. **70:** 33–40

257 Montreuil J. (1980) Primary structure of glycoprotein glycans: basis for the molecular biology of glycoproteins. Adv. Carbohydr. Chem. Biochem. **37:** 157–223

258 Kornfeld R. and Kornfeld S. (1985) Assembly of asparagine-linked oligosaccharides. Annu. Rev. Biochem. **54:** 631–664

259 Vliegenthart J. F. G. and Montreuil J. (1995) Primary structure of glycoprotein glycans. In: Glycoproteins, pp. 13–28, Montreuil J., Vliegenthart J. F. G. and Schachter H. (eds), Elsevier, Amsterdam

260 Schachter H. (1995) Biosynthesis. 2c. Glycosyltransferases involved in the synthesis of N-glycan antennae. In: Glycoproteins, pp. 139–153, Montreuil J., Vliegenthart J. F. G. and Schachter H. (eds), Elsevier, Amsterdam

261 Colley K. J. (1997) Golgi localization of glycosyltransferases: more questions than answers. Glycobiology **7:** 1–13

262 Varki A. (1998) Factors controlling the glycosylation potential of the Golgi apparatus. Trends Cell Biol. **8:** 34–40

263 Cumming D. A. (1991) Glycosylation of recombinant protein therapeutics: control and functional implications. Glycobiology **1:** 115–130

264 Parekh R. B. and Patel T. P. (1992) Comparing the glycosylation patterns of recombinant glycoproteins. Trends Biotechnol. **10:** 276–280

265 Raju T. S., Lerner L. and O'Connor J. V. (1996) Glycoopinion: biological significance and methods for the analysis of complex carbohydrates of recombinant glycoproteins. Biotechnol. Appl. Biochem. **24:** 191–194

266 Finne J. (1982) Occurrence of unique polysialosyl carbohydrate units in glycoproteins of developing brain. J. Biol. Chem. **257:** 11966–11970

267 Troy II F. A. (1995) Sialobiology and the polysialic acid glycotype: occurrence, structure, function, synthesis and glycopathology. In: Biology of the Sialic Acids, pp. 95–143, Rosenberg A. (ed.), New York, Plenum Press

268 Rutishauser U. (1998) Polysialic acid at the cell surface: biophysics in service of cell interactions and tissue plasticity. J. Cell. Biochem. **70:** 304–312

269 Unverzagt C. (1994) Synthesis of a biantennary heptasaccharide by regioselective glycosylations. Angew. Chem. Int. Ed. Engl. **33:** 1101–1103

270 Unverzagt C. (1996) Chemoenzymatic synthesis of a sialylated undecasaccharide by regioselective glycosylations. Angew. Chem. Int. Ed. Engl. **35:** 2350–2353

271 André S., Unverzagt C., Kojima S., Dong X., Fink C., Kayser K. et al. (1997) Neoglycoproteins with the synthetic complex biantennary nonasaccharide or its α2,3/(26-sialylated derivatives: their preparation, assessment of their ligand properties for purified lectins, for tumor cells in vitro, and in tissue sections, and their biodistribution in tumor-bearing mice. Bioconj. Chem. **8:** 845–855

272 Gabius H.-J., Unverzagt C. and Kayser K. (1998) Beyond plant lectin histochemistry: preparation and application of markers to visualize the cellular capacity for protein-carbohydrate recognition. Biotech. and Histochem. **73:** 263–277

273 Rogers J. C. and Kornfeld S. (1971) Hepatic uptake of proteins coupled to fetuin glycopeptide. Biochem. Biophys. Res. Commun. **45:** 622–629

274 Lee R. T., Wong T. C., Lee R., Yue L. and Lee Y. C. (1989) Efficient coupling of glycopeptides to protein with a heterobifunctional reagent. Biochemistry **28:** 1856–1861

275 Gabius S., Kayser K., Hellmann K. P., Ciesiolka T., Trittin A. and Gabius H.-J. (1990) Carrier-immobilized derivatized lysoganglioside GM1 is a ligand for specific binding sites in various human tumor cell types and peripheral blood lymphocytes and monocytes. Biochem. Biophys. Res. Commun. **169:** 239–244

276 Gabius H.-J., Brinck U., Lüsebrink T., Ciesiolka T. and Gabius S. (1991) Glycopeptide-albumin conjugate: its preparation and histochemical ligand properties. Histochem. J. **23:** 303–311

277 Porwoll S., Loch N., Kannicht C., Nuck R., Grunow D., Reutter W. et al. (1998) Cell surface glycoproteins undergo postbiosynthetic modification of their N-glycans by stepwise demannosylation. J. Biol. Chem. **273:** 1075–1085

278 Tauber R., Park C. S., Becker A., Geyer R. and Reutter W. (1989) Rapid intramolecular turnover of N-linked glycans in plasma membrane glycoproteins. Extension of intramolecular turnover to the core sugars in plasma membrane glycoproteins of hepatoma. Eur. J. Biochem. **186:** 55–62

279 Kreisel W., Hanski C., Tran-Thi T. A., Katz N., Decker K., Reutter W. et al. (1988) Remodeling of a rat hepatocyte plasma membrane glycoprotein. De- and reglycosylation of dipeptidyl peptidase IV. J. Biol. Chem. **263:** 11736–11742

280 Volz B., Orberger G., Porwoll S., Hauri H. P. and Tauber R. (1995) Selective reentry of recycling cell surface glycoproteins to the biosynthetic pathway in human hepatocarcinoma HepG2 cells. J. Cell Biol. **130:** 537–551

281 Cacan R. and Verbert A. (1997) Free oligomannosides produced during N-glycosylation process: origin, intracellular trafficking and putative roles. Trends Glycosci. Glycotechnol. **9:** 365–377

282 Kamerling J. P. (1991) Xylose-containing carbohydrate chains derived from N-glycoproteins. Pure Appl. Chem. **63:** 465–472

283 März L., Altmann F., Staudacher E. and Kubelka V. (1995) Protein glycosylation in insects. In: Glycoproteins, pp. 543–563, Montreuil J., Vliegenthart J. F. G. and Schachter H. (eds), Elsevier, Amsterdam

284 Staudacher E. and März L. (1998) Strict order of (Fuc to Asn-linked GlcNAc) fucosyltransferases forming core-difucosylated structures. Glycoconj. J. **15:** 355–360

285 Altmann F. (1996) N-Glycosylation in insects revisited. Trends Glycosci. Glycotechnol. **8:** 101–114

286 Altmann F. (1997) More than silk and honey – or, can insect cells serve in the production of therapeutic glycoproteins? Glycoconj. J. **14:** 643–646

287 Wilson I. B. H., Harthill J. E., Mullin N. P., Ashford D. A. and Altmann F. (1998) Core α1,3-fucose is a key part of the epitope recognized by antibodies reacting against N-linked oligosaccharides and is present in a wide variety of plant extracts. Glycobiology **8:** 651–661

288 Hirschberg C. B. and Snider M. D. (1987) Topography of glycosylation in the rough endoplasmic reticulum and Golgi apparatus. Annu. Rev. Biochem. **56:** 63–87

289 Lennarz W. J. (1987) Protein glycosylation in the endoplasmic reticulum: current topological issues. Biochemistry **26:** 7205–7210

290 Roth J. (1995) Biosynthesis. 4c. Compartmentation of glycoprotein biosynthesis. In: Glycoproteins, pp. 287–312, Montreuil J., Vliegenthart J. F. G. and Schachter H. (eds), Elsevier, Amsterdam

291 Roth J. (1996) Protein glycosylation in the endoplasmic reticulum and the Golgi apparatus and cell-type specificity of cell surface glycoconjugate expression: analysis by protein A-gold and lectin-gold techniques. Histochem. Cell Biol. **106:** 79–92

292 Pavelka M. (1997) Topology of glycosylation – a histochemist's view. In: Glycosciences: Status and Perspectives, pp. 115–120, Gabius H.-J. and Gabius S. (eds), Chapman and Hall, London

293 von Figura K. (1990) Mannose-6-phosphat-Rezeptoren: Ihre Rolle beim Transport lysosomaler Enzyme. Naturwissenschaften **77:** 116–122

294 Ludwig T., le Borgne R. and Hoflack B. (1995) Roles for mannose-6-phosphate receptors in lysosomal enzyme sorting, IGF-II binding and clathrin-coat assembly. Trends Cell Biol. **5:** 202–206

295 Fiedler K. and Simons K. (1995) The role of N-glycans in the secretory pathway. Cell **81:** 309–312

296 Ponnambalam S. and Banting G. (1996) Protein secretion: sorting sweet sorting. Curr. Biol. **6:** 1076–1078

297 Roche A.-C. and Monsigny M. (1996) Trafficking of endogenous glycoproteins mediated by intracellular lectins. Chemtracts (Biochem. Mol. Biol.) **6:** 188–201

298 Gabius H.-J. (1997) Animal lectins. Eur. J. Biochem. **243:** 543–576

299 Leitzgen K. and Haas I. G. (1998) Protein maturation in the endoplasmic reticulum. Chemtracts (Biochem. Mol. Biol.) **11:** 423–445

300 Nichols W. C., Seligson U., Zivelin A., Terry V. H., Hertel C. E., Wheatley M. A. et al. (1998) Mutations in the ER-Golgi intermediate compartment protein ERGIC-53 cause combined deficiency of coagulation factors V and VIII. Cell **93:** 61–70

301 Vollenweider F., Kappeler F., Itin C. and Hauri H.-P. (1998) Mistargeting of the lectin ERGIC-53 to the endoplasmic reticulum of HeLa cells impairs the secretion of a lysosomal enzyme. J. Cell Biol. **142:** 377–389

302 Gahmberg C. G. and Tolvanen M. (1996) Why mammalian cell surface proteins are glycoproteins. Trends Biochem. Sci. **21:** 308–311 and Trends Biochem. Sci. **21:** 491

303 Helenius A., Trombetta E. S., Hebert D. N. and Simons J. F. (1997) Calnexin, calreticulin and the folding of glycoproteins. Trends Cell Biol. **7:** 193–200

304 Parodi A. J. (1996) The UDP-Glc:glycoprotein glucosyltransferase and the quality control of glycoprotein folding in the endoplasmatic reticulum. Trends Glycosci. Glycotechnol. **8:** 1–12

305 Parodi A. J. (1998) The quality control of glycoprotein folding in the endoplasmic reticulum, a trip from trypanosomes to mammals. Braz. J. Med. Biol. Res. **31:** 601–614

306 Fernandez F. S., Trombetta S. E., Hellmann U. and Parodi A. J. (1994) Purification to homogeneity of UDP-glucose: glycoprotein glucosyltransferase from *Schizosaccharomyces pombe* and apparent absence of the enzyme from *Saccharomyces cerevisiae*. J. Biol. Chem. **269:** 30701–30706

307 Jakob C. A., Burda P., te Heesen S., Aebi M. and Roth J. (1998) Genetic tailoring of N-linked oligosaccharides: the role of glucose residues in glycoprotein processing of *Saccharomyces cerevisiae* in vivo. Glycobiology **8:** 155–164

308 Sontheimer R. D., Nguyen T. Q., Cheng S.-T., Lieu T.-S. and Capra D. J. (1995) The unveiling of calreticulin: a clinically relevant tour of modern cell biology. J. Invest. Med. **43:** 362–370

309 Gabius H.-J. (1996) Mannan-binding surface lectin of murine melanoma cells adds new aspect to the multiple intracellular functions of calreticulin and its homologues. Chemtracts (Biochem. Mol. Biol.) **6:** 237–241

310 Coppolino M. G. and Dedhar S. (1998) Calreticulin. Int. J. Biochem. Cell Biol. **30:** 553–558

311 Spiro R. G., Zhu Q., Bhoyroo V. and Söling H. D. (1996) Definition of the lectin-like properties of the molecular chaperone, calreticulin, and demonstration of its copurification with endomannosidase from rat liver Golgi. J. Biol. Chem. **271:** 11588–11594

312 Dairaku K. and Spiro R. G. (1997) Phylogenetic survey of endomannosidase indicates late evolutionary appearance of this N-linked oligosaccharide processing enzyme. Glycobiology **7:** 579–586

313 Fiete D., Beranek M. C. and Baenziger J. U. (1997) The macrophage/endothelial cell mannose receptor cDNA encodes a protein that binds oligosaccharides terminating with SO_4-4-GalNAcβ1,4GlcNAcβ or Man at independent sites. Proc. Natl. Acad. Sci. USA **94:** 11256–11261

314 Gabius H.-J., Engelhardt R. and Cramer F. (1985) Endogenous tumor lectins: a new class of tumor markers and targets for therapy? Med. Hypotheses **18:** 47–50

315 Fiume L., Busi C., Mattioli A. and Spinosa G. (1988) Targeting of antiviral drugs bound to protein carriers. CRC Crit. Rev. Ther. Drug Carr. Syst. **4:** 265–284

316 Kojima S. and Gabius H.-J. (1988) Biodistribution of neoglycoproteins in mice bearing solid Ehrlich tumor. J. Cancer Res. Clin. Oncol. **114:** 468–472

317 Monsigny M., Roche A.-C., Midoux P., Kieda C. and Mayer R. (1988) Endogenous lectins of myeloid and tumor cells: characterization and biological implications. In: Lectins and Glycoconjugates in Oncology, pp. 25–48, Gabius H.-J. and Nagel G. A. (eds), Springer, Heidelberg

318 Gabius H.-J. (1989) Potential participation of tumor lectins in cancer diagnosis, therapy and biology. Adv. Lectin Res. **2:** 87–106

319 Kojima S., Imagawa M. and Gabius H.-J. (1989) Enhancement of clearance of plant lectins as radiopharmaceuticals by chemically glycosylated anti-lectin antibody. Eur. J. Nucl. Med. **15:** 373–375

320 Lee Y. C. and Lee R. T. (eds) (1994) Neoglycoconjugates, Preparation and Applications, Academic Press, San Diego

321 Molema G. and Meijer D. K. F. (1994) Targeting of drugs to various blood cell types using (neo-)glycoproteins, antibodies and other protein carriers. Adv. Drug Deliv. Rev. **14:** 25–50

322 Yamazaki N., Kodama M. and Gabius H.-J. (1994) Neoglycoprotein–liposome and lectin-liposome conjugates as tools

for carbohydrate recognition research. Methods Enzymol. **242:** 56–65
323 Wadhwa M. S. and Rice K. G. (1995) Receptor mediated glycotargeting. J. Drug Target. **3:** 111–127
324 Gabius S., Kayser K., Bovin N. V., Yamazaki N., Kojima S., Kaltner H. et al. (1996) Endogenous lectins and neoglycoconjugates: a sweet approach to tumor diagnosis and targeted drug delivery. Eur. J. Pharm. Biopharm. **42:** 250–261
325 Di Stefano G. and Fiume L. (1997) Liver glycotargeting of antiviral nucleoside analogues. Trends Glycosci. Glycotechnol. **9:** 461–471
326 Gabius H.-J. (1997) Concepts of tumor lectinology. Cancer Invest. **15:** 454–464
327 Kojima S., André S., Korchagina E. Y., Bovin N. V. and Gabius H.-J. (1997) Tyramine-containing poly(4-nitrophenylacrylate) as iodinatable ligand carrier in biodistribution analysis. Pharm. Res. **14:** 879–886
328 Rice K. G. (1997) Glycoconjugate-mediated drug targeting. In: Glycosciences: Status and Perspectives, pp. 471–483, Gabius H.-J. and Gabius S. (eds), Chapman and Hall, London
329 Monsigny M., Quétard C., Bourgerie S., Delay D., Pichon C., Midoux P. et al. (1998) Glycotargeting: the preparation of glyco-amino acids and derivatives from unprotected reducing sugars. Biochimie **80:** 99–108
330 May F. and Weinbrenner H. (1938) Über die Ablagerung des Galaktogens im Warmblüterorganismus. Z. Biol. **99:** 199–216
331 Ashwell G. and Harford J. (1982) Carbohydrate-specific receptors of the liver. Annu. Rev. Biochem. **51:** 531–554
332 Ponpipom M. M., Bugianesi R. L., Robbin I. L., Doebber T. W. and Shen T. Y. (1984) Saccharide receptor-mediated drug delivery. In: Receptor-Mediated Targeting of Drugs, pp. 53–71, Gregoriadis G., Poste S., Senior J. and Trouet A. (eds), Plenum Press, New York
333 Gabius H.-J. (1987) Vertebrate lectins and their possible roles in fertilization, development and tumor biology. In Vivo **1:** 75–84
334 Gabius H.-J. (1991) Detection and functions of mammalian lectins – with emphasis on membrane lectins. Biochim. Biophys. Acta **1071:** 1–18
335 Stahl P. D. (1992) The mannose receptor and other macrophage lectins. Curr. Opin. Struct. Biol. **4:** 49–52
336 Weigel P. H. (1992) Mechanisms and control of glycoconjugate turnover. In: Glycoconjugates: Composition, Structure and Function, pp. 421–497, Allen H. J. and Kisailus E. C. (eds), Marcel Dekker, New York
337 Bourbouze R., Akiki C., Chardon-Loriaux I. and Percheron F. (1982) Mise en évidence de dérivés de l'acide neuraminique dans des glycoproteines végétales. Carbohydr. Res. **106:** 21–30
338 Tretter V., Altmann F., Kubelka V., März L. and Becker W. M. (1993) Fucose α1,3-linked to the core region of glycoprotein N-glycans creates an important epitope for IgE from honeybee venom allergic individuals. Int. Arch. Allergy Immunol. **102:** 259–266
339 Brockhausen I. (1995) Biosynthesis. 3. Biosynthesis of O-glycans of the N-acetylgalactosamine-α-Ser/Thr linkage type. In: Glycoproteins, pp. 201–259, Montreuil J., Vliegenthart J. F. G. and Schachter H. (eds), Elsevier, Amsterdam
340 Hounsell E. F., Davies M. J. and Renouf D. V. (1996) O-linked protein glycosylation structure and function. Glycoconj. J. **13:** 19–26
341 Brockhausen I. and Kuhns W. (1997) Biosynthesis of O-glycans. In: Glycoproteins and Human Diseases, pp. 13–31, Brockhausen I. and Kuhns W. (eds), Springer, Heidelberg
342 Klein A., Carnoy C., Wieruszeski J. M., Strecker G., Strang A. M., van Halbeek H. et al. (1992) The broad diversity of neutral and sialylated oligosaccharides derived from human salivary mucins. Biochemistry **31:** 6152–6165
343 Roussel P. and Lamblin G. (1996) Human mucosal mucins in diseases. In: Glycoproteins and Disease, pp. 351–393, Montreuil J., Vliegenthart J. F. G. and Schachter H. (eds), Elsevier, Amsterdam
344 Lamblin G., Lhermitte M., Klein A., Perini J.-M. and Roussel P. (1991) Diversité des chaînes glycanniques des mucines bronchiques humaines et défense antimicrobienne de la muqueuse bronchique. Synthèse **7:** 1031–1040
345 Green E. D., van Halbeek H., Boine I. and Baenziger J. U. (1985) Structural elucidation of the disulfated oligosaccharide from bovine lutropin. J. Biol. Chem. **260:** 15623–15630
346 van den Eijnden D. H., Neeleman A. P., van der Knaap W. P. W., Bakker H., Agterberg M. and van Die I. (1993) Novel glycosylation routes for glycoproteins: the lacdiNAc pathway. Biochem. Soc. Trans. **23:** 175–179
347 van den Eijnden D. H., Bakker H., Neeleman A. P., van den Nieuwenhof I. M. and van Die I. (1995) Novel pathways in complex-type oligosaccharide synthesis: new vistas opened by studies in invertebrates. Biochem. Soc. Trans. **25:** 887–893
348 Do K.-Y., Do S.-I. and Cummings R. D. (1997) Differential expression of LacdiNAc sequences (GalNAcβ1-4GlcNAc-R) in glycoproteins synthesized by Chinese hamster ovary and human 293 cells. Glycobiology **7:** 183–194
349 Sakiyama T., Kabayama M., Tomita M., Nakamura J.-i., Mukai H., Tomita Y. et al. (1998) Distribution of glycoproteins with β-N-acetylgalactosaminylated N-linked sugar chains among bovine tissues. Biochim. Biophys. Acta **1380:** 268–274
350 Thiem J. (1995) Applications of enzymes in synthetic carbohydrate chemistry. FEMS Microbiol. Rev. **16:** 193–211
351 Guo Z. and Wang P. G. (1997) Utilization of glycosyltransferases to change oligosaccharide structures. Appl. Biochem. Biotechnol. **68:** 1–20
352 Corfield T. (1992) Mucus glycoproteins, super glycoforms: how to solve a sticky problem? Glycoconj. J. **9:** 217–221
353 Jentoft N. (1990) Why are proteins O-glycosylated? Trends Biochem. Sci. **15:** 291–294
354 Devine P. L. and McKenzie I. F. C. (1992) Mucins: structure, function and association with malignancy. BioEssays **14:** 619–625
355 Rose M. C. (1992) Mucins: structure, function and role in pulmonary diseases. Am. J. Physiol. **263:** L413–L429
356 Carlstedt I. and Davies J. R. (1997) Glycoconjugates facing the outside world. Biochem. Soc. Trans. **25:** 214–219
357 Karlsson K.-A. (1991) Glycobiology: a growing field for drug design. Trends Pharmacol. Sci. **12:** 265–272
358 Ofek I. and Doyle R. J. (eds) (1994) Bacterial Adhesion to Cells and Tissues, Chapman and Hall, New York
359 Lee Y. C. and Lee R. T. (1996) Glycobiology in medicine. J. Biomed. Sci. **3:** 221–237
360 Zopf D. and Roth S. (1996) Oligosaccharide anti-infective agents. Lancet **347:** 1017–1021
361 Kaltner H. and Stierstorfer B. (1998) Animal lectins as cell adhesion molecules. Acta Anat. **161:** 162–179
362 Sass P. M. (1998) The involvement of selectins in cell adhesion, tumor progression, and metastasis. Cancer Invest. **16:** 322–328
363 Lindahl U. and Höök M. (1978) Glycosaminoglycans and their binding to biological macromolecules. Annu. Rev. Biochem. **47:** 385–417
364 Carney S. L. and Muir H. (1988) The structure and functions of cartilage proteoglycans. Physiol. Rev. **68:** 858–910
365 Scott J. E. (1993) The nomenclature of glycosaminoglycans and proteoglycans. Glycoconj. J. **10:** 419–421
366 Kresse H. (1997) Proteoglycans – structure and function. In: Glycosciences: Status and Perspectives, pp. 201–222, Gabius H.-J. and Gabius S. (eds), Chapman and Hall, London
367 Casu B. (1985) Structure and biological activity of heparin. Adv. Carbohydr. Chem. Biochem. **43:** 51–134
368 Gallagher J. T., Lyon M. and Steward W. P. (1986) Structure and function of heparan sulphate proteoglycans. Biochem. J. **236:** 313–325
369 Lindahl U. and Kjellén L. (1991) Heparin or heparan sulfate – what is the difference? Thromb. Haemost. **66:** 44–48

370 Salmivirta M., Lidholt K. and Lindahl U. (1996) Heparan sulfate: a piece of information. FASEB J. **11:** 1270–1279
371 Conrad H. E. (1998) Heparin-Binding Proteins, Academic Press, San Diego
372 Ishihara M. and Ono K. (1998) Structure and function of heparin and heparan sulfate: heparinoid library and modification of FGF-activities. Trends Glycosci. Glycotechnol. **10:** 223–233
373 Toida T. and Linhardt R. J. (1998) Structural analysis of heparan sulfate and heparan sulfate oligosaccharides. Trends Glycosci. Glycotechnol. **10:** 125–136
374 Comper W. D. (1990) Extracellular matrix interactions: sulfation of connective tissue polysaccharides creates macroion binding templates and conditions for dissipative structure formation. J. Theor. Biol. **145:** 497–509
375 Laurent T. C. and Fraser J. R. E. (1992) Hyaluronan. FASEB J. **6:** 2397–2404
376 Delpech B., Girard N., Bertrand P., Courel M. N., Chauzy C. and Delpech A. (1997) Hyaluronan: fundamental principles and applications in cancer. J. Intern. Med. **242:** 41–48
377 Itano N. and Kimata K. (1998) Hyaluronan synthase: new directions for hyaluronan research. Trends Glycosci. Glycotechnol. **10:** 23–38
378 Casu B., Petitou M., Provasoli M. and Sinay P. (1988) Conformational flexibility: a new concept for explaining binding and biological properties of iduronic acid-containing glycosaminoglycans. Trends Biochem. Sci. **13:** 221–225
379 Yamada S. and Sugahara K. (1998) Structure of oligosaccharides isolated from heparan sulfate/heparin and substrate specificities of the degrading enzymes of bacterial origin. Trends Glycosci. Glycotechnol. **10:** 95–123
380 Rodén L. and Smith R. (1966) Structure of the neutral trisaccharide of the chondroitin-4-sulfate-protein linkage region. J. Biol. Chem. **241:** 5949–5954
381 Quentin E., Gladen A., Rodén L. and Kresse H. (1990) A genetic defect in the biosynthesis of dermatan sulfate proteoglycan: galactosyltransferase I deficiency in fibroblasts from a patient with progeroid syndrome. Proc. Natl. Acad. Sci. USA **87:** 1342–1346
382 Lind T., Lindahl U. and Lidholt K. (1993) Biosynthesis of heparin/heparan sulfate. Identification of a 70kDa protein catalyzing both the D-glucuronosyl- and the N-acetyl-D-glucosaminyltransferase rections. J. Biol. Chem. **268:** 20705–20708
383 Rodén L. (1970) Structure and metabolism of the proteoglycans of chondroitin sulfates and keratan sulfate. In: Chemistry and Molecular Biology of the Intercellular Matrix, vol. 2, pp. 797–821, Balazs E. A. (ed.), Academic Press, London
384 Evered D. and Whelan J. (eds) (1986) Functions of the Proteoglycans, Ciba Found. Symp., vol. 124, J. Wiley and Sons, Chichester
385 Habuchi H., Habuchi O. and Kimata K. (1998) Biosynthesis of heparan sulfate and heparin: how are the multifunctional glycosaminoglycans built up? Trends Glycosci. Glycotechnol. **10:** 65–80
386 Kusche-Gullberg M., Eriksson I., Pikas D. S. and Kjellén L. (1998) Identification and expression in mouse of two heparan sulfate glucosaminyl N-deacetylase/N-sulfotransferase genes. J. Biol. Chem. **273:** 11902–11907
387 Kusche M., Bäckström G., Riesenfeld J., Petitou M., Choay J. and Lindahl U. (1988) Biosynthesis of heparin. O-Sulfation of the antithrombin-binding region. J. Biol. Chem. **263:** 15474–15484
388 Bourin M.-C. and Lindahl U. (1993) Glycosaminoglycans and the regulation of blood coagulation. Biochem. J. **289:** 313–330
389 Skinner R., Abrahams J.-P., Whisstock J. C., Lesk A. M., Carrel R. W. and Wardell M. R. (1997) The 2.6 Å structure of antithrombin indicates a conformational change at the heparin-binding site. J. Mol. Biol. **266:** 601–609
390 Palmer R. N., Rick M. E., Rick P. D., Zeller J. A. and Gralnick H. R. (1984) Circulating heparan sulfate anticoagulant in a patient with a fatal bleeding disorder. N. Engl. J. Med. **310:** 1696–1699
391 Hart G. W. and Lennarz W. J. (1978) Effects of tunicamycin on the biosynthesis of glycosaminoglycans by embryonic chick cornea. J. Biol. Chem. **253:** 5795–5801
392 Hardingham T. E. and Fosang A. J. (1992) Proteoglycans: many forms and many functions. FASEB J. **6:** 861–870
393 Ruoslahti E. (1988) Structure and biology of proteoglycans. Annu. Rev. Cell Biol. **4:** 229–255
394 Fedarko N. S. (1993) Isolation and purification of proteoglycans. Experientia **49:** 369–383
395 Iozzo R. V. and Murdoch A. D. (1996) Proteoglycans of the extracellular environment: clues from the gene and protein side offer novel perspectives in molecular diversity and function. FASEB J. **10:** 598–614
396 Toole B. P. (1990) Hyaluronan and its binding proteins, the hyaladherins. Curr. Opin. Cell Biol. **2:** 839–844
397 Haynes B. F., Liao H.-X. and Patton K. L. (1991) The transmembrane hyaluronate receptor (CD44): multiple functions, multiple forms. Cancer Cells **3:** 347–350
398 Knudson C. B. and Knudson W. (1993) Hyaluronan-binding proteins in development, tissue homeostasis and disease. FASEB J. **7:** 1233–1241
399 Sherman L., Sleeman J., Herrlich P. and Pouta H. (1994) Hyaluronate receptors: key players in growth, differentiation, migration and tumor progression. Curr. Opin. Cell Biol. **6:** 726–733
400 Yurchenco P. D. and Schittny J. C. (1990) Molecular architecture of basement membranes. FASEB J. **4:** 1577–1590
401 Watanabe H., Kimata K., Line S., Strong D., Gao L. Y., Kozak C. A. et al. (1994) Mouse cartilage matrix deficiency (cmd) caused by a 7bp deletion in the aggrecan gene. Nature Genet. **7:** 154–157
402 Timpl R. (1993) Proteoglycans of basement membranes. Experientia **49:** 417–428
403 Kresse H., Hausser H. and Schönherr E. (1993) Small proteoglycans. Experientia **49:** 403–416
404 Yanagishita M. and Ishihara M. (eds) (1998) Diverse functions and structures on heparan sulfate proteoglycan. Trends Glycosci. Glycotechnol. 10: 1–233
405 Bernfield M., Kokenyesi R., Kato M., Hinkes M. T., Spring J., Gallo R.-L. et al. (1992) Biology of the syndecans: a family of transmembrane heparan sulfate proteoglycans. Annu. Rev. Cell Biol. **8:** 365–393
406 Yanagishita M. and Hascall V. C. (1992) Cell surface heparan sulfate proteoglycans. J. Biol. Chem. **267:** 9451–9454
407 David G. (1993) Integral membrane heparan sulfate proteoglycans. FASEB J. **7:** 1023–1030
408 Elenius K. and Jalkanen M. (1994) Functions of the syndecans – a family of cell surface proteoglycans. J. Cell Sci. **107:** 2975–2982
409 Carey D. J. (1997) Syndecans: multifunctional cell-surface co-receptors. Biochem. J. **327:** 1–16
410 Okayama M., Munesue S., Komaki M., Kusano Y. and Oguri K. (1998) Functional specificity of syndecans. Trends Glycosci. Glycotechnol. **10:** 211–221
411 Veugelers M. and David G. (1998) The glypicans: a family of GPI-anchored heparan sulfate proteoglycans with a potential role in the control of cell division. Trends Glycosci. Glycotechnol. **10:** 145–152
412 Debagge P. L., Lange W., Hellmann T. and Gabius H.-J. (1988) Receptors for sulfated polysaccharides in human placenta. J. Histochem. Cytochem. **36:** 1097–1102
413 Kjellén L. and Lindahl U. (1991) Proteoglycans: structures and interactions. Annu. Rev. Biochem. **60:** 443–475
414 Kohnke-Godt B. and Gabius H.-J. (1991) Heparin-binding lectin from human placenta: further characterization of ligand binding and structural properties and its relationship to histones and heparin-binding growth factors. Biochemistry **30:** 55–65
415 Gabius H.-J., Kohnke-Godt B., Leichsenring M. and Bardosi A. (1991) Heparin-binding lectin of human placenta as a tool for histochemical ligand localization and ligand isolation. J. Histochem. Cytochem **39:** 1249–1256

416 Joubert-Caron R., Lutomski D., Bladier D. and Caron M. (1997) Glycobiology of consciousness. In: Glycosciences: Status and Perspectives, pp. 611–617, Gabius H.-J. and Gabius S. (eds), Chapman and Hall, London

417 Kock N. P., Gabius H.-J., Schmitz J. and Schottelius J. (1997) Receptors for carbohydrate ligands including heparin on the cell surface of *Leishmania* and other trypanosomatids. Trop. Med. Int. Health **2:** 863–874

418 Rostand K. S. and Esko J. D. (1997) Microbial adherence to and invasion through proteoglycans. Infect. Immun. **65:** 1–8

419 Ward H. D. (1997) Glycobiology of parasites: role of carbohydrate-binding proteins and their ligands in the host-parasite interaction. In: Glycosciences: Status and Perspectives, pp. 399–413, Gabius H.-J. and Gabius S. (eds), Chapman and Hall, London

420 Hileman R. E., Fromm J. R., Weiler J. M. and Linhardt R. J. (1998) Glycosaminoglycan-protein interaction: definition of consensus sites in glycosaminoglycan-binding proteins. BioEssays **20:** 156–167

421 Zanetta J.-P. (1998) Structure and function of lectins in the central and peripheral nervous system. Acta Anat. **161:** 180–195

422 Gallagher J. T. and Turnbull J. E. (1992) Heparan sulphate in the binding and activation of basic fibroblast growth factor. Glycobiology **2:** 523–528

423 Lortat-Jacob H. and Grimaux J.-A. (1992) Binding of interferon-γ to heparan sulfate is restricted to the heparin-like domains and involves carboxylic – but not N-sulfated – groups. Biochim. Biophys. Acta **1117:** 126–130

424 Ramsden L. and Rider C. C. (1992) Selective and differential binding of interleukin (IL)-1α, IL-1β and IL-6 to glycosaminoglycans. Eur. J. Immunol. **22:** 3027–3031

425 Nietfeld J. J. (1993) Cytokines and proteoglycans. Experientia **49:** 456–469

426 Tanaka Y., Adams D. H. and Shaw S. (1993) Proteoglycans on endothelial cells present adhesion-inducing cytokines to leukocytes. Immunol. Today **14:** 111–115

427 Webb L. M. C., Ehrengruber M. U., Clark-Lewis I., Baggiolini M. and Rot A. (1993) Binding to heparan sulfate or heparin enhances neutrophil responses to interleukin 8. Proc. Natl. Acad. Sci. USA **90:** 7158–7162

428 Faham S., Hileman R. E., Fromm J. R., Linhardt R. J. and Rees D. C. (1996) Heparin structure and interactions with basic fibroblast growth factor. Science **271:** 1116–1120

429 Taipale J. and Keski-Oja J. (1997) Growth factors in the extracellular matrix. FASEB J. **11:** 51–59

430 Mishra-Gorur K., Delmolino L. M. and Castellot J. J. (1998) Biological functions of heparan sulfates. Trends Glycosci. Glycotechnol. **10:** 193–210

431 Tanaka Y. and Aso M. (1998) Chemokine and cellular adhesion in the context of heparan sulfate proteoglycan. Trends Glycosci. Glycotechnol. **10:** 153–160

432 Lider O., Cahalon L., Gilat D., Hershkoviz R., Siegel D., Margalit R. et al. (1995) A disaccharide that inhibits tumor necrosis factor α is formed from the extracellular matrix by the enzyme heparanase. Proc. Natl. Acad. Sci. USA **92:** 5037–5041

433 Svennerholm L. (1964) The gangliosides. J. Lipid Res. **5:** 145–155

434 Ledeen R. W. and Yu R. (1982) Gangliosides: structure, isolation and analysis. Methods Enzymol. **83:** 139–191

435 Wiegandt H. (1992) Insect glycolipids. Biochim. Biophys. Acta **1123:** 117–126

436 van Echten G. and Sandhoff K. (1993) Ganglioside metabolism, enzymology, topology and regulation. J. Biol. Chem. **268:** 5341–5344

437 Svennerholm L., Ashbury A. K., Reisfeld R. A., Sandhoff K., Tettamanti G. and Toffano G. (1994) Biological function of gangliosides. Progr. Brain Res. **101:** 1–404

438 Kopitz J. (1997) Glycolipids: structure and function. In: Glycosciences: Status and Perspectives, pp. 163–189, Gabius H.-J. and Gabius S. (eds), Chapman and Hall, London

439 Kopitz J. (1997) Glycosphingolipids and disease. Electr. J. Pathol. Histol. 973–08.txt

440 Lloyd K. O. and Furukawa K. (1998) Biosynthesis and functions of gangliosides: recent advances. Glycoconj. J. **15:** 627–636

441 Stults C. L. M., Sweeley C. C. and Macher B. A. (1989) Glycosphingolipids: structure, biological source and properties. Methods Enzymol. **179:** 167–214

442 Karlsson K.-A. (1989) Animal glycosphingolipids as membrane attachment sites for bacteria. Annu. Rev. Biochem. **58:** 309–350

443 Hakomori S. (1981) Glycosphingolipids in cellular interaction, differentiation and oncogenesis. Annu. Rev. Biochem. **50:** 733–764

444 Hakomori S. (1998) Cancer-associated glycosphingolipid antigens: their structure, organization and function. Acta Anat. **161:** 79–90

445 Hata K., Wada T., Hasegawa A., Kisoto M. and Miyagi T. (1998) Purification and characterization of a membrane-associated ganglioside sialidase from bovine brain. J. Biochem. **123:** 899–905

446 Sandhoff K. and Kolter T. (1995) Glykolipide der Zelloberfläche: Biochemie ihres Abbaues. Naturwissenschaften **82:** 403–413

447 Sandhoff K. and Kolter T. (1996) Topology of glycosphingolipid degradation. Trends Cell Biol. **6:** 98–103

448 Kolter T. and Sandhoff K. (1998) Recent advances in the biochemistry of sphingolipidoses. Brain Pathol. **8:** 79–100

449 Brady R. O. and Barton N. W. (1991) Enzyme replacement therapy with macrophage-targeted human placental glucocerebrosidase in Gaucher disease. In: Lectins and Cancer, pp. 237–250, Gabius H.-J. and Gabius S. (eds), Springer, Berlin

450 Stanley P. (1992) Glycosylation engineering. Glycobiology **2:** 99–107

451 Brady R. O., Murray G. J. and Barton N. W. (1994) Modifying exogenous glucocerebrosidase for effective replacement therapy in Gaucher disease. J. Inher. Metab. Dis. **17:** 510–519

452 Nagai Y. and Tsuji S. (1988) Gangliosides in recognitive cell interactions and carbohydrate-mediated biosignaling. In: Lectins and Glycoconjugates in Oncology, pp. 59–66, Gabius H.-J. and Nagel G. A. (eds), Springer, Berlin.

453 Hakomori S. and Igarashi Y. (1995) Functional role of glycosphingolipids in cell recognition and signaling. J. Biochem. **118:** 1091–1103

454 Nagai Y. (1998) Cell regulatory function of glycosphingolipid: carbohydrate recognition and biosignaling. Pure Appl. Chem. **70:** 49–53

455 Song Y., Withers D. A. and Hakomori S. (1998) Globoside-dependent adhesion of human embryonal carcinoma cells, based on carbohydrate-carbohydrate interaction, initiates signal transduction and induces enhanced activity of transcription factors AP1 and CREB. J. Biol. Chem. **273:** 2517–2525

456 Hannun Y. A. and Bell R. M. (1989) Functions of sphingolipids and sphingolipid breakdown products in cellular regulation. Science **243:** 500–507

457 Hooper N. M. (1998) Do glycolipid microdomains really exist? Curr. Biol. **8:** R114–R116

458 Iwabuchi K., Yamamura S., Prinetti A., Handa K. and Hakomori S. (1998) GM3-enriched microdomain involved in cell adhesion and signal transduction through carbohydrate-carbohydrate interaction in mouse melanoma B16 cells. J. Biol. Chem. **273:** 9130–9138

459 Karlsson K.-A. (1995) Microbial recognition of target-cell glycoconjugates. Curr. Opin. Struct. Biol. **5:** 622–635

460 Rauvala H. and Finne J. (1979) Structural similarity of the terminal carbohydrate sequences of glycoproteins and glycolipids. FEBS Lett. **97:** 1–8

461 Feizi T. and Childs R. A. (1985) Carbohydrate structures of glycoproteins and glycolipids as differentiation antigens, tumour-associated antigens and components of receptor systems. Trends Biochem. Sci. **10:** 24–29

462 Fenderson B. A., Eddy E. M. and Hakomori S. (1990) Glycoconjugate expression during embryogenesis and its biological significance. BioEssays **12:** 173–179
463 Hathaway H. J. and Shur B. D. (1997) Transgenic approaches to glycobiology. In: Glycosciences: Status and Perspectives, pp. 507–517, Gabius H.-J. and Gabius S. (eds), Chapman and Hall, London
464 Bosio A., Binczek E. and Stoffel W. (1996) Functional breakdown of the lipid bilayer of the myelin membrane in central and peripheral nervous system by disrupted galactocerebroside synthesis. Proc. Natl. Acad. Sci. USA **93:** 13280–13285
465 Coetzee T., Fujita N., Dupree J., Shi R., Blight A., Suzuki K. et al. (1996) Myelination in the absence of galactocerebroside and sulfatide: normal structure with abnormal function and regional instability. Cell **86:** 209–219
466 Takamiya K., Yamamoto A., Furukawa K., Yamashiro S., Shin M., Okada M. et al. (1996) Mice with disrupted GM2/GD2 synthase gene lack complex gangliosides but exhibit only subtle defects in their nervous system. Proc. Natl. Acad. Sci. USA **93:** 10662–10667
467 Bosio A., Büssow H., Adam J. and Stoffel W. (1998) Galactosphingolipids and axono-glial interaction in myelin of the central nervous system. Cell Tissue Res. **292:** 199–210
468 Chavany C. and Jendoubi M. (1998) Biology and potential strategies for the treatment of GM2 gangliosidoses. Mol. Med. Today **4:** 158–165
469 Sango K., Yamanaka S., Hoffmann A., Okuda Y., Grinberg A., Westphal A. et al. (1995) Mouse models of Tay-Sachs and Sandhoff diseases differ in neurologic phenotype and ganglioside metabolism. Nature Genet. **11:** 170–176
470 Oshima T., Murray G. J., Swain W. D., Longenecker G., Quirk J. M., Cardarelli C. A. et al. (1997) Galactosidase A deficient mice: a model of Fabry disease. Proc. Natl. Acad. Sci. USA **94:** 2540–2544
471 Shimmoto M., Kase R., Itoh K., Utsumi K., Ishii S., Taya C. et al. (1997) Generation and characterization of transgenic mice expressing a human mutant α-galactosidase with an R301Q substitution causing a variant form of Fabry disease. FEBS Lett. **417:** 89–91
472 Sandhoff K. (1977) Biochemie der Sphingolipidspeicherkrankheiten. Angew. Chemie **89:** 283–295
473 Sandhoff K. and Conzelmann E. (1984) The biochemical basis of gangliosidoses. Neuropediatrics Suppl. **15:** 85–92
474 Sandhoff K. and Quintern L. (1988) Zentralnervöse Sphingolipidspeicherkrankheiten. Grundlagen ihrer biochemischen und klinischen Heterogenität. Naturwissenschaften **75:** 123–131
475 Talente G. M., Coleman R. A., Alter C., Baker L., Brown B. I., Cannon R. A. et al. (1994) Glycogen storage disease in adults. Ann. Intern. Med. **120:** 218–226
476 Chen Y.-T. and Burchell A. (1995) Glycogen storage diseases. In: The Metabolic and Molecular Basis of Inherited Disease, 7th ed., vol. 1, pp. 935–965, Sciver C. R., Beaudet A. L., Sly W. S. and Valle D. (eds), McGraw-Hill, New York
477 Lee P. J. and Leonard J. V. (1995) The hepatic glycogen storage diseases – problems beyond childhood. J. Inherit. Metab. Dis. **18:** 462–472
478 Neufeld E. F. and Muenzer J. (1995) The mucopolysaccharidoses. In: The Metabolic and Molecular Basis of Inherited Disease, 7th ed., vol. 2, pp. 2465–2494, Sciver C. R., Beaudet A. L., Sly W. S. and Valle D. (eds), McGraw-Hill, New York
479 Wraith J. E. (1995) The mucopolysaccharidoses: a clinical review and guide to management. Arch. Dis. Child. **72:** 263–267
480 Thomas G. H. and Beaudet A. L. (1995) Disorders of glycoprotein degradation and structure: α-mannosidosis, β-mannosidosis, fucosidosis, sialidosis, aspartylglucosaminuria and carbohydrate-deficient glycoprotein syndrome. In: The Metabolic and Molecular Basis of Inherited Disease, 7th ed., vol. 2, pp. 2529–2561, Sciver C. R., Beaudet A. L., Sly W. S. and Valle D. (eds), McGraw-Hill, New York
481 Michalski J.-C. (1996) Normal and pathological catabolism of glycoproteins. Catabolic pathways. In: Glycoproteins and Disease, pp. 55–97, Montreuil J., Vliegenthart J. F. G. and Schachter H. (eds), Elsevier, Amsterdam
482 Strecker G., Michalski J.-C. and Montreuil J. (1988) Lysosomal catabolic pathway of N-glycosylprotein glycans. Biochimie **70:** 1505–1510
483 Jourdian G. W. (1996) Normal and pathological catabolism of glycoproteins. The enzymes. In: Glycoproteins and Disease, pp. 3–54, Montreuil J., Vliegenthart J. F. G. and Schachter H. (eds), Elsevier, Amsterdam
484 Fuhrmann U., Bause E. and Ploegh H. (1985) Inhibitors of oligosaccharide processing. Biochim. Biophys. Acta **825:** 95–100
485 Schwarz R. T. (1991) Manipulation of the biosynthesis of protein-modifying glycoconjugates by the use of specific inhibitors. Behring Inst. Mitt. **89:** 198–208
486 Elbein A. D. (1991) Glycosidase inhibitors: inhibitors of N-linked oligosaccharide processing. FASEB J. **5:** 3055–3063
487 Legler G. (1993) Zuckeranaloge Inhibitoren für Glycosidasen, Werkzeuge zur Aufklärung der enzymatischen Glycosidspaltung. Naturwissenschaften **80:** 397–409
488 Kaushal G. P. and Elbein A. D. (1994) Glycosidase inhibitors in study of glycoconjugates. Methods Enzymol. **230:** 316–329
489 Jacob G. S. (1995) Glycosylation inhibitors in biology and medicine. Curr. Opin. Struct. Biol. **5:** 605–611
490 Neufeld E. F. (1991) Lysosomal storage diseases. Annu. Rev. Biochem. **60:** 257–280
491 Cragg H., Williamson M., Young E., O'Brien J., Alhadeff J., Fang-Kircher S. et al. (1997) Fucosidosis: genetic and biochemical analysis of eight cases. J. Med. Genet. **34:** 105–110
492 le Coutre P., Demina A., Beutler E., Beck M. and Petrides P. E. (1997) Molecular analysis of Gaucher disease: distribution of eight mutations and the complete gene deletion in 27 patients from Germany. Hum. Genet. **99:** 816–821
493 Maddox L. O., Li P., Bennett A., Descartes M. and Thompson J. N. (1997) Comparison of SSCP analysis and CFLP analysis for mutation detection in the human iduronate 2-sulfatase gene. Biochem. Mol. Biol. Int. **43:** 1163–1171
494 Alkhayat A. H., Kraemer S. A., Leipprandt J. R., Macek M., Kleijer W. J. and Friderici K. H. (1998) Human β-mannosidase cDNA characterization and first identification of a mutation associated with human β-mannosidosis. Hum. Mol. Genet. **7:** 75–83
495 van Pelt J., van Bilsen D. G. J. L., Kamerling J. P. and Vliegenthart J. F. G. (1988) Structural analysis of O-glycosidic type of sialyloligosaccharide-alditols derived from urinary glycopeptides of a sialidosis patient. Eur. J. Biochem. **174:** 183–187
496 Hirabayashi Y., Matsumoto Y., Matsumoto M., Toida T., Iida N., Matsubara T. et al. (1990) Isolation and characterization of major urinary amino acid O-glycosides and a dipeptide O-glycoside from a new lysosomal storage disorder (Kanzaki disease). J. Biol. Chem. **265:** 1693–1701
497 Brady R. O. (1998) Therapy for the sphingolipidoses. Arch. Neurol. **55:** 1055–1056
498 Aly A. M., Higuchi M., Kasper C. K., Kazazian H. H., Antonarakis A. E. and Hoyer L. W. (1992) Hemophilia A due to mutations that create new N-glycosylation sites. Proc. Natl. Acad. Sci. USA **89:** 4933–4937
499 Brennan S. O., Borg J. Y., George P. M., Soria C., Soria J., Caen J. et al. (1988) New carbohydrate site in mutant antithrombin with decreased heparin affinity. FEBS Lett. **237:** 118–122
500 Martinéz J. and Barsigian C. (1987) Carbohydrate abnormalities of N-linked plasma glycoproteins in liver disease. Lab. Invest. **57:** 240–257
501 Turner G. A. (1992) N-Glycosylation of serum proteins in disease and its investigation using lectins. Clin. Chim. Acta **208:** 149–171

502 Brockhausen I. (1993) Clinical aspects of glycoprotein biosynthesis. Crit. Rev. Clin. Lab. Sci. **30:** 65–151
503 Brockhausen I., Schutzbach J. and Kuhns W. (1998) Glycoproteins and their relationship to human disease. Acta Anat. **161:** 36–78
504 van Dijk W., Turner G. A. and Mackiewicz A. (1994) Changes in glycosylation of acute-phase proteins in health and disease: occurrence, regulation and function. Glycosylation Dis. **1:** 5–14
505 van Dijk W., Brinkman-van der Linden E. C. M. and Havenaar E. C. (1998) Glycosylation of α_1-acid glycoprotein (orosomucoid) in health and disease: occurrence, regulation and possible functional implications. Trends Glycosci. Glycotechnol. **10:** 235–245
506 Caldwell S. H., Halliday J. W., Fletcher L. M., Kulaga M., Murphy T. L., Li X. et al. (1995) Carbohydrate-deficient transferrin in alcoholics with liver disease. J. Gastroenterol. Hepatol. **10:** 174–178
507 Seitz G., Stickel F., Fiehn W., Werle E., Simanowski U. A. and Seitz H. K. (1995) Carbohydrate-deficient transferrin. A new, highly specific marker for chronic alcohol consumption. Dtsch. Med. Wschr. **120:** 391–395
508 Schmitt U. M., Stieber P., Jüngst D., Bilzer M., Wächtler M., Heberger S. et al. (1998) Carbohydrate-deficient transferrin is not a useful marker for the detection of chronic alcohol abuse. Eur. J. Clin. Invest. **28:** 615–621
509 Jaeken J., van der Schueren-Lodewyckx M., Casaer P., Snoek L., Corbeel E., Eggermont E. et al. (1980) Familial psychomotor retardation with markedly fluctuating serum prolactin, FSH and GH levels, partial TBG deficiency, increased serum arylsulphatase A and increased CSF protein: a new syndrome? Pediatr. Res. **14:** 179
510 Jaeken J., Carchon H. and Stibler H. (1993) The carbohydrate-deficient glycoprotein syndromes: pre-Golgi or Golgi disorders? Glycobiology **3:** 423–428
511 Winchester B., Clayton P., Mian N., di-Tomaso E., Dell A., Reason A. et al. (1995) The carbohydrate-deficient glycoprotein syndrome: an experiment of nature in glycosylation. Biochem. Soc. Trans. **23:** 185–188
512 Dwek R. A. (1996) Glycobiology: toward understanding of function of sugars. Chem. Rev. **96:** 683–720
513 McDowell G. and Gahl W. A. (1997) Inherited disorders of glycoprotein synthesis: cell biological insights. Proc. Soc. Exp. Biol. Med. **215:** 145–157
514 Kornfeld S. (1998) Diseases of abnormal protein glycosylation: an emerging area. J. Clin. Invest. **101:** 1293–1295
515 Jaeken J., Schachter H., Carchon H., de Cock P., Coddeville B. and Spik G. (1994) Carbohydrate-deficient glycoprotein syndrome type II: a deficiency in Golgi localized N-acetylglucosaminyltransferase II. Arch. Dis. Child. **71:** 123–127
516 Yamashita K. and Ohno K. (1996) Carbohydrate-deficient glycoprotein syndrome. Type I. In: Glycoproteins and Disease, pp. 445–455, Montreuil J., Vliegenthart J. F. G. and Schachter H. (eds), Elsevier, Amsterdam
517 Pohl S., Hoffmann A., Rüdiger A., Nimtz M., Jaeken J. and Conradt H. S. (1997) Hypoglycosylation of a brain glycoprotein (β-trace protein) in CDG syndromes due to phosphomannomutase deficiency and N-acetylglucosaminyltransferase II deficiency. Glycobiology **7:** 1077–1084
518 Panneerselvam K. and Freeze H. H. (1996) Mannose corrects altered N-glycosylation in carbohydrate-deficient glycoprotein syndrome fibroblasts. J. Clin. Invest. **97:** 1478–1487
519 Körner C., Lehle L. and von Figura K. (1998) Carbohydrate-deficient glycoprotein syndrome type I: correction of the glycosylation defect by deprivation of glucose or supplementation of mannose. Glycoconj. J. **15:** 499–505
520 Burda P., Borsig L., de Rijk-van Andel J., Wevers R., Jaeken J., Carchon H. et al. (1998) A novel cabohydrate-deficient glycoprotein syndrome characterized by a deficiency in glucosylation of the dolichol-linked oligosaccharide. J. Clin. Invest. **102:** 647–652
521 de Koning T. J., Dorland L., van Diggelen O. P., Boonman A. M. C., de Jong G. J., van Noort W. L. et al. (1998) A novel disorder of N-glycosylation due to phosphomannose isomerase deficiency. Biochem. Biophys. Res. Commun. **245:** 38–42
522 Niehues R., Hasilik M., Alton G., Körner C., Schiebe-Sukumar M., Koch H. G. et al. (1998) Carbohydrate-deficient glycoprotein syndrome type Ib. Phosphomannose isomerase deficiency and mannose therapy. J. Clin. Invest. **101:** 1414–1420
523 Alton G., Hasilik M., Niehues R., Panneerselvam K., Etchinson J. R., Fana F. et al. (1998) Direct utilization of mannose for mammalian glycoprotein biosynthesis. Glycobiology **8:** 285–295
524 Charuk J. H. M., Tan J., Bernardini M., Haddad S., Reithmeier R. A. F., Jaeken J. et al. (1995) Carbohydrate-deficient glycoprotein syndrome type II. Eur. J. Biochem. **230:** 797–805
525 Jaeken J., Spik G. and Schachter H. (1996) Carbohydrate-deficient glycoprotein syndrome. Type II: an autosomal recessive disease due to mutations in the N-acetylglucosaminyltransferase II gene. In: Glycoproteins and Disease, pp. 457–467, Montreuil J., Vliegenthart J. F. G. and Schachter H. (eds), Elsevier, Amsterdam.
526 Gahl W. A. (1997) Carbohydrate-deficient glycoprotein syndrome: hidden treasures. J. Lab. Clin. Med. **129:** 394–395
527 Stibler H., von Dobeln U., Kristiansson B. and Guthenberg C. (1997) Carbohydrate-deficient transferrin in galactosaemia. Acta Paediatr. **86:** 1377–1378
528 Fukuda M. N. (1996) HEMPAS: a genetic disorder caused by a defect in N-linked oligosaccharide synthesis. In: Glycoproteins and Disease, pp. 299–310, Montreuil J., Vliegenthart J. F. G. and Schachter H. (eds), Elsevier, Amsterdam.
529 Chui D., Oh-Eda M., Liao Y.-F., Panneerselvam K., Lal A., Marek K. W. et al. (1997) α-Mannosidase-II deficiency results in dyserythropoiesis and unveils an alternate pathway in oligosaccharide biosynthesis. Cell **90:** 157–167
530 Tulsiani D. R. P., Broquist H. P., James L. F. and Touster O. (1988) Production of hybrid glycoproteins and accumulation of oligosaccharides in the brain of sheep and pigs administered swaisonine or locoweed. Arch. Biochem. Biophys. **264:** 607–617
531 Scocca J. R., Hartog K. O. and Krag S. S. (1988) Evidence of gene amplification in tunicamycin-resistant Chinese hamster ovary cells. Biochem. Biophys. Res. Commun. **156:** 1063–1069
532 Marth J. D. (1994) Will the transgenic mouse serve as a Rosetta Stone to glycoconjugate function? Glycoconj. J. **11:** 3–8
533 Stanley P. and Ioffe E. (1995) Glycosyltransferase mutants: key to new insights in glycobiology. FASEB J. **9:** 1436–1444
534 Asano M., Furukawa K., Kido M., Matsumoto S., Umesaki Y., Kochibe N. et al. (1997) Growth retardation and early death of β1,4-galactosyltransferase knockout mice with augmented proliferation and abnormal differentiation of epithelial cells. EMBO J. **16:** 1850–1857
535 Lu Q., Hasty P. and Shur B. D. (1997) Targeted mutation in β1,4-galactosyltransferase leads to pituitary insufficiency and neonatal lethality. Dev. Biol. **181:** 257–267
536 Priatel J. J., Sarkar M., Schachter H. and Marth J. D. (1997) Isolation, characterization and inactivation of the mouse *Mgat3* gene: the bisecting N-acetylglucosamine in asparagine-linked oligosaccharides appears dispensable for viability and reproduction. Glycobiology **7:** 45–56
537 Wallis G. A. (1995) The importance of being sulphated. Curr. Biol. **5:** 225–227
538 Leidich S. D., Drapp D. A. and Orlean P. (1994) A conditionally lethal yeast mutant blocked at the first step in glycosyl phosphatidylinositol anchor synthesis. J. Biol. Chem. **269:** 10193–10196
539 Grubb J. H., Kyle J. W., Cody L. B. and Sly W. S. (1993) Large scale purification of phosphorylated recombinant human β-glucuronidase from overexpressing mouse L cells. FASEB J. **7:** A1255

540 Vogler C., Sands M. S., Galvin N., Levy B., Thorpe C., Barker J. et al. (1998) Murine mucopolysaccharidosis type VII: the impact of therapies on the clinical course and pathology in a murine model of lysosomal storage disease. J. Inher. Metab. Dis. **21:** 575–586

541 Paulson J. C. (1996) Leukocyte adhesion deficiency type II. In: Glycoproteins and Disease, pp. 405–411, Montreuil J., Vliegenthart J. F. G. and Schachter H. (eds), Elsevier, Amsterdam

542 Anderson D. C. and Springer T. A. (1987) Leukocyte adhesion deficiency: an inherited defect in the MAC-1, LFA-1, and p150,95 glycoproteins. Annu. Rev. Med. **38:** 175–194

543 Kuijpers T. W., von Lier R. W. A., Hamann D., de Boer M., Thung L. Y., Weening R. S. et al. (1997) Leukocyte adhesion deficiency type 1 (LAD-1)/variant. A novel immunodeficiency syndrome characterized by dysfunctional $\beta 2$ integrins. J. Clin. Invest. **100:** 1725–1733

544 Karsan A., Cornejo C. J., Winn R. K., Schwartz B. R., Way W., Lannir N. et al. (1998) Leukocyte adhesion deficiency type II is a generalized defect of de novo GDP-fucose biosynthesis. Endothelial cell fucosylation is not required for neutrophil rolling on human nonlymphoid endothelium. J. Clin. Invest. **101:** 2438–2445

545 Sturla L., Etzioni A., Bisso A., Zanardi D., de Flora D., Silengo L. et al. (1998) Defective intracellular activity of CDP-D-mannose-4,6-dehydratase in leukocyte adhesion deficiency type II syndrome. FEBS Lett. **429:** 274–278

546 Maly P., Thall A., Petryniak B., Rogers C. E., Smith P. L., Marks R. M. et al. (1996) The (α1,3)fucosyltransferase FucTVII controls leukocyte trafficking through an essential role in L-, E-, and P-selectin ligand biosynthesis. Cell **86:** 643–653

547 Lowe J. B. (1997) Selectin ligands, leukocyte trafficking, and fucosyltransferase genes. Kidney Int. **51:** 1418–1426

548 Varki A. (1997) Selectin ligands: will the real ones please stand up? J. Clin. Invest. **99:** 158–162

549 Wagers A. J., Stoolman L. M., Craig R., Knibbs R. N. and Kansas G. S. (1998) An sLex-deficient variant of HL60 cells exhibits high levels of adhesion to vascular selectins: further evidence that HECA-452 and CSLEX1 monoclonal antibody epitopes are not essential for high avidity binding to vascular selectins. J. Immunol. **160:** 5122–5129

550 Wang C., Eufemi M. and Giartosio A. (1996) Influence of the carbohydrate moiety on the stability of glycoproteins. Biochemistry **35:** 7299–7307

551 Wyss D. F. and Wagner G. (1996) The structural role of sugars in glycoproteins. Curr. Opin. Biotechnol. **7:** 409–416

552 Rudd P. M. and Dwek R. A. (1997) Glycosylation: heterogeneity and the 3D structure of proteins. Crit. Rev. Biochem. Mol. Biol. **32:** 1–100

553 Wyss D. F., Choi J. S., Li J., Knoppers M. H., Willis K. J., Arulamandam A. R. N. et al. (1995) Conformation and function of the N-linked glycan in the adhesion domain of human CD2. Science **269:** 1273–1278

554 de Beer T., van Zuylen C. W. E. M., Leeflang B. R., Hård K., Boelens R., Kaptein R. et al. (1996) NMR studies on the free α subunit of human chorionic gonadotropin. Structural influences of N-glycosylation and the β subunit on the conformation of the α subunit. Eur. J. Biochem. **241:** 229–242

555 Mer G., Hietter H. and Lefevre J.-F. (1996) Stabilization of proteins by glycosylation examined by NMR analysis of a fucosylated proteinase inhibitor. Nature Struct. Biol. **3:** 45–53

556 Thijssen-van Zuylen C. W. E. M., de Beer T., Leeflang B. R., Boelens R., Kaptein R., Kamerling J. P. et al. (1998) Mobilities of the inner core residues and the Man (α1,6) branch of the glycan at Asn78 of the α subunit of human chorionic gonadotropin are restricted by the protein. Biochemistry **37:** 1933–1940

557 Homans S. W. (1994) Conformational studies on oligosaccharides. In: Molecular Glycobiology, pp. 230–257, Fukuda M. and Hindsgaul O. (eds), IRL Press, Oxford

558 Naidoo K. J., Denysyk D. and Brady J. W. (1997) Molecular dynamics simulations of the N-linked oligosaccharide of the lectin from *Erythrina corralodendron*. Protein Eng. **10:** 1249–1261

559 Mukhopadhyay C. (1998) Molecular dynamics simulation of glycoprotein glycans of immunoglobulin G and immunoglobulin M. Biopolymers **45:** 177–190

560 Solís D., Estremera D., Usobiaga P. and Diaz-Mauriño T. (1987) Differential binding of mannose-specific lectins to the carbohydrate chains of fibrinogen domains D and E. Eur. J. Biochem. **165:** 131–138

561 White K. D., Cummings R. D. and Waxman F. J. (1997) Ig N-glycan orientation can influence interactions with the complement system. J. Immunol. **158:** 426–435

562 Noorman F., Barrett-Bergshoeff M. M. and Rijken D. C. (1998) Role of carbohydrate and protein in the binding of tissue-type plasminogen activator to the human mannose receptor. Eur. J. Biochem. **251:** 107–113

563 Spillmann D. and Burger M. M. (1996) Carbohydrate-carbohydrate interactions in adhesion. J. Cell. Biochem. **61:** 562–568

564 Bovin N. V. (1997) Carbohydrate-carbohydrate interaction. In: Glycosciences: Status and Perspectives, pp. 277–289, Gabius H.-J. and Gabius S. (eds), Chapman and Hall, London

565 Quiocho F. A. (1986) Carbohydrate-binding proteins: tertiary structures and protein-sugar interactions. Annu. Rev. Biochem. **55:** 287–315

566 Imberty A., Bourne Y., Cambillau C., Rougé P. and Pérez S. (1993) Oligosaccharide conformation in protein-carbohydrate complexes. Adv. Biophys. Chem. **3:** 71–117

567 Cambillau C. (1995) 3D structure 1. The structural features of protein-carbohydrate interactions revealed by X-ray crystallography. In: Glycoproteins, pp. 29–65, Montreuil J., Vliegenthart J. F. G. and Schachter H. (eds), Elsevier, Amsterdam

568 Rini J. M. (1995) Lectin structure. Annu. Rev. Biophys. Biomol. Struct. **24:** 551–577

569 Weis W. I. and Drickamer K. (1996) Structural basis of lectin-carbohydrate recognition. Annu. Rev. Biochem. **65:** 441–473

570 Bundle D. R. (1997) Antibody-oligosaccharide interactions determined by crystallography. In: Glycosciences: Status and Perspectives, pp. 311–331, Gabius H.-J. and Gabius S. (eds), Chapman and Hall, London

571 Lis H. and Sharon N. (1998) Lectins: carbohydrate-specific proteins that mediate cellular recognition. Chem. Rev. **98:** 637–674

572 Loris R., Hamelryck T., Bouckaert J. and Wyns L. (1998) Legume lectin structure. Biochim. Biophys. Acta **1383:** 9–36

573 Hirabayashi J. (1996) On the origin of elementary hexose. Quart. Rev. Biol. **71:** 365–380

574 Asensio J. L., Cañada F. J., Bruix M., Rodriguez-Romero A. and Jiménez-Barbero J. (1995) The interaction of hevein with N-acetylglucosamine-containing oligosaccharides. Solution structure of hevein complexed with chitobiose. Eur. J. Biochem. **230:** 621–633

575 Siebert H.-C., von der Lieth C.-W., Kaptein R., Beintema J. J., Dijkstra K., van Nuland N. et al. (1997) Role of aromatic amino acids in carbohydrate binding of plant lectins. Laser photo CIDNP (chemically induced dynamic nuclear polarization) study of hevein domain-containing lectins. Proteins **28:** 268–284

576 Asensio J. L., Cañada F. J., Bruix M., Gonzalez C., Khiar N., Rodriguez-Romero A. et al. (1998) NMR investigations of protein-carbohydrate interactions: refined three-dimensional structure of the complex between hevein and methyl β-chitobioside. Glycobiology **8:** 569–577

577 Lee R. T., Gabius H.-J. and Lee Y. C. (1998) Thermodynamic parameters of the interaction of *Urtica dioica* agglutinin with N-acetylglucosamine and its oligomers. Glycoconjugate J. **15:** 649–655

578 van Halbeek H. (1994) NMR developments in structural studies of carbohydrates and their complexes. Curr. Opin. Struct. Biol. **4:** 697–709
579 Peters T. and Pinto B. M. (1996) Structure and dynamics of oligosaccharides: NMR and modeling studies. Curr. Opin. Struct. Biol. **6:** 710–720
580 Poveda A. and Jiménez-Barbero J. (1998) NMR studies of carbohydrate-protein interaction in solution. Chem. Soc. Rev. **27:** 133–143
581 Fischer E. (1894) Einfluß der Configuration auf die Wirkung der Enzyme. Ber. Dt. Chem. Ges. **27:** 2985–2993
582 Poppe L., Brown G. S., Philo J. S., Nikrad P. V. and Shah B. H. (1997) Conformation of sLex tetrasaccharide, free in solution and bound to E-, P- and L-selectin. J. Am. Chem. Soc. **119:** 1727–1736
583 Solís D. and Diaz-Mauriño T. (1997) Analysis of protein-carbohydrate interaction by engineered ligands. In: Glycosciences: Status and Perspectives, pp. 345–354, Gabius H.-J. and Gabius S. (eds), Chapman and Hall, London
584 Gupta D., Dam T. K., Oscarson S. and Brewer C. F. (1997) Thermodynamics of lectin-carbohydrate interactions. Binding of the core trimannoside of asparagine-linked carbohydrates and deoxy analogs to concanavalin A. J. Biol. Chem. **272:** 6388–6392
585 Chervenak M. C. and Toone E. J. (1995) Calorimetric analysis of the binding of lectins with overlapping carbohydrate-binding ligand specificities. Biochemistry **34:** 5685–5695
586 Dam T. K., Cavada B. S., Grangeiro T. B., Sontos C. F., de Sousa F. A. M., Oscarson S. et al. (1998) Diocleinae lectins are a group of proteins with conserved binding sites for the core trimannoside of asparagine-linked oligosaccharides and differential specificities for complex carbohydrates. J. Biol. Chem. **273:** 12082–12088
587 Toone E. J. (1994) Structure and energetics of protein–carbohydrate complexes. Curr. Opin. Struct. Biol. **4:** 719–728
588 Dunitz J. D. (1995) Win some, lose some: enthalpy-entropy compensation in weak intermolecular interactions. Chem. Biol. **2:** 709–712
589 Lemieux R. U. (1996) How water provides the impetus for molecular recognition in aqueous solution. Acc. Chem. Res. **29:** 373–380
590 Bharadwaj S., Kaltner H., Siebert H.-C., Korchagina E. Y., Bovin N. V., Vliegenthart J. F. G. et al., in preparation
591 Gabius H.-J. and Gabius S. (1990) Tumorlektinologie: Status und Perspektiven klinischer Anwendung. Naturwissenschaften **77:** 505–514
592 Welply J. K., Keene J. L., Schmuke J. J. and Howard S. C. (1994) Selectins as potential targets of therapeutic intervention in inflammatory diseases. Biochim. Biophys. Acta **1197:** 215–226
593 Bertozzi C. R. (1995) Cracking the carbohydrate code for selectin recognition. Chem. Biol. **2:** 703–708
594 Gabius H.-J., Kayser K. and Gabius S. (1995) Protein-Zucker-Erkennung. Grundlagen und medizinische Anwendung am Beispiel der Tumorlektinologie. Naturwissenschaften **82:** 533–543
595 Simon P. M. (1996) Pharmaceutical oligosaccharides. Drug Discovery Today **1:** 522–528
596 Cornejo C. J., Winn R. K. and Harlan J. M. (1997) Anti-adhesion therapy. Adv. Pharmacol. **39:** 99–142
597 Lowe J. B. and Ward P. A. (1997) Therapeutic inhibition of carbohydrate-protein interactions in vivo. J. Clin. Invest. **99:** 822–826
598 Stowell C. P. and Lee Y. C. (1980) Neoglycoproteins: the preparation and application of synthetic glycoproteins. Adv. Carbohydr. Chem. Biochem. **37:** 225–281
599 Aplin J. D. and Wriston J. C. (1981) Preparation, properties and applications of carbohydrate conjugates of proteins and lipids. CRC Crit. Rev. Biochem. **10:** 259–306
600 Gabius H.-J. (1988) Tumor lectinology: at the intersection of carbohydrate chemistry, biochemistry, cell biology and oncology. Angew. Chem. Int. Ed. Engl. **27:** 1267–1276
601 Gabius H.-J., Gabius S., Zemlyanukhina T. V., Bovin N. V., Brinck U., Danguy A. et al. (1993) Reverse lectin histochemistry: design and application of glycoligands for detection of cell and tissue lectins. Histol. Histopathol. **8:** 369–383
602 Bovin N. V. and Gabius H.-J. (1995) Polymer-immobilized carbohydrate ligands: versatile chemical tools for biochemistry and medical sciences. Chem. Soc. Rev. **24:** 413–421
603 Roy R. (1996) Synthesis and some applications of chemically defined multivalent glycoconjugates. Curr. Opin. Struct. Biol. **6:** 692–702
604 Lee R. T. and Lee Y. C. (1997) Neoglycoconjugates. In: Glycosciences: Status and Perspectives, pp. 55–77, Gabius H.-J. and Gabius S. (eds), Chapman and Hall, London
605 Gabius H.-J. (1987) Endogenous lectins in tumors and the immune system. Cancer Invest. **5:** 39–46
606 Gabius H.-J., André S., Danguy A., Kayser K. and Gabius S. (1994) Detection and quantification of carbohydrate-binding sites on cell surfaces and in tissue sections by neoglycoproteins. Methods Enzymol. **242:** 37–46
607 Gabius S., Wawotzny R., Martin U., Wilholm S. and Gabius H.-J. (1994) Carbohydrate-dependent binding of human myeloid leukemia cell lines to neoglycoenzymes, matrix-immobilized neoglycoproteins and bone marrow stromal cell layers. Ann. Hematol. **68:** 125–132
608 Gabius H.-J., Engelhardt R., Hellmann K.-P., Hellmann T. and Ochsenfahrt A. (1987) Preparation of neoglycoprotein-enzyme conjugate using a heterobifunctional reagent and its use in solid-phase assay and histochemistry. Anal. Biochem. **165:** 349–355
609 Gabius S., Hellmann K.-P., Hellmann T., Brinck U. and Gabius H.-J. (1989) Neoglycoenzymes: a versatile tool for lectin detection in solid-phase assays and glycohistochemistry. Anal. Biochem. **182:** 447–451
610 Gabius S., Schirrmacher V., Franz H., Joshi S. S. and Gabius H.-J. (1990) Analysis of cell surface sugar receptor expression by neoglycoenzyme binding and adhesion to plastic-immobilized neoglycoproteins for related weakly and strongly metastatic cell lines of murine tumor model systems. Int. J. Cancer **46:** 500–507
611 Gabius H.-J., Kohnke B., Hellmann T., Dimitri T. and Bardosi A. (1988) Comparative histochemical and biochemical analysis of endogenous receptors for glycoproteins in human and pig nerve. J. Neurochem. **51:** 756–763
612 Bardosi A., Dimitri T., Behrends T., Autschbach D. and Gabius H.-J. (1989) Is part of the molecular basis of the perineurial barrier function the lack of endogenous carbohydrate-binding proteins? J. Neurosci. Res. **22:** 65–73
613 Danguy A., Kayser K., Bovin N. V. and Gabius H.-J. (1995) The relevance of neoglycoconjugates for histology and pathology. Trends Glycosci. Glycotechnol. **7:** 261–275
614 Danguy A., Decaestecker C., Genten F., Salmon I. and Kiss R. (1998) Application of lectins and neoglycoconjugates in histology and pathology. Acta Anat. **161:** 206–218
615 Gabius H.-J., Brehler R., Schauer A. and Cramer F. (1986) Localization of endogenous lectins in normal human breast, benign breast lesions and mammary carcinomas. Virch. Arch. [Cell. Pathol.] **52:** 107–115
616 Gabius H.-J. and Bardosi A. (1991) Neoglycoproteins as tools in glycohistochemistry. Progr. Histochem. Cytochem. **22/3:** 1–66
617 Gabius H.-J., Wosgien B., Brinck U. and Schauer A. (1991) Localization of endogenous β-galactoside-specific lectins by neoglycoproteins, lectin-binding tissue glycoproteins and antibodies and of accessible lectin-specific ligands by a mammalian lectin in human breast cancer. Path. Res. Pract. **187:** 839–847
618 Kayser K., Gabius H.-J. and Gabius S. (1991) Biotinylated ligands as markers for receptor-binding sites. An alternative for immunohistochemistry. Zentralbl. Pathol. **137:** 473–478
619 Gabius H.-J. and Gabius S. (1992) Chemical and biochemical strategies for the preparation of glycohistochemical probes and their application in lectinology. Adv. Lectin Res. **5:** 123–157

620 Itzkowitz S. H. (1997) Galectins: multipurpose carbohydrate-binding proteins implicated in tumor biology. Gastroenterology **113:** 2003–2005
621 Kayser K. and Gabius H.-J. (1997) Graph theory and the entropy concept in histochemistry. Theoretical considerations, application in histopathology and the combination with receptor-specific approaches. Progr. Histochem. Cytochem. **32/2:** 1–106
622 Schwarz G., Remmelink M., Decaestecker C., Gielen I., Budel V., Burchert M. et al. (1999) Galectin fingerprinting in tumor diagnosis. Differential expression of galectin-3 and galectin-3-binding sites, but not of galectin-1, in benign versus malignant uterine smooth muscle tumors. Am. J. Clin. Pathol., in press
623 Bardosi A., Dimitri T. and Gabius H.-J. (1988) (Neo)glycoproteins as tools in neuropathology: histochemical patterns of the extent of expression of endogenous carbohydrate-binding receptors, like lectins, in meningiomas. Virchows Arch. [Cell. Pathol.] **56:** 35–43
624 Gabius H.-J., Bodanowitz S. and Schauer A. (1988) Endogenous sugar-binding proteins in human breast tissue and benign and malignant breast lesions. Cancer **61:** 1125–1131
625 Bardosi A., Dimitri T., Wosgien B. and Gabius H.-J. (1989) Expression of endogenous receptors for neoglycoproteins, especially lectins, which allow fiber typing on formaldehyde-fixed, paraffin-embedded muscle biopsy specimens. A glycohistochemical, immunohistochemical and glycobiochemical study. J. Histochem. Cytochem. **37:** 989–998
626 Kayser K., Heil M. and Gabius H.-J. (1989) Is the profile of binding of a panel of neoglycoproteins useful as diagnostic marker in human lung cancer? Path. Res. Pract. **184:** 621–629
627 Kayser K., Gabius H.-J., Rahn W., Martin H. and Hagemeyer O. (1992) Variations of binding of labelled tumor necrosis factor-α, epidermal growth factor, ganglioside GM1, and N-acetylglucosamine, galactoside-specific mistletoe lectin and lectin-specific antibodies in mesothelioma and metastatic adenocarcinoma of the pleura. Lung Cancer **8:** 185–192
628 Kayser K., Bubenzer J., Kayser G., Eichhorn S., Zemlyanukhina T. V., Bovin N. V. et al. (1995) Expression of lectin-, interleukin-2- and histo-blood group-binding sites in prostate cancer and its correlation to integrated optical density and syntactic structure analysis. Analyt. Quant. Cytol. Histol. **17:** 135–142
629 Brinck U., Bosbach R., Korabiowska M., Schauer A. and Gabius H.-J. (1996) Histochemical study of expression of lectin-reactive carbohydrate epitopes and glycoligand-binding sites in normal human appendix vermiformis, colonic mucosa, acute appendicitis and colonic adenoma. Histol. Histopathol. **11:** 919–930
630 Brinck U., Korabiowska M., Bosbach R. and Gabius H.-J. (1998) Detection of inflammation- and neoplasia-associated alterations in human large intestine by plant/invertebrate lectins, galectin-1 and neoglycoproteins. Acta Anat. **161:** 219–233
631 Kayser K., Biechele U., Kayser G., Dienemann H., André S., Bovin N. V. et al. (1998) Pulmonary metastases of breast carcinoma: ligandohistochemical, nuclear and structural analysis of primary and metastatic tumors with emphasis on period of occurrence of metastases and survival. J. Surg. Oncol. **69:** 137–146
632 Kayser K., Bovin N. V., Korchagina E. Y., Zeilinger C., Zeng F.-Y. and Gabius H.-J. (1994) Correlation of expression of binding sites for synthetic blood group A-, B- and H-trisaccharides and for sarcolectin with survival of patients with bronchial carcinoma. Eur. J. Cancer **30A:** 653–657
633 Kayser K., Kayser C., Rahn W., Bovin N. V. and Gabius H.-J. (1996) Carcinoid tumors of the lung: immuno- and ligandohistochemistry, analysis of integrated optical density, syntactic structure analysis, clinical data and prognosis of patients treated surgically. J. Surg. Oncol. **63:** 99–106
634 Coon J. S. and Weinstein R. S. (1986) Blood group-related antigens as markers of malignant potential and heterogeneity in human carcinomas. Hum. Pathol. **17:** 1089–1106
635 Dabelsteen E. (1996) Cell surface carbohydrates as prognostic markers in human carcinomas. J. Pathol. **179:** 358–369
636 Greenwell P. (1997) Blood group antigens: molecules seeking a function? Glycoconjugate J. **14:** 159–173
637 Kim Y. J. and Varki A. (1997) Perspectives on the significance of altered glycosylation of glycoproteins in cancer. Glycoconjugate J. **14:** 569–576
638 Kocourek J. (1986) Historical background. In: The Lectins: Properties, Functions and Applications in Biology and Medicine, pp. 1–32, Liener I. E., Sharon N. and Goldstein I. J. (eds), Academic Press, New York
639 Sharon N. and Lis H. (1987) A century of lectin research (1888–1988). Trends Biochem. Sci. **12:** 488–491
640 Gabius H.-J. (1994) Non-carbohydrate binding partners/domains of animal lectins. Int. J. Biochem. **26:** 469–477
641 Drickamer K. (1995) Increasing diversity of animal lectin structures. Curr. Opin. Struct. Biol. **5:** 612–616
642 Drickamer K. (1997) Making a fitting choice: common aspects of sugar-binding sites in plant and animal lectins. Structure **5:** 465–468
643 Elgavish S. and Shaanan B. (1997) Lectin-carbohydrate interactions: different folds, common recognition principles. Trends Biochem. Sci. **22:** 462–467
644 May A. P., Robinson R. C., Vinson M., Crocker P. R. and Jones E. Y. (1998) Crystal structure of the N-terminal domain of sialoadhesin in complex with 3′ sialyllactose at 1.85 Å resolution. Mol. Cell **1:** 719–728
645 Roberts D. L., Weix D. J., Dahms N. M. and Kim J.-J. P. (1998) Molecular basis of lysosomal enzyme recognition: three-dimensional structure of the cation-dependent mannose-6-phosphate receptor. Cell **93:** 639–648
646 Doyle R. J. (1994) Introduction to lectins and their interactions with microorganisms. In: Lectin-Microorganism Interactions, pp. 1–65, Doyle R. J. and Slifkin M. (eds), Marcel Dekker, New York
647 Powell L. D. and Varki A. (1995) I-type lectins. J. Biol. Chem. **270:** 14243–14246
648 Crocker P. R. and Feizi T. (1996) Carbohydrate recognition systems: functional triads in cell-cell interactions. Curr. Opin. Struct. Biol. **6:** 679–691
649 Kishore U., Eggleton P. and Reid K. B. M. (1997) Modular organization of carbohydrate recognition domains in animal lectins. Matrix Biol. **15:** 583–592
650 Zanetta J.-P. (1997) Lectins and carbohydrates in animal cell adhesion and control of cell proliferation. In: Glycosciences: Status and Perspectives, pp. 439–458, Gabius H.-J. and Gabius S. (eds), Chapman and Hall, London
651 Lasky L. A. (1995) Selectin-carbohydrate interactions and the initiation of the inflammatory response. Annu. Rev. Biochem. **64:** 119–139
652 Ebnet K., Kaldjian E. P., Anderson A. O. and Shaw S. (1996) Orchestrated information transfer underlying leukocyte endothelial interactions. Annu. Rev. Immunol. **14:** 155–177
653 Kansas G. S. (1996) Selectins and their ligands: current concepts and controversies. Blood **88:** 3259–3287
654 Hirabayashi J. (ed.) (1997) Recent topics on galectins. Trends Glycosci. Glycotechnol. **9:** 1–180
655 Bourne Y., Bolgiano B., Liao D., Strecker G., Contau P., Herzberg O. et al. (1994) Crosslinking of mammalian lectin, galectin-1, by complex biantennary saccharides. Nature Struct. Biol. **1:** 863–870
656 Gupta D. and Brewer C. F. (1994) Homogeneous aggregation of the 14kDa β-galactoside-specific vertebrate lectin complex with asialofetuin in mixed system. Biochemistry **33:** 5526–5530
657 Brewer C. F. (1996) Multivalent lectin-carbohydrate cross-linking interactions. Chemtracts (Biochem. Mol. Biol.) **6:** 165–179

658 Gupta D., Kaltner H., Dong X., Gabius H.-J. and Brewer C. F. (1996) Comparative cross-linking activities of lactose-specific plant and animal lectins and a natural lactose-binding immunoglobulin G fraction from human serum with asialofetuin. Glycobiology **6:** 843–849
659 Perillo N. L., Marcus M. E. and Baum L. G. (1998) Galectins: versatile modulators of cell adhesion, cell proliferation and cell death. J. Mol. Med. **76:** 402–412
660 Fearon D. T. and Locksley R. M. (1996) The instructive role of innate immunity in the acquired immune response. Science **272:** 50–54
661 Lu J. (1997) Collectins: collectors of microorganisms for the innate immune system. BioEssays **19:** 509–518
662 Reid K. B. M., Colomb M. G. and Loos M. (1998) Complement component C1 and the collectins: parallel between routes of acquired and innate immunity. Immunol. Today **19:** 56–59
663 Stahl P. D. and Ezekowitz R. A. B. (1998) The mannose receptor is a pattern recognition molecule involved in host defense. Curr. Opin. Immunol. **10:** 50–55
664 Vasta G. R., Ahmed H., Fink N. E., Elola M. T., Marsh A. G., Snowden A. et al. (1994) Animal lectins as self/non-self recognition molecules. Ann. NY Acad. Sci. **712:** 55–73
665 Iwanaga S., Kawabata S.-i. and Muta T. (1998) New types of clotting factors and defense molecules found in horseshoe crab hemolymph: their structures and functions. J. Biochem. **123:** 1–15
666 Parekh R. B., Dwek R. A., Edge C. J. and Rademacher T. W. (1989) N-glycosylation and the production of recombinant glycoproteins. Trends Biotechnol. **7:** 117–122
667 Liu D. T.-Y. (1992) Glycoprotein pharmaceuticals: scientific and regulatory considerations, and the US Orphan Drug Act. Trends Biotechnol. **10:** 114–119
668 Montreuil J. (1975) Recent data on the structure of the carbohydrate moiety of glycoproteins. Metabolic and biological implications. Pure Appl. Chem. **42:** 431–477
669 Gabius H.-J. and Gabius S. (eds) (1997) Glycosciences: Status and Perspectives, Chapman and Hall, London
670 Gabius H.-J. and Romero A. (1998) Structure and function of animal lectins. Carbohydrates in Europe **23:** 24–29
671 Gabius H.-J. and Sinowatz F. (eds) (1998) Special volume on glycosciences. Acta Anat. **161**: 1–274