

Glycosylation

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Protein glycosylation is more abundant and structurally diverse than all other types of post-translational modifications combined. Protein-bound saccharides range from dynamic monosaccharides on nuclear and cytoplasmic proteins, to enormously complex 'recognition' molecules on extracellular N- or O-linked glycoproteins or proteoglycans. Recent elucidation of a few of the myriad functions of these saccharides has finally opened a crack in the door to one the last great frontiers of biochemistry.

Current Opinion in Cell Biology 1992, 4:1017–1023

Introduction

Until recently, protein glycosylation was thought to be largely restricted to luminal or extracellular compartments (reviewed in [1]). However, a multitude of glycosylated proteins are now known to reside within both the nucleoplasm and cytoplasm [2•]. Despite remarkable advances in our understanding of the biosynthesis of complex saccharides [3,4•], and in our ability to structurally characterize glycoproteins [5,6], the functions of protein-bound saccharides largely remain enigmatic.

Major functions of many complex forms of 'extracellular' protein glycosylation appear to involve intermolecular or intercellular interactions that are important only at the multicellular or organismic levels. In contrast, recent circumstantial evidence suggests that simple 'intracellular' glycosylation probably plays a regulatory role that is important to single-cell metabolism or signal transduction. The existence of a large number of glycosylation mutants in cultured cells [7•] suggests that most complex forms of protein glycosylation have no obvious functions in the housekeeping activities of a cell growing in a culture dish. In contrast, very few viable mutants in glycosylation pathways exist in whole organisms (for example [8,9]), suggesting that most such mutants die during development. 'Incorrectly' glycosylated recombinant proteins, which appear to have apparently normal receptor binding properties or enzymatic activities *in vitro*, often fail to function properly in the whole organism [10•].

Given that both the structural diversity and possible functions of protein glycosylation are arguably as great as the polypeptide components themselves (Fig. 1), in this short review I will by necessity focus only on a few selected recent developments that have substantially impacted our current concepts about protein glycosylation. Glycoproteins are classified by the type of protein-saccharide linkages that they possess (Table 1). However, many glycoproteins contain more than one type of saccharide side chain, thus making the classification somewhat arbitrary.

Proteoglycans are the most complex and least understood glycoproteins

Proteoglycans are simply defined as any polypeptide with one or more glycosaminoglycan side chains (Table 1). The role of proteoglycans as mediators of growth factors and as developmentally important cell adhesion molecules [11•,12•,13] have greatly renewed general interest in these complex molecules.

Many eukaryotic proteins are anchored to membranes by glycosylphosphatidylinositols

Over a hundred different membrane proteins are now known to be linked to the membrane by a glycosylphosphatidylinositol (GPI)-anchor (reviewed in [14,15•,16,17]). GPIs from all organisms appear to have a common tri- α -mannosyl core attached to a non-acetylated glucosamine in glycosidic linkage to phosphatidylinositol. GPIs are pre-assembled in the rough endoplasmic reticulum, and are rapidly attached to the nascent polypeptide chain with the concomitant cleavage of a hydrophobic carboxyl-terminal peptide. The steps in the GPI biosynthetic pathway and several GPI structures have largely been elucidated. However, little is known about the enzymes or topology of GPI biosynthesis. GPIs appear to play a role in intracellular targeting of proteins to epithelial surfaces; on lymphocyte receptors they may also be involved in lymphocyte signalling or activation [17]; certain GPIs may act as second messengers for insulin action [16], although this proposed function remains highly controversial.

Nuclear and cytoplasmic glycosylation is simple, abundant and highly dynamic

Structurally, the simplest protein-bound saccharides (Fig. 1) are the nuclear and cytoplasmic glycoproteins, which contain one or more N-acetylglucosamine monosaccharide

Abbreviations

GPI—glycosylphosphatidylinositol.

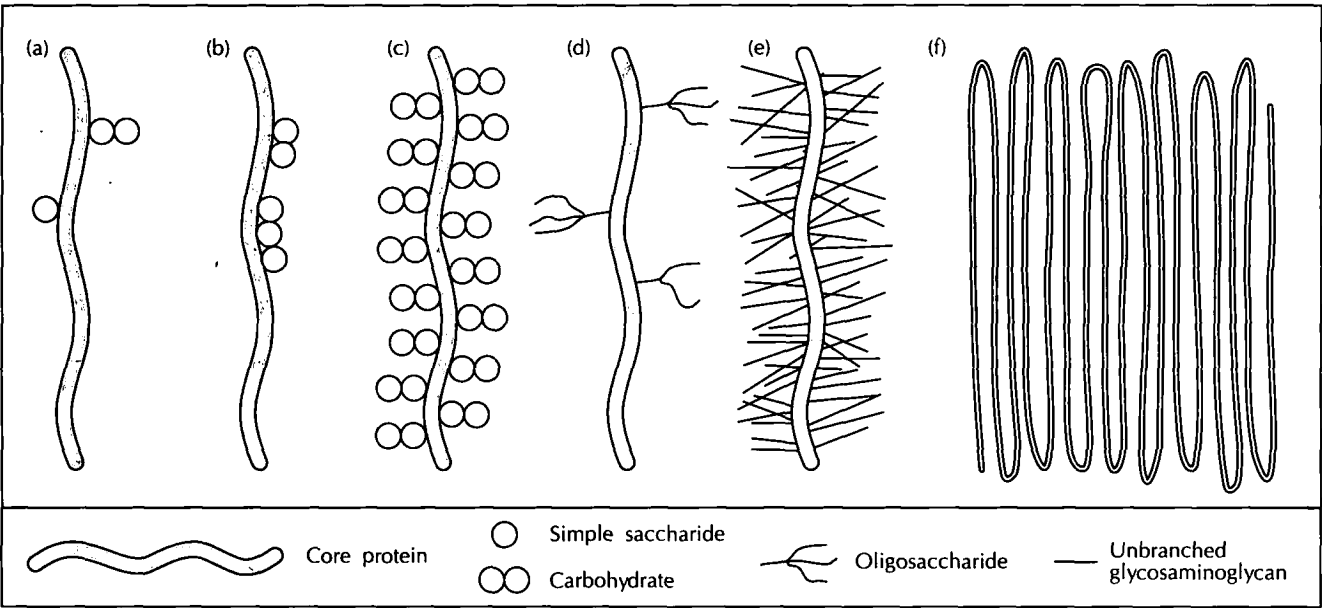


Fig. 1. Spectrum of protein glycosylation: a schematic representation of the range of complexity in protein glycosylation. (a) Collagens and (b) nuclear glycoproteins have only a small number of simple saccharides. (c) Mucins can be more than 50 % carbohydrate with highly charged and clustered structures. (d) Asn-linked glycoproteins generally contain only a few oligosaccharides that are often highly branched and complex. (e) Proteoglycans are among the most complicated molecules in biology and can have from one to over a hundred large glycosaminoglycan side chains. (f) Hyaluronic acid is a relatively simple molecule, made up of many regular, repeating, non-sulfated disaccharide units.

Table 1. Classification of glycoproteins by linkage.		
Glycoprotein	Linkage group	Comments
Collagen	Gal-hydroxylysine	Only in collagens; may also have Glu to form a disaccharide.
O-Linked GlcNAc-	GlcNAc-Ser(Thr)	Not elongated; mostly nucleoplasmic and cytoplasmic; dynamic.
Mucin Type O-Linked	GalNAc-Ser(Thr)	Found in membrane and secretory proteins; mucins most studied can form very complex structures.
Asn-Linked	GlcNAc-Asn	Asn-X-Ser(Thr) attachment site; common GlcNAc ₂ Man ₃ core.
Proteoglycan	Xyl-Ser	Linkage core Gal-Gal-Xyl; one or more glycosaminoglycan side chains.

moieties that are O-glycosidically linked to specific serine or threonine residues (O-GlcNAc) [1,2•]. Physiologically, O-GlcNAc is highly dynamic, abundant in apparently all eukaryotes and appears to have more in common with phosphorylation than it has with the other classical types of protein glycosylation. O-GlcNAc is attached to a multitude of different proteins, which are generally also phosphorylated and multimeric [2•]. The small proportion of O-GlcNAc-bearing proteins identified so far include (older references in [2•]): nuclear pore proteins, many different chromatin proteins, RNA polymerase II and its transcription factors [18•], nuclear oncogene proteins, a nuclear tyrosine phosphatase, viral tegument and fibre proteins [19,20], heat-shock protein related lens α -crystallins [21•], as well as important cytoskeletal proteins such as cytokeratins [22•], talin [23•], erythrocyte band 4.1, and

bovine brain synapsins. O-GlcNAc levels on nuclear and cytoplasmic proteins change rapidly upon lymphocyte activation [24•]. Likewise, pulse-chase studies of O-GlcNAc on cytokeratins indicate that the saccharide is highly dynamic [22•]. O-GlcNAc attachment sites are similar in amino acid sequence to growth-factor kinase phosphorylation sites [2•]. A rat liver cytosolic, O-GlcNAc-polypeptide N-acetylglucosaminyltransferase enzyme has recently been purified to near homogeneity and characterized [25•]. The current view of O-GlcNAc is as follows: it may be as widely distributed on intracellular proteins as phosphate; it appears to behave in a dynamic fashion analogous to phosphorylation/dephosphorylation; it may act as an off switch by sterically blocking certain phosphorylation sites; and it may be involved in mediating reversible intermolecular interactions [2•].

Other less well characterized forms of cytoplasmic or nuclear glycosylation have also been described [1]. More recent ones include: the O-fucosylation of cytosolic proteins in *Dictyostelium* [26]; the O-glucosylation of tyrosine hydroxyl moieties on glycogenin [27•], the primer protein for glycogen synthesis; and O-linked mannose on certain cytosolic proteins that serve as acceptors for a novel cytoplasmic glucose phosphotransferase [28]. Reports of the presence of N-linked oligosaccharides in both the nucleus [29] and cytoplasm [30] continue to appear regularly. However, direct structural proofs for these claims still do not exist.

Extracellular protein glycosylation displays site-specific diversity of complex structures

Although most of the well studied forms of luminal or cell surface glycosylation (Table 1) display a mind-boggling range of complex structures, certain proteins, particularly those of the coagulation/fibrinolytic system, also contain simple, unusual O-glycan moieties on their epidermal growth factor domains, such as O-linked fucose, O-linked Xyl-Glc-(Ser) and Xyl-Xyl-Glc-(Ser) [31–34].

While 'mucin-type' O-linked glycans (Table 1) are probably as abundant on both secretory and membrane glycoproteins as N-linked oligosaccharides, we know much less about them. The enormous complexity of secreted mucins, the prototype molecules of this class, which contain very high carbohydrate content (often > 50% of the dry weight) and can have a molecular weight as large as 6×10^6 , largely prevented their biochemical characterization until the advent of molecular biology (reviewed in [4•]). These amazingly complex molecules typically contain highly clustered GalNAc-Ser(Thr)-linked, highly charged oligosaccharides terminating in sialic acids and/or sulfate moieties. Not only do these important molecules protect epithelial surfaces from mechanical injury or dehydration, but also their almost infinite diversity of carbohydrate structures serve as a front-line defense by competing for adhesion receptors on pathogens, many of which bind to cells via similar cell-surface carbohydrates [35]. Very recently, the enzyme that attaches GalNAc to threonine residues in porcine submaxillary mucins has been purified to homogeneity and characterized [36•]. The surprising finding of this report is that the enzyme appears to have absolute specificity for threonine residues. A similar enzyme specific for serine moieties is undetectable in this tissue, even though earlier analyses indicated that serine is glycosylated in this mucin.

Many membrane-bound, cell surface proteins also contain clustered domains of mucin-type glycosylation [37•]. These mucin domains serve to protect the protein from proteolysis, produce a 'rigid rod' structure to help maintain the active site of the receptor or enzyme above the 'glycocalyx' of the cell, or serve as recognition molecules for cell adhesion. While we typically think of 'mucin-type' O-glycans as short, highly charged oligosaccharides (tetrasaccharides or less) occurring in clusters, many GalNAc-Ser(Thr)-bound saccharides display enormous size and complexity. In many cases, the abnormal expression of a 'mucin-type' glycoprotein, as in the case of epiglycanin

in the mouse TA3 carcinoma, can completely mask other surface recognition molecules, such as histocompatibility antigens, substantially contributing to the malignant properties of a tumor cell [38•].

Intermediate in the spectrum of carbohydrate content (Fig. 1 and Table 1), the N-linked glycoproteins contain complex oligosaccharides attached to the amide group of asparagine via a glycosylamine linkage. The N-linked oligosaccharides are found on nearly all secreted and surface proteins. These oligosaccharides share a common $\text{Man}_3\text{-GlcNAc}_2$ core, but may exhibit astonishing diversity in branching and in outer sugar components [39•]. The only known eukaryotic organism that does not appear to synthesize N-linked oligosaccharides is the asexual intra-erythrocytic stage of the malarial parasite, *Plasmodium falciparum* [40•]. However, this form of the parasite does synthesize a substantial amount of O-glycans, probably including O-GlcNAc [41].

The topography of N-linked oligosaccharide assembly has only been recently understood [42]. Current models indicate that assembly through $\text{Man}_5\text{-GlcNAc}_2\text{-P-P-dolichol}$ occurs on the cytosolic side of the rough endoplasmic reticulum, whereas steps subsequent to this intermediate occur in the lumen. Oligosaccharyltransferase is the key enzyme that donates the pre-assembled dolichol-oligosaccharides to form glycosylamine linkages to accessible asparagine residues in the sequence -Asn-X-Ser(Thr)- (where X is any amino acid except proline) in nascent polypeptides in the lumen of the rough endoplasmic reticulum. Despite intense efforts for many years, this complex, highly insoluble enzyme has eluded characterization (references in [43]). Recently the enzyme has been associated with a protein complex comprising three polypeptides, a 48 kD subunit and the two ribophorins, I and II [44•]. Ribophorin I appears to contain a consensus dolichol-binding sequence, and may serve as the dolichol-binding subunit of the functional enzyme complex. The essential yeast protein, WBP1, has been identified to either be the oligosaccharyltransferase itself, or at least an essential component of the enzyme complex [45•].

After attachment to protein, the N-linked oligosaccharide undergoes a complex series of trimming and elongation reactions leading to the final complex structures present on the mature molecule [3]. This complicated pathway probably arose as a means for generating 'metabolically responsive' structural diversity. Recently, good progress has been made toward understanding the key N-acetylglucosaminyl transferases responsible for the extensive branching of N-linked oligosaccharides [39•]. In contrast, we know almost nothing about the mechanisms controlling site-specific glycosylation. However, it is apparent that the array of structures found at each glycosylation site of a glycoprotein is not only determined by the cell's glycosylation machinery, but also by all levels of protein structure [46] including quaternary structure [47]. For example, the pituitary glycoprotein hormones share a common α -subunit polypeptide but have distinct β -subunits. The pattern of glycosylation is determined by the β -subunits, which specify typical sialylated structures on follitropin, but result in an unusual $\text{SO}_4\text{-GalNAc}$ residue on N-linked oligosaccharides on lutropin ([48]; reviewed in [49•]).

Glycoforms represent unique molecular species with a range of biological activities

The variety of functions of the glycosyl moieties of glycoproteins rival the diversity of functions of the polypeptide backbones themselves. In fact, glycoproteins should not be regarded as a homogeneous population of polypeptides with attached sugars, but rather each glycoform should be seen as a unique population of molecular species in its own right [50,51•]. Many studies have shown that the hydrodynamic volume occupied by saccharide side chains is typically enormous, sometimes even dwarfing or mostly covering the polypeptide component [52]. Protein glycosylation of all types has a pronounced influence on protein conformation or folding. Many seemingly unrelated roles for N-linked oligosaccharides have been deduced from the use of glycosidase inhibitors, lectin-resistant mutants, and/or site-directed mutagenesis [7,53,54]. Human transferrin receptor mutated to prevent glycosylation at Asn251, which normally contains the N-linked oligosaccharide closest to the membrane, is abnormally retained and is rapidly degraded in the rough endoplasmic reticulum by site-specific proteolysis. The mutated receptor also fails to dimerize and form its normal interpeptide disulfide bonds [55]. Human CD4, the T-cell surface N-linked glycoprotein that is required for helper T cell activation and is also the receptor for human immunodeficiency virus, requires at least one of its two N-linked saccharides to fold normally and be expressed at the cell surface [56]. Mutagenesis experiments suggest that N-linked glycosylation of Asn residues 100, 105 and 128 of the human human immunodeficiency virus-1 envelope protein play an important role in its specific fusion activity [57]. Using antibodies specific only for native enzymes, together with kinetic analyses, high-mannose N-linked glycosylation was shown to be an early essential event in the proper folding of two membrane-bound, intestinal brush border enzymes [58]. The very complex and numerous N-linked saccharides of the lysosomal membrane glycoproteins, which contain considerable amounts of large poly-N-acetylactosamines ($\text{Gal}\beta 1-4\text{GlcNAc}\beta 1-3$)_n, appear to play an important role in preventing autolysis of the lysosome [59•].

Glycosylation has attracted the interest of the biomedical/pharmaceutical community

The site-specific glycosylation of recombinant human tissue plasminogen activator (tPA) and human recombinant erythropoietin made in several different cell types has been studied in detail (references in [60,61]), mainly because of its potential importance to the pharmaceutical industry. Strikingly, while several common oligosaccharide structures are present on tPA from the different cell types (as might be expected), all glycoforms of the molecule were found to be cell-type specific ('glycotypes'). In tPA the presence and type of N-linked oligosaccharides at one of its three possible glycosylation sites (Asn184) modulates its binding affinity for lysine. Clearance of tPA from the circulation is modulated by its glycosylation state at Asn117. Recent studies have shown that both the affinity for lysine and fibrin-stimulated enzymatic activities of tPA are mod-

ulated by the complex sialylation and branching of the N-linked oligosaccharides at Asn184 and Asn448 [62•]. Erythropoietin is a medically important hormone that plays a major role in regulating the proliferation and differentiation of erythroid progenitor cells to erythrocytes [61]. One current view is that N-linked sialylated oligosaccharides are essential for *in vivo* activity of the hormone, but neither O-glycans nor N-glycans play a role in its *in vitro* activity [63•].

Immunoglobulins, like most glycoproteins, also display a very large number of glycoforms due to extensive variability in site-specific oligosaccharide structures. N-linked oligosaccharides on immunoglobulin G appear to play a role in the interaction of the molecules with Fc receptors, their activation of complement (C1q binding), the induction of antibody-dependent cellular cytotoxicity and feedback immunosuppression [51•,64]. Recent evidence suggests that the specific under-galactosylation of N-linked oligosaccharides on immunoglobulin G may be directly involved in the etiology of rheumatoid arthritis [51•,64,65•].

Oligosaccharides on laminin, a heavily glycosylated basement membrane protein that is directly involved in cell adhesion, migration, differentiation and neurite outgrowth, appear to be essential for cell spreading but not adhesion [66]. Reconstitution experiments with various glycoforms of laminin suggest that carbohydrate plays a major role in cell-signalling processes associated with spreading and neurite extension.

During recent years, no findings have spurred more interest in glycosylation than the discovery that a family of integral membrane lectins, termed selectins, are transiently expressed on the surfaces of activated endothelial cells and play a key role in the specific homing of leukocytes to sites of inflammation [67•,68•]. Selectins each have an amino-terminal, C-type (Ca^{+2} -dependent) [49•] extracellular domain followed by an epidermal growth factor-like module, multiple copies of the consensus sequence of complement-binding proteins, a transmembrane domain and a short cytoplasmic tail. The major carbohydrate ligands for selectins involved in neutrophil homing appear to be sialyl Lewis X oligosaccharides, i.e. $\text{NeuAc}\alpha (2-3)\text{Gal}\beta (1-4)[\text{Fuc}\alpha (1-3)]\text{GlcNAc}\beta$, or related structures, most probably as part of O-linked chains. The selectins profoundly illustrate that the functions of carbohydrates only reveal themselves when we ask the right questions. For example, in the usual static adhesion assays, the selectin-Sialyl Lewis X interaction did not appear to be strong enough to mediate cell adhesion. Indeed, many studies have shown that tight cell adhesion of neutrophils to activated endothelium is mediated via the integrins and intercellular adhesion molecules [67•]. However, in the rapid flow of the bloodstream, the leukocytes simply move too fast for a stable integrin-intercellular adhesion molecule adhesion to take place. The apparent role of the selectins is to act like a sticky patch of 'velcro' and slow the cells down to a 'slow roll' so that subsequent integrin-mediated adhesion and extravasation can take place. Based upon the initial excitement from these studies many pharmaceutical companies are rapidly developing programs in order to produce carbohydrate-based therapy [69•,70].

Sialic acids display enormous diversity of structure and functions

One important feature of protein-bound saccharides that is often ignored is the inherent complexity of the sialic acids that often terminate oligosaccharides. While N-acetylneuraminic and N-glycolylneuraminic acids are the most common sialic acids, over 30 different types of sialic acids exist in nature, with the most common having additional O-acetyl esters to the hydroxyl groups of 4-, 7-, 8- and 9, or other substituents, such as sulfate [71•, 72•]. With the recent development of methods to assay different sialic acids, we are only just beginning to appreciate both the importance and distribution of these different types of sialic acids. In addition, sialic acids can occur as large homopolymers (degree of polymerization > 200; α 2-8-, α 2-9- or α 2-8/ α 2-9-ketosidic linked) attached to an N-linked or O-linked oligosaccharide core [73•]. Polysialic acids are important oncodevelopmental antigens in human kidney and brain, and appear to enhance the metastatic potential of certain tumors. They also play an important role in the modulation of cell adhesion during brain development.

Trypanosoma cruzi, an obligatory intracellular parasite that causes Chagas' disease, rapidly displays a completely novel trans-sialidase enzyme on its cell surface as it emerges from host macrophages [74••]. This trans-sialidase transfers host sialic acids directly from circulating serum proteins or cells (without high energy intermediates) to the parasite surface where the saccharides play an essential role in binding to new target cells. The trans-sialidase and the well studied neuraminidase of *T. cruzi* have recently been shown to be the same enzyme [75]. Should similar trans-glycosidases prove to be widespread, this discovery could have a major impact on our current views of cell adhesion and glycoconjugate biosynthesis.

Conclusion

In recent years, we have finally begun to understand the biological significance of protein glycosylation. The explosive growth in glycobiology is not only due to major advances in the technology required to study complex glyconjugates, but is also the result of recently acquired knowledge about complex biological systems at the molecular level. Protein glycosylation is one of the last great enigmas of biochemistry/cell biology. Elucidation of all aspects of this most ubiquitous, yet diverse, form of post-translational modification is rapidly progressing. The forthcoming data are relevant to nearly all aspects of biology, including development, immunity and virtually all major diseases.

Acknowledgements

I thank Dr Rodney Willoughby and Ms Lisa Kreppel for critically reading the manuscript.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. HART GW, HALTWANGER RS, HOLT GD, KELLY WG: Glycosylation in the Nucleus and Cytoplasm. *Annu Rev Biochem* 1989, 58:841-874.
2. HALTWANGER RS, KELLY WG, ROQUEMORE EP, BLOMBERG MA, DONG L-YD, KREPPLE L, CHOU T-Y, HART GW: Glycosylation of Nuclear and Cytoplasmic Proteins is Ubiquitous and Dynamic. *Biochem Soc Trans* 1992, 20:264-269.
- A succinct review of the most recent data on O-GlcNAc. Contains the most complete list of O-GlcNAc-bearing proteins, sites of attachment and speculations about possible functions.
3. KORNFIELD R, KORNFIELD S: Assembly of Asparagine-linked Oligosaccharides. *Annu Rev Biochem* 1985, 54:631-664.
4. STROUS GJ, DEKKER J: Mucin-type Glycoproteins. *CRC Crit Rev Biochem Mol Biol* 1992, 27:57-92.
- The most current and excellent detailed review on mucins. Covers recent views of genomic organization, protein and carbohydrate structures, multimeric complexes, biosynthesis and intracellular transport, functions and pathology.
5. ERMENTIN P, WITZEL R, VLEGENTHART JFG, KAMERLING JP, NIMTZ M, CONRADT HS: A Strategy for the Mapping of N-Glycans by High-pH Anion-exchange Chromatography with Pulsed Amperometric Detection. *Anal Biochem* 1992, 203:281-289.
6. ASHFORD DA: Carbohydrate Analysis. *Curr Opin Biotechnol* 1992, 3:45-48.
7. STANLEY P: Glycosylation Engineering. *Glycobiol* 1992, 2:99-107.
- A thoughtful and well written evaluation of methods for the generation of specific glycoforms of glycoproteins: production in different cell types; site-directed mutagenesis; the use of glycosylation inhibitors; or by exogenous treatments with enzymes. Particularly relevant to pharmaceutical interests in recombinant glycoproteins.
8. FUKUDA MN, MASRI KA, DELL A, LUZZATTO L, MOREMEN KW: Incomplete Synthesis of N-Glycans in Congenital Dyserythropoietic Anemia Type II Caused by a Defect in the Gene Encoding α -Mannosidase II. *Proc Natl Acad Sci USA* 1990, 87:7443-7447.
9. PILLER F, LE DEIST F, WEINBERG KI, PARKMAN R, FUKUDA M: Altered O-Glycan Synthesis in Lymphocytes from Patients with Wiskott-Aldrich Syndrome. *J Exp Med* 1991, 173:1501-1510.
10. LIU DT-Y: Glycoprotein Pharmaceuticals: Scientific and Regulatory Considerations, and the US Orphan Drug Act. *Trends Biotechnol* 1992, 10:114-120.
- Essential reading for glycobiologists in the pharmaceutical industry. Presents a well developed discussion of the roles of glycosylation in pharmacokinetics and immunogenicity of glycoprotein pharmaceuticals. Also, contains a thorough discussion of regulatory issues and documents related to glycoprotein therapeutics.
11. HARDINGHAM TE, FOSANG AJ: Proteoglycans: Many Forms and Many Functions. *FASEB J* 1992, 6:861-870.
- Outstanding presentation of the diversity in structure and functions of proteoglycans. Excellent article for bringing the non-specialist up to date on recent developments.
12. YANAGISHITA M, HASCALL VC: Cell Surface Heparan Sulfate Proteoglycans. *J Biol Chem* 1992, 267:9451-9454.
- A more focused review of particular interest to biologists studying cell surfaces and growth factors. Provides a succinct picture of heparan sulfate proteoglycan synthesis, catabolism and functions.
13. TURNBULL JE, FERNIG DG, KE Y, WILKINSON MC, GALLAGHER JT: Identification of the Basic Fibroblast Growth Factor Binding Sequence in Fibroblast Heparan Sulfate. *J Biol Chem* 1992, 267:10337-10341.

14. DOERING TL, MASTERTON WJ, HART GW, ENGLUND PT: Biosynthesis of Glycosyl Phosphatidylinositol Membrane Anchors. *J Biol Chem* 1990, 265:611-614.
 15. FERGUSON MAJ: Glycosyl-phosphatidylinositol Membrane Anchors: The Tale of a Tail. *Biochem Soc Trans* 1992, 20:243-256.
- Presents a concise historical perspective on structures and functions of GPIs. Particularly strong in the presentation of current views of GPI functions.
16. SALTIEL AR: The Role of Glycosyl-phosphoinositides in Hormone Action. *J Bioenerg Biomembr* 1991, 23:29-41.
 17. ROBINSON PJ: Phosphatidylinositol Membrane Anchors and T-Cell Activation. *Immunol Today* 1991, 12:36-41.
 18. REASON AJ, MORRIS HR, PANICO M, MARAIS R, TREISMAN RH, ●● HALTWANGER RS, HART GW, KELLY WG, DELL A: Localization of O-GlcNAc Modification on the Serum Response Transcription Factor (SRF). *J Biol Chem* 1992, 267:16911-16921.
- Excellent examples of fast atom bombardment mass spectrometric and sequencing methods for localizing O-GlcNAc glycosylation. The most heavily glycosylated site on SRF was localized in the region of glycoprotein required for transcription initiation.
19. WHITFORD M, FAULKNER P: A Structural Polypeptide of the Baculovirus *Autographa californica* Nuclear Polyhedrosis Virus Contains O-Linked N-Acetylglucosamine. *J Virol* 1992, 66:3324-3329.
 20. GONZALEZ SA, BURRONE OR: Rotavirus Ns26 Is Modified by Addition of Single O-Linked Residues of N-Acetylglucosamine. *Virology* 1992, 182:8-16.
 21. ROQUEMORE EP, DELL A, MORRIS HR, PANICO M, REASON AJ, ● SAVOY L-A, WISTOW GJ, ZIGLER JS JR, EARLES BJ, HART GW: Vertebrate Lens α -Crystallins are Modified by O-Linked N-Acetylglucosamine. *J Biol Chem* 1992, 267:555-563.
- Demonstrates that the α -crystallins of the eye lens, which are heat-shock proteins in non-lens tissues, are glycosylated by O-GlcNAc. Mass spectrometry and gas-phase sequencing techniques localize attachment sites and confirm the lack of modification of the monosaccharide moieties.
22. CHOU C-F, SMITH AJ, OMARY MB: Characterization and Dynamics of O-Linked Glycosylation of Human Cytokeratin 8 and 18. *J Biol Chem* 1992, 267:3901-3906.
- Pulse-chase analyses demonstrate that O-GlcNAc on cytokeratins is more highly dynamic than the polypeptide backbone. Supports the hypothesis that O-GlcNAc has the hallmarks of a regulatory modification.
23. HAGMANN J, GROB M, BURGER MM: The Cytoskeletal Protein ● Talin Is O-Glycosylated. *J Biol Chem* 1992, 267:14424-14428.
- In addition to describing another important cytoskeletal protein that is glycosylated and localizing the glycosylation-sites, this paper also suggests a possible role for O-GlcNAc in the interaction of talin with vinculin to form the focal contacts attaching actin to the plasma membrane.
24. KEARSE KP, HART GW: Lymphocyte Activation Induces Rapid ● Changes in Nuclear and Cytoplasmic Glycoproteins. *Proc Natl Acad Sci USA* 1991, 88:1701-1705.
- Provides further support for the notion that O-GlcNAc is possibly a regulatory modification by demonstrating rapid changes in O-GlcNAc levels on activated lymphocytes. Similar to early work on phosphorylation in this system.
25. HALTWANGER RS, BLOMBERG MA, HART GW: Glycosylation ● of Nuclear and Cytoplasmic Proteins. Purification and Characterization of a Uridine Diphospho-N-Acetylglucosamine:Polypeptide B-N-Acetylglucosaminyltransferase. *J Biol Chem* 1992, 267:9005-9013.
- Describes the key enzyme that attaches O-GlcNAc to proteins. This glycosyltransferase is completely different from previously described Golgi glycosyltransferases in nearly all of its properties.
26. GONZALEZ-YANES B, CICERO JM, BROWN RD JR, WEST CM: Characterization of a Cytosolic Fucosylation Pathway in *Dictyostelium*. *J Biol Chem* 1992, 267:9595-9605.
 27. CALDER PC: Glycogen Structure and Biogenesis. *Int J Biochem* ● 1991, 23:1335-1352.
- A detailed review of all aspects of glycogen biochemistry. Particularly relevant discussion about glycogen biosynthesis and about the glycogen protein primer.
28. ARCHASE RB, RICHARDSON KL, SRISOMAP C, DRAKE RR, HALEY BE: Resolution of Phosphoglucomutase and the 62 kD Acceptor for the Glucosylphosphotransferase. *Arch Biochem Biophys* 1990, 280:122-129.
 29. EUFEMI M, ALTIERI F, CERVONI L, SPOTO G, FERRARO A: Nuclear Glycoproteins in Higher Vertebrates. *Biochem Int* 1991, 23:35-42.
 30. PEDEMONTE CH, SACHS G, KAPLAN JH: An Intrinsic Membrane Glycoprotein with Cytosolically Oriented N-Linked Sugars. *Proc Natl Acad Sci USA* 1990, 87:9789-9793.
 31. HARRIS RJ, LEONARD CK, GUZZETTA AW, SPELLMAN MW: Tissue Plasminogen Activator Has an O-Linked Fucose Attached to Threonine-61 in the Epidermal Growth Factor Domain. *Biochemistry* 1991, 30:2311-2314.
 32. BUKO AM, KENTZER EJ, PETROS A, MENON G, ZUIDERWEG ERP, SARIN VK: Characterization of a Posttranslational Fucosylation in the Growth Factor Domain of Urinary Plasminogen Activator. *Proc Natl Acad Sci USA* 1991, 88:3992-3996.
 33. HARRIS RJ, LING VT, SPELLMAN MW: O-Linked Fucose Is Present in the First Epidermal Growth Factor Domain of Factor XII But Not Protein C. *J Biol Chem* 1992, 267:5102-5107.
 34. NISHIMURA H, YAMASHITA S, ZENG Z, WALZ DA, IWANAGA S: Evidence for the Existence of O-Linked Sugar Chains Consisting of Glucose and Xylose in Bovine Thrombospondin. *J Biochem (Tokyo)* 1992, 111:460-464.
 35. KARLSSON KA: Animal Glycolipids As Attachment Sites for Microbes. *Chem Phys Lipids* 1986, 42:153-172.
 36. WANG Y, ABERNETHY JL, ECKHARDT AE, HILL RL: Purification ● and Characterization of a UDP-GalNAc:Polypeptide N-Acetyl-galactosaminyltransferase Specific for Glycosylation of Threonine Residues. *J Biol Chem* 1992, 267:12709-12716.
- Excellent characterization of a key enzyme in mucin biosynthesis. Data suggest that the enzyme will glycosylate any exposed threonine residue, but surprisingly has no activity against serine containing peptides.
37. CARRAWAY KL, HULL SR: Cell surface mucin-type glycoproteins ● and mucin-like domains. *Glycobiol* 1991, 1:131-138.
- Excellent short review of the structure and functions of mucin domains present on many important membrane proteins.
38. CODINGTON JF, HAAVIK S: Epiglycanin — A Carcinoma-specific Mucin-type Glycoprotein of the Mouse TA3 Tumour. ● *Glycobiol* 1992, 2:173-180.
- Describes the history and current knowledge of membrane bound mucin-type glycoproteins of carcinomas. One of the best examples of importance to cancer of the 'masking' of normal cell surface receptors by abnormal expression of a glycoprotein.
39. SCHACHTER H: The 'Yellow Brick Road' to Branched Complex ● N-Glycans. *Glycobiol* 1991, 1:453-461.
- A succinct summary of the development and current status of knowledge about the key N-Acetylglucosaminyltransferases that are involved in the extensive branching of N-linked oligosaccharides.
40. DIECKMANN-SCHUPPERT A, BENDER S, ODENTHAL-SCHNITTLER M, ● BAUSE E, SCHWARZ RT: Apparent Lack of N-Glycosylation in the Asexual Intraerythrocytic Stage of *Plasmodium falciparum*. *Eur J Biochem* 1992, 205:815-825.
- Represents the only known example of a eukaryotic cell that does not appear to synthesize N-linked oligosaccharides. Includes an analysis of possible dolichol intermediates and oligosaccharyltransferase.
41. NASIR-UD-DIN , DRAGER-DAYAL R, DECRIND C, HU B-H, DEL ● GIUDICE G, HOESSLI D: *Plasmodium falciparum* Synthesizes O-Glycosylated Glycoproteins Containing O-Linked N-Acetylglucosamine. *Biochem Int* 1992, 27:55-64.
 42. ABEIJON C, HIRSCHBERG CB: Topography of Glycosylation Reactions in the Endoplasmic Reticulum. *Trends Biochem Sci* 1992, 17:32-36.
 43. NOIVA R, KAPLAN HA, LENNARZ WJ: Glycosylation Site-binding Protein Is Not Required for N-Linked Glycoprotein Synthesis. *Proc Natl Acad Sci USA* 1991, 88:1986-1990.
 44. KELLEHER DJ, KREIBICH G, GILMORE R: Oligosaccharyltransferase ● Activity Is Associated with a Protein Complex Composed of Ribophorins I and II and a 48 kD Protein. *Cell* 1992, 69:55-65.
- Provides convincing data that the key enzyme in N-linked glycan synthesis comprises three subunits, including the two ribophorins. Ribophorin I appears to be the dolichol-binding subunit. Represents a major breakthrough in solving a long-term intractable problem.

45. TE HEESSEN S, JANETZKY B, LEHLE L, AEBI M: **The Yeast *wbp1* Is Essential for Oligosaccharyl Transferase Activity *in Vivo* and *in Vitro***. *EMBO J* 1992, 11:2071-2075.

Identifies an essential gene in yeast that is either the oligosaccharyltransferase or required for its activity. Identification of this gene will prove invaluable towards understanding the molecular features of this key enzyme.

46. SWIEDLER SJ, FREED JH, TARENTINO AL, PLUMMER TH JR, HART GW: **Oligosaccharide Microheterogeneity of the Murine Major Histocompatibility Antigens. Reproducible Site-specific Patterns of Sialylation and Branching in Asparagine-linked Oligosaccharides**. *J Biol Chem* 1985, 260:4046-4054.

47. DAHMS NM, HART GW: **Influence of Quaternary Structure On Glycosylation: Differential Subunit Association Affects the Site-specific Glycosylation of the Common β -Chain From Ia and LFA-1**. *J Biol Chem* 1986, 261:13186-13196.

48. SKELTON TP, HOOPER LV, SRIVASTAVA V, HINDSGAUL O, BAENZIGER JU: **Characterization of a Sulfotransferase Responsible for the 4-O-Sulfation of Terminal β -N-Acetyl-D-Galactosamine On Asparagine-linked Oligosaccharides of Glycoprotein Hormones**. *J Biol Chem* 1991, 266:17142-17150.

49. DRICKAMER K: **Clearing Up Glycoprotein Hormones**. *Cell* 1991, 67:1029-1032.

Excellent short review on glycoprotein clearance systems particularly relevant to glycoprotein hormones.

50. RADEMACHER TW, PAREKH RB, DWEK RA: **Glycobiology**. *Annu Rev Biochem* 1988, 57:785-838.

51. RUDD PM, LEATHERBARROW RJ, RADEMACHER TW, DWEK RA: **Diversification of the IgG Molecule by Oligosaccharides**. *Mol Immunol* 1991, 28:1369-1378.

Presents a detailed structural view of the glycoforms of IgG. Describes current concepts with respect to the roles of IgG glycoforms in disease, aging and pregnancy, presenting some plausible models for the involvement of oligosaccharides in the self-association of IgG rheumatoid factors.

52. PERKINS SJ, WILLIAMS AF, RADEMACHER TW, DWEK RA: **The Thy-1 Glycoprotein: A Three-dimensional Model**. *Trends Biochem Sci* 1988, 13:302-303.

53. ELBEIN AD: **Glycosidase Inhibitors: Inhibitors of N-Linked Oligosaccharide Processing**. *FASEB J* 1991, 5:3055-3063.

54. ELBEIN AD: **The Role of N-Linked Oligosaccharides in Glycoprotein Function**. *Trends Biotechnol* 1991, 9:346-352.

55. HOE MH, HUNT RC: **Loss of One Asparagine-linked Oligosaccharide From Human Transferrin Receptors Results in Specific Cleavage and Association with the Endoplasmic Reticulum**. *J Biol Chem* 1992, 267:4916-4923.

56. TIEFT CJ, PROLA RL, CAMERINI-OTERO RD: **The Folding and Cell Surface Expression of Cd4 Requires Glycosylation**. *J Biol Chem* 1992, 267:3268-3273.

57. DEDERA DA, GU R, RATNER L: **Role of Asparagine-linked Glycosylation in Human Immunodeficiency Virus Type 1 Transmembrane Envelope Function**. *Virology* 1992, 187:377-382.

58. DANIELSEN EM: **Folding of Intestinal Brush Border Enzymes. Evidence That High-mannose Glycosylation Is an Essential Early Event**. *Biochemistry* 1992, 31:2266-2272.

59. FUKUDA M: **Lysosomal Membrane Glycoproteins. Structure, Biosynthesis, and Intracellular Trafficking**. *J Biol Chem* 1991, 266:21327-21330.

Definitive review describing the structure, biosynthesis and functions of the heavily glycosylated membrane glycoproteins that appear to not only protect the lysosome from autolysis, but also function in adhesion at the cell surface.

60. CUMMING DA: **Glycosylation of Recombinant Protein Therapeutics: Control and Functional Implications**. *Glycobiol* 1991, 1:115-130.

61. TAKEUCHI M, KOBATA A: **Structures and Functional Roles of the Sugar Chains of Human Erythropoietins**. *Glycobiol* 1991, 1:337-346.

62. HOWARD SC, WITTWER AJ, WELPLY JK: **Oligosaccharides at Each Glycosylation Site Make Structure-dependent Contributions to Biological Properties of Human Tissue Plasminogen Activator**. *Glycobiol* 1991, 1:411-417.

One of the best examples of glycosylation modulating the kinetic properties of an enzyme. Represents one of a select few proteins in which glycosylation has been examined at the single site level.

63. HIGUCHI M, OH-EDA M, KUBONIWA H, TOMONOH K, SHIMONAKA Y, OCHI N: **Role of Sugar Chains in the Expression of the Biological Activity of Human Erythropoietin**. *J Biol Chem* 1992, 267:7703-7709.

The most recent in a series of papers from several laboratories that examine the role(s) of oligosaccharides on erythropoietin. Supports the role of erythropoietin's N-linked glycosylation *in vivo*.

64. FURUKAWA K, KOBATA A: **IgG Galactosylation—Its Biological Significance and Pathology**. *Mol Immunol* 1991, 28:1333-1340.

65. AXFORD JS, SUMAR N, ALAVI A, ISENBERG DA, YOUNG A, BODMAN KB, ROITT IM: **Changes in Normal Glycosylation Mechanisms in Autoimmune Rheumatic Disease**. *J Clin Invest* 1992, 89:1021-1031.

Presents recent clinically relevant data with regard to interrelationship between lymphocytic galactosyltransferase and undergalactosylated IgG in normal and disease conditions.

66. CHANDRASEKARAN S, DEAN JW III, GINIGER MS, TANZER ML: **Laminin Carbohydrates are Implicated in Cell Signaling**. *J Cell Biochem* 1991, 46:115-124.

67. BUTCHER EC: **Leukocyte-Endothelial Cell Recognition: Three (Or More) Steps to Specificity and Diversity**. *Cell* 1991, 67:1033-1036.

Presents a cohesive picture of mechanisms and molecules involved in targeting leukocytes to sites of inflammation. Presents one of the clearest and most cohesive views on the large amount of data on selectins.

68. VARKI A: **Selectins and Other Mammalian Sialic Acid-binding Lectins**. *Curr Opin Cell Biol* 1992, 4:257-266.

A general discussion of sialic acid-binding lectins important in mammalian cells. Presents a general overview by placing the selectins in context of other sialic acid binding proteins.

69. HODGSON J: **Carbohydrate-based Therapeutics**. *Biotechnology* 1991, 9:609-613.

Summarizes current work underway in pharmaceutical companies to develop carbohydrate-based drugs. A good description of the major growth areas.

70. WINKELHAKE JL: **Will Complex Carbohydrate Ligands of Vascular Selectins Be the Next Generation of Non-steroidal Anti-inflammatory Drugs?** *Glycoconjugate J* 1991, 8:381-386.

71. VARKI A: **Diversity in the Sialic Acids**. *Glycobiology* 1992, 2:25-40.

Excellent synopsis of the current structural diversity and nomenclature of sialic acids. A must for people trying to understand oligosaccharide structural diversity.

72. SCHAUER R: **Biosynthesis and Function of N- and O-Substituted Sialic Acids**. *Glycobiology* 1991, 1:449-452.

Lucid description of the biosynthesis of the different types of sialic acids. Provides an excellent historical perspective.

73. TROY FA II: **Polysialylation: From Bacteria to Brains**. *Glycobiology* 1992, 2:5-23.

The definitive review on polysialic acid. Focuses on recent developments in occurrence, structure, function and biosynthesis.

74. SCHENKMAN S, JIANG M-S, HART GW, NUSSENZWEIG V: **A Novel Cell Surface Trans-sialidase of *Trypanosoma cruzi* Generates a Stage-specific Epitope Required for Invasion of Mammalian Cells**. *Cell* 1991, 65:1117-1125.

Describes the initial characterization of a novel trans-sialidase enzyme that transfers sialic acid residues from host glycoconjugates to the surface of trypanosomes emerging from infected macrophages. These sialic acids are essential for parasite adhesion to host macrophages.

75. SCHENKMAN S, PONTES DE CARVALHO L, NUSSENZWEIG V: ***Trypanosoma cruzi* Trans-sialidase and Neuraminidase Activities Can Be Mediated by the Same Enzymes**. *J Exp Med* 1992, 175:567-575.

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