



Biological Roles of Oligosaccharides

These suggestions and data are based on information we believe to be reliable. They are offered in good faith, but without guarantee, as conditions and methods of use of our products are beyond our control. We recommend that the prospective user determine the suitability of our materials and suggestions before adopting them on a commercial scale.

Suggestions for use of our products or the inclusion of descriptive material from patents and the citation of specific patents in this publication should not be understood as recommending the use of our products in violation of any patent or as permission to license to use any patents of ProZyme, Inc.

Biological Roles of Oligosaccharides

Professor Ajit Varki, Glycobiology Program, UCSD Cancer Center, and Division of Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA 92093

This is an abridged version of a review originally published in *Glycobiology* (1993) **3** 97-130, which covered bibliographic material spanning the period 1978-1992. Because of space constraints, only the monographs and reviews cited are listed here. The interested reader is encouraged to consult the original work for the rest of the citations, as well as the earlier literature cited in the monographs and reviews. Since the publication of the review, further examples of these biological roles of oligosaccharides have continued to appear in the literature at an increasing rate.

The ability to accurately sequence the oligosaccharide units of glycoconjugates has revealed a remarkable complexity and diversity of these molecules. However, despite some observations suggesting important biological roles for these sugar chains, a single common theory has not emerged to explain this diversity. Many theories have been advanced regarding the biological roles of oligosaccharides, and have been presented in a number of monographs and review articles. These include a purely structural role, an aid in the conformation and stability of proteins, provision of target structures for microorganisms, toxins and antibodies, masking of such target structures, control of protein and cell half-life, modulation of protein functions, and the provision of ligands for specific binding events which mediate protein targeting, cell-matrix interactions or cell-cell interactions. Most of these discussions have focused either upon one specific type of glycoconjugate or upon one specific theory of function. In this overview, an attempt is made to consider together all of these theories regarding all of the major types of glycoconjugates. Although the emphasis of this review is somewhat biased towards the glycoprotein oligosaccharides of higher animal cells, the principles that emerge may be generally applicable to all types of organisms.

The difficulty in predicting specific rules for oligosaccharide functions: N- and O-linked sugar chains as examples.

Of all the different types of glycosylation, N-asparagine-linked sugar chains are the easiest to manipulate in experimental systems. Feasible approaches include the enzymatic or chemical removal of completed chains, prevention of initial glycosylation, alteration of processing, elimination of specific glycosylation sites and the study of natural variants and genetic mutants. Such approaches have been used to explore the biological roles of these sugar chains on a variety of glycoproteins. A summary of some such studies is reported in Table I, and a shorter list of similar experiments for O-linked oligosaccharides is presented in Table II. It can be seen that the consequences of altering these types of glycosylation range from being essentially undetectable, to the complete loss of particular functions, or even loss of the entire protein itself. Even within a given group of proteins, (e.g. cell surface receptors or enzymes), the effects of altering glycosylation are highly variable and quite unpredictable. Furthermore, the same modification in glycosylation can have a dramatically opposite effect on function *in vivo* versus *in vitro* (for example, see the

Table I. Some examples of the effects of altered N-linked oligosaccharides on the biosynthesis, transport & functions of glycoproteins

PROTEIN	EFFECT OF LACK / ALTERATION OF OLIGOSACCHARIDES
ENZYMES	
Most lysosomal enzymes ^{a,b,c,d}	Loss of targeting signal for delivery of enzymes to lysosomes.
Yeast Acid Phosphatase ^{a,d}	Loss of activity and conformation. Increased susceptibility to denaturation and proteolysis.
HMG-CoA Reductase ^b	>90% decrease in activity (direct or indirect effect?).
Yeast Carboxypeptidase Y ^d	Reduced transport rate, especially at higher temperatures. No change in sorting, stability or activity.
Propapain ^d	N-glycosylation of Pro-region require for transport & secretion.
Purified lysosomal α -L-fucosidase ^a	No effect upon activity or conformation. Shift of pH optimum, decreased acid and heat stability.
Pancreatic Ribonuclease B ^{a,e}	Folding kinetics unchanged, increased susceptibility to proteases.
HORMONES/CYTOKINES/GROWTH FACTORS	
Glycoprotein hormones (HCG, LH, TSH, Prolactin) ^{a,b,c,d,e}	Complex effects. Altered combining of α - and β -subunits. In some cases, agonist converted into antagonist by loss of glycosylation. Altered glycosylation causes changes in specificity, affinity and intracellular signalling <i>in vitro</i> . Effects on <i>in vivo</i> action are different, because of altered clearance.
Granulocyte/macrophage colony stimulating factor (GM-CSF) ^{a,c,d}	Graded increase in receptor binding and activity with decreased glycosylation. However, increased antigenicity and rapid clearance of unglycosylated form.
Erythropoietin ^{a,c,d,e}	Complex effects. Failure of secretion, decreased stability, and decreased biological activity if multiple glycosylation sites are eliminated. Desialylation and/or less branched oligosaccharides give increased activity <i>in vitro</i> but decreased activity <i>in vivo</i> .
Vascular Endothelial Growth Factor ^b	Markedly decreased rate and efficiency of secretion. No loss of binding or of activity in increasing vascular permeability.
Interferon-gamma ^a	No change in antiviral activity or target cell specificity.
VIRAL GLYCOPROTEINS	
HIV virus glycoprotein (GP120) ^{a,b,c,d}	Prevention of glycosylation or prevention of glucose removal results in markedly lowered fusion activity and decreased infectivity.
Some viral coat proteins ^{a,d,e}	Variable alterations in antigenicity; increased reactivity with polyclonal antisera upon removal of chains.

Biological Roles of Oligosaccharides

Some (not all) viral coat proteins^{b,c,d}

Complex and variable effects. Complete loss of glycosylation can result in misfolding, retention in the ER, proteolytic degradation and loss of virion production. Alteration of individual sites show varying effects on cell surface expression. Alteration in late processing has little effect upon expression or virion production.

PLASMA PROTEINS/COAGULATION FACTORS

Immunoglobulin D^a

Removal causes loss of binding by IgD Receptors.

Immunoglobulins^{a,b,c,d,e}

Complex effects. Altered secretion (variable). Alteration of several secondary effector functions of the Fc region. Agalactosyl IgG is associated with granulomatous diseases. Variable region glycosylation can enhance antigen binding.

Tissue-type plasminogen activator^{a,d,e}

Increased enzymatic activity and/or lysine-binding of certain glycoforms. Glycosylation alters conversion to two-chain form.

Antithrombin III β - natural variant with decreased carbohydrate^e

Increased antithrombin activity. Enhanced heparin binding(?)

Plasminogen^{d,e}

Variations in glycosylation or sialylation associated with differences in cell-surface binding, circulatory half-time and fibrinolytic activity.

Von Willebrand Factor^a

Variable effects on multimeric structure and altered function. Increased susceptibility to proteases

Fibrinogen^{a,d,e}

Altered polymerization/aggregation. Sialic acids are low affinity calcium binding sites that can influence fibrin assembly.

Corticosteroid-binding globulin^{b,e}

Acquisition of binding activity requires glycosylation.

Thyroxine-binding globulin^a

Decreased stability. Small change in affinity for thyroxine and immunoreactivity.

Folate-binding protein^b

Initial glycosylation required for acquisition of binding function.

MATRIX PROTEINS

Fibronectin^{a,b}

Increased susceptibility to proteases.

Bone and platelet osteonectin^e

Differences in glycosylation alter collagen-binding properties.

MEMBRANE PROTEINS / RECEPTORS

EGF receptor^{b,c}

Glycosylation-dependent acquisition of ligand-binding capacity. Thereafter, oligosaccharides not required for binding.

Insulin and Insulin-like Growth Factor-I receptors^{c,d}

Loss of binding with complete de-glycosylation only. Partial changes in binding affinity/specificity with altered glycosylation. Some studies show loss of tyrosine kinase activity.

Basic Fibroblast Growth Factor Receptor^a

Loss of binding of bFGF.

Lymphocyte CD2^{a,d}

Single glycosylation site required for binding of CD2 to CD58 on another cell. Postulated that glycosylation is required for stabilizing domain 1, which is involved in adhesion.

MHC Class II molecules^d

Variable loss of antigen presenting function. No loss of peptide-binding function of purified protein.

Glucose transporters^{a,d}

Increase in Km for glucose. Partial or complete loss of activity.

Erythrocyte Band 3 protein^a

Increased aggregation. No change in CD spectra, proteolytic susceptibility or anion transport activity.

β -2 Adrenergic Receptors^{c,d}

No change in expression of ligand affinity. Uncoupling of adenylate cyclase.

Transferrin Receptor expressed in various cell types^d

Decreased dimer expression. Decreased binding affinity in intact cells. Elimination of glycosylation sites causes partial ER retention and degradation.

VIP receptor^a

No change in expression. Decreased binding affinity.

Membrane Class I MHC protein.

Increased lateral mobility in the membrane.

Vasopressin Receptor of LLC-PK1 cells^{b,c}

Inhibition of biosynthesis and internalization.

Low Density Lipoprotein Receptor^{b,c}

No change in expression or in recycling. ~50% decrease in ligand binding capacity. Redistribution between intracellular and surface.

Asialoglycoprotein receptor^{a,b}

No change in expression, binding or turnover.

CD4 protein^{b,c,d}

Loss of one glycosylation site tolerated. Loss of both sites results in ER retention and degradation.

Thyrotropin receptor^d

Expression unchanged. Elimination of certain sites causes loss of binding.

PDGF receptor^c

Glycosylation not required for acquisition of ligand-binding capacity.

MISCELLANEOUS

Jack bean concanavalin A^{d,e}

N-linked oligosaccharide keeps the precursor in inactive form, until it is deglycosylated during later processing.

Cobra venom Factor^a

Complete deglycosylation abolishes complement activation.

Lactotransferrin^a

Loss of Iron-binding activity.

Spinach Chloroplast Coupling Factor^a

ATPase activity intact. Photophosphorylation activity lost.

Many secreted animal proteins^c

Prevention of glucose removal slows secretion, while blocking of late processing accelerates secretion in many glycoproteins.

Plant glycoproteins secreted by sycamore cells in culture^d

Prevention of glycosylation causes marked decrease in secretion, whereas altered processing has little effect.

Note: Unless otherwise stated, the proteins are of mammalian origin. a Enzymatic or chemical removal of N-linked oligosaccharides

b Prevention of N-linked glycosylation by tunicamycin (can have pleiotropic effects on other proteins in the same cell)

c N-linked glycosylation processing inhibitor(s). (can have pleiotropic effects on other proteins in the same cell)

d Genetically engineered change in glycosylation. e Natural genetic variant or defect.

Biological Roles of Oligosaccharides

Table II. Some examples of the effects of altered O-linked oligosaccharides on the biosynthesis, structure, transport and functions of glycoproteins.

PROTEIN	EFFECT OF LACK / ALTERATION OF OLIGOSACCHARIDES
GM-CSF ^d	Single O-linked chain protects against polymerization, denaturation, and loss of certain activities.
Submaxillary gland mucin (molecular modelling studies)	Native and asialo-mucin are found to be highly extended random coils. Removal of the carbohydrate side chains causes collapse to chain with dimensions typical of denatured globular proteins.
Intestinal brush border Lactase-phlorizin hydrolase (LPH) ^d	Natural variant glycoform without O-glycosylation has identical Km value but a 4-fold higher Vmax.
Arctic Fish antifreeze glycoprotein ^a	O-linked oligosaccharides required for freezing-point depression.
Erythropoietin ^c	No effect of elimination on biosynthesis or biological activity. Small effect on secretion rate (?).
Glycophorin A ^{a,d}	Polymorphic changes in amino acids surrounding three O-linked sites give rise to the MN blood group antigens. Intact, unmodified oligosaccharides are required for full antigenicity.
LDL Receptor ^c	Multiple O-linked oligosaccharides: some are required for stable expression on the cell surface – but not required for LDL binding.
β-HCG ^c	No change in assembly or secretion, hormonal activity or immunoreactivity

Note: Unless otherwise stated, the proteins are of mammalian origin. a Enzymatic or chemical removal of O-linked oligosaccharides

b Prevention of O-linked glycosylation by competitive inhibitors (can have pleiotropic effects on other proteins in the same cell)

c Genetically engineered change in O-glycosylation d Natural genetic variant or defect.

studies on erythropoietin and the gonadotrophic hormones referred to in Table I). Thus, the biological roles of oligosaccharides appear to range from trivial to crucial, depending upon the glycoconjugate, the oligosaccharide structure, the biological context, and the question being asked.

Can one make any sense out of these diverse and confusing findings? Most prior attempts have focused upon either a single type of glycosylation or upon a single theory of their function. An attempt is made here to undertake a global overview of most of the known facts concerning the biological roles of oligosaccharides of major classes of glycoconjugates in eukaryotic cells, and to synthesize some general principles for understanding them. Much of the actual data is presented in table form and is necessarily simplified and/or incomplete. For details, the reader is referred to the monographs and reviews listed at the end, the articles cited in the original review, as well as the earlier literature cited in the monographs and reviews.

'Structural', 'protective' and 'stabilizing' roles of oligosaccharides.

There is little doubt that some oligosaccharides are important in the physical maintenance of tissue structure, integrity and porosity (Table III). It is also clear that the 'coating' of oligosaccharides on many glycoproteins can protect the polypeptide from recognition by proteases or antibodies (Tables I, II and III) and that the coating of glycoconjugates covering a whole cell can present a 'glyco-calyx' of substantial proportions. Another well-accepted function of the oligosaccharide units of glycoproteins is that they are involved in the initiation of correct polypeptide folding in the rough endoplasmic reticulum (ER), and in the subsequent maintenance of protein solubility and conformation (see examples in Tables I and II). Many proteins that are incorrectly glycosylated fail to fold properly and/or fail to exit the ER, and are consequently degraded. On the other hand, there are many examples of proteins for which the prevention or alteration of glycosylation has little apparent consequence to their synthesis, folding, or delivery to a final location (see Tables I and II). Likewise,

there are examples wherein removal of oligosaccharides from a mature protein does not drastically alter sensitivity to proteolysis or immune recognition, nor their functions. These observations exemplify a recurring theme: that while supporting evidence can be found for many theories regarding oligosaccharide function, exceptions to each can equally well be observed.

While important, the development of these 'structural', 'protective' and 'stabilizing' functions during evolution should not have required the complexities of structure found in naturally occurring oligosaccharide units. In keeping with this, inhibitors that affect the later steps of oligosaccharide processing generally do not interfere with these types of functions. In these situations then, the oligosaccharides are analogous to the 'axle grease' of an automobile. While its absence would markedly affect the ability of the entire vehicle to function, the fine details of composition of the grease should not be critical to the turning of the axle. Thus, while these roles of oligosaccharides are vital to the basic structure and function of the organism, they cannot explain the extensive structural diversity seen in nature.

The 'organizational' and 'barrier' functions.

The extra-cellular matrix consists of a variety of glycoconjugates, each of which have been shown to have binding sites for various types of sugar chains, e.g. the heparin-binding domains of fibronectin and collagen. Recently the role of such oligosaccharide binding domains in the organization of the matrix has been clearly demonstrated *in vitro* (Table III). Thus, for example, the chondroitin sulfate chain of the proteoglycan decorin is required for the organization of fibronectin in the extracellular matrix of CHO cells, which in turn dictates the phenotype of the cells in culture.

Oligosaccharides as specific receptors for noxious agents: the 'traitorous' functions.

Certain oligosaccharides can act as highly specific receptors for a variety of viruses, bacteria and parasites (Table IV). They can also be receptors for many plant and bacterial tox-

Biological Roles of Oligosaccharides

ins and serve as antigens for autoimmune and alloimmune reactions. In most of these instances, there is exquisite specificity for the sequence of the oligosaccharide involved. Thus, for example, the influenza viral hemagglutinins specifically recognize the type of sialic acid, its modifications and its linkage to the underlying sugar chain, while cholera toxin binds with great specificity to G_{M1} ganglioside and not to related structures. Likewise, incompletely synthesized (or partially degraded) oligosaccharides such as the Tn antigen can behave as autoantigens in man. There is little doubt about the extreme specificity of this group of 'functions' of oligosaccharides (Table IV). However, they are of little benefit to the organisms that synthesize them.

Oligosaccharide sequences that protect from microorganisms and antibodies: the 'masking' and 'decoy' functions.

Just as certain oligosaccharides act as 'traitorous' signposts for microbial and immune attack, others can serve to abrogate these detrimental reactions (Table V). Thus, the addition of specific monosaccharides or modifications masks the sequences recognized by microorganisms, toxins, or autoimmune antibodies. For example, the addition of a single O-acetylerster to the 9-position of terminal sialic acid residues abrogates binding of the highly pathogenic influenza A viruses, and the extension of the oligosaccharide chain of GM1 would prevent binding of cholera toxin. Likewise, the addition of galactose and sialic acid to the Tn antigen would abolish its autoimmune reactivity. Oligosaccharides sequences on soluble glycoconjugates such as the mucins can also act as 'decoys' for microorganisms and parasites. Pathogenic organisms attempting to gain access to mucosal membranes might first encounter their cognate oligosaccharide ligands attached to soluble mucins. Upon binding to these sequences, they could then be swept away by ciliary action, leaving the mucosal cells untouched. Thus, the host may successfully turn the specificity of the pathogen receptor to its own advantage.

Oligosaccharides as specific receptors for 'symbiotic' functions.

The possible evolutionary interplay between the 'traitorous' and 'masking' functions of oligosaccharides is discussed below. In contrast to these functions of sugar chains, there are some cases in which symbiotic relationships of animals with micro-organisms appear to be aided by interactions involving specific oligosaccharides. Thus, certain commensal gut bacteria in animals and some root-nodule forming bacteria in plants appear to mediate their binding to host cell surfaces via specific sugar sequences. In these cases, inter-species recognition via oligosaccharides serves a function useful to both organisms involved. It is possible that such interactions are much more common, and that they have not been carefully sought after.

Effects of oligosaccharides on the biological functions of proteins: 'On-off' and 'tuning' functions.

Glycosylation can substantially modulate the interaction of peptides with their cognate ligands or receptors (Tables I and VI). Some cell surface receptors for growth factors appear to acquire their binding functions in a glycosylation-dependent manner during biosynthesis. This might limit unwanted early interactions with a growth factor synthesized in the same cell. Glycosylation of a ligand can also potentially mediate such an 'on-off' or 'switching' effect. When the hormone β -HCG is deglycosylated, it still binds to its receptor with similar affinity, but fails to transmit a signal via stimulation of adenylate cyclase: thus, an agonist is converted into an antagonist.

In most cases, such effects of glycosylation are not all or none, but are partial or relative. In these 'tuning' functions, the activity of glycosylated growth factors or hormones is modulated over a significant range by the presence and extent of glycosylation. For example, the cytokine GM-CSF is most active in the unnatural non-glycosylated form derived from recombinant technology. Naturally occurring preparations of GM-CSF contain a range of 'glycoforms',

Table III. Cell surface and matrix structure: Some examples of the 'structural' 'organizational' and 'barrier' functions

OLIGOSACCHARIDE SEQUENCES	INTERACTION WITH	EXAMPLES OF BIOLOGICAL EFFECTS
Heparan sulfate chains of proteoglycans	Fibronectin, Laminin, collagen, etc.	Organization of basement membrane and extracellular matrix. Filtering function of renal glomerulus. Cell adhesion to matrix.
Chondroitin / dermatan sulfate chains of proteoglycans	Extracellular fibronectin.	Deposition of fibronectin in the matrix: ?contact inhibition of cell growth.
Dermatan sulfate chain of human blood protein pre-alpha inhibitor	Covalent cross-linking to heavy chain via esterification	A novel mechanism for cross-linking of two peptide subunits of a protein
Sialic acid and sulfate esters on glycoprotein	Other negatively charged residues	Determinant of net negative charge of cell surfaces and proteins: modulates cell-cell interactions and viscosity of secretions
Sialic acid and sulfate esters on glycoproteins	Other negatively charged residues.	Determinant of net negative charge of cell surfaces and proteins: modulates cell-cell interactions and viscosity of secretions.
Glycolipids on epithelial cells	Other components of apical membranes, e.g. GPI anchored proteins	High local concentration on outer leaflet facing the lumen of organs: protective or barrier function?
Polylectosamine chains of laminin oligosaccharides	Bound by 65 Kda elastin receptor in a β -galactoside-specific manner?	Organization of matrix elastin deposition?
Gastric Mucus (major component is gastric mucin, a glycoprotein with many O-linked sialylated and sulfated chains	Water, Ions and ? self-association.	HCl, secreted by gastric glands traverses the mucus layer without acidifying it because of 'viscous fingering'. HCl in the lumen (pH 2) cannot diffuse back to the epithelium because of the high viscosity of gastric mucus gel. Prevents the stomach from digesting itself.

Biological Roles of Oligosaccharides

Table IV. Some examples of oligosaccharides as specific receptors for noxious agents, antigens for autoimmune responses, or as facilitators of infectious and malignant disease: Some examples of the 'traitorous' functions

OLIGOSACCHARIDE SEQUENCES	RECOGNITION BY	BIOLOGICAL EFFECTS
Terminal sialic acids	Receptors for a variety of virus hemagglutinins Recognition is affected by linkage and/or substitutions of the sialic acids.	First step in infectious process of viruses. Consequences range from lethal disease to mild self-limited infection.
Terminal sialic acids	<i>Trypanosoma cruzi</i> trans-sialidase (has both neuraminidase and sialyltransferase activity).	Parasite utilizes host sialic acids and transfers them blocking antigenicity and complement activation.
Specific gangliosides on cell surfaces	Receptors for several bacterial toxins e.g. <i>Vibrio cholerae</i> B subunits binds GM1 ganglioside.	Cell binding and delivery of toxic subunits (e.g. A subunit of - <i>Vibrio cholerae</i>).
Specific oligosaccharide sequences of glycolipids and glycoproteins on mucosal surfaces	Fimbrial proteins of pathogenic or commensal bacteria; binding receptors of certain mycoplasma, chlamydiae, protozoa and fungi.	First step in infectious process of pathogenic organisms. Also helps to maintain localization of normal 'commensal' organisms?
Cell surface heparan sulfate chains of CMP-sialic acid ('serum resistance factor') found in blood	Receptor for Herpes Simplex virus ?Surface transferase on pathogenic <i>Neisseria gonorrhoeae</i> .	Initiates infection. Sialic acid is transferred to bacterial surface, blocking antigenicity and complement activation
N-linked oligosaccharides on gastric parietal cell proteins	Required for reactivity of some auto-antibodies in autoimmune gastritis and pernicious anemia.	Antibodies associated closely with mucosal atrophy, parietal cell loss - likely to be involved in pathogenesis.
Erythrocyte and platelet glycoconjugates e.g. Polylactosamine chains on red blood cells ('I' antigen)	Antigens for spontaneously appearing 'cold agglutinins' and other autoimmune antibodies.	Autoimmune destruction of cells, e.g. autoimmune hemolytic anemia.
Incomplete oligosaccharide structures resulting from infectious agents or malignant transformation	Natural or induced antibodies against the incomplete structures e.g. Tn antigen.	?Autoimmune damage to cells and organs. Also beneficial because of anti-tumor effects?
Sialylated, fucosylated lactosaminoglycans on leukocytes	Ligands for selectin molecules on endothelial cells. System can work 'too well' under certain conditions of post-ischemic reperfusion or inflammation.	Toxic or inflammatory injury to tissues mediated by leukocytes exiting the blood-stream in excess.
Highly branched and sialylated N-linked oligosaccharides	Expressed in increased amounts on transformed cells.	Enhances tumorigenicity and metastatic capability of tumor cells?
Sialylated, fucosylated lactosaminoglycans on tumor cells	Ligands for selectin molecules on endothelial cells.	Enhances metastatic capability of tumor cells via selectin-mediated binding?
Sialic acids and sialyloligosaccharides on many microbial surfaces	Blocks generation and reactivity of many antimicrobial antibodies and facilitates binding of complement regulatory protein H.	Protects micro-organism from host antibodies and alternate pathway of complement.
Neural glycosphingolipids	Naturally occurring monoclonal or polyclonal antibodies.	Peripheral neuropathy (in some cases, it is not clear if the antibody is the cause or consequence of the disease).

Table V. Oligosaccharide sequences that protect from microorganisms and immune reactions: Some examples of the 'masking' functions

OLIGOSACCHARIDE SEQUENCES	MASKS RECOGNITION OF:	MASKS RECOGNITION BY:
9-O-acetylation	Terminal sialic acids.	Influenza A & B Viruses (generates recognition by other viruses).
9-O-acetylation	Terminal sialic acids.	Bacterial and viral sialidases.
4-O-acetylation		
Terminal sialic acids on glycoproteins and cell surfaces	β -Gal and β -GalNAc residues.	Asialoglycoprotein receptor Macrophage Gal/GalNAc lectin.
Terminal sialic acids on O-linked oligosaccharides	Core O-linked oligosaccharides (T & Tn antigens).	Natural antibodies to T & Tn antigens.
β -Gal residues	β -GlcNAc residues.	Chicken Hepatic Lectin.
Cell surface heparan sulfate	Underlying 'activators'?	Alternate pathway of complement activation.
Heterogenous oligosaccharides found on secreted mucins and in milk	Gut epithelial surface membrane oligosaccharides.	By various microbial pathogens. Soluble molecules act as inhibitors or 'decoys'.

each of which show substantial differences in binding affinity and signal transduction (Tables I and VI). Another example is seen in the addition of polysialic acid to the neural cell adhesion molecule (N-CAM), which normally mediates homophilic binding with identical molecules on other cells (Table XI). In the embryonic form, the oligosaccharides of N-CAM carry extended highly anionic polysialic acid chains that markedly reduce its capacity for homophilic binding. During development, the length of these chains is altered, and in the adult state they are much shorter. Thus, adult N-CAM is capable of more effective

homophilic binding, presumably because there is no longer a need for as much neural plasticity.

The function of proteins can also be 'tuned' not by oligosaccharides on the receptors or ligands themselves but by those on other neighboring structures. An excellent example of this is the modulation of growth factor receptors by gangliosides which are discrete and separate entities from the receptor proteins (Table VI). Thus, the precise type of ganglioside present in a membrane can have a substantial effect on the tyrosine phosphorylation activity of the EGF receptor and the insulin receptor. While the precise mech-

Biological Roles of Oligosaccharides

anism is uncertain and some conflicting data has been published, it is clear that the specific oligosaccharide sequence of the ganglioside is required, rather than simply its net charge or size.

Since these 'tuning' effects of oligosaccharides are usually partial and rarely absolute, a skeptic might raise questions about their importance. However, when taken together, such partial effects could have a dramatic effect upon the final biological outcome. Consider two different cell types with the identical number and types of growth factor receptors, that are presented with the identical concentrations of the same growth factors. If the signal delivered to a cell was based only upon the primary receptor-ligand interactions, there should be no difference in response between

these two cells. However, if differences existed in the glycosylation state of the ligands, receptors or membrane gangliosides, each could have a significant positive or negative quantitative effect upon the final signals delivered to the cell. Furthermore, glycosaminoglycan chains on the surface and in the matrix surrounding the cells could also modulate the action of each growth factor differently. Taken together, the combined effects upon the different receptor-ligand generated signals would be quite different for the two different cells. These differences would be amplified by the overlap and cross-talk between the intracellular signalling mechanisms of many receptors. Thus, the final effects of the same ligands at the same concentrations on the two different cells could be dramatically different.

Table VI. Effects of oligosaccharides on the biological functions of proteins: Some examples of 'on-off' and 'tuning' functions

PEPTIDE(S)	TYPE OF OLIGOSACCHARIDES	BIOLOGICAL EFFECT(S)
Glycoprotein Hormones e.g. β -HCG	N-linked oligosaccharides on the hormones.	Required for signal transduction: removal results in conversion to an antagonist. Role in normal biology not fully defined.
Hematopoietic growth factors (e.g. GM-CSF & erythropoietin)	N-linked oligosaccharides on the factors.	Binding affinities and biological activities change substantially with differing degrees of glycosylation.
Interleukin-1 and Interleukin-2	Binding to high mannose-type oligosaccharides on other molecules.	Generates a surface membrane-bound form, of uncertain function. The cognate oligosaccharides show immunosuppressive actions <i>in vitro</i> .
Several proteases, protease activators, and protease inhibitors (other than Antithrombin III and Heparin co-factor II, see Table XII)	Interaction with heparin / heparan sulfate chains.	Varying degrees of positive or negative modulation of activities.
Some growth factor receptors b, c, d	N-linked glycosylation dependant activation: completion of disulfide bonds and native conformation requires partial processing of oligosaccharide (?)	Prevents premature association of growth factor made in the same cell with its cognate receptor?
Some growth factor receptors (EGF receptor, Insulin receptor)	Up or down-regulation of tyrosine phosphorylation by specific gangliosides present in the same plasma membrane. Effects on growth control?	Permits modulation of the function of receptors without altering their number or affinity for the ligand. Role in normal biology not yet well defined.
Unknown	Neolacto- and G_{M3} gangliosides. Granulocytic or monocytic differentiation of myeloid their expression.	Exogenous addition of these gangliosides triggers the appropriate differentiation response. Role in normal biology not well defined precursors correlates with
Transcription factors, cytosolic proteins and RNA polymerase	O-linked GlcNAc residues at multiple sites.	Indirect evidence suggests that expression may be rapidly regulated inversely with phosphorylation at the same sites - a mechanism for modulation of activities?

Table VII. Maintaining local concentrations of specific molecules: Some examples of the "sink" or "depot" functions

OLIGOSACCHARIDE SEQUENCES	MOLECULES BOUND AND LOCALLY CONCENTRATED	PROPOSED BIOLOGICAL EFFECTS
Matrix and/or basement membrane heparan sulfate chains	FGFs, GM-CSF and other cytokines in cell basement membranes or intercellular matrix	Maintains local concentration and availability of growth factor to the relevant cells. Protection of factors from proteolysis. Release upon injury to endothelium stimulates angiogenesis?
Cell surface and matrix heparan sulfate chains	Superoxide dismutase	Maintenance of local concentration of the enzyme in tissues?
Secretory granule proteoglycans	Binding to other components of the granule e.g. enzymes	Permits packaging of materials into storage granules? Protects, localizes and modifies activity of bound molecules after secretion?
Chondroitin sulfate chain of Macrophage Colony Stimulating Factor (M-CSF)	Specific and selective binding of oligosaccharide to matrix Type V Collagen	Maintains high local concentration of this form of M-CSF in bone marrow matrix and other sites of action?
Cell surface sialic acids	Facilitates high local binding and retention of complement regulatory protein H on cell surfaces	Prevents activation of the alternate pathway of complement on homologous surfaces (requires unmodified sialic acid side chain)
Sialic acid residues of Fibrinogen	Calcium ("low affinity" binding site)	At physiological Ca^{++} concentrations, facilitates proper fibrin polymerization
Surface sialic acids on synaptosomes	Sodium?	Removal of sialic acid markedly changes kinetics of Na^+ dependant uptake of the Gamma-aminobutyric acid and D-aspartate
Pig Gastric Mucus (major component is gastric mucin, a glycoprotein with many O-linked sialylated and sulfated chains.	Water and Bicarbonate Ions	Bicarbonate ions secreted by the gastric epithelium are trapped in the mucus gel, establishing a gradient from pH 1-2 at the lumen to pH 6-7 at the cell surface.

Biological Roles of Oligosaccharides

Table VIII. Intra- and inter-cellular trafficking of proteins: Some examples of 'targetting' and 'clearance' functions

OLIGOSACCHARIDE SEQUENCES	LECTIN	PROPOSED ROLE IN PROTEIN TRAFFICKING RECOGNIZED
Phosphorylated high-mannose-type oligosaccharides on lysosomal enzymes & other proteins	Cation-independent M6P Receptor. Cation-dependent M6P Receptor.	Trafficking of newly synthesized lysosomal enzymes to lysosomes. Salvage of secreted phosphorylated enzymes?
Exposed terminal β -Gal residues on mammalian plasma proteins	Asialoglycoprotein Receptor.	Clearance from circulation: determinant of half-life? Abnormalities in liver diseases, but significance unproven in normal.
Exposed terminal β -GlcNAc residues on avian plasma proteins	Chicken Hepatic lectin.	Clearance from circulation: determinant of half-life?
Terminal 4-O-sulfated β -GalNAc residues on glycoprotein hormones	Specific Receptor in the Non-parenchymal Liver cells.	Rapid clearance from circulation: determinant of short half-life?
Mannose-rich oligosaccharides of endogenous and exogenous origin	Macrophage Man/Fuc Receptor.	Clearance of proteins from circulation? 'lectinophagocytosis' of pathogens?
Gal/GalNAc terminated oligosaccharides of endogenous and exogenous origin	Macrophage Gal/GalNAc Receptor.	Clearance of proteins from circulation? 'lectinophagocytosis' of pathogens?
N-linked high mannose-type oligosaccharides of endogenous and exogenous origin	Circulating 'Core-specific' lectin.	Opsonization of pathogens, allowing complement activation and phagocytosis. Clearance of proteins from circulation

Glycosylation can, therefore, be a general mechanism for generating important functional diversity while utilizing a limited set of receptor-ligand interactions.

The 'sink' or 'depot' function of oligosaccharides.

Another recently appreciated function of oligosaccharides appears to be that of acting as a protective storage depot for certain biologically important molecules (see Table VII). It has long been known that a variety of growth factors could be purified by affinity chromatography on immobilized glycosaminoglycan chains such as heparin. It now appears that the specific growth factors may bind tightly and specifically to certain glycosaminoglycan chains *in vivo*. This serves to localize the growth factors in question to the extra-cellular matrix surrounding the cells that need to be stimulated, and prevents diffusion of the factors to distal sites. There is also evidence that such a 'sink' can protect the growth factors from non-specific proteolysis, thus prolonging their active lives. A similar role may be played by the glycosaminoglycan chains found in secretory granules that appear to bind and protect protein contents after secretion, and to modulate their subsequent functions. As indicated in Table VII, there are other classic examples where oligosaccharides may act as 'sinks' or 'depots' for a variety of biological important molecules, ranging from water to complement regulatory proteins.

'Intra-cellular trafficking' functions of oligosaccharides'.

The best understood example is the role of mannose-6 phosphate residues in targeting newly synthesized lysosomal enzymes to their final destination in the lysosomes (Table VIII). In this case, the human disease states in which phosphorylation is deficient (I-cell disease and pseudo-Hurler polydystrophy) are characterized by a failure of lysosomal enzyme targeting in several cell types. This provides conclusive evidence for the importance of the oligosaccharide modification in mediating this pathway. However, even this elegantly precise function of oligosaccharides appears to have its exceptions: mannose-6-phosphate is not absolutely required for lysosomal enzymes trafficking in some lower eukaryotes, nor is it essential in certain cell types in mammals. As indicated in

Table VIII, many other endocytic receptors that recognize specific carbohydrate sequences have been described. However, any role in intra-cellular trafficking (i.e. during the biosynthesis of glycoconjugates) has yet to be defined.

The role of oligosaccharides in regulating the 'clearance' or 'turn-over' of proteins and whole cells: 'inter-cellular trafficking'.

The effects of glycosylation on the susceptibility of proteins to proteolysis has been mentioned, and can affect their turn-over and half-life in the single cell. In the intact animal, recognition of oligosaccharide sequences by certain receptors can result in removal of the glycoconjugate or even the whole cell from the circulation. There are several well-documented examples of such 'inter-cellular trafficking' receptors (Table VIII). Many of these interactions are highly specific for the oligosaccharide sequences recognized, suggesting that their biological roles are equally specific. While rational theories have been put forward to explain the functions of many of these clearance pathways (Table VIII), the lack of naturally occurring mutants in these receptors in an intact animal makes it difficult to obtain definitive proof.

'Hormonal' actions of oligosaccharides.

It is now recognized that free oligosaccharides can themselves have biological effects in various systems, thus acting like hormones. The best documented examples are the 'oligosaccharins' of plants, which induce specific responses in a manner highly dependent upon the structure of the sugar chain. Likewise, free high-mannose chains can have strong immuno-suppressive effects in *in vitro* mammalian systems in a manner dependent upon the specific structure of the sugar chain. Other less-well defined oligosaccharides or glycopeptides with proposed biological effects are known (Table IX).

Role of oligosaccharides in 'cell:cell and cell:matrix recognition'.

Since all cells are covered with a dense coating of sugars, it has long been predicted that oligosaccharides must be critical determinants of 'cell-cell interactions'. In fact, there

Biological Roles of Oligosaccharides

are relatively few examples in which such functions have been clearly defined (Table X). Perhaps the best-documented example is that of the selectin family of receptor proteins that mediate the adhesion of leukocytes to endothelial cells (L-Selectin), the recognition of leukocytes by stimulated or wounded endothelium (E-Selectin), and the interactions of activated platelets or endothelium with leukocytes (P-Selectin). In each case, the minimal carbohydrate ligands involved in recognition appear to be sialylated fucosylated sugar chains, such as Sialyl Lewis^X and Sialyl Lewis^a. However, biologically relevant recognition may require specific glycoconjugates that 'present' multiple copies of such oligosaccharides in a specialized fashion, i.e. in a proper spacing on the linear polypeptide chain, or in the proper three dimensional context. We have recently suggested that oligosaccharides closely spaced together on a polypeptide might be packed tightly together, generating a 'clustered saccharide patch' for specific recognition. This would allow the generation of uncommon recognition markers utilizing common oligosaccharides. Alternatively, specific modifications of the oligosaccharides (e.g. sulfation) might create unique binding sites.

Do any general principles emerge?

The discussion so far makes it clear that the oligosaccharide units of glycoconjugates have many and varied functions. It also leads to the conclusion that while all of the theories about their functions appear to be correct, exceptions to each can also be found. A corollary of this conclusion is that it is difficult to predict *a priori* the functions of a given oligosaccharide on a given glycoconjugate. Fortunately, some other worthwhile principles do emerge from this analysis.

Temporal and spatial differences in the expression of oligosaccharides: the same structure can have multiple roles.

Expression of specific types of glycosylation on different glycoconjugates in different tissues at different times during development imply that these structures have diverse and different roles in the same organism. For example, while mannose-6-phosphate containing oligosaccharides are

clearly involved in targeting lysosomal enzymes, mannose-6-phosphate has since been found on a variety of apparently unrelated proteins, including proliferin, thyroglobulin, the EGF receptor, and the TGF- β precursor. While the significance of these observations is uncertain, they raise the possibility that mannose-6-phosphate containing oligosaccharides have other biological roles. Likewise, 9-O-acetylation of sialic acids is found on a variety of different glycoconjugates, in a variety of different tissues at different times in development.

Can the interplay between 'traitorous' and 'masking' functions result in the formation of 'junk' oligosaccharides?

The 9-O-acetylerster group that abrogates binding of the highly pathogenic influenza A viruses to sialic acids simultaneously generates a specific binding site for the less pathogenic influenza C virus and coronaviruses (see Tables IV and V). Such O-acetylated acids are frequently found on mucosal surfaces of mammals. Perhaps the mammalian organism first attempted to mask the sialic acid receptor for the highly pathogenic Influenza A virus by adding an O-acetyl group to it. However, subsequent selection could have resulted in the appearance of micro-organisms (Influenza C and coronaviruses) capable of binding specifically to the 'masking' structure.

Since micro-organisms and parasites evolved in parallel with their multicellular hosts, they may have had to constantly adapt themselves to bind to each new 'masking' structure evolved by the host. In response, the host organism may have found it most efficient to generate new masking structures, perhaps because the existing structure had already evolved a vital function elsewhere within the organism. Having committed itself to this course of action, the host would then be left with no choice but to keep the underlying 'scaffolding' upon which the latest 'mask' was placed. Thus, yet another layer of complexity would have been added to its oligosaccharides. Such cycles of interaction between micro-organisms and hosts could explain in part some of the extremely complex and extended sugar chains found on mucosal surfaces and secreted mucins. It also leads

Table IX. Biologically active oligosaccharides and glycopeptides: Some examples of 'hormonal' actions

OLIGOSACCHARIDE SEQUENCES RECOGNIZED	TARGET TISSUES OR CELLS/ COGNATE RECEPTOR	REPORTED BIOLOGICAL EFFECTS
Specific β -glucan oligosaccharide of <i>Phytophthora</i> fungal cell walls	Various plants Unknown receptor	Elicitor of production of Phytoalexins (plant antibiotics against fungi)
Exo-polysaccharide of <i>Rhizobian</i> species. Specific sulfated, acylated or fucosylated sequences	Alfalfa or Soybean Root cells Unknown receptor	Elicits Root nodulation in host: determinant of symbiotic relationships between <i>Rhizobium</i> and plants
Oligosaccharides derived from sycamore cells	Tissue culture "callus" of tobacco cells or duckweed cells. Unknown receptor	Selective induction of vegetative budding or floral growth: hypothesized to modulate organ development in the intact plant
Free Heparin/heparan chains from adjacent cells e.g. smooth muscle cells and endothelial cells	Unknown receptor in nucleus? Eventually works via c-fos and c-myc in endothelial cells.	Inhibition of cell growth.
Sialylated fucosylated glycopeptide from brain cells	Various animal cell types Receptor of 150Kda?	Growth inhibition of a variety of cell types.
A sialoglycopeptide circulating in with sepsis or trauma	Unknown	Induces endogenous proteolysis in rat muscle, similar to that patients occurring in patients

Biological Roles of Oligosaccharides

to the possibility that 'junk' oligosaccharides do exist akin to 'junk' DNA. While serving a general (and important) function as a scaffolding, they may have no other specific definable role.

Inter-species variations in glycosylation.

The existence of species-specific variations in glycoconjugate structure indicates that some oligosaccharides do not play fundamental and universal roles in all biological systems (Table XII). For example, when the N-linked glycosylation on a conserved protein such as gamma glutamyl transpeptidase was examined from a variety of species, marked differences were seen in the sequence of the sugar chains. Such variations between species in the glycosylation of similar proteins or cells implies that these sugar chains cannot be crucial for the basic functions of these proteins or cells. On the other hand, such diversity in glycosylation could certainly be involved in generating the many obvious differences in morphology and function that are observed between species. Such differences could also reflect differing selection pressures resulting from different pathogens that infect the different species (see below).

Intra-species variations in glycosylation.

Genetic polymorphisms with no known biological consequence are quite common in proteins. For example, in Sweden alone, at least 9 different albumin variants

were found, with a combined prevalence of 1:1700 in the population. The genetic polymorphisms in glycosylation that are recognized as 'blood-groups' also have somewhat limited consequences for the normal biology of humans. While substantial intra-species polymorphism in oligosaccharide structure can exist without obvious reason in laboratory animals, this may be of more importance in the wild state where protection against certain microorganisms or toxins could prove to be of survival value to individuals expressing a specific oligosaccharide structure (Table XII).

Terminal sequences, unusual structures and modifications of oligosaccharides are more likely to mediate specific biological roles.

Since the biological roles of oligosaccharides can range from those that are trivial to those that are critical for the development, growth, function or survival of an organism, the challenge is to predict which are more likely to mediate specific or crucial biological roles. Review of the matter suggests that terminal sugar sequences, unusual structures, or modifications of the oligosaccharides are more likely to be involved in such specific roles (Table XI). For example, the high-mannose oligosaccharide structures of lysosomal enzymes are identical to those found on a wide variety of proteins, ranging from the immunoglobulin IgM in mammals to the lectin soy-bean agglutinin in plants. However, the addition of phosphomonoester residues to one

Table X. Lectin-carbohydrate and carbohydrate-carbohydrate interactions: Some examples of 'cell:cell and cell:matrix recognition'

LECTIN RECOGNIZED	OLIGOSACCHARIDE SEQUENCES	PROPOSED BIOLOGICAL FUNCTIONS
E-Selectin (ELAM-1)	Sialylated fucosylated polylectosaminoglycans on specific leukocyte glycoconjugates?	Primary adhesion of granulocytes, monocytes and certain lymphocytes to acutely or chronically inflamed endothelium.
P-Selectin (GMP-140 / PADGEM/ CD62)	Sialylated fucosylated polylectosaminoglycans (on a specific myeloid glycoprotein?) (sulfated ligands as well?)	Primary adhesion of granulocytes, monocytes and certain lymphocytes to activated platelets or endothelium.
L-Selectin (LAM-1, MEL-14 antigen, LHR)	Sialylated, fucosylated, sulfated oligosaccharides (on specific glycoproteins?) (other sulfated ligands as well?)	Adhesion of granulocytes to inflamed endothelium and of naive lymphocytes to endothelium of high-endothelial venules: a determinant of lymphocyte trafficking
CD44 (Hermes, Pgp-1)	Hyaluronic Acid	Adhesion of many cell types to endothelial cells and matrix. Additional determinant of lymphocyte trafficking
CD22 β Lectin of B-lymphocytes	α 2-6 linked sialic acids on lactosamine units of specific glycoproteins e.g. CD45	Early step in interactions of B-cells with activated T-cells or activated B-cells. Cross-linking of CD45?
Macrophage sialic acid-specific receptor (sialoadhesin)	Sialic acids on surface glycoconjugates, in the sequence Sia α 2-3Gal β 1-3GalNAc.	Interaction of bone marrow macrophages with hematopoietic precursors and lymph node macrophages with lymphocytes?
Unknown, on mammalian sperm	α -linked Gal residues on O-linked oligosaccharide of egg protein ZP3	Sperm-egg recognition. Inducer of acrosome reaction? Competing theory with surface β -galactosyltransferase
Cell surface/soluble β -galactoside-binding of cell types	β -galactoside and / or polylectosamine residues on other cells or in the matrix.	Implicated in cell-cell and cell-matrix interactionslectins in a variety in development and tissue organization. Also may serve as receptors for certain immunoglobulins. Role in tumor metastasis?
Cerebellar soluble lectin	31Kda endogenous glycoprotein ligand?	Mediates contact guidance of neuron migration by astrocytes?
Extracellular matrix proteins (e.g. laminin, fibronectin, thrombospondin)	Binding to heparan sulfate chains on cell surface proteoglycans.	Role in mediating cell adhesion, differentiation, spreading or invasion.
Unknown receptor in epithelium of sandfly midgut	Lipophosphoglycan of <i>Leishmania</i> only in non-infective stage promastigotes	Allow stage-specific adhesion of non-infective stage promastigotes and selective release of infective promastigotes
Gal-specific lectin of <i>Bradyrhizobium japonicum</i>	Unknown ligand on plant cells	Facilitates symbiotic interactions?
CARBOHYDRATE 1	CARBOHYDRATE 2	
Multimillion Molecular weight aggregation factor (MAF)	MAF	Mediates cell-cell recognition in the sponge <i>Microciona prolifera</i> .
<i>B. Fragilis</i> capsular polysaccharide A Lewis ^x structure	<i>B. Fragilis</i> capsular polysaccharide B Lewis ^x structure	Strong ionic interactions create a complex, aggregated polymer – a virulence factor? mediates compaction of the morula-stage embryo

Biological Roles of Oligosaccharides

Table XI. Unique or unusual types of glycosylation more frequently mediate specific biological roles. some examples

MOLECULE(S) MODIFIED GLYCOSYLATION	UNUSUAL OR UNIQUE TYPE OF	PROPOSED BIOLOGICAL ROLE(S)
Lysosomal Enzymes	Mannose 6-phosphate on high mannose-type oligosaccharides.	Intracellular trafficking of enzymes by binding to mannose 6-phosphate receptors.
Neural Cell Adhesion Molecule (N-CAM)	Polysialic acid	Inhibition of homotypic binding between N-CAM molecules and general inhibition of intercellular adhesion involving other binding systems. Positive effects also?
Endothelial and mast-cell heparan sulfate proteoglycans	3-O-sulfation of selected GlcNAc residues in specific pentasaccharides.	Antithrombin-III binding and activation, resulting in anticoagulation.
Extracellular matrix dermatan sulfate proteoglycans	4-O-S-GalNAc and 2-O-S-GIA residues in three sequential disaccharide repeats	Heparin Co-factor II binding and activation, resulting in anticoagulation
Cell surface and matrix Heparan sulfate	2-O-S-IdoA residues within defined sequences	Required for high affinity binding of bFGF to its cell surface receptor
Pituitary glycoprotein hormones	GalNAc 4-SO ₄ terminating antennae of N-linked oligosaccharides.	Effects upon turnover, plasma half-life and bioactivity of the hormones.
<i>Rhizobium</i> polysaccharides	Sulfation and acylation of β 1-4 linked glucosamine residues	Symbiotic host-specificity: elicitation of root hair deformation and nodulation
Several neural cell adhesion molecules glucuronic acid residues.	HNK-1 epitope/L1 epitope: sulfated	Some evidence for direct involvement in cell:cell adhesion.

or two of the mannose units results in the generation of the highly specific phosphomannosyl recognition marker that dictates trafficking of the enzyme to the lysosome.

Unusual oligosaccharides or modifications are also more likely to arise from interactions with micro-organisms and other noxious agents.

While terminal or outer sugars and their modifications may be more involved in specific biological roles within the organism, they are also most likely to vary as a result of host-pathogen interactions. However, the two functions need not be mutually exclusive. For example, it is possible that while O-acetylation of sialic acids on mucosal surfaces may play a protective role in host-microbial interaction, the temporal and spatial gradients of expression of O-acetylation found in the developing nervous system may play important roles in the process of development in the brain. The challenge then is to predict and sort out which of these two completely distinct roles are to be assigned to a given oligosaccharide structure in a given tissue.

In some cases of sporadic autoimmune reactions to oligosaccharides, the antigenic structures are normally present in adult tissues. However, there are examples of oligosaccharide structures which, when expressed post-natally by the organism, result universally in an immune response. The best examples in humans are the conversion of N-acetyl-neuraminic acid to N-glycolyl-neuraminic acid, and the expression of Gal α 1-3 Gal sequences (Table XI). In these cases, the structures are not expressed in normal adults, but can appear in disease states such as cancer, causing immune reactions due to newly-induced or pre-existing antibodies. In at least one case (N-glycolyl-neuraminic acid), expression actually does occur in the normal fetus, but is then suppressed post-natally in the normal adult. While the oligosaccharides in question must have no normal functions in the adult, it is likely that their expression in the fetus is required, and is a case of ontogeny recapitulating phylogeny.

Is there a common theme to the varied functions of oligosaccharides?

The evidence reviewed here indicates that, while all of the diverse theories regarding oligosaccharides functions are correct, exceptions to almost every theory can also be found. In the final analysis, the only common feature of all of these functions is that they either mediate 'specific recognition' events, or that they provide 'modulation' of biological processes. In so doing, they help to generate the functional diversity required for the evolution and development of different types of cells, tissues, organs and species. There is a limited number of genes available in the genome for the generation of diversity. Thus, it should not be surprising that an oligosaccharide structure resulting from the action of a single gene product could be utilized to generate a wide variety of functions in different tissues at different times in the life cycle of the organism. However, even complete knowledge about the structure, biosynthesis and expression of a particular type of structure does not necessarily give us clues to its specific functions. The challenge is to design experiments to differentiate between the trivial and crucial functions mediated by a given oligosaccharide.

Approaches to uncovering specific biological roles of oligosaccharides.

Some functions of oligosaccharides are discovered serendipitously. In most cases, the investigator who has elucidated complete details of the structure and biosynthesis of a specific oligosaccharide is still left without knowing its functions. If it is possible to make educated guesses about the role of the oligosaccharide in question, this can sometimes lead to a definitive experiments. However, conclusive proof of the biological roles of an oligosaccharide sequence often requires analysis of mutants that are defective in such a structure. It is therefore useful to consider the lessons that have been learned to date by studying such mutants.

Biological Roles of Oligosaccharides

Genetic or acquired defects in glycosylation are easily obtained in cultured cells, but have somewhat limited consequences.

Tissue culture cell lines with mutations in a variety of specific steps in the biosynthesis of N-linked oligosaccharides, glycosaminoglycans, O-linked oligosaccharides and glycosphospholipid anchors have been obtained, including some with defects in very early steps in the biosynthetic pathways. Mutants affecting the biosynthesis of dolichol sugars, sugar nucleotides or sugar nucleotide transport into the Golgi apparatus have also been obtained, and have pleiotropic effects on the biosynthesis of multiple types of glycoconjugates in the same cell. Likewise, cell lines can be grown in the presence of global inhibitors of the biosynthesis and processing of several types of oligosaccharides. In most cases, the abnormalities in glycosylation seem to have limited consequences to the growth and maintenance of these cells. This suggests that many (though not all) aspects of glycosylation are of limited importance in the day-to-day housekeeping functions of the single cell, when it is in a protected environment, under optimal conditions of growth. However, some of these mutants do show alterations in density-dependent growth inhibition, and others

demonstrate changes in tumorigenicity or metastatic behavior when injected into athymic mice. This suggests that many of the more specific biological roles of oligosaccharides need to be uncovered by studying mutations in the intact multicellular organism.

Genetic defects in glycosylation are rare in intact organisms, but have highly variable consequences.

In contrast to the situation *in vitro*, genetic defects in glycosylation are surprisingly rare in intact organisms. There are few other biochemical pathways in which naturally-occurring mutants in mouse and man are so uncommon. In the few instances in which glycosylation mutants have been observed in intact complex multicellular organisms, the consequences have been highly variable (Table XII). In humans, the effects of genetically altered glycosylation range from severe lethal diseases such as I-cell disease to apparently unremarkable consequences such as the ABO blood group polymorphisms. A likely possibility is that the great majority of these mutants cause lethal aberrations that prevent completion of embryogenesis. Another possibility is that mutations in glycosylation remain undetected because of alternate or 'fail-safe' mechanisms that ensure that vital biological functions are carried out by more than one pathway.

Table XII. Genetic defects or polymorphisms in glycosylation are uncommon in higher animals, but have variable consequences. Some examples

GENETIC DEFECT/VARIATION	BASIC DEFECT IN GLYCOSYLATION	BIOLOGICAL CONSEQUENCE(S)
Partial or complete deficiency of Lysosomal Enzyme: UDP-GlcNAc GlcNAc-6-phosphotransferase Partial deficiency of xylosylprotein 4-β Galactosyltransferase ?Decreased conversion of GDP-fucose to GDP-mannose (General fucose deficiency)	Partial or complete failure of phosphorylation of mannose residues on lysosomal enzymes. Decreased production of glycosaminoglycan chains with core Galβ1-4Xyl linkage. Low levels of GDP-fucose, low levels of fucose on all glycoconjugates.	Partial or complete failure of lysosomal enzyme trafficking in I-cell disease (Mucopolidosis II) and Pseudo-Hurler polydystrophy (MLIII). Progeroid syndrome with delayed mental development, and multiple connective tissue abnormalities. Leukocyte Adhesion Deficiency Type II. Low fucose on many glycoconjugates results in lack of ligands for selectins, and poor leukocyte adhesion. Bombay Blood group type. Infections, short stature, mental abnormalities, skeletal defects. Paroxysmal Nocturnal Hemoglobinuria (PNH): Failure of GPI anchoring /secretion of several cell surface proteins on blood cells – hemolytic anemia, increased infections, progression to malignancy
Defects at multiple steps in assembly of the glycosphospholipid (GPI) anchor precursor (?acquired genetic defect in hematopoietic stem cell) Mosaic deficiency of UDP-Galactose: GalNAc 3-β galactosyltransferase affecting a subset of cells (usually acquired) Hereditary opsonic defect	Lack of pre-formed donor for GPI-anchoring of proteins Decrease in Galβ1-3GalNAc of O-linked oligosaccharides. Increase in GalNAc-O-Ser (Tn antigen). Point mutation in serum mannose-binding protein Point mutation creates a new N-linked glycosylation site.	Polyagglutinability of red cells by all normal human sera: variable hemolytic anemia. Can be seen as a precursor to leukemias. Heterozygous state causes low opsonization of pathogens. Increased infections in childhood Decreased function of Factor VIII, leading to bleeding disorder.
Hemophilia A variant	Additional glycosylation site created by three base deletion	Type II Hereditary Angioneurotic Edema
C1 inhibitor Ta	New glycosylation site and altered signal peptidase cleavage	No obvious phenotype(?)
Albumin Redhill	Marked decrease of Galα1-3 Galβ1-4 GlcNAc sequences terminating glycoprotein and glycolipid oligosaccharides.	No obvious abnormality results. All humans have a natural antibody (up to 1% of circulating IgG) against Galα1-3Galβ1-4GlcNAc sequences.
Deficiency of UDP-Gal: 3-α Galactosyltransferase (in humans, apes and old world monkeys) Polymorphic expression of active or null alleles for UDP-Gal: H-precursor 3-α Galactosyltransferase (B-enzyme) and UDP-GalNAc: H-precursor 3-α N-acetylgalactosaminyltransferase (A enzyme) Primary enzymatic basis not fully defined	Polymorphism expression of A- and B- and O-blood groups structures terminating glycoprotein and glycolipid oligosaccharides. Polymorphic expression of N-acetyl and N-glycolyl-neuraminic acid on the erythrocyte gangliosides of dogs and cats.	No obvious abnormality results. Humans have natural antibodies against the blood groups sequences that they do not express. No grossly obvious consequences in dogs. Possibly related to the geographic co-migration of dogs with humans, and subsequent breeding patterns. In cats, this accounts for a major blood group system.

Note: unless otherwise stated, the defects reported in this Table were found in humans.

Biological Roles of Oligosaccharides

Table XIII. Altered oligosaccharides in diseases without a known primary defect in glycosylation. Some examples

GLYCOCONJUGATE(S) AFFECTED	CHANGE IN OLIGOSACCHARIDES	BIOLOGICAL EFFECT(S)
Plasma fibrinogen in hepatoma and in congenital dysfibrinogenemias	Increased branching or number of N-linked oligosaccharides and increased sialic acid content.	Prolonged thrombin time and reptilase time. Inhibition of coagulation.
Plasma membrane and secreted proteins in Cystic Fibrosis	Generalised increase in fucosylation and sulfation.	? contribute to change in physical properties of secreted glycoproteins.
CD43 (leukosialin, sialophorin) in Wiskott-Aldrich syndrome	Altered branching of O-linked oligosaccharides.	Decreased expression (due to altered glycosylation?).
Serum IgG immunoglobulin	Decreased galactosylation of N-linked oligosaccharides.	A general feature of many chronic granulomatous diseases (Rheumatoid arthritis, Crohn's disease, tuberculosis, etc.)
Several plasma proteins	Abnormal N-linked glycosylation of some glycoproteins ?Primary or secondary defect in glycosylation.	'Carbohydrate Deficient Glycoprotein Syndrome'. Growth abnormalities, characteristic fat accumulations, abnormal electrophoretic mobility of certain serum glycoproteins, due to ?altered glycosylation.
Dolichol oligosaccharides	Altered Processing and accumulation of Dolichol-linked mannosyl-oligosaccharides.	Neuronal Ceroid-lipofuscinosis ?Primary or secondary defect in humans, dogs and sheep.

Note: unless otherwise stated, the defects reported in this Table were found in humans.

Creating mutants in glycosylation in intact organisms: a challenge for the future.

To explore these issues, it appears necessary to create mutants in glycosylation in intact animals. Several possible approaches could be taken towards this goal. Antibodies or lectins specific for certain oligosaccharide sequences could be expressed in transgenic animals or injected into specific developing tissues. However, since such molecules are multivalent, they may disrupt development or other functions simply by causing unwanted cell-cell adhesion. Alternatively, the molecular cloning of glycosyltransferases allows overexpression, or the creation of 'knockout' mice lacking a specific sugar sequence. However, the consequences could be lethal.

An alternate approach uses the fact that many microbial degradative enzymes are highly specific for certain outer sugar chain sequences. Thus, direct injection of specific endoneuraminidase into developing neural tissues yielded dramatic phenotypic changes, suggesting specific roles for polysialic acids. Expression in transgenic mice of a viral sialic acid-specific 9-O-acetyltransferase under the control of specific promoters caused abnormalities either early or late in development. In principle, the latter approach could be generalized to any situation where a cDNA is available encoding a specific oligosaccharide-degrading enzyme.

Future Prospects.

The future now appears bright for the understanding of many new biological roles of oligosaccharides. Until recently, mainstream research was focused either upon the molecular biology of the single cell, or upon the physiology of whole organs or organisms. In both these disparate areas, the roles of oligosaccharides tend to be less prominent, and can often be ignored or bypassed. However, the future of biology and biotechnology now lies in studies of cell-cell interactions, embryonic development, tissue organization and morphogenesis, and in the integration of these studies with the molecular physiology and pharmacology of organs and organisms. In these arenas, the biological roles of oligosaccharides seem to be critical, and their under-

standing becomes crucial to further progress.

MONOGRAPHS AND REVIEWS

1. Gottschalk, A. (1972) Glycoproteins: Their Composition, Structure and Function. Elsevier Publ. Co., New York.
2. Horowitz, M. and Pigman, W. (1982) The Glycoconjugates. Academic Press, New York.
3. Rosenberg, A. and Schengrund, C.-L. (1976) Biological Roles of Sialic Acid. Plenum Press, New York.
4. Sweeley, C. C. (1979) Cell Surface Glycolipids. ACS, Washington, D.C.
5. Lennarz, W. J. (1980) The Biochemistry of Glycoproteins and Proteoglycans, Plenum Press, New York.
6. Ginsburg, V. and Robbins, P. (1981) Biology of Carbohydrates, Vol 1, J. Wiley, New York.
7. Schauer, R. (1982) Sialic Acids, Chemistry, Metabolism, and Function, Springer-Verlag, New York.
8. Ivatt, R. J. (1984) The Biology of Glycoproteins, Plenum Press, New York.
9. Ginsburg, V. and Robbins, P. (1985) Biology of Carbohydrates, vol 2, J. Wiley, New York.
10. Liener, I. E., Sharon, N., Goldstein, I. J. (1986) The Lectins: Properties, Functions, and Applications in biology and Medicine, Academic Press, Orlando.
11. Margolis, R. U. and Margolis, R. K. (1989) Neurobiology of Glycoconjugates, Plenum Press, New York.
12. Bock, G. and Harnett, S. (1989) Carbohydrate Recognition in Cellular Function. Ciba Foundation symposium No.145, Wiley, New York.
13. Sharon, N. and Lis, H. (1989) Lectins, Chapman and Hall, London.
14. Evered, D. and Whelan, J. (1989) The Biology of hyaluronan. Ciba Foundation symposium No.143, Wiley, New York
15. Ginsburg, V. and Robbins, P. (1991) Biology of Carbohydrates, Vol 3, J. Wiley, New York.
16. Fukuda, M. (1992) Cell Surface Carbohydrates and Cell Development, CRC Press, Boca Raton.
17. Allen, H. J. and Kisailus, E.C. (1992) Glycoconjugates: Composition, Structure, and Function, Dekker, New York.
18. Roth, J., Rutishauser, U. and Troy, F. (1992) Polysialic Acids, Birkhauser Verlag, Basel.
19. Sharon, N. and Lis, H. (1982) Glycoproteins: research booming on long-ignored ubiquitous compounds. *Mol. Cell. Biochem.* **42**, 167-187
20. Berger, E. G., Buddecke, E., Kamerling, J. P. Kobata, A., Paulson, J. C. and Vliegenthart, J. F. G. (1982) Structure, biosynthesis and functions of glycoprotein glycans. *Experientia* **38**, 1129-1162

Biological Roles of Oligosaccharides

21. Olden, K., Parent, J. B. and White, S. L. (1982) Carbohydrate moieties of glycoproteins. A re-evaluation of their function. *Biochim. Biophys. Acta* **650**, 209-232
22. Aplin, J. D. and Hughes, R. C. (1982) Complex carbohydrates of the extracellular matrix structures, interactions and biological roles. *Biochim. Biophys. Acta* **694**, 375-418
23. West, C. M. (1986) Current ideas on the significance of protein glycosylation. *Mol. Cell Biochem.* **72**, 3-20
24. Rademacher, T. W., Parekh, R. B. and Dwek, R. A. (1988) Glycobiology. *Annu. Rev. Biochem.* **57**, 785-838
25. Paulson, J. C. (1989) Glycoproteins: what are the sugar chains for. *Trends Biochem. Sci.* **14**, 272-276
26. Cumming, D. A. (1991) Glycosylation of recombinant protein therapeutics: control and functional implications. *Glycobiology* **1**, 115-130
27. Elbein, A. D. (1991) The role of N-linked oligosaccharides in glycoprotein function. *Trends Biotechnol.* **9**, 346-352
28. Drickamer, K. and Carver, J. (1992) Upwardly mobile sugars gain status as information-bearing macromolecules. *Curr. Opin. Struct. Biol.* **2**, 653-654
29. Rasmussen, J. R. (1992) Effect of glycosylation on protein function. *Curr. Opin. Struct. Biol.* **2**, 682-686
30. Stanley, P. (1992) Glycosylation engineering. *Glycobiology* **2**, 99-107
31. Fukuda, M. (1992) Function of Carbohydrate Moieties: Membrane and Nonsecretory Glycoproteins. Glycoconjugates: composition, structure, and function (Allen, H. J. and Kisailus, E. C., eds) pp. 379-402, Marcel Dekker, Inc., New York
32. Olden, K., Yeo, T., and Yeo, K. (1992) Function of the Carbohydrate Moieties of Secretory Glycoconjugates. Glycoconjugates: composition, structure, and function (Allen, H. J. and Kisailus, E. C., eds) pp. 403-420, Marcel Dekker, Inc., New York
33. Cummings, R. D. (1992) Synthesis of Asparagine-linked Oligosaccharides: Pathways, Genetics, and Metabolic Regulation. Glycoconjugates: composition, structure, and function (Allen, H. J. and Kisailus, E. C., eds) pp. 333-360, Marcel Dekker, Inc., New York
34. Kobata, A. (1991) Function and pathology of the sugar chains of human immunoglobulin G. *Glycobiology* **1**, 5-8
35. Kobata, A. (1992) Structures and functions of the sugar chains of glycoproteins. *Eur. J. Biochem.* **209**, 483-501
36. Hart, G. W. (1992) Glycosylation. *Curr. Opin. Cell Biol.* **4**, 1017-1023
37. Devine, P. L. and McKenzie, I. F. C. (1992) Mucins: Structure, function, and associations with malignancy. *BioEssays* **14**, 619-625
38. Carraway, K. L. and Hull, S. R. (1991) Cell surface mucin-type glycoproteins and mucin-like domains. *Glycobiology* **1**, 131-138
39. Jentoft, N. (1990) Why are proteins O-glycosylated? *TIBS* **15**, 291-294
40. Hilkens, J., Ligtenberg, M. J. L., Vos, H. L. and Litvinov, S. V. (1992) Cell membrane-associated mucins and their adhesion-modulating property. *Trends Biochem. Sci.* **17**, 359-363
41. Rose, M. C. (1992) Mucins: Structure, function, and role in pulmonary diseases. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **263**, L413-L429
42. Carraway, K. L., Fregien, N., Carraway, K. L., III and Carraway, C. A. C. (1992) Tumor sialomucin complexes as tumor antigens and modulators of cellular interactions and proliferation. *J. Cell Sci.* **103**, 299-307
43. Hakomori, S. (1981) Glycosphingolipids in cellular interaction, differentiation, and oncogenesis. *Annu. Rev. Biochem.* **50**, 733-764
44. Hakomori, S. (1986) Tumor associated glycolipid antigens, their metabolism and organization. *Chem. Phys. Lipids* **42**, 209-233
45. Schengrund, C. -L. (1990) The role(s) of gangliosides in neural differentiation and repair: A perspective. *Brain Res. Bull.* **24**, 131-141
46. Hannun, Y. A. and Bell, R. M. (1989) Functions of sphingolipids and sphingolipid breakdown products in cellular regulation. *Science* **243**, 500-507
47. Hakomori, S. (1990) Bifunctional role of glycosphingolipids. Modulators for transmembrane signaling and mediators for cellular interactions. *J. Biol. Chem.* **265**, 18713-18716
48. Shayman, J. A. and Radin, N. S. (1991) Structure and function of renal glycosphingolipids. *Am. J. Physiol. Renal, Fluid Electrolyte Physiol.* **260**, F291-F302
49. Zeller, C. B. and Marchase, R. B. (1992) Gangliosides as modulators of cell function. *Am. J. Physiol. Cell Physiol.* **262**, C1341-C1355
50. Schnaar, R. L. (1991) Glycosphingolipids in cell surface recognition. *Glycobiology* **1**, 477-485
51. Marcus, D. M. (1984) A review of the immunogenic and immunomodulatory properties of glycosphingolipids. *Mol. Immunol.* **21**, 1083-1091
52. Saito, M. (1989) Bioactive sialoglycosphingolipids (gangliosides): Potent differentiation-inducers for human myelogenous leukemia cells. *Dev. Growth Diff.* **31**, 509-522
53. Hook, M., Kjellen, L. and Johansson, S. (1984) Cell-surface glycosaminoglycans. *Annu. Rev. Biochem.* **53**, 847-869
54. Ruoslahti, E. (1988) Structure and biology of proteoglycans. *Annu. Rev. Cell Biol.* **4**, 229-255
55. Burgess, W. H. and Maciag, T. (1989) The heparin-binding (fibroblast) growth factor family of proteins. *Annu. Rev. Biochem.* **58**, 575-606
56. Ruoslahti, E. (1989) Proteoglycans in cell regulation. *J. Biol. Chem.* **264**, 13369-13372
57. Ruoslahti, E. and Yamaguchi, Y. (1991) Proteoglycans as modulators of growth factor activities. *Cell* **64**, 867-869
58. Kjellén, L. and Lindahl, U. (1991) Proteoglycans: Structures and interactions. *Annu. Rev. Biochem.* **60**, 443-475
59. Jackson, R. L., Busch, S. J. and Cardin, A. D. (1991) Glycosaminoglycans: Molecular properties, protein interactions, and role in physiological processes. *Physiol. Rev.* **71**, 481-539
60. Klagsbrun, M. and D'Amore, P. A. (1991) Regulators of angiogenesis. *Annu. Rev. Physiol.* **53**, 217-239
61. Hardingham, T. E. and Fosang, A. J. (1992) Proteoglycans: Many forms and many functions. *FASEB J.* **6**, 861-870
62. Gallagher, J. T., Turnbull, J. E. and Lyon, M. (1992) Patterns of sulphation in heparan sulphate: Polymorphism based on a common structural theme. *Int. J. Biochem.* **24**, 553-560
63. Yanagishita, M. and Hascall, V. C. (1992) Cell surface heparan sulfate proteoglycans. *J. Biol. Chem.* **267**, 9451-9454
64. Rifkin, D. B. and Moscatelli, D. (1989) Recent developments in the cell biology of basic fibroblast growth factor. *J. Cell Biol.* **109**, 1-6
65. Bhavanandan, V. P. and Davidson, E. A. (1992) Proteoglycans: Structure, Synthesis, Function. Glycoconjugates: composition, structure, and function (Allen, H. J. and Kisailus, E. C., eds) pp. 167-202, Marcel Dekker, Inc., New York
66. Wight, T. N., Kinsella, M. G. and Qwarnström, E. E. (1992) The role of proteoglycans in cell adhesion, migration and proliferation. *Curr. Opin. Cell Biol.* **4**, 793-801
67. Laurent, T. C. and Fraser, J. R. E. (1992) Hyaluronan. *FASEB J.* **6**, 2397-2404
68. David, G. (1992) Structural and functional diversity of the heparan sulfate proteoglycans. *Adv. Exp. Med. Biol.* **313**, 69-78
69. Zhou, F., Höök, T., Thompson, J. A. and Höök, M. (1992) Heparin protein interactions. *Adv. Exp. Med. Biol.* **313**, 141-153
70. Hascall, V. (1981) Proteoglycans: Structure and Function In Ginsburg, V. and Robbins, P. (eds) *Biology of Carbohydrates*, Vol 1, J. Wiley, New York.
71. Esko, J. D. (1991) Genetic analysis of proteoglycan structure, function and metabolism. *Curr. Opin. Cell Biol.* **3**, 805-816

Biological Roles of Oligosaccharides

72. Gallagher, J. T. and Turnbull, J. E. (1992) Heparan sulphate in the binding and activation of basic fibroblast growth factor. *Glycobiology* **2**, 523-528
73. Schauer, R. (1985) Sialic acids and their role as biological masks. *Trends Biochem. Sci.* **10**, 357-360
74. Schauer, R. (1991) Biosynthesis and function of N- and O-substituted sialic acids. *Glycobiology* **1**, 449-452
75. Troy, F.A., II (1992) Polysialylation: From bacteria to brains. *Glycobiology* **2**, 5-23
76. Varki, A. (1992) Diversity in the sialic acids. *Glycobiology* **2**, 25-40
77. Corfield, T. (1992) Bacterial sialidases—Roles in pathogenicity and nutrition. *Glycobiology* **2**, 509-521
78. Low, M. G. (1989) Glycosyl-phosphatidylinositol: A versatile anchor for cell surface proteins. *FASEB J.* **3**, 1600-1608
79. Lisanti, M. P. and Rodriguez-Boulant, E. (1990) Glycophospholipid membrane anchoring provides clues to the mechanism of protein sorting in polarized epithelial cells. *TIBS* **15**, 113-118
80. Lisanti, M. P., Rodriguez-Boulant, E. and Saltiel, A. R. (1990) Emerging functional roles for the glycosyl-phosphatidylinositol membrane protein anchor. *J. Membr. Biol.* **117**, 1-10
81. Cross, G. A. M. (1990) Glycolipid anchoring of plasma membrane proteins. *Annu. Rev. Cell Biol.* **6**, 1-39
82. Ferguson, M. A. J. (1992) Lipid anchors on membrane proteins. *Curr. Opin. Struct. Biol.* **1**, 522-529
83. Yednock, T. A. and Rosen, S. D. (1989) Lymphocyte homing. *Adv. Immunol.* **44**, 313-378
84. Brandley, B. K., Swiedler, S. J. and Robbins, P. W. (1990) Carbohydrate ligands of the LEC cell adhesion molecules. *Cell* **63**, 861-863
85. Bradbury, M. G. and Parish, C. R. (1991) Characterization of lymphocyte receptors for glycosaminoglycans. *Immunology* **72**, 231-238
86. Feizi, T. (1991) Carbohydrate differentiation antigens: Probable ligands for cell adhesion molecules. *TIBS* **16**, 84-86
87. Picker, L. J. and Butcher, E. C. (1992) Physiological and molecular mechanisms of lymphocyte homing. *Annu. Rev. Immunol.* **10**, 561-591
88. Stoolman, L. M. (1992) Selectins (LEC-CAMs): lectin-like receptors involved in lymphocyte recirculation and leukocyte recruitment. Cell surface carbohydrates and cell development (Fukuda, M., ed) pp. 71-98, CRC Press Inc., Boca Raton
89. Varki, A. (1992) Selectins and other mammalian sialic acid-binding lectins. *Curr. Opin. Cell Biol.* **4**, 257-266
90. Feizi, T. (1992) Cell-cell adhesion and membrane glycosylation. *Curr. Opin. Struct. Biol.* **1**, 766-770
91. McEver, R. P. (1992) Leukocyte-endothelial cell interactions. *Curr. Opin. Cell Biol.* **4**, 840-849
92. Lasky, L. A. (1992) Selectins: Interpreters of cell-specific carbohydrate information during inflammation. *Science* **258**, 964-969
93. Bevilacqua, M. P. and Nelson, R. M. Selectins. *J. Clin. Invest.* (1993) **91**, 379-387
94. Hynes, M. A., Dodd, J., and Jessel, T. M. (1989) Carbohydrate Recognition, Cell Interactions and Vertebrate Neural Development. In Margolis, R. U. and Margolis, R. K. (eds) *Neurobiology of Glycoconjugates*, Plenum Press, New York.
95. Sharon, N. and Lis, H. (1989) Lectins as cell recognition molecules. *Science* **246**, 227-234
96. Barondes, S. H. (1988) Bifunctional properties of lectins: Lectins redefined. *TIBS* **13**, 480-482
97. Stahl, P. D. (1992) The mannose receptor and other macrophage lectins. *Curr. Opin. Immunol.* **4**, 49-52
98. Zhou, Q. and Cummings, R. D. (1992) Animal lectins: a distinct group of carbohydrate binding proteins involved in cell adhesion, molecular recognition, and development. Cell surface carbohydrates and cell development (Fukuda, M., ed) pp. 99-126, CRC Press Inc., Boca Raton
99. Zanetta, J. -P., Kuchler, S., Lehmann, S., Badache, A., Maschke, S., Marschal, P., Dufourcq, P. and Vincendon, G. (1992) Cerebellar lectins. *Int. Rev. Cytol.* **135**, 123-154
100. Zanetta, J. -P., Kuchler, S., Lehmann, S., Badache, A., Maschke, S., Thomas, D., Dufourcq, P. and Vincendon, G. (1992) Glycoproteins and lectins in cell adhesion and cell recognition processes. *Histochem. J.* **24**, 791-804
101. McCoy, J. P. J. and Chambers, W. H. (1991) Carbohydrates in the functions of natural killer cells. *Glycobiology* **1**, 321-328
102. Lee, Y. C. (1992) Biochemistry of carbohydrate-protein interaction. *FASEB J.* **6**, 3193-3200
103. Hughes, R. C. (1992) Lectins as cell adhesion molecules. *Curr. Opin. Struct. Biol.* **2**, 687-692
104. Lotan, R. and Raz, A. (1988) Lectins in cancer cells. *Ann. NY Acad. Sci.* **551**, 385-398
105. Leffler, H., Masiarz, F. R. and Barondes, S. H. (1989) Soluble lactose-binding vertebrate lectins: A growing family. *Biochemistry* **28**, 9222-9229
106. Lotan, R. (1992) Beta-Galactoside-Binding Vertebrate Lectins: Synthesis, Molecular Biology, Function. *Glycoconjugates: composition, structure, and function* (Allen, H. J. and Kisailus, E. C., eds) pp. 635-672, Marcel Dekker, Inc., New York
107. Kornfeld, S. (1986) Trafficking of lysosomal enzymes in normal and disease states. *J. Clin. Invest.* **77**, 1-6
108. von Figura, K. and Hasilik, A. (1986) Lysosomal enzymes and their receptors. *Annu. Rev. Biochem.* **55**, 167-193
109. Kornfeld, S. and Mellman, I. (1989) The biogenesis of lysosomes. *Annu. Rev. Cell Biol.* **5**, 483-525
110. Kornfeld, S. (1992) Structure and function of the mannose 6-phosphate/insulinlike growth factor II receptors. *Annu. Rev. Biochem.* **61**, 307-330
111. Varki, A. (1992) Role of oligosaccharides in the intracellular and intercellular trafficking of mammalian glycoproteins. Cell surface carbohydrates and cell development (Fukuda, M., ed) pp. 25-69, CRC Press, Ann Arbor
112. Farquhar, M. G. (1991) Protein traffic through the Golgi complex. *Intracellular Trafficking of Proteins* (Steer, C. J. and Hanover, J., eds) Cambridge Univ. Press, New York
113. Freeze, H. H. (1986) Modifications of lysosomal enzymes in *Dictyostelium discoideum*. *Mol. Cell. Biochem.* **72**, 47-65
114. Freeze, H. H. (1992) Developmental glycobiology of *Dictyostelium discoideum*. Cell Surface Carbohydrates and Cell Development (Fukuda, M., ed) pp. 285-317, CRC Press Inc., Boca Raton
115. Olson, T. S. and Lane, M. D. (1989) A common mechanism for post-translational activation of plasma membrane receptors. *FASEB J.* **3**, 1618-1624
116. Ashwell, G. and Harford, J. (1982) Carbohydrate-specific receptors of the liver. *Annu. Rev. Biochem.* **51**, 531-554
117. Ashwell, G. and Steer, C. J., Hepatic recognition and catabolism of serum glycoproteins. *JAMA* **246**, 2358-2364
118. Drickamer, K. (1991) Clearing up glycoprotein hormones. *Cell* **67**, 1029-1032
119. Ezekowitz, R. A. and Stahl, P. D. (1988) The structure and function of vertebrate mannose lectin-like proteins. *J. Cell Sci. Suppl.* **9**, 121-133
120. Schwartz, A. L. (1990) Cell biology of intracellular protein trafficking. *Annu. Rev. Immunol.* **8**, 195-229
121. Dennis, J. W. and Laferte, S. (1987) Tumor cell surface carbohydrate and the metastatic phenotype. *Cancer Metastasis. Rev.* **5**, 185-204

Biological Roles of Oligosaccharides

122. Dawson, G. (1990) Glycosphingolipid function in cancer. *Cancer Cells* **2**, 327-328
123. Feizi, T. (1985) Demonstration by monoclonal antibodies that carbohydrate structures of glycoproteins and glycolipids are onco-developmental antigens. *Nature* **314**, 53-57
124. Lloyd, K. O. and Old, L. J. (1989) Human monoclonal antibodies to glycolipids and other carbohydrate antigens: Dissection of the humoral immune response in cancer patients. *Cancer Res.* **49**, 3445-3451
125. Kim, Y. S. (1992) Altered glycosylation of mucin glycoproteins in colonic neoplasia. *J. Cell. Biochem. 50 Suppl.* **16G**, 91-96
126. Sairam, M. R. (1989) Role of carbohydrates in glycoprotein hormone signal transduction. *FASEB J.* **3**, 1915-1926
127. Kobata, A. (1988) Structures, function, and transformational changes of the sugar chains of glyco hormones. *J. Cell Biochem.* **37**, 79-90
128. Baenziger, J. U. and Green, E. D. (1988) Pituitary glycoprotein hormone oligosaccharides: structure, synthesis and function of the asparagine-linked oligosaccharides on lutropin, follitropin and thyrotropin. *Biochim. Biophys. Acta* **947**, 287-306
129. Fukuda, M., Sasaki, H. and Fukuda, M. N. (1990) Structure and role of carbohydrate in human erythropoietin. *Adv. Exp. Med. Biol.* **271**, 53-68
130. Baenziger, J. U. and E. D. Green. (1991) Structure, Synthesis and function of the Asparagine-linked Oligosaccharides on Pituitary Glycoprotein Hormones In Ginsburg, V. and Robbins, P. (eds) *Biology of Carbohydrates*, Vol 3, J. Wiley, New York.
131. Fukuda, M. N. (1991) HEMPAS disease: genetic defect of glycosylation. *Glycobiology* **1**, 9-16
132. Rosse, W. F. (1990) Phosphatidylinositol-linked proteins and paroxysmal nocturnal hemoglobinuria. *Blood* **75**, 1595-1601
133. Chrispeels, M. J. and Raikhel, N. V. (1991) Lectins, lectin genes, and their role in plant defense. *Plant Cell* **3**, 1-9
134. Etzler, M. E. (1992) Plant Lectins: Molecular Biology, Synthesis, and Function. *Glycoconjugates: composition, structure, and function* (Allen, H. J. and Kisailus, E. C., eds) pp. 521-540, Marcel Dekker, Inc., New York
135. Bundle, D. R. and Young, N. M. (1992) Carbohydrate-protein interactions in antibodies and lectins. *Curr. Opin. Struct. Biol.* **2**, 666-673
136. Darvill, A., Augur, C., Bergmann, C., Carlson, R. W., Cheong, J. J., Eberhard, S., Hahn, M. G., Ló, V. -M., Marfà, V., Meyer, B., Mohnen, D., O'Neill, M. A., Spiro, M. D., van Halbeek, H., York, W. S. and Albersheim, P. (1992) Oligosaccharins – Oligosaccharides that regulate growth, development and defence responses in plants. *Glycobiology* **2**, 181-198
137. Ryan, C. A. and Farmer, E. E. (1991) Oligosaccharide signals in plants: A current assessment. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **42**, 651-674
138. Nap, J-P. and Bisseling, T. (1990) Developmental biology of a plant-prokaryote symbiosis: the legume root nodule. *Science* **250**, 948-954
139. Fisher, R. F. and Long, S. R. (1992) Rhizobium – plant signal exchange. *Nature* **357**, 655-660
140. Ofek, I. and Sharon, N. (1990) Adhesins as lectins: Specificity and role in infection. *Curr. Top. Microbiol. Immunol.* **151**, 91-114
141. Karlsson, K. A. (1989) Animal glycosphingolipids as membrane attachment sites for bacteria. *Annu. Rev. Biochem.* **58**, 309-350
142. Lingwood, C. A. (1992) Bacterial adhesins/glycolipid receptors. *Curr. Opin. Struct. Biol.* **2**, 693-700
143. Gilboa-Garber, N. and Garber, N. (1992) Microbial Lectins. *Glycoconjugates: composition, structure, and function* (Allen, H. J. and Kisailus, E. C., eds) pp. 541-592, Marcel Dekker, Inc., New York
144. Wick, M. J., Madara, J. L., Fields, B. N. and Normark, S. J. (1991) Meeting review. Molecular Cross Talk Between Epithelial Cells and Pathogenic Microorganisms. *Cell* **67**, 651-659
145. 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
146. Roseman, S. (1970) The synthesis of carbohydrates by multiglycosyltransferase systems and their potential function in intercellular adhesion. *Chem. Phys. Lipids* **5**, 270-297
147. Shur, B. D. (1989) Expression and function of cell surface galactosyltransferase. *Biochim. Biophys. Acta* **988**, 389-409
148. Clausen, H. and Hakomori, S. (1989) ABH and related histo-blood group antigens; immunochemical differences in carrier isotypes and their distribution. *Vox Sang.* **56**, 1-20
149. Montreuil, J. (1980) Primary structure of glycoprotein glycans: basis for the molecular biology of glycoproteins. *Adv. Carb. Chem. Biochem.* **37**, 157-223



1933 Davis Street, Suite 207
San Leandro, CA 94577-1258

TOLL FREE (800) 457-9444
PHONE (510) 638-6900
FAX (510) 638-6919

E-MAIL info@prozyme.com
WEB www.prozyme.com