EXHIBIT S
PART 2

Lodish Decl. in Support of Opposition to Roche's Motion for Summary Judgment of Invalidity for Double Patenting Over Claim 10 of the '016 Patent

Table XII. *continued*

Genetic defect/variation	Basic defect in glycosylation	Biological consequence(s)	References
IgG cryoglobulin	*N*-Linked glycosylation in first heavy chain hypervariable region	Precipitation of immunoglobulin in the cold, leading to vascular problems	(991)
Type I procollagen in a case of osteogenesis imperfecta	?New *N*-linked glycosylation site in carboxy-terminal peptide	Cause of increased fragility of bones?	(992)
Saposin B in a case of congenital deficiency	Point mutation eliminates a new *N*-linked glycosylation site	Unmasking of proteolytic site causes rapid turnover, resulting in deficiency	(993)
Haemophilia A variant	Point mutation creates a new *N*-linked glycosylation site	Decreased function of Factor VIII, leading to bleeding disorder	(994)
C1 inhibitor Ta	Additional glycosylation site created by three-base deletion	Type II hereditary angioneurotic edema	(271)
Albumin Redhill	New glycosylation site and altered signal peptidase cleavage	No obvious phenotype(?)	(995)
Protein S (Heerlen polymorphism)	Loss of glycosylation site	No change in protein C binding. No phenotype	(290)
Deficiency of UDP-Gal: 3-α-galactosyltransferase (in humans, apes and Old World monkeys)	Marked decrease of Galα1-3Galβ1-4GlcNAc sequences terminating glycoprotein and glycolipid oligosaccharides	No obvious abnormality results. All humans have a natural antibody (up to 1% of circulating IgG) against Galα1-3-Galβ1-4GlcNAc sequences	(996–999)
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-acetylgalactos-aminyltransferase (A enzyme)	Polymorphism expression of A and B and O blood groups structures terminating glycoprotein and glycolipid oligosaccharides	No obvious abnormality results. Humans have natural antibodies against the blood group sequences that they do not express	90, 123, 148, 1000–1003)
Polymorphic expression of Sd^a antigen in humans	Polymorphism in expression of GalNAcβ1-4[NeuAcα2-3]Galβ1-4GlcNAc	No obvious abnormality results	(1004, 1005)
Polymorphic expression of UDP-Gal: Galα1-4 galactosyltransferases (the P blood group system). Some individuals lack the enzyme(s) (blood group p)	Polymorphism in the expression of P, P1 and P^k blood group structures terminating glycoprotein and glycolipid oligosaccharides	No obvious abnormality results. Individuals with some P blood groups are at greater risk for urinary tract infections with *E. coli* carrying specific P-fimbriae, because they express the cognate oligosaccharide ligand on their urothelial surfaces	(140, 141, 444–446, 449, 450)
Primary enzymatic basis not fully defined	Polymorphic expression of *N*-acetyl and *N*-glycolyl-neuraminic acid on the erythrocyte gangliosides of dogs and cats	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	(1006–1008)
Differing levels of expression of ganglioside biosynthetic enzymes in the livers of different inbred strains of mice	Differences in the overall pattern of ganglioside expression in the liver and other organs	No grossly obvious consequences	(1009–1012)

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

Unusual oligosaccharides or modifications are also more likely to arise from interactions with microorganisms and other noxious agents

The constant balance between the 'traitorous' and 'masking' functions of oligosaccharides has been discussed above (see Tables IV and V). In most cases, it is the terminal or outer sugars and their modifications that are involved in these life-and-death interactions. Consequently, while such structures 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 some cases of sporadic autoimmune reactions to oligosaccharides, the antigenic structures are normally present in adult tissues (e.g. antibodies against peripheral nerve glycolipids seen in some individuals with multiple myeloma). However, there are examples of oligosaccharide structures which when expressed postnatally by the organism result universally in an immune response. The best examples in humans are the conversion of *N*-acetylneuraminic acid to *N*-glycolylneuraminic acid (1040, 1041) and the expression of Galα1-3 Gal sequences (see Table XII). In these cases, the structures are not expressed in normal adults, but can appear in

disease states such as cancer, resulting in immune reactions due to newly induced or pre-existing antibodies. In at least one case (*N*-glycolylneuraminic acid), it is clear that expression actually does occur in the normal fetus, but is then suppressed post-natally in the normal adult. The oligosaccharides in question evidently must have no normal functions in the adult. However, it is likely that their expression in the fetus is a required event and is a case of ontogeny recapitulating phylogeny.

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

We have reviewed the evidence that all of the diverse theories regarding the functions of oligosaccharides are correct, but that 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 that is 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 such 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 before us 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 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.

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

The essential pathways of biosynthesis of most of the major classes of oligosaccharides have now been worked out and involve a large number of gene products, including many families of glycosyltransferases. Tissue culture cell lines with mutations in a variety of specific steps in the biosynthesis of *N*-linked oligosaccarides, glycosaminoglycans, *O*-linked oligosaccharides and glycophospholipid anchors have been obtained, including some with defects in very early steps in the biosynthetic pathways (for examples, see 30,71,1043,1044). 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 (for example, see 1042). In most of these situations, the abnormalities in glycosylation seem to have limited consequences to the growth and maintenance of these tissue culture cell lines. 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. Of note, however, some of these mutants do show alterations in density-dependent growth inhibition and others demonstrate changes in tumorigenicity or metastatic behaviour when injected into athymic mice (1045). 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 (see 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 the ABO blood group polymorphisms. Glycosylation mutants in intact mice are even more uncommon. The rarity of such naturally occurring mutations could be explained in several ways. It is possible that they do occur frequently, but have little detectable biological consequence. A more likely possibility is that the great majority of them cause lethal aberrations that prevent completion of embryogenesis. A third 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. In this regard, it is worth noting that the congenital absence of a variety of highly conserved proteins in humans (e.g. glycophorin A, haptoglobin, prekallikrein, myeloperoxidase, coagulation factor XII and high molecular weight kininogen) are also known to have little biological or pathological consequence. Likewise, many 'knockout' experiments involving highly conserved proteins such as cellular proto-oncogenes have surprisingly limited consequences in the intact mouse (1047, 1049).

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

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

Glycoconjugate(s) affected	Change in oligosaccharides	Biological effect(s)	References
Plasma fibrinogen in hepatoma and in congenital dysfibrinogenaemias	Increased branching or number of *N*-linked oligosaccharides and increased sialic acid content	Prolonged thrombin time and reptilase time. Inhibition of coagulation	(278–280)
Plasma membrane and secreted proteins in cystic fibrosis	Generalized increase in fucosylation and sulphation	?Contribute to change in physical properties of secreted glycoproteins	(1013, 1014)
CD43 (leukosialin, sialophorin) in Wiskott–Aldrich syndrome	Altered branching of *O*-linked oligosaccharides	Decreased expression (due to altered glycosylation?)	(1015–1020)
Serum IgG immunoglobulin	Decreased galactosylation of *N*-linked oligosaccharides	A general feature of many chronic granulomatous diseases (rheumatoid arthritis, Crohn's disease, tuberculosis, etc.)	(34, 248, 254)
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	(1021–1032)
Dolichol oligosaccharides	Altered processing and accumulation of dolichol-linked mannosyl-oligosaccharides	Neuronal Ceroid-lipofuscinosis. ?Primary or secondary defect in humans, dogs and sheep	(1033–1036)

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

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. If such an intervention blocks early embryogenesis, the consequences may not be available for analysis (study of first-generation chimeric animals may give some information in gene-deletion experiments). However, even if live homozygous animals are observed with overexpression or with gene deletions, care must be taken in interpreting the results. The consequences seen could be the result of interference with other competing glycosylation pathways, or may be due to non-specific physical effects of grossly altered glycosylation in all tissues of the organism.

An alternate approach makes use of 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 (901, 905), suggesting specific roles for polysialic acids, and injection of heparanase into the developing embryo caused randomization of left–right axis formation (1048). Expression in transgenic mice of a viral sialic acid-specific 9-*O*-acetylesterase under the control of specific promoters caused abnormalities either early or late in development (940). In principle, the latter approach could be generalized to any situation where a cDNA is available encoding a specific oligosaccharide-degrading enzyme. Thus, rather than interfering with the basic genetic and cellular machinery responsible for the synthesis of specific oligosaccharides, one might eliminate them selectively after normal synthesis by expression of a degradative enzyme as a cell surface molecule. Specific promoters should limit expression of the enzyme and allow the analysis of tissue-specific functions of oligosaccharides in later stages of development, side-stepping an early lethal event that could have occurred with a gene 'knockout' experiment. In the short term, such approaches are likely to generate even more new questions than immediate answers. However, it is much better to have many incomplete clues to the biological roles of oligosaccharides than to have extensive and specific structural information only, and no way to pursue their relevance.

Future prospects

As recently suggested, modern progress in glycobiology 'has finally opened a crack in the door to one of the last great frontiers of biochemistry' (36). The future now appears bright for the understanding of many new biological roles of oligosaccharides. Until recently, mainstream research was focused either on the molecular biology of the single cell, or on the physiology of whole organs or organisms. In both of 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 understanding becomes crucial to further progress.

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Abbreviations

bFGF, basic fibroblast growth factor; CHO, Chinese hamster overy; EGF, epidermal growth factor; ER, endoplasmic reticulum; GM-CSF, granulocyte/macrophage colony-stimulating factor; HCG, human chorionic gondotrophin; HNK-1/L1, the antigenic epitope recognized by the HNK-1 antibody; I-CAM, intercellular adhesion molecule; LDL, low-density lipoprotein; LFA-1, leukocyte function antigen 1; MHC, major histocompatability complex; N-CAM, neural cell adhesion molecule; SSEA, stage-specific embryonic antigen; TGF, transforming growth factor.

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