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Preface

Glycobiology involves studies of complex carbohydrates and posttranslational modifications of proteins, and has become an important interdisciplinary field encompassing chemistry, biochemistry, biology, physiology, and pathology. Although initial research was directed toward elucidation of the different carbohydrate structures and the enzymes synthesizing them, the field has now moved toward identifying the functions of carbohydrates. The protocols described in *Glycobiology Protocols* form a solid basis for investigations of glycan functions in health and disease. The cloning of many of the genes participating in glycosylation processes has helped to enhance our knowledge of how glycosylation is controlled, but has also added another dimension of complexity to the great heterogeneous variety of the structures of the oligosaccharides of glycoproteins, proteoglycans, and glycolipids. A family of similar enzyme proteins exists for each glycosylation step. Glycosyltransferases are extremely specific for both the nucleotide sugar donor and the acceptor substrate, but many other factors control sugar transfer, including the localization and topology of enzymes, cofactors, possible chaperone proteins, and the availability of sugar acceptor substrates. The analysis of the intracellular organization of glycosylation and of the factors controlling the activities of the participating enzymes in the cell are important areas that need more research efforts. Another challenge for future research is to understand the glycodynamics of a cell, that is, how the cell responds to stimuli leading to biological and pathological changes in terms of alterations in glycosylation, and how this affects the biology of the cell. Because complex carbohydrates have many demonstrated and postulated tissue-specific functions, they can have an impact on the pathology of diseases such as cancer, inflammatory bowel diseases, and cystic fibrosis. Specific carbohydrate structures have been shown to be closely linked to tumor metastasis and invasiveness, inflammation, and immune functions.

The 24 chapters of *Glycobiology Protocols* highlight important methodological progress in the field of glycobiology, and will help scientists to answer specific questions on glycoprotein structures, on the biosynthesis of glycoconjugates, and on the functions of lipid- or protein-bound carbohydrates. This volume is meant to help students, postdoctoral fellows, and senior scientists, whether new or established in the field of glycobiology.

The biosynthesis of GlcNAc-Asn-linked and Man-*O*-Ser/Thr-linked oligosaccharides involves dolichol-phospho-sugars as both sugar donors and

acceptor substrates. Because both the enzymes involved and their substrates are in a lipid phase they are very difficult to study, and this area has therefore been neglected in the past. Useful reagents and methods that overcome the problem inherent in assays of water-insoluble biomolecules have been suggested. Chapter 1 (by N. Gao) demonstrates a new fluorescence-based method to study dolichol pyrophosphate oligosaccharides. Chapter 2 (by J. S. Rush and C. J. Waechter) and Chapter 3 (by J. S. Schutzbach) describe how the enzymes utilizing dolichyl substrates can be purified and characterized. Chapter 3 emphasizes the importance of assaying membrane-bound enzymes within their membrane environment. In Chapter 4, T. Endo and H. Manya address the unusual mammalian type of *O*-glycosylation where the first enzyme utilizes dolichol-phospho-mannose as the donor substrate, as well as the following reaction that utilizes nucleotide sugar as the donor substrate. This chapter highlights the fact that assay systems established in yeast cannot necessarily be used in mammals, owing to differences in enzyme topology and/or specific substrates. The other unusual types of *O*-glycosylation (*O*-Fuc and *O*-Glc) also require specific acceptor substrates, at least for the first sugar added to the protein, with the donor substrates being nucleotide sugars. A. Nita-Lazar and R. Haltiwanger (Chapter 5) describe how enzymes involved in these unusual *O*-glycosylations can be assayed.

Sialic acids are a family of terminal acidic sugars of glycoproteins and glycolipids that have multiple functions in determining the overall structure and properties of carbohydrates and the exposure of recognition determinants on the cell surface. These acidic sugars exhibit great structural diversity and play important roles in the immune system, apoptosis, and in providing receptors for microbes. The nature and amount of relatively labile sialic acid linkages are regulated by biosynthesis and degradation. In Chapter 6, J. P. Kamerling and G. J. Gerwig summarize the state-of-the-art methods by which sialic acids can be isolated and analyzed, utilizing chemical methods combined with gas chromatography and mass spectrometry. S. Schrader and R. Schauer report, in Chapter 7, an assay for a *trans*-sialidase from trypanosomes that facilitates analyses of a large number of samples, and can be applied to study the pathology of trypanosomal parasitic diseases.

The biggest hurdle in the field of glycobiology has been to show unequivocally that carbohydrates have specific functions. Many biochemists still consider protein-bound glycans as unwanted modifications that interfere with protein functions rather than having a direct and recognized role. Some of the problems in studying functions are the marked heterogeneity of carbohydrate structures of glycoproteins (especially in mucus glycoproteins) and the fact that the glycosylation inhibitors in use (e.g., tunicamycin) do not prevent the

synthesis of any one specific structure, but rather have a nonspecific effect and inhibit glycosylation in general. The discovery of mammalian lectins has greatly influenced our vision of the role of carbohydrates. Thus, carbohydrates not only regulate protein functions, but also have defined roles by themselves. The functions of sialic acid-containing carbohydrate determinants in cell adhesion are addressed in Chapter 23 (by M. E. Beauharnois et al.), which deals with selectin-carbohydrate interactions, and in Chapter 24 (by N. Bock and S. Kelm), which describes how the specificity of sialic acid-binding lectins in the immune system can be determined. B. S. Ireland et al. report in Chapter 22 on an assay for the lectin-like function of chaperones in the endoplasmic reticulum, which is important for our understanding of protein folding.

A sensitive, efficient, and accurate analysis of protein-bound carbohydrates of glycoproteins or proteoglycans is essential for continued progress in glycobiology. In most cases, only small amounts of materials for these analyses can be obtained, and new tools for the separation of oligosaccharides and their analysis by enzymatic methods combined with chromatography and mass spectrometry have been developed. In Chapter 8, C. Robbe et al. describe the analysis of mucin-type *O*-glycans by mass spectrometry methods. This group of researchers has accomplished the detailed structural analysis of relatively small amounts of underivatized, highly heterogeneous mucin-type *O*-glycans from complex mixtures. The exquisite approach by Royle et al. (Chapter 9) to study glycan structures involves the release of *N*-glycans from the protein, treatments with specific glycosidases, and separations by high-performance liquid chromatography using a battery of glycan standards.

The field of glycosyltransferases has been advanced by intense gene-cloning efforts during the past 20 yr, by crystallography of an increasing number of enzymes, and by methods of protein modeling. Therefore, we now have a better understanding of the characteristics and functions of enzyme-active sites, and the relationships among enzymes from different species that have similar activities. As a consequence, the dogma of "one linkage, one enzyme" has been modified to "one linkage, one enzyme family." The molecular modeling described by A. Imberty et al. in Chapter 10, illustrates that enzyme-modeling efforts have to be based on specific expertise and require specialized computer hardware and an ability to utilize specially designed programs. Because relatively few glycosyltransferases have been crystallized to date, the modeling methods, in combination with biochemical methods, are indispensable to obtain knowledge of enzyme mechanisms. The design of specific inhibitors will benefit greatly from computer modeling.

Biochemical methods for assaying specific mammalian glycosyltransferases involved in the biosynthesis of *N*- and *O*-glycans of glycoproteins have been described in Chapter 11 by F. Dall'Olio et al., in Chapter 12 (by M. Prorok-

Hamon et al.), and in Chapter 14 (by I. Brockhausen et al.). The methods presented in Chapter 12 can also be applied to measure the distribution and activity of a glycosyltransferase in cultured cells. Chapter 14 addresses the dynamic state of glycan structures and biosynthesis that can be altered by cytokines, in inflammation, or in disease. The protocol presented for bone cell studies can be used to relate biological phenomena to glycosylation in many other biological systems. Complementing these sometimes tedious enzyme assays are protocols on RT-PCR (Chapters 11 and 13). J. J. García-Vallejo et al. (Chapter 13) have designed and compiled a large library of mammalian DNA sequences that are useful for measuring the expression levels of glycosyltransferases by RT-PCR in a sensitive, efficient, and reliable fashion. Thus, many of the enzymes involved in glycan synthesis of glycolipids and glycoproteins, as well as many sulfotransferases and mannosidases, can be investigated by this method.

The lipopolysaccharides of Gram-negative bacteria are essential for bacteria and important for our encounters with bacteria. Chapter 15 (by C. L. Marolda et al.) describes a protocol to rapidly characterize lipopolysaccharides, as well as the outer carbohydrate O-chains. The method can be applied to assess the effects of gene modifications in bacteria. In the past, it has been difficult to study the biosynthesis of these lipopolysaccharides because polyprenol-phosphate intermediates are involved, which are similar to the dolichol-linked intermediates in *O*-Man- or *N*-glycan biosynthesis. In Chapter 16 (by I. Brockhausen et al.), the synthesis of a novel substrate analog that can serve as a substrate in facile assays of an O-chain glycosyltransferase is described.

Extracellular glycoconjugates, such as mucins or proteoglycans, have essential functions for tissue homeostasis but are difficult to investigate because of their large sizes, charges, and abundance of a heterogeneous mixture of large glycans. K. J. Rees-Milton and T. P. Anastassiades (Chapter 17) describe a method to quantify anionic glycoconjugates with a dye-binding method. This protocol can be useful for the analysis of the alterations of proteoglycans in arthritis or other conditions. Chapter 18 (by P. Argueso and I. Gipson) describes the analysis of small amounts of large mucins, utilizing antibodies against specific mucin peptides. These mucins can be isolated from mucus secretions or from cellular material. Antibodies as well as lectins, can also be used to detect mucins in tissues. F. Kan reports on sensitive ultrastructural analyses (Chapter 19) using colloidal-gold labeling that has successfully demonstrated the subcellular and extracellular distribution of zona pellucida glycoproteins.

Glycolipids are difficult to study because of their hydrophobic character. C. Lingwood et al. (Chapter 20) have established methods by which glycolipid mimics can be synthesized and utilized to study glycolipid function. Finally,

glycosidases are essential for the metabolic handling of glycocolipids and abnormalities in these enzymes can lead to severe pathological conditions. J. Callahan and A. Skomorowski (Chapter 21) describe how a lysosomal storage disease (Krabbe disease), characterized by deficiency in galactocerebrosidase, can be diagnosed. This simple protocol is suitable for a routine laboratory test.

The protocols in *Glycobiology Protocols* contain specific methods for the analysis of the structures or functions of glycoconjugates, as well as of glycosyltransferases and glycosidases involved in the biosynthesis of glycans. The methods described for a specific system can usually be modified for investigations of similar biomolecules and tissues or cell types.

I am grateful to all contributors for taking time and effort to share their valuable expertise.

Inka Brockhausen

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