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GLYCOPINION

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Research into carbohydrates conjugated to proteins and lipids is now entering a new era. In this article Dr Minoru Fukuda reflects on previous research, focusing on the impact it has made on other areas of science and assessing the role that it now plays in glycobiology. In the future it is clear that molecular biology will play an increasingly important role in the extension of the field.

The development of a particular field and its influence on related areas of research can be accelerated rapidly by a small number of significant discoveries. In this case, the elucidation of the processing pathway of *N*-glycans in the endoplastic reticulum and Golgi complex had an important impact on cell biology.

The field of glycobiology is developing very rapidly at the moment. In this article we can see that past discoveries and recent technological developments in both glycoconjugate research and molecular biology have contributed to this. It is also a fact that rapid progress often results from creative interactions between two previously independent disciplines.

Letters or comments relating to this article would be received with interest by Pauline Rudd, Assistant to the Special Advisory Editor, R. A. Dwek

The biology of glycoconjugates

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The development of a particular field can be assessed by evaluating the impact of discovery on that field. However, more importantly, a particular field could become much more important if its developments influence research in other fields and disciplines and, most prominently, if it influences science in general.

The detailed description of the *N*-glycan processing pathway made a huge impact on cell biology, and it is worthwhile looking back to see how this happened. First, extensive studies of the biosynthesis of glycans attached to proteins or lipids revealed that the majority of complex and hybrid oligosaccharide chains are formed by stepwise additions to a precursor which, in its turn, becomes an acceptor for a particular glycosyl transferase. Each enzyme requires a unique acceptor or set of acceptors which eventually dictates the final product. The orderly addition of sugars is orchestrated by the glycosyl transferases which are present in the Golgi complex. Thus a glycosyltransferase in the medial Golgi adds *N*-acetylglucosamine to a precursor acceptor that was made in the first part of the Golgi complex. Similarly, a galactosyltransferase in the *trans* Golgi waits for an acceptor made in the medial Golgi. The association of a specific glycosyltransferase with a particular part of the ER or the Golgi complex opened up the possibility of identifying a cellular subcompartment by the glycosyl transferases it contained. This can be done by measuring enzyme activity or visualizing the glycosyltransferases using antibodies.

N-Glycans undergo considerable transformation during their processing (Kornfeld and Kornfeld (1985) *Ann Rev Biochem* **54**:631–64; see also Fig. 1). *N*-Glycans are attached to protein acceptors through a precursor sugar linked to dolichol. Once this precursor sugar, which contains a block of nine mannose residues, is attached to a protein it is gradually trimmed at the ER and then at the Golgi complex. In the medial and *trans* Golgi, the trimmed precursor sugar chain acquires residues other than mannose. This transition changes the susceptibility of the carbohydrate chain to endo-β-*N*-acetylglucosaminidase H. The oligomannose carbohydrate and its trimmed forms are susceptible to endo-H, while more processed molecules are not. If the protein is not susceptible to endo-H, it can therefore be concluded that the protein passed through the *cis* Golgi. This method has been used extensively to determine how far the protein is transported in the Golgi, particularly in experiments with engineered proteins or during processing studies.

The isolation of endo-*β*-*N*-acetylglucosaminidases and the elucidation of their specificities and functions within the cell required detailed structural analysis of their oligosaccharide substrates which, in turn, depended heavily on the purification and characterization of exoglycosidase

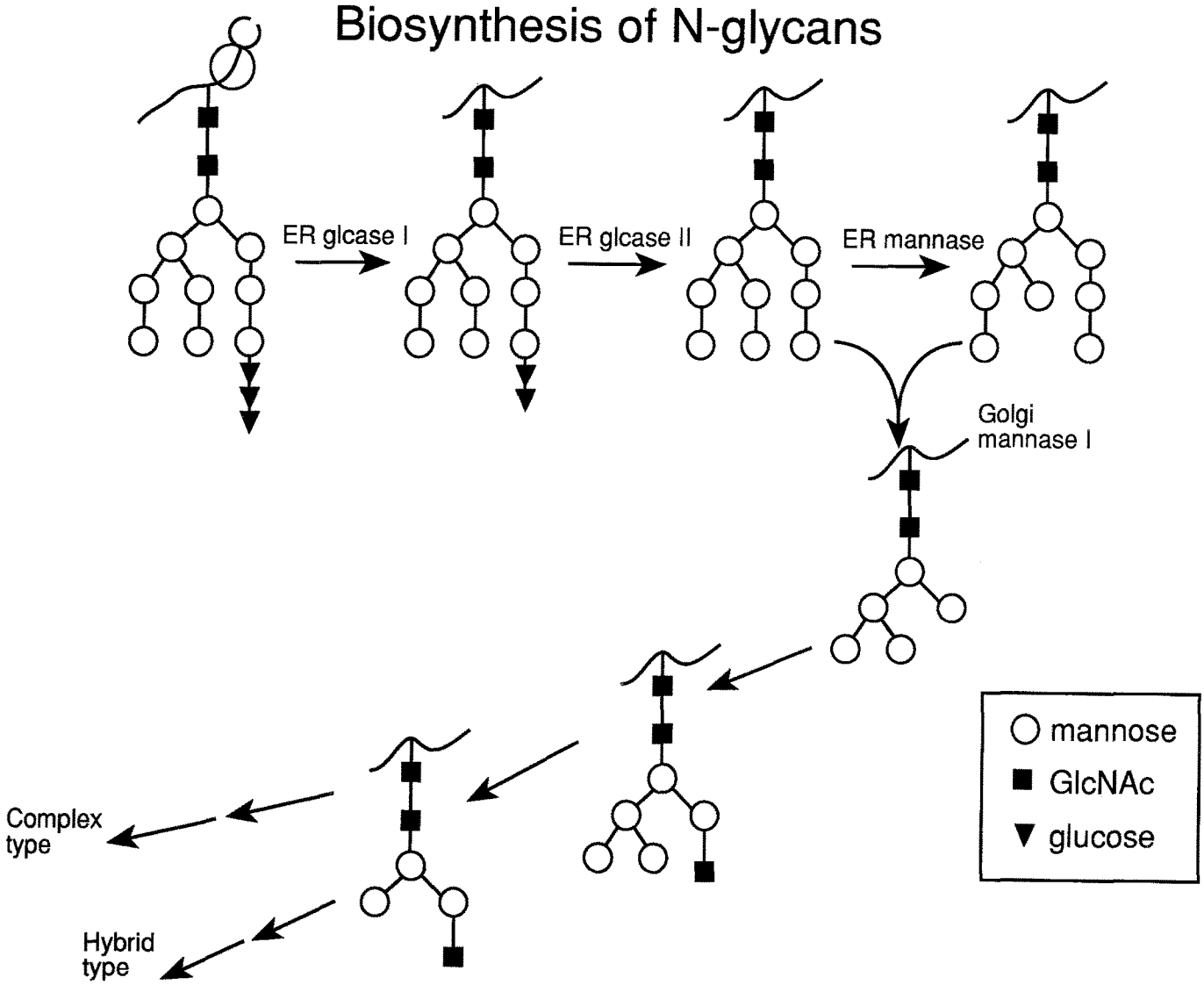

Figure 1.

sequencing enzymes. This is a further illustration of the way in which glycoconjugate research has contributed to the progress of cell biology.

Lectin–carbohydrate interactions

We are now entering an era where we can discuss the roles of carbohydrates in cell recognition. It is now evident that there are two main structural features which influence the recognition of oligosaccharides attached to glycoproteins. The first is the nature of the oligosaccharides, particularly the terminal residues; the second is the degree of branching which different sugars adopt. Specific expression of a particular carbohydrate depends on the ability of the cell type to express the glycosyltransferases that catalyse the necessary reactions. The levels of these enzymes is under cellular control and can alter during development, differentiation and disease. Glycosylation changes therefore reflect changes in the environment of the cell, and may in turn influence its interaction with receptors.

Cell type specific glycoproteins are now known to play a role in cell recognition in a number of systems including mouse embryonal development, sperm–egg interaction and mating factor in yeast. Of major significance is the recent discovery of leucocyte–endothelial cell interactions. Here it has been shown that oligosaccharides on the cell surface glycoproteins of leucocytes are ligands for selectins on endothelial cells and platelets, allowing a role for sugars in cell–cell adhesion.

Two important pieces of work preceded this discovery. First, Kurt Drickamer's pioneering work demonstrated that there is a conserved amino acid sequence in the carbohydrate recognition domains (CRDs) of different

calcium dependent animal lectins. When the primary protein sequences of the selectins were derived from their cDNA sequences, it was found that they too contain the sequence conserved in the animal lectins suggesting that selectins might also recognize carbohydrate ligands. Second, the fine specificity was defined in a most elegant experiment by John Lowe's group. CHO cells, which normally lack the sialyl Le^X structure, were transfected with cDNA encoding fucosyltransferase while, in a control experiment, a fucosyltransferase which produces neutral Le^X was introduced into similar cells. Only those cells expressing sialyl Le^X bound to E-selectin. (Lowe, Stoolman, Nair, Larsen, Berhend and Marks (1990) *Cell* **63**:475–84.)

Recently, the mechanism of this interaction has been compared with the calcium dependent lectin–carbohydrate interactions of a mannose binding protein CRD (Weis, Drickamer and Hendrickson (1992) *Nature* **360**:127–134). Both depend on the formation of a coordination complex in which one Ca^{2+} ion is ligated to five carboxylate side chains of amino acids in the protein. The Ca^{2+} ion in mannose binding protein also ligates the 3- and 4-OH in the mannose ring; however, in the selectins the Ca^{2+} ion can coordinate with the 2- and 3-OH groups of fucose which forms part of the oligosaccharide ligand, sialyl Lewis X, allowing a different specificity. This event is possible because the 2- and 3-OHs of fucose can be superimposed on the 3- and 4-OHs of mannose. Finally, each calcium coordination complex is stabilized by hydrogen bonds between the sugar and the protein.

The future: the interface with cell and molecular biology

The power of molecular biology to address questions in glycobiology is now widely recognized. For example, the mannose binding protein CRD includes two glutamic acid–asparagine pairs which are required to recognize mannose residues. The specificity of the domain can be engineered to accept galactose by constructing a mutant in which one pair, (Glu 185 and Asn 187) are replaced with Gln and Asp; the altered sequence conforms with that found in galactose binding domains (Drickamer (1992) *Nature* **360**:183–86).

As far as the biosynthesis of oligosaccharides is concerned, we have now reached the point where each glycosylation step can be carried out *in vitro* by isolating the cDNA or gene encoding glycosyl transferases. Over a dozen cDNA sequences are already known, and it is expected that before long most of the others will be determined. (Paulson and Colley (1989) *J Biol Chem* **264**:17615–18; Schachter (1993) in *Molecular Glycobiology* (ed. Fukuda and Hindsgaul) Oxford University Press, in press.) This will make the large scale enzymatic synthesis of specific oligosaccharides a realistic alternative to difficult and elaborate chemical synthesis and also encourage research into glycosylation inhibitors.

The effects of this research into glycosyl transferases will be far reaching. For example, it is possible to investigate the function of a particular glycoconjugate by using down regulators or anti-sense technology to reduce transferase levels, thereby preventing its synthesis. Normal biosynthesis of glycoconjugates can also be interrupted by using sugar analogues as glycosylation inhibitors. The function of particular carbohydrate structures can also be probed by introducing a cDNA encoding a glycosyltransferase that was otherwise absent in the cell, as exemplified by Lowe's group.

Now that the technology exists to knock out specific genes encoding glycosyltransferases other experiments become possible. The abolition of particular glycoforms of glycoproteins in mice or *Drosophila*, for example, may allow a means of probing function *in vivo*. Some glycosyltransferases undergo dramatic changes in transcription levels during development and differentiation as well as in tumorigenesis. The development of cDNA technology may allow ways of probing the regulation of these changes at the transcription level and of determining whether a particular transcription factor is involved.

While much previous glycoconjugate research was chemically based, the advent of molecular biology is presently allowing the significance of these molecules to be appreciated in their biological context. Thus glycobiology is now entering a new era promising significant impact on cell biology and molecular biology in general.

Glycoprotein analysis: an EMBO practical course organized by the Glycobiology Institute, Oxford University, in Conjunction with Oxford Glycosystems Ltd, 6–18 September 1992

Sixteen people from fourteen European countries attended the Practical Course which was held in the recently opened Rodney Porter Building on the University Campus. This houses the Glycobiology Institute of which Professor Raymond Dwek is the Director. The course was designed to introduce the participants to both structural and functional aspects of glycobiology and to encourage them to think about the implications of glycoprotein structure for biology. Although it was intensely practical, the supporting lectures were intended to give a strong academic background to the work.

During the course, each person isolated and sequenced the oligosaccharides associated with a particular glyco-

protein. *O*- and *N*-linked sugars were released by hydrazine using the new Oxford GlycoSystems (OGS) GlycoPrep1000 and, after reduction and radiolabelling, analysed first by high voltage paper electrophoresis, to determine the ratios of differently charged sugars, and then by sequential exo-glycosidase sequencing of the desialylated oligosaccharide pools. This involves determining changes in the hydrodynamic volume of the oligosaccharides by gel permeation chromatography using the GlycoMap 1000 (OGS).

Supporting lectures by members of the Institute and OGS dealt with the chemistry of hydrazinolysis, the structure, glycobiology and biosynthesis of glycoproteins, the use of NMR, mass spectrometry and glycosidases to elucidate oligosaccharide structure and conformation, and described the latest developments in rapid sequencing technology. Some applications of high performance anion exchange chromatography and capillary electrophoresis to oligosaccharide and glycoprotein analysis were demonstrated.

Three visiting lecturers dealt with specific areas of glycobiology: Dr Michael Ferguson of Dundee University described his own research on glycosyl phosphatidyl inositol (GPI) membrane anchors. He also assisted the participants with practical experiments using GPI detection kits supplied by OGS. Professor Ghislain Opdenakker, of the University of Leuven, described the bioactivity of t-PA, stressing the importance of glycosylation in the activation of plasminogen. Genetic approaches to the study of glycosylation were discussed by Dr David Roberts of the Department of Genetics in Oxford. Other lectures from members of the Glycobiology Institute concentrated on the role of glycosylation in disease and, importantly, on the structure and function of carbohydrate recognition proteins.

The participants were accommodated in Exeter College, Oxford University, and were welcomed at the start of the course at a reception given by Chapman & Hall. In spite of the need to work long hours there was still enough time and energy for a punting expedition on the Cherwell, for many relaxing meals in the historic dining rooms at Exeter College and, most importantly, for the exchange of many ideas and the formation of new friendships.

The members of the Glycobiology Institute and OGS who put in many extra hours of work, both before and during the Course, were responsible for making it such a successful and stimulating event.

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Structure and function of glycoconjugates: a course organized by the Laboratoire de Chimie biologique, Lille, 6–19 September 1992

During 6–19 September, 1992, a course on 'Structure and Function of Glycoconjugates' held at the Université des Sciences et Technologies de Lille and organized by the Laboratoire de Chimie biologique (UMR n° 111 du CNRS, Director: Professor André Verbert).

This course was the eighth of a series of biennial international courses initiated in 1978 by Professor Jean Montreuil. The main purpose is to give to young European researchers an opportunity to acquire a good overview of current problems and to pick the 'hot-points' in the field of structure, metabolism and functions of glycoconjugates.

This has been achieved by presenting about 30 lectures given by outstanding experts in the field: A. Dell (London, UK), H. Egge (Bonn, Germany), F. Hemming (Nottingham, UK), B. Hoflack (EMBL, Heidelberg, Germany), R. C. Hughes (London, UK), D. Joziasse (Amsterdam, The Netherlands), L. Lehle (Regensburg, Germany), U. Lindhal (Uppsala, Sweden), M. Monsigny (Orléans, France), J. Montreuil (Villeneuve d'Ascq, France), G. Rebel (Strasbourg, France), G. Reuter (Kiel, Germany), Ph. Roussel (Lille, France), H. Schachter (Toronto, Canada), J. Shaper and N. Shaper (Baltimore, USA), N. Sharon (Rehovot, Israel), L. Shaw (Kiel, Germany), G. Tettamanti (Milan, Italy), D. Van den Eijnden (Amsterdam, The Netherlands), J. F. G. Vliegenthart (Utrecht, The Netherlands) and eight seminars related to technical aspects of glycoconjugate methodologies, given by members of our Laboratory. This was augmented by six half-days of laboratory work during which participants could get acquainted with specific methods or technologies (separation, purification and structural analyses of glycans, use of lectins, NMR and mass spectrometry, molecular modelling, metabolic studies and molecular biology approaches).

This year there were 34 participants, from Austria, Belgium, France, Germany, Ireland, Italy, The Netherlands, Poland, Romania, Spain, Sweden, Switzerland, Czechoslovakia, UK and Ukraine. Thus, in addition to the scientific aspects, this workshop was also an opportunity to establish new collaborations among participants and/or teachers, and to forge new friendships.

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