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GLYCOSYLTRANSFERASES. EARLY HISTORY, DEVELOPMENT AND FUTURE PROSPECTS*

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The importance of polysaccharides as sources of energy and storage materials in plants and animals has long been recognised. However, it is only comparatively recently that biochemists, cell biologists, immunologists and molecular geneticists have come to appreciate the crucial role played by carbohydrates in a multiplicity of cell surface phenomena and as covalently attached units that are essential to the correct conformation and biological functioning of many proteins. An understanding of the formation, inter-relationships and genetic control of these carbohydrate structures is obviously dependent upon a knowledge of their pathways of biosynthesis. It is therefore timely that an issue of *Carbohydrate Research* should be devoted to papers on the enzymes that catalyse the biosynthesis of glycosidic linkages in simple glycosides, oligosaccharides, polysaccharides and the carbohydrate moieties of glycosphingolipids, glycoproteins and proteoglycans.

EARLY HISTORY

The description glycosyltransferase defines enzymes that catalyse the transfer of a glycosyl residue from a donor to an acceptor substrate. Earlier workers established that some exoglycosidases[1] and phosphorylases[2–4] catalyse the transfer of sugar moieties to suitable acceptors to form di- and oligo-saccharides by a reversal of hydrolytic or phosphorolytic reactions. Indeed in the 1940s transglycosylation from sugar phosphates was advanced as a possible general mechanism for the biosynthesis of polysaccharides *in vivo*[3,4]. However, the role of phosphorylases is now acknowledged to be primarily degradative and present concepts of the mode of biosynthesis of glycosidic linkages stem from the characterisation by Luis Leloir and his colleagues[5] in 1950 of a new class of compound containing a sugar esterified to a nucleoside diphosphate. Following this discovery the central role of the nucleotide sugars as donor substrates for the enzymes involved in the biosynthesis of both simple and complex carbohydrate structures soon became apparent.

The first nucleotide sugar to be characterised, uridine 5′-α-D-glucopyranosyl diphosphate ("uridine diphosphate D-glucose"), was obtained from yeast[5] but this discovery was followed rapidly by the isolation of similar compounds from animals,

*Dedicated to Luis Leloir on the occasion of his 80th birthday.

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plants and microorganisms[6]. By the early 1960s over sixty different nucleotide sugars had been characterised containing hexoses, alditols, amino sugars or uronic acids as the sugar moieties[7] and uridine, guanosine, cytidine, adenosine or thymidine as the nucleoside bases[8]. The first indications of the role of the nucleotide sugars as the glycosyl donors in transfer reactions emerged in 1953 from the observations of Dutton and Storey[9], who identified UDP-glucuronic acid as the donor for the biosynthesis of phenyl β-D-glucopyranosiduronic acid catalysed by liver extracts, and of Leloir and Cabib[10] who implicated UDP-D-glucose in the formation of trehalose phosphate catalysed by yeast extracts. Independently Buchanan[11] suggested that UDP-D-glucose might serve as the glucose donor in the synthesis of sucrose phosphate; a suggestion that was confirmed by Cardini *et al.*[12] using wheat germ extracts as the enzyme source. The involvement of the sugar nucleotides in the biosynthesis of more complex polysaccharides was heralded by the work of Glaser and Brown who demonstrated the enzyme-catalysed transfer of *N*-acetyl-D-glucosamine from UDP-*N*-acetyl-D-glucosamine to both hyaluronic acid[13] and chitin[14]. Subsequently UDP-D-glucose was shown to be the sugar donor for glycogen synthesis in animals[15] and for callose formation in plants[16]. These tentative beginnings thus demonstrated the importance of the nucleotide sugars as donor substrates for glycosyltransferases involved in simple glycoside, disaccharide and complex carbohydrate biosynthesis.

In the late 1960s investigations on the biosynthesis of bacterial cell surface polysaccharides disclosed a second class of donor substrate involved in transglycosylation reactions. The usual reaction involving transfer from a nucleotide sugar:

$$\text{XDP-sugar} + \text{acceptor} \rightarrow \text{sugar-acceptor} + \text{XDP}$$

leads to the release of a nucleoside diphosphate (where X may be uridine, guanosine, adenosine or thymidine). Hence the observations by Strominger and his colleagues that in the biosynthesis of peptidoglycans the transfer of pentapeptidyl-*N*-acetylmuramic acid from its UDP derivative generated UMP and not UDP[17] suggested that some different mechanism might be operating. Eventually a complex pathway was unravelled which revealed the part played by lipid-linked sugars as intermediates in glycosyltransferase reactions. The sequence of events was shown to involve first the transfer of the pentapeptidyl-*N*-acetylmuramyl phosphate from its UDP-derivative to an endogenous phospholipid with the formation of an *N*-acetylmuramyl-(pentapeptide)-*P*-*P*-lipid complex and the release of UMP, then the transfer of *N*-acetylglucosamine from UDP-*N*-acetylglucosamine to the *N*-acetylmuramyl residue to form a disaccharide pentapeptide-lipid complex and finally, after further modification to the peptide part, transfer of the disaccharide pentapeptide from the lipid carrier to the growing end of the linear peptidoglycan[18]. Soon after the involvement of lipid carriers had been demonstrated in peptidoglycan synthesis, Weiner *et al.*[19] and Wright *et al.*[20] detected the occurrence

of lipid-linked intermediates in the biosynthesis of lipopolysaccharides of Gram-negative bacteria. The core polysaccharide of the complex lipopolysaccharide structure is built up by the sequential addition of sugar residues but the outer O-specific side chains, responsible for the antigenic properties of the polysaccharides, are first built up in repeating tri- or tetra-saccharide units on a phospholipid intermediate and subsequently transferred to the core region[21]. The lipid intermediates involved in both peptidoglycan[22] and O-antigen[20] synthesis were identified as the phosphoric monoesters of a C_{55} polyisoprenoid alcohol. Similar compounds were implicated in the synthesis of mannan in *Micrococcus lysodeikticus*[23], in teichoic acid synthesis in *Staphylococcus lactis*[24] and in other bacterial polysaccharides[18]. The disclosure of the part played by lipid-intermediates in complex carbohydrate assembly in bacterial polysaccharides stimulated a search for a function for lipid complexes in the glycosyl transfer systems in higher organisms. Once again seminal observations came from the laboratory of Leloir[25,26] which eventually led to the demonstration of the important part played by long chain isoprenyl alcohols, the dolichols, as carriers of the monosaccharides and oligosaccharides transferred by enzymes involved in the assembly of asparagine-linked oligosaccharide chains in glycoproteins[27]. Hence the group of enzymes generally understood today by the term glycosyltransferase are those that utilise either nucleotide sugars or lipid-linked sugars as their donor substrates.

DEVELOPMENT

The discovery of the natural donor substrates for the glycosyltransferases made practicable for the first time systematic investigations of the occurrence, properties and regulation of the enzymes involved in the biosynthesis of glycosidic linkages *in vivo*. Over the last three decades these studies have generated a vast literature and in this short introduction it is possible to comment on only a few salient features. For a detailed account of general progress in the field up to 1971 the reader is referred to the review of Nikaido and Hassid[28] and for more recent articles on mammalian glycosyltransferases to the comprehensive surveys of Schachter[29], Beyer *et al.*[30] and Berger *et al.*[31].

One of the most striking features of the glycosyltransferases is the stringency of their specificity requirements that permit complex oligosaccharide structures to be precisely assembled in the absence of a template. Insofar as the donor substrates are concerned, all glycosyltransferases exhibit specificity towards both the sugar moiety and the nucleotide or lipid carrier. In plants and microorganisms the same sugar may be carried by more than one nucleotide although each glycosyltransferase shows a marked preference, or absolute specificity, for only one carrier. For example in plants UDP-D-glucose is the glucosyl donor for sucrose biosynthesis[12], whereas ADP-D-glucose is the donor for starch biosynthesis[32] and GDP-D-glucose the donor for cellulose biosynthesis[33]. A plausible explanation for the use of several different nucleotides as carriers of monosaccharides, first

advanced by Ginsburg[7], is that this diversity provides a means for the independent control of various metabolic pathways. In mammalian tissues the segregation of pathways appears to be under a different form of control because the sugars commonly occurring in the oligosaccharide chains of glycoproteins, glycolipids and proteoglycans have so far been found associated each with only one nucleotide carrier[29–31]. Thus, although there is a family of mammalian galactosyltransferases capable of catalysing the addition of D-galactose in either α- or β-anomeric configuration to different positional linkages in a variety of acceptor sugars, the donor substrate for this family of enzymes is invariably UDP-D-galactose. Uridine diphosphate sugars also serve as donor substrates for the formation of glycosides of glucose, *N*-acetylglucosamine, *N*-acetylgalactosamine, xylose and glucuronic acid, whereas guanosine diphosphate sugars serve as nucleotide donors for the synthesis of mannosides and fucosides. The donor used by the sialyltransferases differs from the other nucleotide carriers in being a monophosphate, CMP-sialic acid, and certain of the mannosyl- and glucosyl-transferases involved in glycosylation steps in the biosynthesis of *N*-linked oligosaccharide chains of glycoproteins utilise as substrates the lipid-linked sugars dolichyl mannosyl phosphate and dolichyl glucosyl phosphate[27].

Even before the discovery of the appropriate enzymes, ideas on the strict acceptor specificity of the glycosyltransferases were developed from a knowledge of the behaviour of certain complex heterosaccharide structures exhibiting antigenic properties. Sequential degradation of the oligosaccharide chains of human ABH and Lewis blood group specific glycoproteins with exoglycosidases revealed that removal of a single terminal non-reducing sugar resulted in a loss of a particular antigenic property and the development of a new specificity[34]. This procedure could be repeated sequentially down the chain for as long as the appropriate specific exo-glycosidases and antibodies were available; demonstrating that each new carbohydrate structure exposed could function as an antigenic determinant and that the antigenicity of each structure is masked by the addition of a single sugar unit. This pattern of activity suggested that biosynthesis proceeded by a reversal of the degradation pathway and that each specific structure is built up sequentially by the addition of single sugar units. Since the blood group specific determinants are precisely defined carbohydrate structures[35,36] it was apparent that the process could not be random and that the product of one transfer reaction must be the preferred substrate for the next transferase in the series. Predictions from these studies that the primary products of the human *ABO, H* and *Lewis* blood group genes were glycosyltransferase enzymes adding the final sugar to precursor structures to complete the biosynthesis of each determinant[37] were later confirmed by the characterisation of the appropriate human glycosyltransferases[38,39]. Similar studies on the enzymes in pig submaxillary glands demonstrated that the biosynthesis of a blood group A reactive structure resulted from the concerted action of five distinct glycosyltransferases[40].

Extensive pioneering studies on the glycosyltransferases involved in the

biosynthesis of mammalian glycoproteins[41,42] enabled the maxim to be formulated that serial transfer of sugar units, with the product of one enzyme reaction becoming the requisite substrate for the next reaction, is the general mechanism of synthesis of oligosaccharide chains[42,43]. This concept has now to be modified slightly with respect to the asparagine-linked oligosaccharides[27] but sequential addition appears to be the normal pathway for the biosynthesis of *O*-linked oligosaccharide chains[29–31] and for the assembly of the oligosaccharide moieties of glycolipids[44].

Early indirect evidence suggested that each glycosidic linkage is formed by the action of a single highly specific glycosyltransferase and this led Hagopian and Eylar[45] to propose the "one enzyme–one linkage" hypothesis which suggests that there are as many glycosyltransferases as there are different types of glycosidic linkages. This concept, when coupled with the "one gene–one enzyme" hypothesis originating from the classical work of Garrod, Bateson, Beadle and Tatum, suggests that one gene is ultimately responsible for the synthesis of one glycosidic linkage. A vast number of different glycosyltransferases have now been identified and the precise specificity of several have been established for highly purified preparations[30]; as a broad generalisation, the concept of a different glycosyltransferase for each type of linkage appears to be valid. There are, however, some instances where the relationships between gene, glycosyltransferase and glycosidic linkage are more complex than this simple concept might suggest.

A case in point is lactose synthetase. This was the first mammalian galactosyltransferase to be characterised and the mechanism for lactose synthesis was shown to proceed by the straightforward transfer of D-galactose from UDP-D-galactose to the O-4 position of D-glucose[46]. However, subsequent attempts by Brodbeck and Ebner[47] to purify soluble lactose synthetase from milk led to the isolation of two proteins, A and B. Neither component individually had the capacity to synthesise lactose but combination of the two proteins restored catalytic activity. Further studies revealed that the A component was identical with the (1→4)-β-D-galactosyltransferase that catalyses the transfer of D-galactose to terminal non-reducing 2-acetamido-2-deoxy-D-glucopyranosyl groups in glycoproteins[48], and that the B component was α-lactalbumin[49], a protein produced exclusively by the mammary gland. The physiological role of α-lactalbumin is to inhibit the transfer of galactose to *N*-acetylglucosamine and to stimulate the synthesis of lactose. The lactose synthetase A protein has been the subject of considerable study and it was the first glycosyltransferase to be purified to homogeneity[50]. Evidence of continued interest in this enzyme is provided by the fact that it forms the subject of two papers in this issue[51,52]. To date no other protein with a specifier function for a glycosyltransferase has been described and it is conceivable that the mechanism has been evolved uniquely to ensure production of lactose by the mammary gland. However, it has to be borne in mind that other specialised tissues, or cell types, might produce proteins which modify the specificity of ubiquitous glycosyltransferases in order to synthesise di- or oligo-saccharides appropriate to the functioning of that tissue or cell type.

Another apparent exception to the rule of one gene–one enzyme–one glycosidic linkage is the observation that the blood group *B* gene associated (1→3)-α-D-galactosyltransferase has some capacity to transfer *N*-acetyl-D-galactosamine[53] and the (1→3)-*N*-acetyl-α-D-galactosaminyltransferase product of the allelic blood group A gene has some ability to transfer D-galactose[54]; hence both enzymes have the potential to synthesise A and B antigenic determinants. However, from a genetic point of view it is perhaps more surprising that the enzymic products of allelic genes should have major differences in their substrate specificities than that they should share some overlapping functions. No other polymorphic genetic loci encoding glycosyltransferases with qualitatively different donor substrate specificities have yet been recognised in mammalian sources but examples are known in the plant kingdom[55]. The topic of the dual function of the *B* gene-associated transferase is further addressed in a paper in this issue[56].

The Lewis blood group system provides another example of a seemingly anomalous glycosyltransferase. An α-L-fucosyltransferase present only in those individuals carrying an *Le* gene appears to transfer L-fucose not only to the O-4 position of subterminal *N*-acetylglucosamine residues to form Le^a and Le^b structures[38,39] but also to the O-3 position of D-glucose in lactose and in milk oligosaccharides[57,58]. The ability to transfer the donor sugar to either *N*-acetylglucosamine or D-glucose resembles the specificity shift found for the lactose synthetase system[47,48] but this is the first example of a glycosyltransferase adding the donor sugar to two different positions in the acceptor molecules. Whether the *Le* gene product is indeed an enzyme with a dual function or whether a modifier of some kind is involved is a question that remains to be answered.

Despite the very precise specificities of the glycosyltransferases which permit complex heterosaccharide structures to be synthesised with high fidelity, considerable heterogeneity is found in the oligosaccharide chains of glycoproteins. The information available on the preferred pathways of action of the glycosyltransferases and the knowledge that certain products become "chain-stoppers" enables explanations to be put forward for this heterogeneity[29–31]. At a branch point, competition between two or more glycosyltransferases for the same acceptor substrate may occur and the subsequent development of that oligosaccharide chain will depend upon which enzyme is successful. The first sugar linked to protein-bound serine or threonine residues in *O*-linked chains is *N*-acetylgalactosamine and the structure formed is a possible substrate for either a sialyltransferase with the formation of α-Neu*p*^{5}Ac-(2→6)-α-D-Gal*p*NAc-Thr/Ser or a galactosyltransferase with the formation of β-D-Gal*p*-(1→3)-α-D-Gal*p*NAc-Thr/Ser. If the sialyltransferase acts first, the structure formed is not a substrate for the β-galactosyltransferase and the chain cannot be extended whereas if the β-galactosyltransferase acts first the structure becomes the acceptor for further sequential sugar additions. Schachter *et al.*[59] studied the biosynthesis of pig and sheep submaxillary mucins and found that although the glands of both animals contain the galactosyltransferase the ratio of sialyltransferase to galactosyltransferase is much higher in ovine glands with a

consequence that 95% of the oligosaccharide chains in ovine submaxillary mucin terminate with the structure α-Neu*p*5Ac-(2→6)-α-D-Gal*p*NAc-Thr/Ser, whereas in porcine glands longer oligosaccharide chains are synthesised. Similarly heterogeneity of the terminal groupings in the blood group-specific glycoproteins leading to the possibility of multiple specific determinants associated with ABH and Lewis blood groups being carried on the same macromolecule[37] can be explained on the basis of the competition of more than one glycosyltransferase for certain precursor structures and the fact that, once some structures are formed, they are not suitable substrates for any of the remaining glycosyltransferases[38,39]. Beyer *et al.*[30] studied the pathways of biosynthesis of the non-reducing terminal sequences frequently found in glycoproteins by use of highly purified sialyl-, fucosyl- and *N*-acetylgalactosaminyl-transferases; they confirmed the importance of the order in which the transferases act and the fact that the actions of certain glycosyltransferases are mutually exclusive. Two papers in this issue make further contributions to our knowledge of the fine specificity of glycosyltransferases[60,61].

The discovery of the biosynthesis of oligosaccharide chains by the concerted action of a series of glycosyltransferases led to the concept of multi-glycosyltransferase systems[43] and the idea that the enzymes involved in the formation of a particular oligosaccharide chain might be arranged along physically separate assembly lines[29]. Recently an alternative suggestion was put forward to account for the way in which terminal glycosylation might be controlled when two enzymes competing for the same substrate were simultaneously present. In blood group AB individuals the puzzling observation was made that the polyglycosylpeptides isolated from red cell membranes carry either A or B determinants, but not both, despite the fact that each peptide carries an average of three or four determinants[62]. Experimentally it was confirmed that when polyglycosylpeptides from group O cell membranes were used as acceptor molecules in the presence of both the α-(1→3)-*N*-acetyl-D-galactosaminyl- and α-(1→3)-D-galactosyl-transferases, the A and B determinants were found on different polyglycosylpeptides[63]. As an explanation, it was suggested that once the enzyme–acceptor complex is formed it does not dissociate until all the H acceptor sites are substituted with the monosaccharide transferred by the bound glycosyltransferase. The validity and generality of this idea will need to be explored in greater detail but it offers an intriguing alternative to intracellular compartmentalisation.

One of the most exciting developments in the last decade has been the elucidation of the pathways of biosynthesis of asparagine-linked oligosaccharides[27,29–31,64]. The story that has unfolded is so remarkable that it would have been difficult to believe but for the wealth of evidence to support it. The overall pattern appears to be similar in the plant and animal kingdoms. Very briefly stated, a branched oligosaccharide with the structure $Glc_3Man_9GlcNAc_2$ is built up sequentially on the lipid carrier dolichyl phosphate. The first seven sugars (two GlcNAc and five Man residues) are derived from the nucleotide sugars UDP-GlcNAc and GDP-Man, whereas the next seven sugars (four Man and three Glc residues) are derived from

the lipid carriers Dol-*P*-Man and Dol-*P*-Glc. The oligosaccharide is then transferred to an asparagine residue in a newly synthesised protein with the release of dolichyl phosphate. The oligosaccharide chains then undergo a series of degradation, or "processing", steps catalysed by a number of specific exoglycosidases. Removal of three glucose units leaves the "high-mannose" chains which in some glycoproteins are not further processed. Other chains are more extensively degraded and then built up into "complex-type" chains with a constant core structure of $Man_3GlcNAc_2$. To achieve this core structure, four further mannose units are removed from the $Man_9GlcNAc_2$ structure followed by the transfer of GlcNAc to a remaining mannose unit; only after this additional residue is added are two more mannose units removed. Further transfers of *N*-acetylglucosamine, galactose, sialic acid and fucose may then be catalysed by a series of glycosyl transfer reactions. The outer chains of these complex-type structures may be relatively short or large and highly branched. The unravelling of these highly complex pathways has been a major tour de force resulting from intense activity in many different laboratories[27,29–31,64]. Two papers in this issue relate to activities of glycosyltransferases involved at certain stages in the biosynthesis of *N*-linked chains; one is concerned with the enzyme catalysing mannosyl transfer to dolichyl phosphate[65] and the other with the effect of the addition of a "bisecting" *N*-acetylglucosamine residue on the binding of biantennary complex oligosaccharides to lectins[66].

The vital importance of glycosylation for the biological activity of many proteins is becoming increasingly apparent as attempts are made to genetically engineer functional proteins for medical and industrial purposes. The introduction of reagents that inhibit synthesis and processing of *N*-linked chains[27] has made it possible to study the effects of alterations of glycosylation on the function of proteins. The results of perturbation of processing on the activity of the neurotoxin responsive Na^+ channels in mouse neuroblastoma cells forms the subject of a report in this issue[67]. The glycosylation of a fungal glucosyltransferase itself is also considered in a paper[68] demonstrating that antibodies raised against the purified enzyme are specific for the carbohydrate moiety of the glycoprotein. This is an aspect that has to be explored before antibodies to glycosyltransferases are used for the localisation of the enzymes in tissue slices or on cell surfaces, because antibodies specific for carbohydrate structures are likely to detect a wider range of molecules than simply the glycosyltransferase which was used as the immunogen.

Although the glycosyltransferases involved in the biosynthesis of polysaccharides such as glycogen, starch and cellulose have been extensively studied[28], problems of regulation and initiation of synthesis remain to be solved. One paper in this issue invokes a unique guanosyl oligonucleotide as an activator of cellulose synthesis is *Acetobacter xylinium*[69] and the authors of a second paper conclude that a covalently linked protein functions as a primer of glycogen synthesis by the liver[70]. These two papers should each provide a stimulus for further research in these areas.

FUTURE PROSPECTS

Although very substantial developments have taken place in the last three decades in our knowledge of the occurrence and properties of the glycosyltransferases, many facets of the role of these enzymes in the biosynthesis and regulation of carbohydrate structures have still to be clarified. As with all forms of research, the most exciting advances are likely to be those not yet anticipated—but certain areas are obviously ready for exploitation. A number of the enzymes necessary for the biosynthesis of known glycosidic linkages remain to be identified and relatively few of the glycosyltransferases that have been characterised have yet been purified to homogeneity. The introduction of affinity chromatographic methods[30] has been a major advance in the purification of the glycosyltransferases, but progress in the isolation of pure enzyme proteins in amounts sufficient for structural studies is frequently hampered by the very low concentrations in which the enzymes occur in tissues and body fluids.

Isolation of the genes encoding the glycosyltransferases, followed by insertion of the DNA into microorganisms, may be one route by which larger quantities of enzyme proteins will eventually be obtained. Successful procedures for cloning bacterial glycosyltransferase genes have been described[71,72] and a start has been made on eukaryotic genes with the report of the isolation of a cDNA clone that produces a fusion protein reacting with an antibody raised against a sialyltransferase from rat liver[73]. The isolation of the *ABO* blood group genes would enable an answer to be obtained to the vexed questions of how the allelic *A* and *B* genes encode enzymes with qualitatively different sugar specificities and whether the *O* gene encodes an enzymically inactive protein. This system should also serve as a model for the determination of the extent of alteration in the base composition of the DNA, and hence of the amino acid sequence of the protein, necessary to change the specificity of the glycosyltransferase from one sugar to another. The suggestion that the secretor gene, *Se*, is not, as earlier thought, a regulator gene controlling the expression of the blood group *H* gene but a second structural gene encoding an α-(1→2)-L-fucosyltransferase[74] is also amenable to exploration by molecular genetic techniques.

Despite the spectacular advances in the information concerning the pathways of biosynthesis of *N*-linked oligosaccharide chains, the factors that direct the terminal processing reactions and regulate the subsequent glycosylation events are only just beginning to be understood[27]; considerable progress can therefore be expected in deciphering the signals that determine which of the many possible structures the mature oligosaccharides will assume. Early biochemical analysis of subcellular fractions demonstrated glycosyltransferase activities in the endoplasmic reticulum and Golgi apparatus[29] and more recent studies have shown that the enzymes catalysing terminal glycosylation occur in different Golgi subcellular fractions from those involved in the earlier steps[75]. The availability of antibodies to the purified glycosyltransferases is now making possible the precise localisation of

the enzymes by immunoelectron microscopy[76] and this technique can be expected to find much wider application in the future.

The oligosaccharide units of glycoproteins and/or glycolipids have been implicated in a wide variety of normal biological functions including cell–cell recognition, cell adhesion, cell differentiation, the clearance of glycoproteins from the blood, the compartmentalisation of lysosomal enzymes, the binding of antibodies and lectins to cell surfaces and the binding of toxins and microorganisms to host cells[30,31]. Purified glycosyltransferases provide powerful tools for probing many of these structure–function relationships since removal of sugars from glycoproteins or glycolipids with exoglycosidases, followed by reglycosylation enables the importance of the monosaccharide added, and the linkage attachment of that sugar, to be assessed in relation to biological activity. A simple early example of this technique was the demonstration that ceruloplasmin was rapidly cleared from the circulation after neuraminidase treatment and that the elimination could be prevented by resialylation of the glycoprotein with a sialyltransferase[77]. A report in this issue describes the use of a similar technique to examine the role of sialic acid in the binding of erythrocytes to macrophages[78] and numerous other examples could be quoted. The availability of a wider range of highly purified glycosyltransferases of precisely established acceptor specificity should enable this method to be extended to the elucidation of many of the unresolved questions relating to the functions of oligosaccharides.

As more glycosyltransferases are discovered, there is an increasing demand for the well-characterised oligosaccharide substrates that are needed in specificity studies; it is to be anticipated that more extensive use will be made of purified glycosyltransferases for the biosynthesis of oligosaccharides[31,32], especially for those structures which have yet to be synthesised by chemical procedures. Two previously undescribed glycosyltransferases involved in the biosynthesis of human blood group characters are reported in this issue[79,80] but the enzyme responsible for this formation of the P_1 antigen in the blood group P system remains to be identified. A further profitable approach to the study of glycosyltransferases under controlled conditions is the use of cultured cell lines. Enzymes occurring in cultured cells are described in two papers in this issue[67,81] and an extension of this approach, which has already proved effective in providing information on the genesis of cell surface oligosaccharides[82], is the isolation of mutant cell lines.

This catalogue of applications of the glycosyltransferases, and of methods used to investigate their distribution, properties and functions could be extended still further, especially in relation to changes in enzyme expression occurring in disease states. Suffice it to say that the discovery of the glycosyltransferases opened up an area of research which is now being rapidly developed and becoming of increasing importance in biochemistry and medicine.

REFERENCES

1 K. NISIZAWA AND Y. HASHIMOTO, in W. PIGMAN AND D. HORTON (Eds.), *The Carbohydrates*, Vol. 2A, Academic Press, New York, 1970, pp. 241–300, and references cited therein.
2 M. DOUDOROFF, H. A. BARKER, AND W. Z. HASSID, *J. Biol. Chem.*, 168 (1947) 725–732.

3 C. F. CORI, G. SCHMIDT, AND G. T. CORI, *Science*, 89 (1939) 464–465.
4 C. S. HANES *Proc. R. Soc. London, Ser. B*, 128 (1940) 421–450.
5 R. CAPUTTO, L. F. LELOIR, C. E. CARDINI, AND A. C. PALADINI, *J. Biol. Chem.*, 184 (1950) 333–350.
6 W. Z. HASSID, E. F. NEUFELD, AND D. S. FEINGOLD, *Proc. Natl. Acad. Sci. U.S.A.*, 45 (1959) 905–915, and references cited therein.
7 V. GINSBURG, *Adv. Enzymol.*, 26 (1964) 35–38, and references cited therein.
8 E. CABIB, *Annu. Rev. Biochem.*, 32 (1963) 321–354, and references cited therein.
9 G. J. DUTTON AND I. D. E. STOREY, *Biochem. J.*, 53 (1953) xxxvii–xxxviii.
10 L. F. LELOIR AND E. CABIB, *J. Am. Chem. Soc.*, 75 (1953) 5445–5446.
11 J. G. BUCHANAN, *Arch. Biochem. Biophys.*, 44 (1953) 140–149.
12 C. E. CARDINI, L. F. LELOIR, AND J. CHIRIBOGA, *J. Biol. Chem.*, 214 (1955) 149–155.
13 L. GLASER AND D. H. BROWN, *Proc. Natl. Acad. Sci. U.S.A.*, 41 (1955) 253–260.
14 L. GLASER AND D. H. BROWN, *J. Biol. Chem.*, 228 (1957) 729–742.
15 L. F. LELOIR AND C. E. CARDINI, *J. Am. Chem. Soc.*, 79 (1957) 6340–6341.
16 D. S. FEINGOLD, E. F. NEUFELD, AND W. Z. HASSID, *J. Biol. Chem.*, 233 (1958) 783–788.
17 J. S. ANDERSON, M. MATSUHASHI, M. A. HASKIN, AND J. L. STROMINGER, *Proc. Natl. Acad. Sci. U.S.A.*, 53 (1965) 881–889.
18 J. L. STROMINGER, Y. HIGASHI, H. SANDERMANN, K. J. STONE, AND E. WILLOUGHBY, in R. PIRAS AND H. G. PONTIS (Eds.), *Biochemistry of the Glycosidic Linkage*, Academic Press, New York, 1972, pp. 135–154.
19 I. M. WEINER, T. HIGUCHI, L. ROTHFIELD, M. SALTMARSH-ANDREW, M. J. OSBORN, AND B. HORECKER, *Proc. Natl. Acad. Sci. U.S.A.*, 54 (1965) 228–235.
20 A. WRIGHT, M. DANKERT, P. FENNESSEY, AND P. W. ROBBINS, *Proc. Natl. Acad. Sci. U.S.A.*, 57 (1967) 1798–1803.
21 M. J. OSBORN, *Annu. Rev. Biochem.*, 38 (1969) 501–538, and references cited therein.
22 Y. HIGASHI, J. L. STROMINGER, AND C. C. SWEELEY, *Proc. Natl. Acad. Sci. U.S.A.*, 57 (1967) 1878–1884.
23 M. SCHER, W. J. LENNARZ, AND C. C. SWEELEY, *Proc. Natl. Acad. Sci. U.S.A.*, 59 (1968) 1313–1320.
24 D. BROOKS AND J. BADDILEY, *Biochem. J.*, 113 (1969) 635–642; 115 (1969) 307–314.
25 N. H. BEHRENS, A. J. PARODI, AND L. F. LELOIR, in R. PIRAS AND H. G. PONTIS (Eds.), *Biochemistry of Glycosidic Linkage*, Academic Press, New York, 1972, pp. 189–192.
26 A. J. PARODI, N. H. BEHRENS, L. F. LELOIR, AND H. CARMINATTI, *Proc. Natl. Acad. Sci. U.S.A.*, 69 (1972) 3268–3272.
27 R. KORNFELD AND S. KORNFELD, *Annu. Rev. Biochem.*, 54 (1985) 631–664, and references cited therein.
28 H. NIKAIDO AND W. Z. HASSID, *Adv. Carbohydr. Chem. Biochem.*, 26 (1971) 351–483.
29 H. SCHACHTER, in M. I. HOROWITZ AND W. PIGMAN (Eds.), *The Glycoconjugates*, Vol. 2, Academic Press, New York, 1978, pp. 87–181.
30 T. A. BEYER, J. E. SADLER, J. I. REARICK, J. C. PAULSON, AND R. L. HILL, *Adv. Enzymol.*, 52 (1981) 24–175.
31 E. G. BERGER, E. BUDDECKE, J. P. KAMERLING, A. KOBATA, J. C. PAULSON, AND J. F. G. VLIEGENTHART, *Experientia*, 38 (1982) 1129–1258.
32 E. RECONDO AND L. F. LELOIR, *Biochem. Biophys. Res. Commun.*, 6 (1961) 85–88.
33 A. D. ELBEIN, G. A. BARBER, AND W. Z. HASSID, *J. Am. Chem. Soc.*, 86 (1964) 309–310.
34 W. M. WATKINS, *Immunology*, 5 (1962) 245–266.
35 W. T. J. MORGAN, *Proc. R. Soc. London, Ser. B*, 151 (1960) 308–347.
36 K. O. LLOYD, E. A. KABAT, AND E. LICERIO, *Biochemistry*, 7 (1968) 2976–2990.
37 W. M. WATKINS AND W. T. J. MORGAN, *Vox Sang.*, 4 (1959) 97–119.
38 W. M. WATKINS, in A. GOTTSCHALK (Ed.), *Glycoproteins*, Elsevier, Amsterdam, 1972, pp. 830–891, and references cited therein.
39 V. GINSBURG, *Adv. Enzymol.*, 36 (1972) 131–149, and references cited therein.
40 E. J. MCGUIRE, in D. AMINOFF (Ed.), *Blood and Tissue Antigens*, Academic Press, New York, 1970, pp. 461–478.
41 S. ROSEMAN, in E. ROSSI AND E. STOLL (Eds.), *Biochemistry of Glycoproteins and Related Substances, Part II*, Karger, Basel, 1968, pp. 244–269, and references cited therein.
42 R. G. SPIRO, *Annu. Rev. Biochem.*, 39 (1970) 599–638, and references cited therein.
43 S. ROSEMAN, *Chem. Phys. Lipids*, 5 (1970) 270–297.

44 S. BASU AND M. BASU, in M. HOROWITZ (Ed.), *The Glycoconjugates*, Vol. 3, Academic Press, New York, 1982, 265–284, and references cited therein.
45 A. HAGOPIAN AND E. H. EYLAR, *Arch. Biochem. Biophys.*, 128 (1968) 422–433.
46 W. M. WATKINS AND W. Z. HASSID, *J. Biol. Chem.*, 237 (1962) 1432–1440.
47 U. BRODBECK AND K. E. EBNER, *J. Biol. Chem.*, 241 (1966) 762–764.
48 K. BREW, T. C. VANAMAN, AND R. L. HILL, *Proc. Natl. Acad. Sci. U.S.A.*, 59 (1968) 491–497.
49 U. BRODBECK, W. L. DENTON, N. TANAHASHI, AND K. E. EBNER, *J. Biol. Chem.*, 242 (1967) 1391–1397.
50 I. P. TRAYER AND R. L. HILL, *J. Biol. Chem.*, 246 (1971) 6666–6675.
51 D. K. PODOLSKY AND K. J. ISSELBACHER, *Carbohydr. Res.*, 149 (1986) 225–239.
52 E. G. BERGER, E. AEGERTER, T. MANDEL, AND H.-P. HAURI, *Carbohydr. Res.*, 149 (1986) 23–33.
53 P. GREENWELL, A. D. YATES, AND W. M. WATKINS, *Proc. Int. Symp. Glycoconjugates, VIth*, (1979) 268–269.
54 A. D. YATES AND W. M. WATKINS, *Biochem. Biophys. Res. Commun.*, 109 (1982) 958–965.
55 J. VAN BREDERODE AND O. MASTENBROCK, *Theor. Appl. Genet.*, 64 (1983) 151–153.
56 P. GREENWELL, A. D. YATES, AND W. M. WATKINS, *Carbohydr. Res.*, 149 (1986) 149–170.
57 P. H. JOHNSON, A. D. YATES, AND W. M. WATKINS, *Biochem. Biophys. Res. Commun.*, 100 (1981) 1611–1618.
58 J.-P. PRIEELS, D. MONNOM, M. DOLMANS, T. A. BEYER, AND R. L. HILL, *J. Biol. Chem.*, 256 (1981) 10456–10463.
59 H. SCHACHTER, E. J. MCGUIRE, AND S. ROSEMAN, *J. Biol. Chem.*, 246 (1971) 5321–5328.
60 H. T. DE HEIJ, P. L. KOPPEN, AND D. H. VAN DEN EIJNDEN, *Carbohydr. Res.*, 149 (1986) 85–100.
61 H. BAUBICHON-CORTAY, M. SERRES-GUILLAUMOND, P. LOUISOT, AND P. BROQUET, *Carbohydr. Res.*, 149 (1986) 209–223.
62 J. VIITALA, K. K. KARHI, C. G. GAHMBERG, J. FINNE, J. JÄRNEFELT, G. MYLLYLÄ, AND T. KRUSIUS, *Eur. J. Biochem.*, 113 (1981) 259–265.
63 J. VIITALA, *Glyconconjugate J.*, 2 (1985) 149–161.
64 S. C. HUBBARD AND R. J. IVATT, *Annu. Rev. Biochem.*, 50 (1981) 555–583.
65 J. W. JENSEN AND J. S. SCHUTZBACH, *Carbohydr. Res.*, 149 (1986) 199–208.
66 S. NARASIMHAN, J. C. FREED, AND H. SCHACHTER, *Carbohydr. Res.*, 149 (1986) 65–83.
67 M. NEGISHI AND M. C. GLICK, *Carbohydr. Res.*, 149 (1986) 185–198.
68 J. H. PAZUR, D. K. DEHOFF, F. J. MISKIEL, AND C. R. BAUMRUCKER, *Carbohydr. Res.*, 149 (1986) 137–147.
69 P. ROSS, Y. ALONI, H. WEINHOUSE, D. MICHAELI, P. WEINBERGER-OHANA, R. MAYER, AND M. BENZIMAN, *Carbohydr. Res.*, 149 (1986) 101–117.
70 C. R. KRISMAN, R. GEREMIA, AND W. J. WHELAN, *Carbohydr. Res.*, 149 (1986) 35–45.
71 E. S. CREEGER AND L. I. ROTHFIELD, *Adv. Enzymol.*, 83 (1982) 326–353.
72 S. K. KADAM, A. REHEMTULLA, AND K. E. SANDERSON, *J. Bacteriol.*, 161 (1985) 277–284.
73 J. WEINSTEIN, E. L. UJITA, K. MCENTEE, AND J. C. PAULSON, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, 44 (1985) 690.
74 R. ORIOL, J. DANILOVS, AND B. R. HAWKINS, *Am. J. Hum. Genet.*, 33 (1981) 421–431.
75 W. G. DUNPHY AND J. E. ROTHMAN, *J. Cell. Biol.*, 97 (1983) 270–275.
76 J. ROTH, D. J. TAATJES, J. M. LUCOCQ, J. WEINSTEIN, AND J. C. PAULSON, *Cell*, 43 (1985) 287–295.
77 J. HICKMAN, G. ASHWELL, A. G. MORELL, C. J. A. VAN DEN HAMER, AND I. H. SCHEINBERG, *J. Biol. Chem.*, 245 (1970) 759–766.
78 S. KELM, A. K. SHUKLA, J. C. PAULSON, AND R. SCHAUER, *Carbohydr. Res.*, 149 (1986) 59–64.
79 F. PILLER, D. BLANCHARD, M. HUET, AND J. P. CARTRON, *Carbohydr. Res.*, 149 (1986) 171–184.
80 P.-I. CHENG AND A. DE VRIES, *Carbohydr. Res.*, 149 (1986) 253–261.
81 K. K. DAS, M. BASU, S. BASU, AND C. H. EVANS, *Carbohydr. Res.*, 149 (1986) 119–135.
82 J. FINNE, M. M. BURGER, AND J. P. PRIEELS, *J. Cell Biol.*, 92 (1982) 277–282.