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So what do your sugars do?

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Up to about fifteen years ago the above question was quite often asked of glycoconjugates enthusiasts. It was always a silly question, the answer being 'How many doings do you want to hear about?' Since then our understanding of the structures, biosynthesis and varied functions of the glycoconjugates has developed incredibly and come to have a major impact on much of present-day biological and medical research.

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Introduction

The two fundamentally unique aspects of the glycoconjugates were already well-established when I started out as a student forty-odd years ago. My supervisor Bill Whelan inspired his students to share a fascination with starch and glycogen. Although homopolymers, the highly branched structures of these polysaccharides and the intricate pathways involved in their assembly and degradation echo the complexity of structure and metabolism of the heteroglycans of glycoproteins, as discovered later. Indeed, as it turned out, starch and glycogen are themselves glycoproteins: initiation of homoglycan assembly first requires formation of a glucosyl-tyrosine linkage in a protein primer [1]. Our departmental chief Walter Morgan with Winifred Watkins, and Elvin Kabat and his colleagues, were engrossed in their work showing how specific arrangements of branched sugars in the human blood group ABO antigens provide unique information that can be deciphered by proteins such as antibodies or lectins [2].

Heteroglycans: problems and solutions

In 1975, in collaboration with Robin Marshall, I helped organize the IGO Symposium held at the University of Sussex in Brighton. The abstracts presented at this symposium provide a good snap-shot of the still fragmentary knowledge of the glycan moieties of the glycoconjugates and their biosynthesis available at that time. Some partial oligosaccharide structures were described, notably the then novel Fucα1,3 Gal linkage in a human milk oligosaccharide by Akira Kobata and Galα1,4 Galβ1,4 GlcNAc in blood group P_1 active glycolipids by the groups of Walter Morgan and Don Marcus as well as several reports on oligosaccharides isolated from the urine of various lysosomal storage disease patients and now seen to be derived from the Man_3 $GlcNAc_2$ core structure of N-glycans. These successes were no doubt due to the easier purification by paper chromatography or GLC of single components from mixtures of glycolipids or oligosaccharides. Structural analysis of their counterparts in glycoproteins used unfractionated glycopeptides, or even the whole glycoprotein, and techniques such as sequential Smith degradation with sodium metaperiodate. Not suprisingly, the averaged structures of the N-glycans of glycoproteins presented in Brighton in general have not stood the test of time, pioneering though they were. The situation with the O-glycans was somewhat better due to their easy release by alkaline β-elimination. For instance, the structures of the full-length O-linked tetrasaccharide NeuNAcα2,3 Galβ1,3 [NeuNAcα2,6] GalNAc and it's mono- and de-sialylated derivatives, obtained from glycophorin of the human red cell membrane, were already known [3] and soon afterwards were shown to fit biochemically with the pathway of assembly of O-glycan core I structures [4].

In the absence of reliable structural information and some notion of their degree of heterogeneity, the task of elucidating the biosynthetic pathways of the N-glycans was daunting. However, some insight into what was to come was given at Brighton by separate reports of the loss of a specific glycosyltransferase in lectin-resistant CHO cells from Pamela Stanley and Harry Schachter and in BHK cells from Tony Meager and myself. By using different exogenous acceptors for transferase assays with whole cell lysates, Harry and Pam demonstrated for the first time that the wild-type cells contain at least two N-acetylglucosaminyltransferases, only one of which (now called GlcNAc transferase I) was deleted in the

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lectin-resistant cells. Similar results were obtained at the same time by Stuart Kornfeld. Recently, Paul Gleeson and his colleagues cloned the mutant CHO and BHK enzymes and showed that the loss of activity in each case is due to point mutations affecting the catalytic domain of the enzyme [5,6].

Within the next four years leading up to the IGO conferences at Woods Hole and Kiel, the situation had been transformed. The availability of endo-glycosidases and new methods for separation of individual glycans [7] provided substances for reliable carbohydrate sequencing, either by enzyme-linked methods using highly puried exo-glycosidases, or instrument-led methods such as NMR and MS. The major processing pathway of the N-glycans mediated by novel exo-glucosidases and mannosidases was elucidated during this time by Stuart Kornfeld and Phil Robbins and their colleagues. This discovery, together with identification of branch-specific enzymes in N-glycan biosynthesis and earlier information derived for pathways involved in assembly of O-glycans [4,8], provided essential evidence for the existence of subtle but rather stringent controls governing glycan structures. It was an astute realization that when several transferases compete for one particular oligosaccharide to add sugar residues at various positions, the addition of a key monosaccharide by one glycosyltransferase activity present in excess could reduce substrate recognition by others sufficiently to block any further additions. Further, the action of one transferase could provide a substrate for other competing enzymes further down the line of glycan assembly. The conclusion from such work was that variation in glycan structures, although extensive, was not infinite encouraging the growing belief that carbohydrate structures can breed true and therefore could play roles in recognition phenomena, for example at a particular time and place during development.

This period of research in glycoconjugates illustrates one of those occasions when progress in biochemical research was complemented or even led by contemporaneous developments in chemistry. For example, the detailed structures of the N-glycans of egg-white ovalbumin and thyroglobulin by Akira Kobata's group [7] predicted the nature of a common precursor as well as the order of mannose removal in the major processing pathway. Although not so obvious at the time, the structures of oligomannose glycans of human IgM determined by Rosalind Kornfeld's group [9] may have been the first sighting of an alternative processing pathway involving endo-mannosidase subsequently discovered by Robert Spiro [10]. Most impressive was the contribution of synthetic carbohydrate chemistry, notably by Roger Jeanloz and his colleagues Chris Warren and Annette Herscovics, to the elucidation of the structure and anomericity of glycosylpolyisoprenol phosphates involved in glycan biosynthesis.

Recognizing carbohydrates

All good things, such as post-doctoral wanderings, must come to an end and by 1970 I had responsibility to develop some individual line of research. At that time NIMR was a hot-bed of immunological research. One of the dramatic discoveries, by Martin Raff and later extended by many others, was the capping of surface glycoproteins of lymphocytes by specific antibodies such as anti-Thy 1 or plant lectins such as PHA, and it's link to activation of the recently discovered T- or B-lymphocyte sub-sets. Equally exciting news was coming from Max Burger and others showing that cells transformed either by oncogenic viruses or chemical carcinogens were agglutinated by plant lectins, such as wheat germ agglutinin, whereas normal cells were not. These findings indicated roles for glycoconjugates in important cell surface interactions: the roles appeared to depend on the presence of particular carbohydrates at the cell surface, since not all lectins were active in capping lymphocytes or in agglutinating tumour cells. Conversely, glycosylation of surface glycoconjugates was seen to be cell-specific, even in cells derived from a common progenitor, since some lectins capped glycoproteins of T- but not B-lymphocytes and *vice versa*. Thirdly, changes in sugar composition of cell surface glycoconjugates accompanied the change to malignancy, one striking feature of which was often reduced adhesiveness and increased motility of the transformed cells.

Although it had been long appreciated that inter-cellular adhesion and agglutination, cell–cell recognition, contact inhibition of growth and motility, cell attachment to a growth substratum and other important physiological phenomena are mediated at the cell surface the cell-adhesion molecules involved were very ill-defined in 1970. The discovery of the major classes of cell-adhesion molecules (CAMs), namely the integrins and the members of the immunoglobulin and cadherin superfamilies, was some distance away. Similarly, the matrix molecules involved in cell attachment to substrata were undefined, although the 'spreading factor' of serum, later re-named plasma fibronectin, was already in use. 'Spreading factor' was so-called because an inert surface such as a glass coverslip coated with it promoted the rapid spreading of cells following their initial attachment. Spreading was associated with cytoskeletal reorganization and formation of focal contacts at the ventral surface of the substrate-attached cells. Antibodies directed against plasma membranes detached the cells from the substratum, showing spreading was mediated by fibronectin receptors on the cell surface but the nature of these receptors was unknown [11].

Despite this lack of knowledge, or perhaps because of it, the idea that cell-surface carbohydrates might be implicated in cell–cell and cell–substratum interactions was attractive and experimentally approachable. In an influential review paper [12] based on extensive experimental work from his group, Saul Roseman suggested that glycosyltransferases on cell-surfaces might function non-catalytically to engage with carbohydrate 'substrates' on apposing cell-surfaces to mediate intercellular adhesions. He also showed that cells would adhere to certain carbohydrates e.g. thiogalactoside linked to inert surfaces by aminohexyl or other linker arms, perhaps

mimicking cell–substratum interactions. Given the then emerging evidence for the exacting oligosaccharide substrate specificity of glycosyltransferases, such interactions could clearly be very precise and account for selective adhesions that had been established long before by embryologists such as Holfreter and Moscona. The remarkable phenomenon of 'sorting-out' by which cells from different tissues segregate by tissue type was widely believed to be relevant to histogenesis. The list of potential candidates for carbohydrate-binding partners in adhesive interactions was enlarged with the then recent discovery by Gil Ashwell and Anatole Morell of the hepatic galactose-binding lectin, a protein without any enzymic activity [13]. Although it seemed probable at the time and later became established that this lectin is a prototype for receptor-mediated endocytosis and trafficking of circulatory glycoproteins to lysosomes, the notion that lectins may be stably expressed at the cell-surface and participate in adhesive interactions was very attractive in the 1970s. This view was of course completely vindicated by the discovery ten years later of the selectins involved in leukocyte adhesion, and the galectin family of mammalian lectins of which more later.

Already in 1970 it was known that many plant lectins, after binding to cell-surface carbohydrates of mammalian cells, entered cells by endocytosis and blocked cell proliferation. Therefore, a proportion of cell mutants selected for resistance to cytotoxic lectins would be expected to exhibit altered glycosylation of surface glycoconjugates such that lectin binding was abolished. The adhesive properties of these cells would be an interesting test of the Roseman hypothesis. Tony Meager and I [14] chose ricin to select a panel of BHK mutants, mainly because of its extreme cytotoxicity and relatively well-established mechanism of action. At the same time Pamela Stanley and Stuart Kornfeld used several plant lectins including ricin to select CHO mutants. Biochemical assays quickly established a range of glycosylation defects leading to lectin resistance and subsequent screens have produced many more, recessive or dominant when crossed with wild-type cells [15]. One defect in GlcNAc transferase I was mentioned previously: glycan analysis showed that in these cells N-glycan processing was blocked at the $Man_5GlcNAc_2$ stage and complex N-glycans were undetectable. Peter Vischer, Paul Gleeson and I found other BHK mutants to be defective in GlcNAc transferase II and processing mannosidase II activities, leading to replacement of complex N-glycans by hybrid-type glycans containing core tri- or penta-mannoside respectively.

To our relief the ricin-resistant cells with changed surface glycosylation did show abnormal growth and adhesive properties. Studies with John Edwards showed that several of the cell lines formed less strong intercellular aggregates when single cell suspensions obtained by EDTA- or trypsin-treatment of monolayer cultures were re-suspended in calcium-containing medium [16]. A little later Sergio Pena noticed that these cells had a rounded morphology when grown as monolayers, unlike the well-spread fibroblastic appearance of the wild-type cells [17]. He then discovered that the cells produced fibronectin as efficiently as wild-type cells and as active in promoting adhesion of wild-type cells to substratum. By contrast, the mutants were unable to adopt a fully spread morphology, shown by wild-type cells, when plated onto inert surfaces coated with fibronectin from either mutant or wild-type cells. These results indicated two interesting points: the glycosylation defects in the mutant cells, although having no effect on the biological activity of the fibronectin secreted from the cells, did block some other cell-surface component involved in fibronectin-mediated adhesions. Was this the fibronectin receptor itself? When the fibronectin-binding integrin $\alpha_5\beta_1$ was finally identified, and specific antibodies became available, Tamami Koyama tested this by analysing the molecule obtained from wild-type and ricin-resistant BHK cells [18]. The wild-type receptor subunits contained mainly complex-type N glycans whereas, as expected, the subunits from the GlcNAc transferase I-deficient mutant cells contained only endo H-sensitive high mannose chains. Nevertheless, the mutant subunits assembled into a heterodimer which was expressed normally at the cell surface and bound as well as wild-type receptor to fibronectin in solid state binding assays. Thus, the glycosylation defect when expressed on the $\alpha_5\beta_1$ integrin did not account for the reduced adhesiveness of mutant cells on fibronectin. An alternative explanation for our observations then came from Anne Woods and John Couchman [19]. They showed that the GlcNAc transferase I-deficient cells possess a cell surface heparan sulphate with dramatically altered properties compared to wild-type. These included decreased sulphation, reduced affinity for fibronectin in binding assays and a shorter half-life at the cell surface. We were encouraged by these findings since earlier John and Anne had found that BHK and other cells required both the cell-binding and at least one heparin-binding domain of fibronectin for focal adhesion formation [20]. By chance, Stamatis Stamatoglou, who at that time was a colleague studying adhesion molecules in liver development and cancer [21], had reported even earlier and independently a relationship between under-sulphation of cell-surface heparan suphates, fibronectin binding and adhesion [22]. Recent work has shown directly that syndecan-2, specifically a micro-domain enriched in 2-O-sulphated iduronides essential for fibronectin binding, participates selectively with integrin $\alpha_5\beta_1$ in induction of stress fibres [23]. In retrospect, it is not surprising that a glycosylation block such as GlcNAc transferase I-deficiency had pleiotropic effects that contributed indirectly to abnormal cellular behaviour. For example, mutant N-glycosylation might affect the activity, multimerization or proper direction to intracellar compartments of transferases and other enzymes involved in proteoglycan biosynthesis. It is an interesting question whether the lethality or developmental abnormalities in null mice lacking N-glycan transferase genes [24] are partly due to down-stream alterations in proteoglycan structures, known to be crucial activators of developmentally important growth factors [25].

Science and serendipity

In early 1987 an unusual event occurred. A graduate student Louise Foddy declared she had collected enough data for her thesis to be presented (successfully) the following September. She wanted to fill in the time doing something more biological than her previous projects and did not wish to take an extended holiday. For graduate students this is even more unusual. We decided to look again at the cell agglutination experiments with the ricin-resistant mutants done by John Edwards several years before. In the meantime there had been an explosion in defining CAM structures and the mechanisms of cell–cell and cell–substratum adhesions [26]. How did the agglutination of disaggregated cells, removed from their growth substratum, relate to the adhesions formed between cells *in vivo*? We knew that most of the mutant cell lines we had tested, like the wild-type cells, were contact inhibited in monolayer culture suggesting that intercellular junctions formed relatively normally. Indeed, dye-spreading experiments had shown early on that the mutant cells formed gap junctions, almost as well as the wild-type. The small reduction in gap junction activity could be explained by the weak adhesion of the mutant cells to substratum, given the known interdependence of cell–cell and cell–substratum adhesions [26]. Perhaps the explanation for John's observations was as follows: the agglutination assays required incubation of single cell suspensions in a gyratory shaker for several hours to allow the surface to 'repair' after EDTA or trypsin treatment. During this time the cells may have secreted a galactose-binding lectin which then could agglutinate wild-type cells but not mutant cells lacking surface galactosylated proteins. Louise passed a detergent lysate of BHK cells through immobilized β-galactoside columns and eluted the bound fraction with lactose-containing solution. A clean band of 30 kDa was detected after SDS-PAGE. Rabbit antibodies raised against this protein, which we called CBP30, readily blotted it in BHK wild-type or mutant cell extracts. However, we were chagrined to find that the antibody did not blot CBP30 in extracts of hamster kidney. Then we realized that BHK is short-hand for baby hamster kidney and sure enough late embryonic kidney gave a strong CBP30 signal that rapidly decayed after birth to very low levels [27]. The follow-up to these observations have kept me and a succession of superb colleagues busy ever since.

In connection with serendipity in science, the following story is relevant. In the mid-1980s, an undergraduate student Elizabeth Monis came to the laboratory for six months on secondment from Brunel University and needed a short project. Some years before Terry Butters, a long-time colleague, had become interested in evolutionary aspects of N-glycosylation and had been studying glycosylation in insect cells lines, mainly mosquito [28]. He had found that the majority of the N-glycans of these cells were endo-H sensitive oligomannosides and complex-type glycans were undetectable. However, a small fraction of the mannose-labelled glycans were endo-H resistant and assumed to be the core Manα 1,3 [Manα 1,6] Manβ 1,4 GlcNAcβ 1,4 GlcNAc-R structure. This was puzzling since the cells had no detectable GlcNAc transferase I and in absence of this enzyme the end-product of mannosidase processing would be a penta-mannose carrying two extra mannose residues on the Manα 1,6-arm of the core structure. In mammalian cells removal of these residues by mannosidase II requires the prior action of GlcNAc transferase I [8]. Perhaps insect cells contained a new mannosidase that could remove these two residues directly. Therefore, Liz incubated extracts of mosquito cells with a radiolabelled Man_5 GlcNAc oligosaccharide and separated the products using methods developed by Gary Mills, who was profiling the glycans of ricin-resistant mutants at that time. As negative control, Liz used BHK cells. To our surprise, the pentamannose substrate, and indeed all of the processing oligomannose $Man_{5–9}$ GlcNAc intermediates, were rapidly converted to Manα 1,3 [Manα 1,6] Manβ 1,4 GlcNAc tetrasaccharide by the BHK extracts. Perversely, the mosquito extracts were completely unable to process beyond Man_5 GlcNAc [29]. Pedro Bonay, a Venezuelan graduate student, later purified the mannosidase from rat liver. It turned out to be a novel Co^{2+}-dependent enzyme, a dimer of 110 kDa subunits, resistant to the mannosidase II inhibitor swainsonine. Using sub-cellular fractionation and EM in collaboration with Jurgen Roth, Pedro localized the enzyme in the endoplasmic reticulum, Golgi and endosomal compartments of liver [30]. The role of this enzyme in N-glycan processing is still unclear. An enzyme of similar properties called mannosidase III was described by Michiko Fukuda and her colleagues in many tissues, except erythroid cells, of mannosidase II null mice [31]. The null mice showed a complete loss of complex glycans in erythroid cells, and interestingly exibited dyserythropoiesis. Strikingly, complex-type glycans were found in all other tissues which led Michiko to suggest that the Co^{2+}-dependent enzyme may bypass the normal processing pathway and allow formation of complex-type glycans in absence of mannosidase II. However, if the Monis-Bonay enzyme and mannosidase III are the same entity it is puzzling that GlcNAc transferase I-deficient BHK cells, which contain the enzyme, accumulate large amounts of the pentamannosidic core structure.

Towards a developmental glycobiology

When CBP30 was cloned [32], it was found to be the hamster homologue of galactose-binding proteins of similar size previously described in other species. Later these proteins were collectively incorporated into the galectin family of mammalian β-galactoside-binding proteins [33]. CBP30 was re-named galectin-3 [reviewed in 34–39].

We found galectin-3 is secreted from cells, in a novel pathway avoiding the ER-Golgi route used by glycoproteins including potential binding partners [40–43]. Extracellularly, it

can bind to cell surface glycoproteins such as integrin subunits [44] or to matrix glycoproteins such as laminin or fetal fibronectin [45]. Like several other galectins, galectin-3 binds preferentially to polylactosamine glycans, but shows additional affinity for glycans capped with sialylα2,3 or blood group ABO-like structures [45,46]. So what is it doing in kidney?

Immuno-histochemistry of neonatal hamster kidney [27] had localised the protein to the collecting ducts and connecting segments of distal tubules, epithelia that derive from the Wolffian duct by repeated rounds of branching. As found earlier in hamsters, galectin-3 is highly regulated during development of the human or mouse metanephric kidney. In mouse it is very scarce before (embryonic day) E14 during the steady-state phase of ureteric expansion and becomes maximally expressed at E16 when the mature nephrons start to form [47, S Bullock, T Johnson, Q Bao, RC Hughes, PJD Winyard and AS Woolf, to be published]. Perhaps, one role for galectin-3 is in limiting the growth of the ureteric tree? Therefore, we added galectin-3 exogenously to explant cultures of embryonic mouse kidney and found that it blocks ureteric branching *in vitro* and in a manner reversible by sugar inhibitors. The mechanism of the blockade was suggested by other results. *In vivo* the protein is first localised at low levels in human or mouse kidney at the apical domains of actively proliferating ureteric bud branch tips. However, as the lineage matures into medullary collecting ducts galectin-3 is up-regulated and re-distributed to the basolateral domain, that portion of the polarized cell surface engaged in adhesive interactions with basement membranes to stabilize tubular organization [47,48, Bullock et al., to be published]. In other experiments, MDCK cells derived from collecting duct epithelia that form cysts when grown in collagen gels, were found to be sensitive to galectin-3 added exogenously to the basal surface. The steady-state growth of wild-type MDCK cysts, but not ricin-resistant MDCK cells lacking surface galactose, was strongly inhibited [49,50]. Evidently, adhesive interactions between the basal surface of the epithelium and basement membranes are stabilized by the lectin, possibly by linking matrix proteins such as laminin to adhesive glycoproteins such as integrins on the ventral surface of the epithelial cells or by activation of the latter receptors in some way. Importantly, this is a specific effect since galectin-1, or galectin-3 mutants lacking affinity for blood group A-like structures [46], are completely inactive in the kidney explant or MDCK cyst assays (Bullock et al., to be published). We believe these studies may have relevance for understanding, and perhaps treatment, of human cystic diseases. For example, in human renal dysplasia where the dysplastic tubules often terminate in massive cysts, galectin-3 expression is confined to the apical aspect of the cystic epthelia [48] where any role at basal surfaces of the kind discussed above is obviously compromised.

What next?

In 1970 the US National Academy of Sciences convened a distinguished panel to appraise the state of the art in the biological sciences and to visualize future trends [51]. This exercise resulted in a masterly account of the major achievements up to that time, but understandably there was no hint from the panel that they expected to be reading whole genomes by the millennium. Progress always outstrips prediction.

Taking a short term view new structures will continue to enhance understanding of carbohydrate-binding specificity and it's biological significance. Structures of the C-type lectins and the galectins are already defining the molecular basis for sugar-lectin binding [52–55]. The first structures for an N-glycan transferase [56] and a processing glycosidase [57] were reported recently and those for other members of the galactosyl-, sialyl- and GlcNAc-transferase families as well as the multiple polypeptide/α-GalNAc transferases cannot be far behind. Differences in substrate specificity of enzymes within families [see 58,59] are likely in many cases to derive from small differences in non-conserved residues on a largely conserved structural background within the combining site: in the extreme case only four nucleotide substitutions are found in the cDNAs encoding the human blood-group A or B transferases [60]. Based on this knowledge, enzymes and lectins with interesting new specificities might be produced by site-directed mutation, to provide tools for studying important carbohydrate-mediated events. For example, the role of endosomal pH in cargo-release and receptor recycling may be further defined using acid-insensitive mutants of the asialoglycoprotein receptor [61]. Similarly, the role of ER mannosidases in calnexin/calreticulin-mediated quality control of polypeptide folding and glycoprotein trafficking might be dissected using cells carrying a mutant yeast ER mannosidase broadened in it's specificity to resemble that of Golgi mannosidases [62].

Although the general pathways of glycan assembly are rather well established from *in vitro* studies, challenging questions remain about their regulation inside the cell. The discovery of alternative pathways including the endo-mannosidase or mannosidase III by-pass discussed earlier for N-glycans is particularly intriguing. Are the 'minor' pathways simply a fail-safe mechanism in case something amiss happens to a major pathway, or a more 'primitive' one in phylogeny or during development? Do the structurally distinct processing intermediates generated in 'minor' pathways have some biological function, for example in intra-cellular trafficking or as substrates for novel transferases or endogenous lectins? Are the 'minor' pathways normally used specifically for glycosylation of particular proteins, in preference to the major pathway? Many years ago David Williams and Bill Lennarz showed that the Man_5 $GlcNAc_2$ oligosaccharide moiety of native bovine ribonuclease B was a substrate for processing enzymes, presumably GlcNAc transferase-I and -II and

mannosidase II, present in detergent lysates of rat liver Golgi fractions. In the presence of UDP-GlcNAc, the glycan of the native or denatured RNAase was rapidly converted to an endo-H resistant form after treatment [63]. However, lysates of the homologous bovine Golgi membranes were only able to carry out glycan remodelling of the denatured protein. Is the failure to remodel the native protein due simply to species-related differences in transferases and processing glycosidases? Alternatively, is there another regulatory layer governing terminal glycosylation of folded or partially folded glycoproteins within Golgi compartments, perhaps determined by as yet undiscovered ancillary proteins? This provocative study deserves re-examination with other native glycoproteins.

Contributions from the new glycobiology can be expected to have major impact on advances in developmental biology and immunology. There is already substantial evidence for developmentally-regulated glycosylation affecting the functions of CAMs and matrix molecules [reviewed in 64], the best-known being the influence of polysialylation of Ig-domain V on the adhesive properties of NCAM. A recent structure [65] helps in understanding how such glycosylation influences homophilic interactions of NCAM mediated through Ig-domains I and II. More generally, perhaps a principal role of endogenous lectin families is to act as sensors of regulated changes in glycosylation of adhesion proteins, leading to modulation of their activities. In line with this possibility, the increased surface expression of galectin-3 in activated mouse peritoneal macrophages during peritonitis has been correlated with altered glycosylation of receptor glycoproteins, including the Mac-1 integrin implicated in macrophage extravasation [40,44]. The galectins are attractive candidates for such roles, given their widespread distribution and dynamic patterns of expression during development [66,67]. Recent work, especially of Francoise Poirier and her colleagues, shows that null mutant mice lacking either or both galectin-1 and -3 are viable and have no overt abnormalities [66]. However, subtle effects are seen on close examination that could very likely be due to effects on adhesive mechanisms. Thus, absence of galectin-1 leads to deficits in axon pathfinding by primary olfactory neurons [68], consistent with other data showing the lectin regulates initial axonal outgrowth in peripheral nerve after axotomy [69]. Null mice lacking galectin-3 have significantly reduced abilty to retain neutrophils during an inflammatory response, perhaps due to mis-functioning of adhesion mechanisms at the inflammatory sites [70]. In this connection the observed binding of galectin-3 to the heavy-chain of CD98 [44], a widely expressed cell-surface transmembrane heterodimeric glycoprotein, might be relevant. At cell surfaces CD98 forms complexes with several integrins and clustering of these complexes by CD98-specific antibodies stimulates integrin-dependent cell signaling and adhesion [71] raising the possibility that galectin-3 functions as an endogenous activator of CD98-integrin complexes. Whatever the mechanisms turn out to be, the growing evidence that galectins influence crucial extracellular activities and are associated with the progression of cystic disease as discussed previously, tumour metastasis [72], fibrosis [73,74] and other serious diseases makes them attractive therapeutic targets.

The roles of the glycan chains of the proteoglycans in differentiation and morphogenesis will continue to excite developmental biologists [25]. So far most attention has been directed at heparan sulphate proteoglycans, long known to show great structural diversity generated by complex patterns of deacetylation, sulphation and epimerization of the precursor polysaccharide. Recent work, however, has drawn attention to the chondroitin sulphates as well [75]. For example, during mouse hindbrain development pathfinding by sub-sets of sensory axons appears to be regulated by extracellular chondroitin-6-sulphate [76]. In the case of heparan sulphates, specifically glycosylated domains within the heparan sulphate chains can bind various growth factors, limiting their extracellular diffusion and establishing morphogenetic gradients across developmental fields. Discovery of novel enzymes involved in minor structural modifications of chondroitin glycosaminoglycans, required for binding of morphogens, seems very likely. Recent studies suggest that heparan sulphate, or specific oligosaccharide fragments derived from it, can activate some growth factors for example FGFs [77–79]. Activation appears to involve assembly of signaling complexes with primary cell surface receptors. Elucidation of these signaling pathways, and the development of small oligosaccharide agonists or antagonists of the pathways, may suggest new therapeutic leads for control of certain diseases. For example, deficits in EXT genes involved in heparan sulphate biosynthesis are linked with human hereditary multiple exotoses [80], perhaps due to some failure in signalling by the Hedgehog family of bone morphogens that might be corrected by small molecular agonists.

Conclusion

Whatever the future holds for glycobiology, it will be exciting and unexpected. I can find only four very brief comments on the glycoconjugates in Handler ([51], pages 103, 121, 123) as follows: the variety of structures possible in heteroglycans is enormous, an example is the blood group substances; in the nervous system complex polymers may constitute a mechanism for recognition between matching neurons; understanding of the protein-polysaccharides is meager; study of their synthesis and degradation is in it's infancy. Times have changed!

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