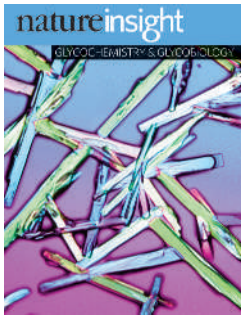

GLYCOCHEMISTRY & GLYCOBIOLOGY

Cover illustration
Polarized light micrograph showing crystalline glucose, one of the simplest sugars. (Courtesy of Science Photo Library.)

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Carbohydrates have long been underappreciated by the scientific community, and many scientists approach the complex structures and elaborate nomenclature of carbohydrates with trepidation. Like amino acids and nucleic acids, sugars are abundant in nature: many natural products contain oligosaccharides that are vital for their biological activity, and carbohydrates have key roles in a broad range of biological processes, including signal transduction and immune responses.

Although fewer scientists work with carbohydrates than with other biopolymers, researchers in this field have been prolific. Chemists and biochemists have developed new methods to rapidly synthesize oligosaccharides, enabling them to generate complex polysaccharides and analogues of natural products that have increased activity *in vivo*. Biologists have explored the physiological roles of various sugars, discovering that many have essential roles in all of the major organ systems and are involved in several disease states. In addition to extending our knowledge of how the natural world works, these findings have been used to develop carbohydrate-based drugs and vaccines, some of which show great promise for treating or preventing various diseases, including malaria, cancer and AIDS.

The number of chemists, biochemists and biologists in this field is steadily increasing, and this interest underscores the fact that there are so many discoveries to be made. To quote Ajit Varki (see page 1023), it is "a fertile area for the new generation of young scientists".

In this Insight, we present a collection of reviews that highlights some of the hottest areas in glycochemistry and glycobiology, including the chemical synthesis of carbohydrates, their biological functions and the therapeutic potential of carbohydrate-based drugs and vaccines. We hope that you enjoy it.

We are pleased to acknowledge the financial support of Vastox and Dextra Laboratories in producing this Insight. As always, *Nature* carries sole responsibility for all editorial content and peer review.

Joshua Finkelstein, Senior Editor

REVIEWS

nature insight

Glycan-based interactions involving vertebrate sialic-acid-recognizing proteins

Ajit Varki[1]

All cells in nature are covered by a dense and complex array of carbohydrates. Given their prominence on cell surfaces, it is not surprising that these glycans mediate and/or modulate many cellular interactions. Proteins that bind sialic acid, a sugar that is found on the surface of the cell and on secreted proteins in vertebrates, are involved in a broad range of biological processes, including intercellular adhesion, signalling and microbial attachment. Studying the roles of such proteins in vertebrates has improved our understanding of normal physiology, disease and human evolution.

Among the few seemingly universal biological findings is the fact that all the cells of every species studied so far are covered with a dense coating of glycans (oligosaccharides or polysaccharides), which was originally revealed by electron microscopists and named the 'glycocalyx'[1]. These sugar chains are often covalently attached to underlying proteins or lipids, and form various structures that are cell-type specific and developmentally regulated, typically changing in response to environmental cues[2–4], and causing disease when genetically deficient[5]. In eukaryotic cells, the main mechanisms by which cell surfaces become glycan-coated occur in the endoplasmic reticulum–Golgi biosynthetic pathway[3,4,6,7]. Other complex machineries fulfil similar functions in prokaryotes[8]. The probable evolutionary reasons for this prominence of cell-surface glycosylation are discussed elsewhere[9,10]. Given that glycans have dominated cell surfaces for billions of years, it is reasonable to predict that many cellular interactions are mediated or modulated by these molecules. It should also not be surprising that these biological roles range widely, from highly specific 'lock-and-key' type mechanisms all the way to relatively non-specific effects such as negative charge repulsion — and everything in between.

This review discusses principles involved in understanding and classifying the cellular interactions that are mediated by glycans, and then focuses on sialic acids and the proteins that recognize them. Particular attention is paid to vertebrate sialic-acid-recognizing proteins, such as the selectins and the Siglecs. Such molecules are presented as examples of different models of glycan-mediated cellular interactions, an area of increasing biological interest and importance.

General principles of glycan-mediated cellular interactions

There are various classes of cell-surface glycan, including N- and O-linked glycans, glycosphingolipids, glycosaminoglycans, glycophospholipid anchors and lipo-oligo/polysaccharides. Each of these has characteristic core or linkage regions, and they often present varying types of terminal or internal sequence[2–4]. The structural complexity of glycans is much greater than that of proteins and lipids, and the details of their structural features are not reviewed here. Another unusual feature is that in aqueous solution most glycans present an ensemble of many different conformations, only one of which might be relevant to a given interaction[11]. Interactions involving glycans can include recognition by other glycans (carbohydrate–carbohydrate interactions)[12], as well as recognition by certain proteins[13]. This review focuses on the latter class of interaction.

Most glycan–protein interactions involve the recognition and locking into place of one of a specific glycan's many conformations in the protein's binding pocket. This is, in part, why most glycan-based interactions require multivalency to achieve the avidity sufficient for biologically meaningful interactions[13]. There are also many intermediate situations in which cell-surface glycans have relative specificity for certain surface proteins, modulating rather than dictating their activity[12]. Glycan-binding proteins can themselves be glycosylated, potentially affecting any stage in their life cycle, from their initial folding, their delivery to the cell surface and their ability to interact with other molecules at the surface, to their turnover.

Glycan-binding proteins have traditionally been classified according to the type of glycan they recognize, or on the basis of their biological roles. As discussed elsewhere[14], they may be better classified on the basis of their structural and evolutionary relationships into two broad groups: lectins[13,15], which can be further subclassified into evolutionarily related subgroups with common ancestors, and glycosaminoglycan-binding proteins, which seem to have evolved by convergent evolution to bind the acidic sulphated sequences found in glycosaminoglycans (see page 1030). This evolutionary approach is useful and most logical when seeking to understand orthologous functions between organisms[15]. Glycan-binding proteins comprise a very large group of molecules that cannot be reviewed in the available space. The rest of this review therefore focuses on one class of glycan (the sialic acids) and the proteins in nature that recognize them. Particular attention is then given to vertebrate sialic-acid-recognizing lectins — that is, those intrinsic to the multicellular organisms that synthesize sialic acids.

The sialic acids

The sialic acids are a family of sugars with a shared nine-carbon backbone that are typically found attached to the terminal positions of several classes of cell-surface and secreted glycan molecules (Fig. 1). Although they are probably evolutionarily ancient, sialic acids are most prominent in the deuterostome lineage (which comprises vertebrates and 'higher' invertebrates)[16,17]. Interestingly, several of the rare non-deuterostome organisms that express sialic acids are microbial pathogens that invade vertebrates, apparently because this is a successful form of 'molecular mimicry'[18]. Having emerged as major cell-surface molecules of deuterostomes during the Cambrian expansion about 530 million years ago, the sialic acids have become greatly diversified, such that there are more than 50 structural variations in nature[16,17] (see Fig. 2

[1]Departments of Medicine and Cellular & Molecular Medicine, Glycobiology Research and Training Center, University of California at San Diego, La Jolla, California 92093-0687, USA.

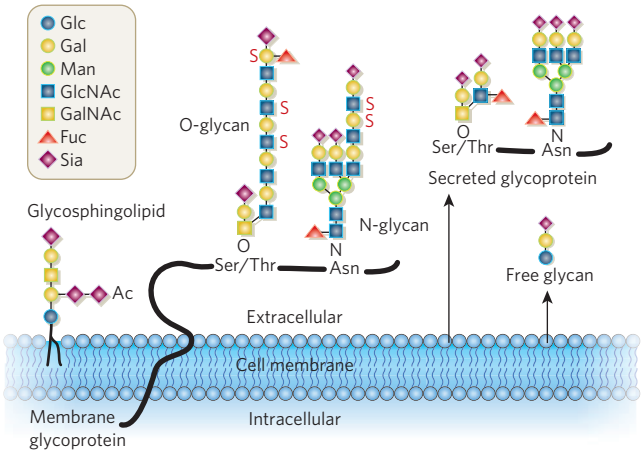

Figure 1 | Sialic acids on cell-surface and secreted molecules. Sialic acids (Sia) are typically found at the terminal position of N- and O-linked glycans attached to the cell surface and to secreted glycoproteins, as well as on glycosphingolipids expressed at the cell surface. Ac, *O*-acetyl ester; Fuc, fucose; Gal, galactose; GalNAc, *N*-acetylgalactosamine; Glc, glucose; GlcNAc, *N*-acetylglucosamine; Man, mannose; S, sulphate ester.

for two examples), each of which can be attached to underlying glycans by means of various α-linkages from the C2 position[19]. This remarkable diversity is expressed in a cell-type and developmentally regulated manner, and often changes in response to environmental cues. Thus, sialic acids are in a position to mediate specific interactions governed by the binding of proteins that are intrinsic to the same species. However, the relative lack of conservation of sialylation patterns even between related species[20] suggests that diversity may also be driven by the pathogens that exploit sialic acids as targets for recognition[9,10,14]. Of special note is the fact that the evolutionary lineage from which humans descended suffered an unusual genetic defect[21] in the production of one of the common Old World primate sialic acids, *N*-glycolylneuraminic acid (Neu5Gc) — secondarily causing accumulation of the precursor sialic acid *N*-acetylneuraminic acid (Neu5Ac) (Fig. 2).

Details about sialic acids[16,17] and the usefulness of metabolic incorporation of unnatural sialic acid precursors into mammalian cells[22] are discussed elsewhere. Included in this review are examples of interactions involving the three main classes of vertebrate glycan that carry sialic acids: N-glycans, O-glycans and glycosphingolipids (Fig. 1) — as well as certain bacterial polysaccharides that can display them[18].

Sialic-acid-binding proteins in organisms without sialic acids

Given their terminal location and diversity, it is not surprising that many pathogens have evolved binding proteins or toxins specific for certain sialic acids, often with additional specificity for the linkage and/or the underlying sugar chain (see Table 1 for examples). Mysteriously, many multicellular sialic-acid-deficient organisms (such as plants, arthopods and molluscs) also express sialic-acid-binding proteins, which have a remarkable degree of specificity for certain types of sialic acid and their linkages (Table 1). These lectins might serve to recognize sialic acids on pathogens, or on other species that display them. It is also possible that some of these lectins recognize other related acidic sugars (such as keto-deoxyoctulosonic acids), and have sufficient cross-specificity to be identified and classified as sialic-acid-binding lectins. Regardless of the reasons, such proteins can be extremely useful tools with which to study sialic acids[20,23].

Sialic-acid-binding proteins in organisms with sialic acids

Until the 1980s it was thought that sialic acids functioned primarily to provide negative charge and hydrophilicity to vertebrate cell surfaces, to mask subterminal galactose residues from recognition by certain receptors, and to act as receptors for pathogens and toxins. Although examples of the latter role are widespread and show elegant structural specificity, they obviously cannot explain the persistence of sialic acids in all deuterostomes for more than 500 million years. Indeed, sialic acids are essential for early embryonic development in mice[24], but not for embryogenesis in *Drosophila* or *Caenorhabditis elegans*. Thus, sialic acids must have intrinsic functions in vertebrates that go beyond providing negative charge and hydrophilicity, and masking underlying residues. In fact, during the past few decades several vertebrate proteins have been found that mediate specific recognition events involving sialic acids[25,26]. The rest of this review focuses on these vertebrate sialic-acid-recognizing proteins, as examples of glycan–protein interactions. These lectins can be either attached to the cell surface or secreted into the extracellular space, and recognition can involve sialic acids on either the same cell or an opposing cell surface.

Factor H

The first vertebrate sialic-acid-recognizing protein discovered was complement factor H, which negatively regulates the alternative complement pathway. This pathway is constantly being activated ('ticking over') in the circulating blood plasma, but amplification is continually held in check by factor H, a protein present at high levels in blood plasma[27]. When plasma is exposed to a complement-activating surface (such as a foreign bacterium) the alternative pathway evades fluid-phase factor H and amplifies itself on the surface to be attacked, initiating a cascade of events in the innate immune system. Given the promiscuous nature of targets for the pathway, the question arose as to why amplification does not also occur on 'self' surfaces. One explanation is the presence of endogenous species-specific complement-controlling proteins on the plasma membranes of host self surfaces. A second mechanism involves soluble factor H being recruited to such surfaces to continue its role of dissociating the alternative pathway convertase enzyme. This recruitment is supported by anion-binding domains of factor H that specifically recognize sialic acids and/or certain sulphated glycosaminoglycans on vertebrate cell surfaces[27–29]. Further studies must define the precise nature of the factor-H interaction with cell-surface sialic acids that mediate this regulatory function. Of course, several successful microbial pathogens also cover their own surfaces with sialic acids[18]. These can recruit host factor H, providing the same protection that host cells enjoy.

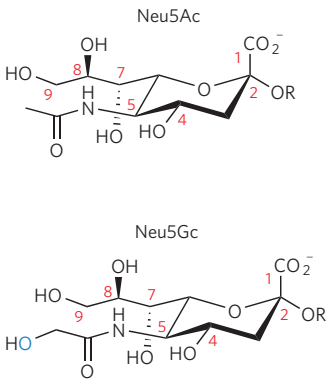

Figure 2 | Two major sialic acids in mammalian cells. Sialic acids share a nine-carbon backbone, a carboxylic acid at the C1 position, and various α-glycosidic linkages to the underlying sugar chain (R) from the C2 position. Various substitutions at the C4, C5, C7, C8 and C9 positions combine with linkage variation to generate the diversity of sialic acids in nature. The structures shown here are the most prevalent sialic acids found in mammalian cells. The only difference between the two is the additional oxygen atom in the *N*-glycolyl group of Neu5Gc (blue). This modification of the precursor sialic acid Neu5Ac cannot occur in humans because of a genetic mutation that occurred during human evolution.

Selectins

The second group of sialic-acid-binding proteins discovered was the selectins, which are involved in many cell–cell interactions in immunity, haemostasis and inflammation[30,31]. In classic studies, the homing of lymphocytes into lymph nodes through high endothelial venules was shown to be dependent on sialic acids[32]. This turned out to involve recognition of sialylated ligands by leukocyte (L)-selectin, which is constitutively expressed on many types of leukocyte. Independent evidence showed that leukocyte adhesion to activated endothelial cells mediated by endothelial (E)-selectin also involved sialic acids[33]. However, E-selectin is not constitutively expressed, and requires transcriptional induction by various inflammatory stimuli. Parallel studies led to the discovery of platelet (P)-selectin[34], which is stored in the alpha granules of platelets and in Weibel–Palade granules of endothelial cells. This is rapidly mobilized to the cell surface in response to various stimuli, again mediating adhesion to sialic-acid-containing ligands on leukocytes[35,36]. Cloning of the three selectin molecules revealed them to be products of a small cluster of homologous genes[37].

All three types of selectin require sialic acids and nearby α1-3(4)-linked fucose residues for most recognition processes[30,31,33,35,36,38]. This combination is typically found in a terminal glycan motif known as 'sialyl-LewisX' (SLeX; Table 1), which was initially thought to be the shared ligand for all selectins. In fact, SLeX is necessary but not sufficient to generate optimal binding by selectins[38]. As with the Arg-Gly-Asp-Ser motif for integrin recognition, placing SLeX in the context of other structures is important for biologically relevant selectin recognition. Thus, certain leukocyte glycoproteins carrying SLeX in specific arrangements seem to be optimal ligands for E-selectin, and functionally significant recognition by L- or P-selectin requires additional sulphate esters[30,31]. With L-selectin, optimal recognition involves certain sulphated forms of SLeX, with the sulphate typically at the C6 position of *N*-acetylglucosamine (GlcNAc) residues. This form is constitutively expressed by sialomucin-like proteins of the high endothelial venules of lymph nodes[31,39]. Such ligands are also induced on endothelium at other sites during inflammation.

A crucial high-affinity molecule for P-selectin recognition has also been discovered[40], cloned and named P-selectin glycoprotein ligand-1 (PSGL-1)[41]. This sialomucin polypeptide is expressed on all leukocytes, but becomes specialized for selectin recognition on neutrophils and monocytes. In this case, the sulphate component is not on the sugar chain but on adjacent tyrosine residues. Thus, optimal PSGL-1-binding sites for P-selectin (and also for L-selectin) comprise a short O-linked sugar chain bearing an SLeX motif adjacent to two or three sulphated tyrosine residues, all contained within a short sequence at the amino-terminal region of PSGL-1 (refs 42–44). Conclusive evidence for the importance of sialic acid, fucose, PSGL-l and sulphated GlcNAc or tyrosine residues in generating these complex recognition motifs has been obtained by biochemical synthesis[45] and by genetic ablation of genes for the corresponding biosynthetic enzymes in the mouse[39,46]. Many further complexities have since been discovered in the biosynthesis and specialization of the selectin-recognition motifs, and there is now substantial evidence that combinatorial biosynthesis of such recognition motifs is crucial for the formation of biologically relevant selectin ligands.

Meanwhile, studies of selectin-deficient mice[46,47] further confirmed the roles of selectins in the initial interactions among leukocytes, endothelium and platelets, which are involved in mediating various processes in acute and chronic inflammation, in reperfusion injury, and in haemostasis. Owing to space limitations, detailed descriptions are not feasible here. A further fact of biophysical interest is that interactions of P-selectin with PSGL-1 have been shown to involve novel 'catch' bonds, which help to explain the 'rolling' behaviour shown by leukocytes when they encounter endothelial surfaces[48].

A serendipitous finding was that an unrelated glycan (a glycosaminoglycan known as heparan sulphate) can also act as a ligand for L- and P-selectin[49,50], and that a highly sulphated version of this molecule known as heparin is a potent inhibitor of their interactions[50–53]. Although the dominant system of natural selectin recognition seems to be dependent on sialic acid and fucose residues, there is some evidence that heparan-sulphate-mediated interactions can be physiologically significant[53]. How can two such disparate glycan classes be recognized by the same selectin-binding site? A likely explanation is that heparan sulphate and heparin display a high density of carboxylic acid groups, as is found in sialic acids, and a high density of 6-O-sulphated GlcNAc residues, as is found in L-selectin ligands. Thus, some combination of carboxylic acid and sulphate ester groups seems to be presented in 'clustered saccharide patches' by these glycosaminoglycans, mimicking the natural ligand that is presented by PSGL-1 or by some of the sulphated sialomucins recognized by L-selectin[38]. Regardless of whether this is physiologically significant, it has direct relevance to medical practice, as heparin is a well-recognized therapeutic agent and has been used for more than 50 years as an anticoagulant (see page 1046). Because inhibition of L- and P-selectin can be achieved at clinically acceptable heparin concentrations[54,55], these findings have direct therapeutic significance[56], which is currently being pursued. Indeed, it is possible that these and other biological effects of heparin might explain its usefulness as an anti-inflammatory agent[53], in addition to its value as an anticoagulant.

Selectins also have an important role in haematogenous carcinoma metastasis (the spread of epithelial cancers through the bloodstream). SLeX was originally discovered as an antigen prominently expressed on some malignant tumours, particularly tumours of epithelial origin (carcinomas)[57] — a finding associated with poor prognosis[58]. Several investigators subsequently found that E-selectin on activated endothelial cells could bind carcinoma cells that expressed SLeX[58]. However, E-selectin is not constitutively expressed on endothelium. So others pursued the involvement of P- and L-selectin. It seems that the sulphated sialylated mucins of circulating carcinoma cells (and possibly other glycans such as heparan sulphate and/or sulphatides) mimic natural selectin ligands, initiating interactions with platelets and leukocytes[59,60]. This was reminiscent of classic studies showing that platelets and leukocytes facilitate the survival and spread of malignant cells after their entry into the bloodstream[56]. Studies in selectin-deficient mice confirmed a pivotal role of these glycan-binding proteins in various models of cancer metastasis[56,59,60]. Furthermore, when heparin was used as an inhibitor of P- and L-selectin, it markedly decreased metastasis in these model systems. This helps to explain the classic finding that heparin inhibits carcinoma metastasis[61] in a manner apparently independent of its anticoagulant effects[56].

Selectins are also involved in biomedically important processes other than cancer and inflammation, particularly reperfusion injury (which occurs when blood flow is interrupted and then restored in situations such as stroke, heart attack and traumatic shock)[62]. Therefore, selectins are a promising target for the prevention of such injuries. It is unfortunate that early attempts at clinical trials failed, for various scientific, practical and/or

Table 1 | Selective recognition of sialic acids by sialic-acid-binding proteins

Cognate glycan	Intrinsic	Extrinsic (pathogen-binding)	Extrinsic (unknown function)
α2-6 β1-4 β R	CD22 (Siglec-2)	Human influenza A	*Sambucus nigra* agglutinin
α2-3 β1-4 β R	Sialoadhesin (Siglec-1)	Avian influenza A	*Maackia amurensis* leukoagglutinin
α2-3 β1-4 β R, α1-3	E-selectin	*Pseudomonas aeruginosa* mucoid strain 8830, *Anaplasma phagocytophilium*	None known
α2-3 β1-3 α/β R, α2-6	Myelin-associated glycoprotein (Siglec-4)	*Plasmodium falciparum* Merozoite EBA-175	*Maackia amurensis* haemagglutinin

■ GlcNAc ◆ Sia ▲ Fuc ● Gal ■ GalNAc

Only a few examples of sialic-acid-bearing glycan termini are shown here, along with examples of cognate sialic-acid-binding proteins. 'Intrinsic' refers to binding proteins made by the same organism that synthesizes the sialylated structures. The third structure is SLeX.

Table 2 | Differences in sialic acid biology in humans compared with other hominids

Gene	Human-specific changes	Possible consequences for humans	Refs
CMAH	Human-specific *Alu* transposon-mediated exon deletion/frame-shift/inactivation. Loss of cytosine 5′-phosphate (CMP)–Neu5Gc production	Loss of cell-surface Neu5Gc expression, excess of Neu5Ac on cell surfaces. Humans have anti-Neu5Gc antibodies but metabolically incorporate Neu5Gc *in vivo* (from dietary sources), and cultured human cells incorporate Neu5Gc from animal serum or feeder layers (*in vitro*)	21
SIGLEC1	Increased ligand density in humans, enhanced frequency and altered expression pattern in human macrophages	Altered responses to sialic-acid-expressing pathogens?	92
SIGLEC5/14	Restoration of 'essential arginine residue' for sialic acid recognition; expression suppressed on T cells	Hyper-responsive phenotype of human T cells — a possible role in disease propensity?	74, 93
SIGLEC6	Placental expression?	Unknown	94
SIGLEC7/9	Amino-acid changes in the sialic-acid-recognizing domain, allowing Neu5Ac recognition	Altered control of innate immune response by neutrophils? Enhanced susceptibility to sialic-acid-bearing pathogens?	95
SIGLEC11	Human-specific gene conversion, altered binding, new expression in microglia	Altered response of microglia to infections? Altered interactions of microglia with neural cells?	96
SIGLEC12	Human-specific mutation of 'essential arginine residue' decreasing sialic acid recognition	Unknown	97
SIGLEC13	Human-specific *Alu* transposon-mediated gene deletion	Unknown	98
ST6GAL1	Altered expression of Siaα2-6Galβ1-4GlcNAcβ1 termini in certain cell types	Protection from avian influenza virus. Susceptibility to human influenza virus	99

Of the fewer than 60 genes known to be involved in sialic acid biology[20,86], a surprisingly large number show human-specific changes compared with the closely related great apes (chimpanzees, gorillas and orangutans). Most of the differences involve changes in the Siglec family of intrinsic sialic-acid-binding lectins. The biological significance and relevance to human evolution of these changes are being investigated. Some possible consequences are given above.

commercial reasons. However, the basic principles remain unchanged, and further translational studies in this area are definitely warranted.

Siglecs

The negative charge of the sialic acids is broadly sufficient to explain their role in recognition involving factor H and selectins. Indeed, in both instances sulphate esters can replace sialic acids. Thus, until the 1990s there was no evidence for vertebrate sialic-acid-binding lectins dependent on multiple structural features of sialic acids for recognition. Two independent lines of work resulted in the discovery of such molecules. First, sheep erythrocyte rosetting by macrophages was traced to the presence of a cell-surface protein known as sialoadhesin[63]. While the sialic-acid-binding properties of this molecule were being characterized, independent evidence emerged that CD22 on B cells mediated adhesion specifically through the recognition of sialic acids in α2-6 linkage[64]. Subsequent cloning of sialoadhesin[65] revealed that CD22 and sialoadhesin belonged to a subfamily of immunoglobulin (Ig)-related proteins that shared homology at their N-terminal V-set and adjacent C2-set Ig domains (which were named because of their homology to the variable and constant domains of Igs), along with other known molecules termed CD33 and myelin-associated glycoprotein (MAG). These were then shown to have sialic-acid-dependent binding properties[66]. On the basis of these common structural and functional features the family was eventually termed the Siglecs (for sialic-acid-recognizing Ig-superfamily lectins). Homology searches of mouse and human genomes revealed the presence of an extended family of sialic-acid-recognizing molecules that could all be defined as Siglecs (see refs 14, 67, 68 for reviews).

Siglec binding requires recognition of not only the sialic acid's negative charge but also the side chain that comprises the C7, C8 and C9 positions, and its 5-*N*-acyl group. Furthermore, Siglecs often interact specifically with the sialic acid linkage to the underlying sugar chain (Table 1). In some cases, such as MAG, an extended specificity has been found to exist that depends on various aspects of the underlying glycan structure[69,70]. Several reviews on the expression and functions of Siglecs have recently been published[14,67,68].

In a nutshell, Siglecs fall into two evolutionarily distinct categories. The first comprises molecules conserved between mammalian species, including sialoadhesin (Siglec-1), CD22 (Siglec-2), MAG (Siglec-4), and the newly discovered Siglec-15 (T. Angata, personal communication). The second category is the CD33-related Siglecs (CD33rSiglecs), members of which are characterized by general homology to one another, the presence of tyrosine-based signalling motifs in the cytosolic tail, and rapid evolution by various mechanisms[14,67,68]. It has been suggested that CD33rSiglecs are involved in recognizing sialic acids as self, and thus in inhibiting responses of the immune system. They may also be involved in the recognition of pathogens that express sialic acids[71,72]. A few CD33rSiglecs lack these cytosolic signalling motifs. Instead, they display a charged residue within the transmembrane domain, which they use to recruit the adaptors DAP-10 and DAP-12, proteins that contain potentially activating motifs[73,74]. Siglec-5 and -14 are paired CD33rSiglecs with negative and positive signalling potential, respectively, that are undergoing a fascinating form of concerted genomic evolution[74].

MAG is the most highly conserved Siglec; it is specifically expressed in glial cells and can recognize gangliosides (glycolipids that contain sialic acids and/or sulphate esters at specific positions). This recognition is crucial for the long-term stability of myelin, as is shown by defects in mutant mice that are null for either MAG or its glycan targets[69,70]. There is also evidence to suggest that MAG is partly responsible for the inhibition of neuronal sprouting and reconnection after injury. Thus, interfering appropriately with MAG could potentially improve nerve regeneration after events such as spinal cord injury[75]. Extensive work on CD22 has shown that it acts a negative modulator of signalling through the surface antigen receptor of B cells (see refs 76–78 for examples). This requires recognition of α2-6-linked sialic acids on CD22 itself, and probably on other molecules. The role of sialoadhesin in macrophages is still being investigated, but it seems to both modulate adaptive immune responses[79] and assist in recognition and/or phagocytosis of sialic-acid-expressing pathogens[71,72]. The functions of the evolutionarily conserved Siglec-15, recently discovered by Angata and colleagues, are unknown.

Thus, Siglecs are the largest family of sialic-acid-binding molecules, and much further work is required to define their functions. One unusual feature is that naturally occurring Siglec binding sites are typically 'masked' by the large pool of sialic acids present on the same cell surface[80]. Although such '*cis*' recognition may be of biological significance and may change during activation, '*trans*'-recognition can also occur between cells, as long as the density of sialic acid on the opposing cell is high enough[81]. In this regard, it is interesting that many successful vertebrate pathogens (particularly human pathogens) coat themselves with a high density of sialic acids[18] and can be recognized by CD33rSiglecs[71,72]. It has been suggested (but not proved) that this is a form of molecular mimicry in which the pathogens are mimicking self in order to down-regulate the responses of innate immune cells[14].

L1CAM

Another Ig-superfamily member that has been reported to recognize sialic acids is L1 cell-adhesion molecule (L1CAM), which is widely

expressed in the nervous system, and is involved in many aspects of neural development and function[82]. Various human genetic disorders with prominent nervous system defects are caused by mutations in L1CAM. Sialic-acid-recognizing properties of L1CAM were demonstrated by one research group, which proposed that it has specificity for α2-3-linked sialic acids presented on CD24, a heavily glycosylated cell-surface molecule[82,83]. Unlike in Siglecs, this sialic-acid-binding property is not attributable to one of the Ig domains, but seems to derive from a fibronectin type-III repeat in another region of L1CAM[82,83]. Further studies of this recognition system are needed.

Uterine agglutinins

Soluble extracts of the endometrial lining of rat and human uterus contain a haemagglutinin (a protein capable of agglutinating erythrocytes), whose binding is mediated by recognition of sialic acids[84]. Erythrocytes are probably not the natural ligands for this agglutinin, which presumably recognizes other local sialic-acid-bearing ligands, mediating some as yet unknown biological functions. Interestingly, the agglutinin has also been shown to bind to sperm in a sialic-acid-dependent manner[85]. An unexpected finding is that the human uterine agglutinin preferentially recognizes Neu5Gc[85], the sialic acid absent in humans because of a genetic mutation[21]. It remains to be seen whether this apparent evolutionary loss of natural ligands can explain unusual aspects of human female reproductive biology[86]. This agglutinin needs to be cloned and further characterized.

Sperm sialic-acid-binding proteins

It has been reported that the binding of bovine sperm to egg zona pellucida glycoproteins is mediated by α2-3-linked sialic acids[87]. Studies in this area indicate the presence of a sperm plasma membrane sialic-acid-recognizing protein. Use of a specific enzyme inhibitor suggested that a neuraminidase released from cortical granules might also participate in the block to polyspermy by removing sialic acid from the zona pellucida. Further studies are needed to define the role of sialic acid recognition in such interactions.

Laminin G domains

Abundant evidence indicates that interactions of the G domains of laminins with glycans displayed on α-dystroglycan are important in mediating interactions in the nervous system, at muscle–nerve junctions and in skeletal muscle[88]. One unusual glycan found on α-dystroglycan is a sialic-acid-containing sequence attached to the protein through an O-linked mannose residue[89]. Some evidence suggests that these sialic acids are involved in recognition[89]. However, this does not seem to be universal to all laminin G-domain interactions, and further studies are needed.

CD83

One study showed indirect evidence for sialic acid binding by the Ig-superfamily member CD83 (ref. 90), a marker for mature dendritic cells that is also essential for the generation of $CD4^+$ T cells. The sequences of CD83 do not show homology to Siglecs, and this finding needs further investigation.

How evolution changed human sialic acid recognition

The hominid evolutionary lineage that gave rise to humans suffered a genetic defect in the production of the major Old World primate sialic acid, Neu5Gc[21]. As an isolated incident, this could simply have been due to a random drift of a mutation that occurred in a small population of ancestral hominids[21]. However, multiple differences in sialic acid biology have since been found between humans and our closest evolutionary cousins, the great apes[86] (Table 2). Although it is always difficult to reconstruct evolutionary events, a reasonable scenario is depicted in Fig. 3. Whatever the true sequence of events, it is clear that human evolution was associated with a 'sialoquake' in which a number of the genes associated with sialic acid biology underwent significant

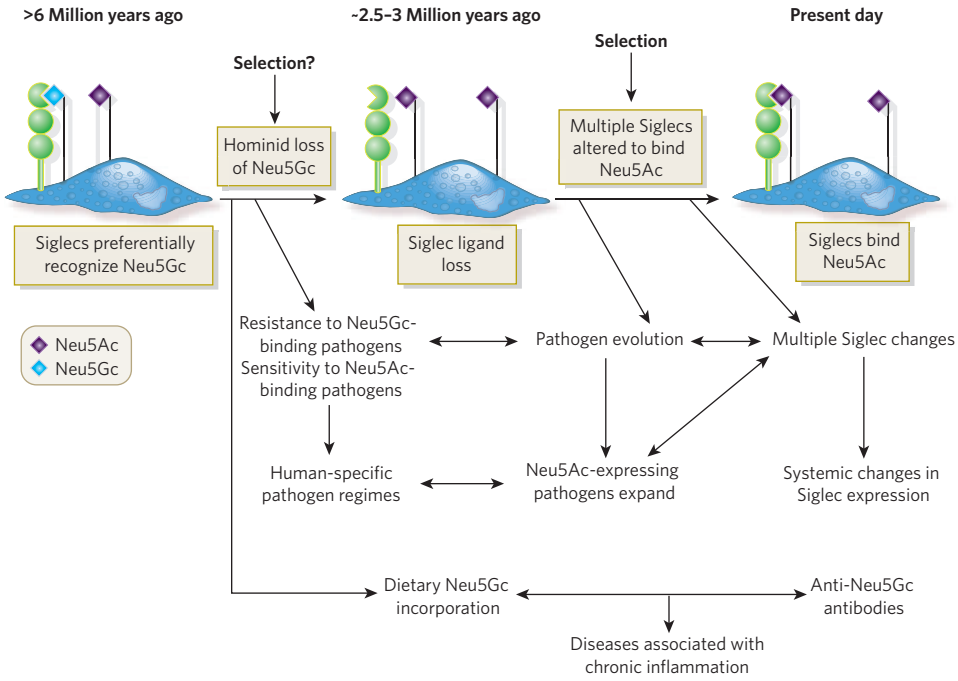

Figure 3 | Proposed scenario for occurrence of multiple changes in sialobiology during human evolution. Initial loss of Neu5Gc in human evolution could have occurred randomly or through selection as the result of a pathogen preferentially recognizing Neu5Gc. This would have led to sudden loss of Neu5Gc-binding sites for several CD33rSiglecs (green), probably generating unusual immune activation. All of the other changes in sialic acid biology could have resulted from evolutionary adjustments to these events (see Table 1 for details of human-specific Siglec changes). The Siglecs are depicted as binding sialic acids on the same cell surface. Binding could also occur to high densities of sialic acids on other cell surfaces, including those of Neu5Ac-expressing pathogens. Also noted are additional possibilities for evolutionary changes in the range of pathogens that humans suffer from (pathogen regimes), as well as the unusual phenomenon of metabolic incorporation of dietary Neu5Gc into human endothelia and epithelia in the face of an anti-Neu5Gc antibody response. The latter process potentially facilitates diseases associated with chronic inflammation, such as atherosclerosis and cancer. Note that there have been no reports of pathogens that synthesize Neu5Gc.

changes in expression or function (see Table 2). This occurred in a biological system in which some rapid evolution was expected[14,67,68,86]. It remains to be seen what the consequences of all of these changes are for human populations today. Some possibilities are included in Table 2.

Future prospects

Glycan–protein interactions span a wide variety of biological processes, and only a very small subset (the sialic-acid-binding vertebrate proteins) have been considered here. The biology of such interactions remains incompletely understood, partly because of the small number of laboratories that are currently focused on their study. This is not surprising, because the present generation of established cellular and molecular biologists were not exposed to information about glycan biology and biochemistry during their training. This makes it a fertile area for the new generation of young scientists interested in gaining a more complete understanding of biology and disease at a molecular level.

Apart from learning more about known vertebrate sialic-acid-recognizing proteins, it is interesting to speculate as to whether there are any as yet undiscovered ones. In this regard, all known sialic-acid-binding features of vertebrate proteins (other than those of sialoadhesin and CD33rSiglecs) were discovered only after the protein had already been identified and characterized for other reasons. In other words, discovery of sialic acid binding was serendipitous, and related to observations such as unexpected loss of binding upon sialidase treatment. Thus, it is difficult to predict how many sialic-acid-binding proteins are yet to be discovered. After all, sialic acids have been consistently expressed at a high density on the cell surfaces of the deuterostome lineage for more than 500 million years. It is therefore reasonable to predict that they were co-opted for a variety of different recognition functions over evolutionary time. Rather than awaiting discovery of new sialic-acid-binding motifs and proteins by further chance observations, one could take a systematic approach to identifying sialic-acid-binding properties in tissue extracts, or in libraries of existing cDNAs and proteins. The use of glycan arrays[91] could also facilitate such an exploration. Meanwhile, *in vivo* manipulation of sialic-acid-binding protein functions is becoming increasingly feasible as a result of genetic modifications in mice[4,46] and by directed modification of sialic acids using various chemically synthesized precursors[22]. All of the above suggestions can also be broadly applied to glycan-binding proteins in general. It is probable that we have only scratched the surface of this class of biological recognition processes. A new generation of young scientists trained in understanding glycan structure and function must exploit these exciting opportunities. ■

Note added in proof: Ravetch and colleagues recently reported that the small fraction of IgG that binds to *Sambucus nigra* agglutinin (that is, that carries α2-6-linked sialic acids) mediates the inhibition of autoimmune responses, possibly through a sialic-acid-recognizing receptor on macrophages[100].

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