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REVIEW

From glycophenotyping by (plant) lectin histochemistry to defining functionality of glycans by pairing with endogenous lectins

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Abstract
About 60 years ago, the efforts to identify blood group-specific haemagglutinins in plant extracts by broad-scale testing were beginning to make a large panel of these proteins available as laboratory tools. Their ability to 'read' cell surface signals like antibodies do was the reason for W. C. Boyd to call them lectins, from Latin *legere* (to read). These proteins turned out to be as widely present in nature as glycans (polysaccharides or carbohydrate chains of cellular glycoconjugates) are. Since carbohydrates have the virtue to facilitate high-density coding in a minimum of space and lectins (initially mostly from plants called phytohaemagglutinins) turned out to be receptors for glycans, their pairing made many applications possible. Most prominently, these proteins were instrumental to map glycome complexity and sites of product generation during glycan assembly in the cell. The detection of mammalian (tissue) lectins and the emerging evidence for intimate molecular recognition between this class of receptors and their (glycoconjugate) counterreceptors substantiate that understanding the rules of the sugar code is presently a major challenge.

Keywords Galectin · Glycocalyx · Glycosylation · Haemagglutinin · Sugar code

Introduction

The history of research on carbohydrates teaches an intriguing lesson on the role of patience in basic science: it takes a long time from the discovery of a class of biocompounds to understanding its natural significance, on all its levels. Having started with work on ubiquitous (homo)polysaccharides and then glycoproteins (mucins), the virtues of sugars to be the most versatile biochemical platform for (high-density information) coding had not been realized in this early phase. Instead, tremendous problems encountered in carbohydrate chemistry, for example to synthesize oligomers and purify them, became a reason to explain why "the subject of the chemical structure of these tissue elements (glycoproteins and mucoproteins) seems to be a very neglected branch of science" (Levene 1925). It required the development of sophisticated analytical procedures for determining glycan structure in chemistry and for glycophenotyping of biomaterials in histochemistry and cell biology. As will be outlined in this review, work on receptors for sugars began around the time the first glycoprotein was detected. However, these two lines of research started to converge only about 60 years ago. To give physiological meaning to the obviously enormous structural complexity of glycans found in cellular glycoconjugates, that is to crack the sugar code, is now a major challenge.

Considering natural occurrence, carbohydrates are the most abundant organic compounds on Earth. In sheer quantity, annual production of the homopolymer cellulose and chitin surpasses that of any other type of biopolymer by far, that for chitin being of the order of at least 10 gigatons (Merzendorfer 2009). Originally named 'fungin' by H. Braconnot in 1811, a term that reflects its occurrence in fungi, the Greek word for a covering, i.e., chiton, is the etymological root for the name 'chitin'. It was meant to give the 'covering', for example, found on chockchafers (May bug: *Melolontha melolontha*, a scientific name) (Odier 1829). The biochemical nature of this covering was identified after its hydrolysis with concentrated hydrochloric acid. The product of polymer degradation to its monomer *N*-acetylglucosamine (GlcNAc) and of its de-*O*-acetylation received the name "Glycosamin" (Ledderhose 1876, 1879, 1880; Hofmann 1929; Haworth

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et al. 1939). In the homopolymer chitin, GlcNAc is (uniformly) linked via a β1,4-glycosidic bond, as glucose (Glc) is in cellulose.

What cellulose is for plant cell walls, chitin is for bacteria, fungi and invertebrates: an equivalent "of a steel lattice in reinforced concrete, which makes the skeletal structures remarkably tough and durable" (Merzendorfer 2009). "This seeming simplicity, perhaps even dullness of structure, is probably one of the reasons why biochemists for a number of years lost interest in carbohydrates" (Sharon 1975). Interestingly, pioneered by studies of Chambers (1940), a "sugary coating of cells", a sweet extracellular husk rich in complex (poly)saccharides (or glycocalyx, a term introduced into the literature by Bennett in 1963), is typical for "very many types of cells", among them cell walls or the zona pellucida (Gasic and Gasic 1962; Bennett 1963; Rambourg et al. 1966; Ito 1969). "In the brief space of half an hour", as Bennett noted in his seminal lecture, "one cannot discuss very adequately all the variants which the glycocalyx can present, nor all the ways in which it might participate in the physiological functions of the cell" (Bennett 1963). Prophetically, the glycocalyx was assumed to have variability and significance beyond mechanical and protective roles assigned to the homopolysaccharides cellulose and chitin—but if it does, then dealing with glycans becomes a necessity.

"Another important reason why biochemists shied away from the study of carbohydrates stemmed from the many chemical problems encountered in dealing with these materials" (Sharon 1975). Carbohydrates, so-called because carbon (Latin 'carbo' = coal) and water (Greek 'hydor') in formal terms appear in a stoichiometric proportion, i.e., $C_n(H_2O)_m$ (with $n \leq m$), not only build homopolymers. They are capable, too, of serving as toolbox for the synthesis of the glycans of cellular glycoconjugates (glycoproteins, glycolipids and proteoglycans).

In nature, these have been discovered to be associated to a scaffold, first in the case of protein for mucin (Eichwald 1865; for details on the current status of mucin biochemistry, please see Corfield 2015), thereafter for sphingolipids that build cerebrosides (Thudichum 1874) and gangliosides (Klenk 1942, 1970) with glycans. Since the individual building blocks of the glycan part can theoretically be connected in many ways, the carbohydrate oligomers presented by glycoconjugates will reach an unsurpassed degree of structural complexity, thus rightly called complex (poly)saccharides. Ironically, the access to a multitude of isomers, a boon for high-density coding but a huge problem for analysis, seriously slowed down progress in this field, as noted above. This tremendous impact justifies to have a look at the chemical basis of the exceptional versatility.

In structural terms, 'letters' of the alphabets of life are joined to form 'words', as graphically summarized in Fig. 1. In the case of nucleotides (for nucleic acids) and amino acids (for proteins), the first and second alphabets of life, this is done by virtue of a single chemical mode, that is, phosphodiester or peptide bonding, with nearly invariably uniform usage of connection points (Fig. 1a, b). Chemical synthesis thus is straightforward, and the determination of the sequence is complete, when the spatial order of the letters has been defined. In numbers, the 20 proteinogenic amino acids will generate a total of 6.4×10^7 hexapeptides. This number is by far surpassed when calculating with sugars, and examining Fig. 1c gives the answer to the question why carbohydrates, the third alphabet of life, are predestined to encode biochemical messages in a minimum of space.

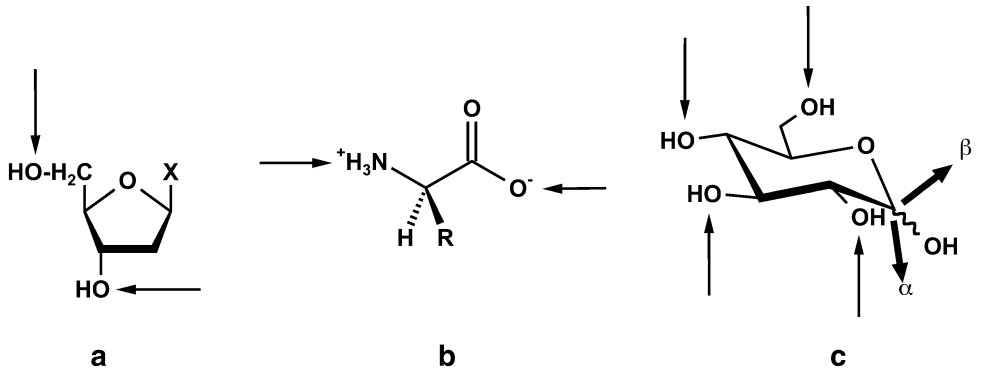


Fig. 1 Illustration of the linkage points for oligomer formation of nucleic acids (**a**), proteins (**b**) and glycans (**c**). In contrast to the letters of the first and second alphabets of life (**a, b**), each carbohydrate unit can engage any of its hydroxyl groups for letting the chain grow or introduce branches (**c**). The anomeric position (α/β) is the donor site for conjugation, any other hydroxyl group can serve as acceptor, as symbolized by arrows (from Rüdiger and Gabius 2009, with permission; please see also; Gabius and Roth 2017)

The concept of the sugar code

Carbohydrates present a series of hydroxyl groups, thus more than a single pair of connecting points is used to yield glycans. As the number of arrows in Fig. 1c that symbolize linkage points highlights, this variability opens the way to far more structural isomers than obtained with amino acids and nucleotides. Theoretically, working with an alphabet of only six types of hexoses, up to 1.05×10^{12} hexasaccharides can be devised (Laine 1997). As can be deduced from Fig. 1a–c, there is much more to do in glycan characterization than to determine the sequence that is sufficient to define nucleic acids and peptides: each glycosidic linkage is in one of two (anomeric) positions (α or β), and this connection of the anomeric center of one residue is then possible to any of the chemically equivalent hydroxyl groups of an acceptor sugar moiety. As consequence, each disaccharide unit needs to be defined by the (1) sequence, (2) anomeric position and (3) connection points, each combination yielding a distinct 'word'. Explicitly, two glucose moieties have 11 possibilities to become a diglucoside. Evidently, glycans are special, offering unsurpassed potential for structural variability.

In addition, the ring size can vary. Besides the thermodynamically favored pyranose (*p*) of a hexose such as Gal*p*, its furanose (*f*) form can be a part of glycans: galactofuranose (Gal*f*), the five-membered ring form, is found in polysaccharides and glycoconjugates of bacteria, fungi and protozoa (never in mammals) (Peltier et al. 2008; Richards and Lowary 2009; Tefsen et al. 2012). As consequence, Gal*f*, is one indicator of a foreign glycosignature, a target for innate immunity (please see below in the second paragraph of chapter on lectin–glycan pairing).

To achieve glycan synthesis, devising elegant chemical protocols to guide formation of desired bonding or recruiting enzymes such as glycosyltransferases are necessary (Oscarson 2009). In metaphoric terms, 'writing' messages in a minimum of space with a biochemical alphabet will, therefore, ideally be done with carbohydrates, and nature has not missed this opportunity. In fact, the glycan part of cellular glycoconjugates can be considered as a biochemical means to embody a large panel of signals for communication.

Considering human glycosylation, at least 500–600 genes are devoted to sugar activation, transport, glycan assembly, remodeling and substitution (Brockhausen and Schachter 1997; Reuter and Gabius 1999; Nairn et al. 2008; Patsos and Corfield 2009; Zuber and Roth 2009; Moremen et al. 2012; Cummings and Pierce 2014; Corfield and Berry 2015; Hennet and Cabalzar 2015; Neelamegham and Mahal 2016; Corfield 2017). This machinery is capable of generating a currently mapped glycome size of more than 3000 determinants, with additional complexity on the level of oligosaccharides in glycosaminoglycans (Buddecke 2009; Cummings 2009). Looking further at the widely documented ubiquity of glycans as part of cellular glycoconjugates in all three urkingdoms of life and the variety of at least 41 types of linking a sugar to a protein, these facts strongly argue in favor of fundamental physiologic significance for glycan-based information coding, a biochemical mission beyond being a source of energy by catabolism or mechanical stability (Sharon 1975; Spiro 2002; Gabius 2009, 2015; Gabius and Roth 2017).

The first aspect of this mission comes into effect in a rather passive way after the site-specific introduction of a sugar into a protein chain. Besides affecting physicochemical properties such as solubility, charge and viscosity or stabilizing a distinct conformation (Sharon and Lis 1997), the sugar moiety has a bearing on protein properties on the biochemical level. By masking a distinct functional group of the protein, the carbohydrate can block the glycoprotein's route of processing. Introducing an *O*-glycan is known to be able to confer resistance to proteolysis, for example, precluding otherwise rapid release of the binding domain of the low-density lipoprotein receptor from its extracellular stalk (Kingsley et al. 1986; Jentoft 1990). A clinically relevant case underlying hyperphosphatemia and ectopic calcification has been delineated for a defect in mucin-type *O*-glycosylation by one of the 20 polypeptide: *N*-acetylgalactosaminyltransferases (GalNAc-Ts), i.e., GalNAc-T3. The presence of the *O*-GalNAc modification at Thr178 protects the phosphaturic effector fibroblast growth factor 23 from action of proprotein convertase and hereby favors its secretion, a cause of familial tumoral calcinosis when deficient (Topaz et al. 2004; Ichikawa et al. 2005; Kato et al. 2006; Goetz et al. 2010). In addition to affecting protein processing, distinct types of glycan modification, for example, the addition of a fucose moiety (in α1,6-linkage) to the core of complex-type *N*-glycans by fucosyltransferase-8, can serve as molecular switches that alter glycan shape and hereby protein features (André et al. 2009). In its absence, dysregulation of receptor activation and signaling was delineated in the case of transforming growth factor-β (Schachter 2005; Wang et al. 2005; Honke and Taniguchi 2009) and the rate of hepatic clearance of neoglycoproteins from blood was affected (Unverzagt et al. 2002). Even glycoprotein stability, as for instance shown for the human epidermal growth factor receptor (Contessa et al. 2008; Gabius et al. 2012), is sensitive to modifications in *N*-glycosylation. In such instances, the glycan makes its presence felt by modulating distinct aspects of the protein's properties, as is common for post-translational modifications (Karsdal et al. 2010; Nussinov et al. 2012; Lothrop et al. 2013).

What singles out glycans from the large number of biochemical means to implement substitutions into proteins is

the mentioned vast potential for structural variability (and also their common occurrence on the surface of membranes presented by sphingolipid anchors; Kopitz 2009, 2017; Ledeen and Wu 2015; Schengrund 2015). Since "carbohydrates are ideal for generating compact units with explicit informational properties", "the significance of the glycosyl residues (of glycoconjugates) is to impart a discrete recognitional role on the protein" (Winterburn and Phelps 1972). When considering glycan determinants as biochemical messages, as done above, they will not only block accessibility to a distinct protein region but will need to be 'read' and then translated. These processes constitute a highly versatile route of information flow, a central part of the concept of the sugar code. As consequence, glycans then become bioactive ligands (counterreceptors) within carbohydrate–protein recognition. Of note, the abundance of hydroxyl groups of carbohydrates offers a wealth of contact sites for hydrogen bonding to proteins. The study of recognition phenomena with cells (erythrocytes) was instrumental to track down proteins that are receptors for glycans. Experimentally, a biological sample and a suspension of red blood cells are mixed, and formation of aggregates is interpreted as evidence for the presence of a bridging factor that causes the visible haemagglutination.

From haemagglutination to lectins

Based on this activity, a detected compound is operationally called a haemagglutinin (Elfstrand 1898). Applying this assay led to the first observations on surface recognition phenomena relevant in our context. They were directed by constituents of rattlesnake (*Crotalus durissus*) venom (Mitchell 1860; Mitchell and Reichert 1886) and of extracts of *Ricinus communis* seeds (Stillmark 1888), actually the starting points of lectin research. As Mitchell and Reichert (1886) wrote, the red blood corpuscles, in the presence of venom, "fuse together into irregular masses acting like soft elastic material". By the way, his groundbreaking work earned him the honor to have a rattlesnake species, i.e., the speckled rattlesnake *Crotalus mitchelii*, named after him. Also, serum compounds called heterohaemolysins or heteroagglutinins when active in cross-species assays, later characterized as natural antibodies, were capable to bring about a clustering of red blood cells (Creite 1869). Interestingly, this type of erythrocyte agglutination was found to depend on the source of the blood, thus on surface properties characteristic for the donor. These insights were crucial to form the concept of blood group systems and to make transfusion safe, by blood group typing and predicting fatal incompatibilities (Landsteiner 1900, 1901; for historical reviews, please see Hughes-Jones and Gardner 2002; Schwarz and Dorner 2003). Making reliable and robust reagents for this purpose available and eventually elucidating the biochemical nature of the blood group material by hapten assays were aims that gave work direction. Because plant seed extracts and mammalian serum both contained similar activities, their study in parallel was tempting. In fact, testing plant extracts in a pilot study revealed activity profiles that differed depending on the species used as donor of the blood cells (Landsteiner and Raubitschek 1907). As highlighted by Boyd (1963), "Landsteiner had observed fairly early that these seed extracts did not always agglutinate the blood of different species equally and wrote, probably in 1914, a paper entitled "Pflanzliche Hämagglutinine" on the subject. This paper reached the stage of page proof, but was never published"—but served as personal incentive for W.C. Boyd, in his own words, "to test seeds for blood group specificity" in more detail.

Following this line of research, plant agglutinins were indeed discovered with a level of specificity for the different ABO blood groups that respective serum antibodies harbor against these epitopes (for historical reviews, please see Boyd 1963; Kocourek 1986; Kilpatrick and Green 1992). By running haemagglutination assays with saline extracts from seeds of 99 or 262 plant species up to a broad survey of 2663 plant seeds, antibody-like selectivity among ABO-type erythrocytes could be observed (Renkonen 1948; Boyd and Reguera 1949; Allen and Brilliantine 1969). In the largest study, 90 extracts (3%) were found to 'read' surface determinants accordingly, what leads to a blood group type-specific erythrocyte bridging as antibodies do, up to the first detection of an anti-M lectin activity (Allen and Brilliantine 1969). Selecting an epitope like an antibody but coming from a different source (from plants) prompted to propose "the word lectin from Latin *lectus*, the past principle of *legere* meaning to pick, choose or select" (Boyd and Shapleigh 1954) or to read for this antibody-like activity. Experiments to answer the question on the biochemical basis that underlies (cell) aggregation by lectins followed interaction analysis. They were designed in analogy to the strategy successfully applied to characterize the specificity of serological reactions, as described by Landsteiner (1945). Respective assays came up with glycocompounds (polysaccharides or highly glycosylated mucins) as cognate binders, and mucins and simple sugars were shown to interfere with the agglutinating activity of phytohaemagglutinins and of the blood group H(0)-specific eel (*Anguilla anguilla*) serum like haptens do in serology (Sumner and Howell 1936; Koulumies 1950; Krüpe 1950; Watkins and Morgan 1952). Of note, J. B. Sumner and S. F. Howell had suggested already in 1935 that, when testing redissolved haemagglutinin of jack bean (*Canavalia ensiformis*) extract for activity, "our previous negative results were possibly due to an inhibiting action of the sucrose solution employed to dissolve the concanavalin A", this plant's lectin (Sumner and Howell 1935). Likely with a touch of understatement, W. M. Watkins described

her feelings when planning the pioneering hapten inhibition of haemagglutination that "we decided, with no great expectation of the outcome, to screen them (anti-H reagents) for inhibition of the agglutination of O cells with the component sugars present in the blood group-active substances. Somewhat to our surprise one of the many reagents, that from the eel, *Anguilla anguilla*, was quite strongly inhibited by L-fucose and to a greater extent by α-methyl L-fucoside and not by the other monosaccharides… The failure of many human anti-H and anti-A antibodies to be inhibited by the simple sugars told us that the complete determinant structures recognized by these antibodies must be larger than the monosaccharides to which the lectins bound. Nevertheless, these simple experiments pointed to the carbohydrate nature of the blood-group determinants and gave the first indications that for each specificity one of the component sugars was playing a more dominant role than the others" (Watkins 1999). Simple sugars were also found to inhibit blood group A- and O-specific haemagglutination by plant seed extracts originally described by Renkonen (1948), especially those seen with extracts from *Lotus tetragonolobus* and *Vicia cracca* (Morgan and Watkins 1953). These phytohaemagglutinins defined central parts of ABO blood group epitopes and were hereby defined as receptors for monosaccharides (Watkins 1966, 1999). Taken together, these reports are the cornerstone for the concept of productive lectin–glycan recognition.

This connection between the name 'lectin' and the target specificity to glycans has been kept since then. It is now the common term for a distinct class of carbohydrate-binding proteins. Lectins ('antibody-like substance') are separated from antibodies (of historical importance, the use of synthetic neoglycoproteins as antigens revealed the specificity of antibodies raised against sugar epitopes so that immunization against capsular polysaccharides of bacteria became a practical perspective, a proof of principle for glycan–protein recognition of clinical relevance in adaptive immunity; Göbel and Avery 1929; Avery and Göbel 1929, 1931; Göbel et al. 1934). They are distinguished from enzymes that use glycans as substrates such as glycosyltransferases, from sensor/transport proteins for free mono- to oligosaccharides and from carbohydrate-binding modules of glycoside hydrolases that degrade cell wall and storage polysaccharides (Barondes 1988; Gabius et al. 2011). Proceeding from recognizing the lectins' molecular selectivity, two discoveries nearly 60 years ago fueled interest in this class of proteins:

1. A partially purified haemagglutinin activity from bean (*Phaseolus vulgaris*) called phytohaemagglutinin (PHA), "employed originally as means of separating the leukocytes from whole blood" due to its erythrocyte-agglutinating ability, "was found to be a specific initiator of mitotic activity" (Nowell 1960). Binding of a plant lectin to the cell surface can, therefore, trigger an intracellular post-binding response, here the activation of resting leukocytes (for review on mitogenic lectins, please see Borrebaeck and Carlsson 1989).
2. A wheat-germ preparation enriched for lipase activity was tested, "working on the theory that if isolated normal and tumor cells of comparable derivation respond differentially to the same substance under carefully controlled conditions, the nature of the response and of the substance causing the response might throw light on the chemical structure of the tumor cell membrane… We have found a substance: …it causes the agglutination of the tumor cells while the normal cells remain almost completely isolated" (Aub et al. 1963). Noting the obvious possibility of an analogy to lectin binding to "antigenic groups on the surfaces of red blood cells", the authors concluded that "perhaps we, too, are dealing with such an antigenic reaction" (Aub et al. 1963), and, indeed, GlcNAc-specific wheat germ agglutinin (WGA) and its glycan-dependent binding were later described to underlie cell bridging (Burger and Goldberg 1967).

The introduction of affinity chromatography to achieve efficient (often one step) purification in 1965 (Agrawal and Goldstein 1965) paved the way to making plant lectins widely available, for thorough biochemical characterization and for applications. The elucidation of their profile of glycan specificity, as exemplarily shown in Table 1, underlined their antibody-like capacity for target binding so that lectins quickly became popular tools for histochemical glycan detection and glycophenotyping, superior to chemical methods of visualizing glycocompounds in their specificity (Schrével et al. 1981; Roth 2011).

In sum, agglutination of erythrocytes by activities different from serum antibodies, their (antibody-like) ability for selecting their targets and the biochemical nature of their ligands as glycans were crucial to realize that proteins have developed to complete a glycan-receptor (lectin) recognition system. In the first phase of research with purified lectins, respective work continued to focus on proteins obtained mostly from plant seeds.

Lectin histochemistry

Plant (and also few invertebrate) lectins have been and continue to be very valuable to map the glycome on the surface of cells and in tissue sections. For tissue sections, leguminous lectins, prominently the mentioned mannose/glucose-specific concanavalin A (Bittiger and Schnebli 1976), were the first to serve as laboratory tools for localization of glycans based on their specificity (Avrameas 1970; Bernhard and Avrameas 1971; Roth and Thoss 1974; Roth and Franz

Table 1 Specificity profile of plant/fungal/mammalian lectins used for histochemical glycophenotyping

Lectin name (common name)	Abbreviation	Monosaccharide specificity	Potent glycan ligands
Canavalia ensiformis (jack bean)	ConA	Man/Glc	GlcNAcβ2Manα6(GlcNAcβ2Manα3) Manβ4GlcNAc
Lens culinaris (lentil)	LCA	Man/Glc	*N*-Glycan binding enhanced by core fucosylation
Triticum vulgare (wheat germ)	WGA	GlcNAc/Neu5Ac	$(GlcNAc)_n$, Manβ4GlcNAcβ4GlcNAcβ,N-Asn, terminal GlcNAcα/β in *O*-glycans and truncated immature *N*-glycans, *O*-GlcNAcylated peptides
Phaseolus vulgaris erythroagglutinin (kidney bean)	PHA-E	[a]	Bisected complex-type *N*-glycans: Galβ4GlcNAcβ2Manα6(GlcNAcβ2-Manα3) (GlcNAcβ4)Manβ4GlcNAc
Phaseolus vulgaris leukoagglutinin (kidney bean)	PHA-L	[a]	Tetra- and tri-antennary *N*-glycans with β6-branching
Viscum album (mistletoe)	VAA	Gal	Galβ2(3)Gal, Galα3(4)Gal, Galβ3(4)GlcNAc without/with α2,6-sialylation, Fucα2Gal
Galectin-3 (human)	Gal-3	Gal	(Gal/Neu5Acα3)Galβ3(4)GlcNAc, LacNAc repeats without/with α2,3(6)-sialylation, ABH histo-blood group epitopes, GalNAcβ4GlcNAc (LacdiNAc), core 1 (TF antigen) and core 2/4 mucin-type *O*-glycans, ganglioside GM1 pentasaccharide
Lycopersicon esculentum (tomato)	LEA	[a]	Core and stem regions of high-mannose-type *N*-glycans $(GlcNAcβ3Galβ4GlcNAcβ3Gal)_n$ of complex-type *N*-glycans[b]
Maackia amurensis-I (leukoagglutinin)	MAA-I	[a]	Neu5Acα3Galβ4GlcNAc/Glc, 3′-sulfation tolerated
Sambucus nigra (elderberry)	SNA	Gal/GalNAc	Neu5Acα6Gal/GalNAc, clustered T_n antigen
Polyporus squamosus (polypore mushroom)	PSL	[a]	Neu5Acα6Galβ4Glc(NAc) (over 300-fold more active than LacNAc, not reactive with free Neu5Ac); 6′-sulfation tolerated; 6-sialyl T_n not reactive
Siglec-2 (CD22; human)	CD22	[a]	Neu5Acα6Galβ3(4)GlcNAc (without/with 6-*O*-sulfation of GlcNAc), sT_n antigen, 9′-*O*-acetylation blocks binding
Arachis hypogaea (peanut)	PNA	Gal	Galβ3GalNAcα/β
Dolichos biflorus (horse gram)	DBA	GalNAc	GalNAcα3GalNAcα3Galβ4Galβ4Glc > A-tetrasaccharide
Glycine max (soybean)	SBA	GalNAc	GalNAcα3Galβ6Glc
Macrophage galactose-type lectin (CD301; human)	MGL	GalNAc	T_n and s/suT_n antigens (T_n presentation by Tyr active), core 5/6 mucin-type *O*-glycans, β4-linked GalNAc in LacdiNAc or in chains of GD2/GM2/Forssman-type glycosphingolipids

[a]No monosaccharide known as ligand

[b]Dual reactivity documented by Kawashima et al. (1990) and Oguri (2005)

1975; for examples on binding profiles, please see Table 1). Examples of illustrations resulting from processing tissue sections by plant histochemistry are given in Fig. 2a, c, e. Before long, this experimental approach was extended from testing a single labeled protein to a panel of lectins on serial sections to measure glycan complexity. Hereby, the questions are answered whether distribution of various glycan classes is spatially distinct or affected by differentiation. Indeed, non-uniform regional patterns are common observations, as documented in Fig. 3. It shows examples for glycan detection in adult chicken eye (for details, please see Manning et al. 2017a, 2018).

Taken to the level of electron microscopy, applying protocols of lectin staining facilitated ultrastructural glycan localization. Saccharide epitope mapping of the glycocalyx, for example, became possible (Roth et al. 1972), and also thorough monitoring of the products of the intracellular glycosylation machinery. Intriguingly, since its components were biochemically studied at that time, the exciting opportunity arose to let lectin histochemistry complement

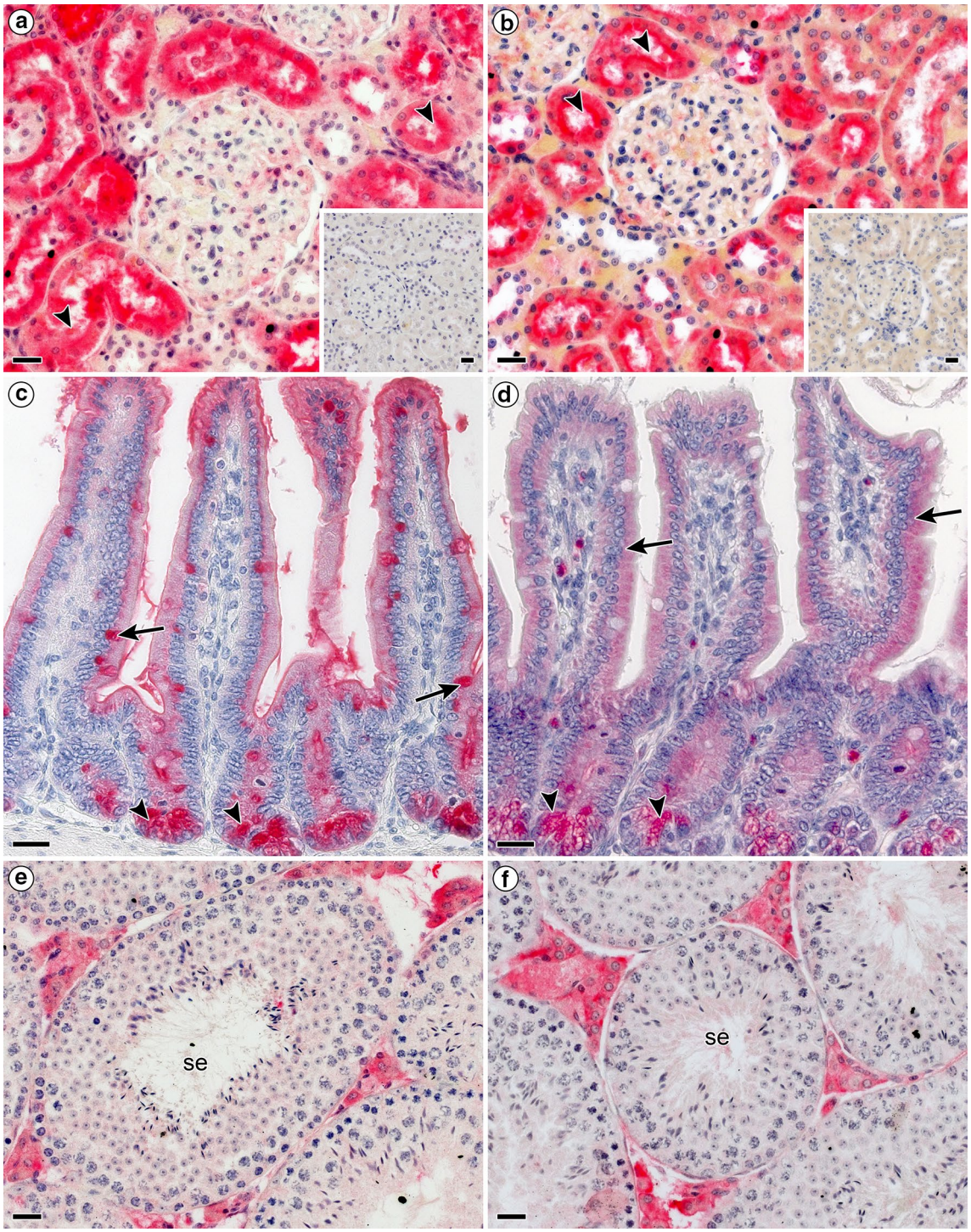


Fig. 2 Illustrations of lectin histochemical staining profiles in cross sections of rat kidney cortex (**a, b**), murine jejunum (**c, d**) and testis (**e, f**). Specificities and acronyms of the applied lectins are given in Table 1. VAA (**a**) and Gal-3 (**b**) strongly stain epithelial cells and their brush borders (arrowheads) in proximal tubules. Blocking binding to accessible sites by co-incubation of the lectins with 100 mM lactose precluded lectin-dependent signals (insets to **a, b**). **c, d** SBA binds to epithelial cells of glandulae intestinales (arrowheads) and goblet cells (arrows) (**c**). MGL-dependent positivity in epithelial cells of glandulae intestinales (arrowheads) and, comparatively weaker, in the epithelial lining of villi intestinales (arrows) (**d**). Applying PSL (**e**) and CD22 (siglec-2) (**f**) yields intense staining in the cytoplasm of Leydig cells located between seminiferous tubules (se). Scale bars 20 µm

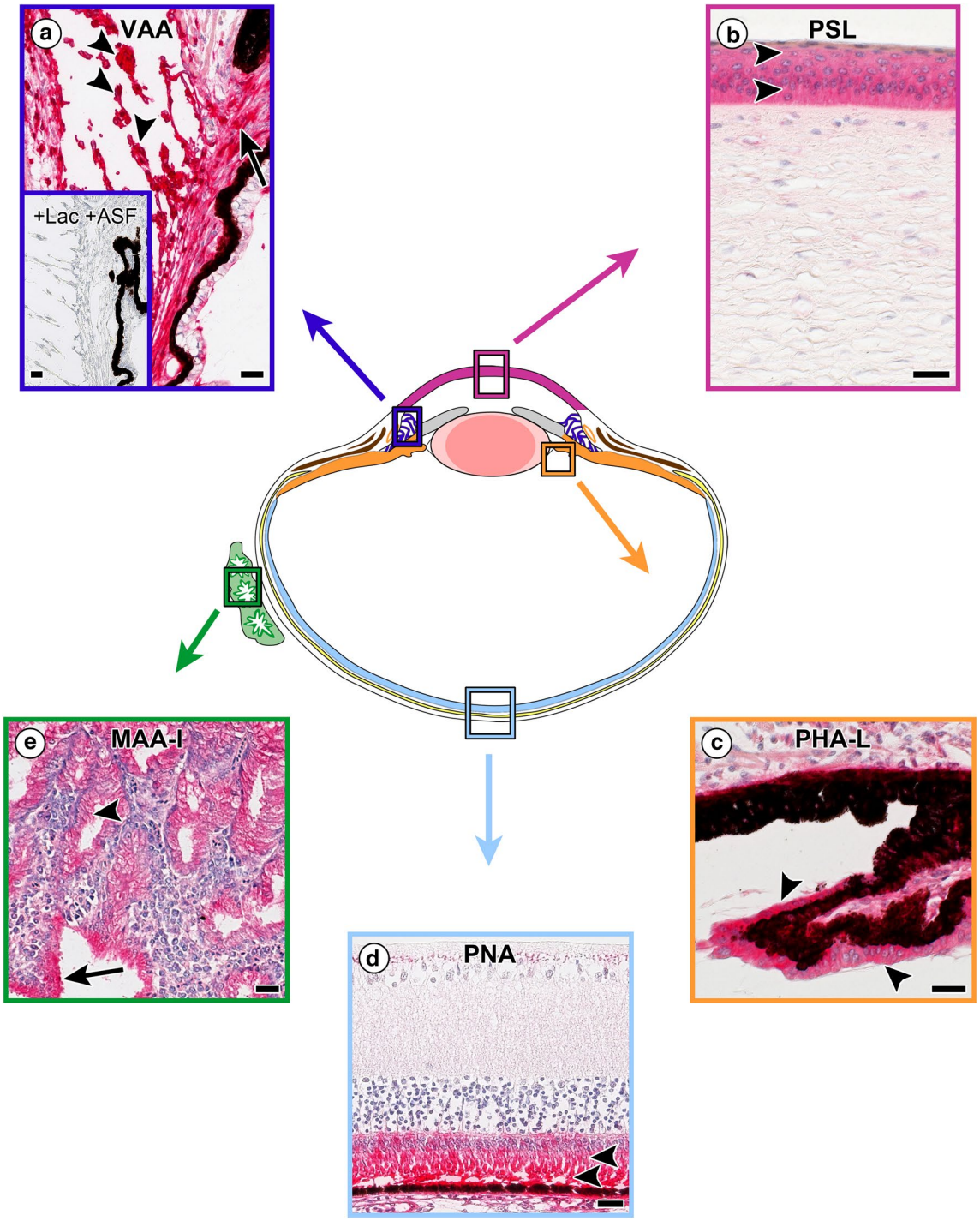


Fig. 3 Illustrations of lectin histochemical staining profiles in distinct regions of eyes of 3-month-old chickens, as given by color coding in the scheme (center). Specificities of applied lectins are given in Table 1. **a** VAA strongly stains endothelial cells of the trabecular reticulum in the iridocorneal angle (arrowheads) and the stroma of the ciliary body (arrow). Co-incubation of lectin with a mixture of the haptenic inhibitors 75 mM lactose and the glycoprotein asialofetuin (1 mg/mL) saturates the lectin's binding site and thus makes interaction with tissue glycoconjugates impossible (inset to **a**). **b** PSL binds to the corneal epithelium (arrowheads). **c** PHA-L binding is seen in the non-pigmented epithelium of the ciliary body (arrowheads). **d** PNA strongly associates to the photoreceptor layer of the retina (arrowheads). **e** MAA-I positivity in the epithelial linings of ducts (arrow) and tubuli (arrowhead) of the Harderian gland. Scale bars 20 µm. For details, please see Manning et al. 2017a, 2018

immunohistochemistry on glycosyltransferases. Strategically combined, application of lectin and immunohistochemistry gave structure to routes of glycan assembly, and this at an unprecedented level of microscopical resolution (Roth 1987, 1993, 1996; Pavelka 1997). Positions of glycosylation steps in the assembly line could hereby be related to the architecture of the endoplasmic reticulum and the Golgi apparatus, a complex system of cisternae (Dröscher 1998), revealing topologically fine-structured patterns that ensure precision in glycan maturation (Roth et al. 1988; Taatjes and Roth 1991). Assignments of starting mucin-type *O*-glycosylation by GalNAcylation of protein, mentioned above to *cis*-cisternae of the Golgi apparatus (Roth 1984) and of α2,6-sialylation of *N*-glycans to be mentioned below to *trans*-cisternae (Roth et al. 1985), attest the level of sophistication of the method and the high-level spatial order of glycan assembly.

Equally important, native cells can be exposed to lectins to determine interactions with the surface (Nicolson 1974) and processed with labeled lectins to yield lectin staining. This will then be measured by various methods such as FACScanning as well as classical fluorescence or confocal laser scanning microscopy (for examples of respective illustrations, please see Manning et al. 2017b). These techniques that obtain a molecular fingerprint have enabled to relate distinct features of glycosylation detected by lectin binding to certain cellular characteristics, e.g., the status of differentiation or the effect of switching on/off distinct genes such as oncogenes or tumor suppressors. Fully in line with the concept of the sugar code, the glycophenotype turned out to be a sensitive indicator for single-site alterations, first interpreted phenomenologically. Status of sialylation and degree of branching belonged to features often encountered on lists of changes (for examples, please see Patsos et al. 2009; André et al. 2014). The increase of *N*-glycan size had initially been detected by paper electrophoresis of radiolabeled glycans obtained by hydrazinolysis of membrane glycoproteins or by serial lectin affinity chromatography of these glycans together with glycosidase application and methylation analysis, using polyoma- or Rous sarcoma virus-transformed

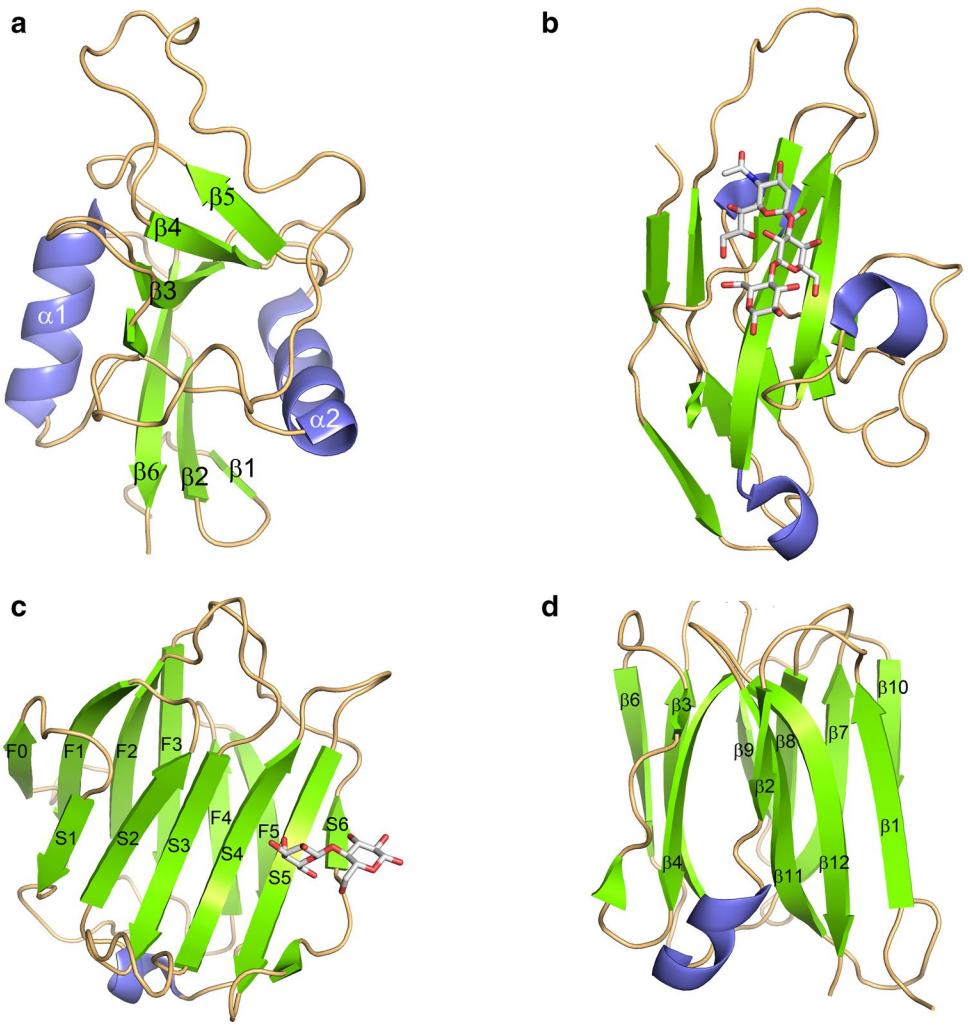
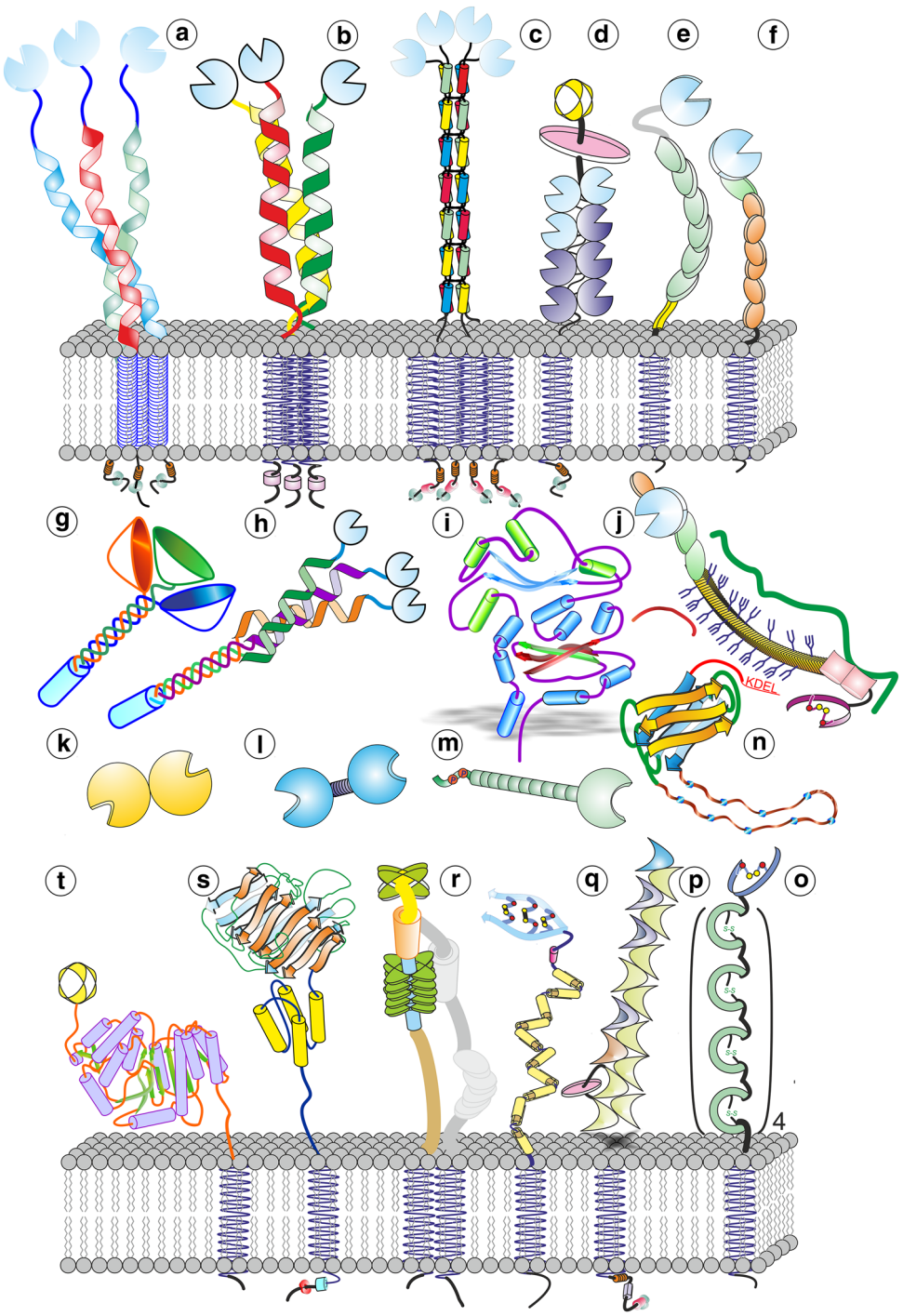


Fig. 4 Illustration of four types of common folds present in families of human lectins. **a** C-type CRD (human asialoglycoprotein receptor; PDB code 1DV8). **b** I-type CRD (human siglec-5 in complex with 3′-sialyllactose; PDB code 2ZG3). **c** β-Sandwich (N-terminal CRD of human galectin-8 in complex with lactose; PDB code 4BMB). **d** β-Prism I fold (human ZG16p lectin; PDB code 3APA)



baby hamster kidney cells as starting material (Yamashita et al. 1984; Pierce and Arango 1986).

Such alterations occurred in glycans of glycoproteins and glycolipids. In human neuroblastoma (SK-N-MC) cells or activated effector T cells, onset of differentiation/activation has been found to be accompanied by an enzymatic desialylation step that converts the hexasaccharide chain of ganglioside GD1a to the pentasaccharide of ganglioside GM1 (Ledeen et al. 2012, 2018). Working with pancreatic adenocarcinoma (Capan-1) cells, re-expression of the tumor suppressor $p16^{INK4a}$ is a switch to down-regulate the extent of *N*-glycan α2,6-sialylation (André et al. 2007). One way such a change can make its manifestation tangible is by the ensuing decrease of charge repulsion, e.g., between matrix glycoproteins such as fibronectin and integrins, what will favor cell attachment (a more adhesive behavior had been reported for hyposialylated β_1-integrin toward fibronectin; Semel et al. 2002). In our context, it is suggested to assume that changes in plant and fungal

◂**Fig. 5** Illustration of the versatility of modular design of mammalian lectins. Aggregation of transmembrane C-type lectin subunits by coiled-coil (**a, b**) or four-helical bundle (**c**) motifs in the cases of the asialoglycoprotein (Ashwell) receptor (**a**) and langerin (CD207; **b**) as well as DC-SIGN (CD209; **c**). **d** Combination of tandem-repeat-type display of C-type lectin(-like) domains with a β-trefoil lectin domain in the mannose macrophage receptor (CD206; see also **t**). Combination of a C-type lectin domain with EGF-like section(s) in thrombomodulin (CD141; **e**) and E-selectin (CD62E; **f**). Triple-helical association of subunits in ficolin (**g**) and mannan-binding lectin (**h**), a member of the collectins (lectins with collagen-like regions). **i** Distantly related intelectin with its fibrinogen-like lectin domain capable for oligomerization without the collagen-like stem. **j** Integration of a C-type lectin domain into multimodular hyalectan versican. The three types of galectin design as non-covalently associated homodimer (**k**), covalently connected bivalent tandem-repeat-type protein (**l**) and chimera-type protein with lectin domain associated to an N-terminal tail with non-triple-helical collagen repeats and a sequence with two sites for serine phosphorylation (**m**). **n** Association of the β-sandwich (lectin) fold with a P-domain and retention signals of the ER chaperone calreticulin. **o–r** Molecular puzzle with a V-set Ig-like CRD and 16 C2 set Ig-like modules in sialoadhesin (siglec-1, CD169; **o**), with a fibronectin type II domain and 15 P-type lectin(-like) domains in the cation-independent mannose-phosphate receptor (CD222; **p**), the terminal link domain for hyaluronan binding and a stem of variable length in CD44 (**q**) and the lectin site of CD11b with FG-GAP repeats and the I-domain (**r**) that associates with β_2-integrin (in gray) to form the $\alpha_M\beta_2$-integrin (CD11b-CD18 complex). **s** β-Sandwich fold of the cargo transporter ERGIC-53 with its stalk and routing signals. **t** β-Trefoil lectin domain of GalNAc-T2, an example of combination of a lectin domain with an enzymatic center

lectin binding may mean more than a restructuring of charge presentation, that is, a re-writing of glycan-encoded signals. Its consequence is detected by plant lectins as tools, and this analytical pairing could be a model for a functional interaction in situ with tissue lectins. This reasoning is an evidently strong incentive to search for cognate endogenous lectins that are capable to 'read' these (and many other) glycan determinants.

Historically, the line of research resulting from this reasoning, indeed, led to endogenous lectins. Driven by the hypothesis of a functional role of glycans either as routing or as recognition signal, efforts were directed to isolate the first mammalian lectins by affinity chromatography with a glycoprotein as bioactive ligand, so successfully applied for plant lectins. In detail, observations that "exposed, terminal galactosyl residues were identified as specific determinant for hepatic recognition and uptake" of serum glycoproteins were the basis to set up the purification of the membrane-embedded asialoglycoprotein receptor from rabbit liver by affinity chromatography on resin-immobilized asialo-orosomucoid at a yield of 50–75 mg/kg of tissue (Hudgin et al. 1974). Since "complex carbohydrate-containing molecules may function in synaptic recognition and transmission through establishment of cell–cell contacts and possibly also as mediators of communication between the surface and the interior of the cell", the authors of the report on the first soluble vertebrate lectin (Teichberg et al. 1975) reasoned that "in line with these ideas, the presence in neural tissue of enzymes and proteins capable of interacting with saccharides is to be expected" (a research area that continues to attract considerable attention; Higuero et al. 2017). Thus, they performed haemagglutination assays, found them revealing activity that was inhibited by β-galactosides and purified the protein responsible for aggregating cells from electric organ tissue of *Electrophorus electricus* at a yield of 400 mg/kg of tissue (Teichberg et al. 1975), the first *ga*(lactose-binding)*lectin*. Like plants (and bacteria or viruses, here often called adhesins or haemagglutinins; Holgersson et al. 2009), vertebrates, therefore, express lectins, and "after the genetic code was deciphered, the next important code to solve will be the one for cellular recognition" (Barondes 1997), that is, giving glycome analysis a functional dimension by studying glycan pairing with endogenous lectins to crack the sugar code (Gabius 2017).

Glycan–lectin pairing

To translate the noted diversity of glycan-encoded signals into specific cellular effects, ubiquity of occurrence and extent of structural variety of lectins should be on par with those of glycans. In contrast to the immunoglobulin fold of antibodies of adaptive immunity with its versatility by engineered imprinting, lectins should, therefore, have arisen more than once to meet this requirement. As graphically documented in recent Galleries of Lectins (Solís et al. 2015; Manning et al. 2017b), more than a dozen protein folds have acquired ability to accommodate oligosaccharides. Figure 4 illustrates the basic structural motif shared in a lectin family, here for C-type lectins, galectins, siglecs and β-prism proteins. Starting from an ancestral fold, the ensuing evolutionary divergence of the respective gene generated families of lectins that have a carbohydrate recognition domain (CRD) in common (Gabius 1987, 1997; Cooper 2002; Angata and Brinkman-van der Linden 2002; Houzelstein et al. 2004; Gready and Zelensky 2009; Mayer et al. 2017; Vasta et al. 2017). Mechanisms toward divergence operated on two main features of the proteins: (1) the fine structure of the CRD and (2) its presentation in a modular context.

Sequence differences in the site of binding the ligand account for letting distinct fine-specificities arise. They can, for example, 'read' pathogen-associated molecular patterns. The disaccharide LacdiNAc is a characteristic of schistosome surfaces that is selectively recognized by one of the galectins, i.e., galectin-3, a putative sensor within the innate immunity system for parasitic helminth infection (van den Berg et al. 2004). Another component of foreign glycosignature referred to above, i.e., galactofuranose present in polysaccharides, is traced by a different lectin (intelectin) with specially adapted binding site as part of innate immunity (Wesener et al. 2015). In parallel, the homologous proteins

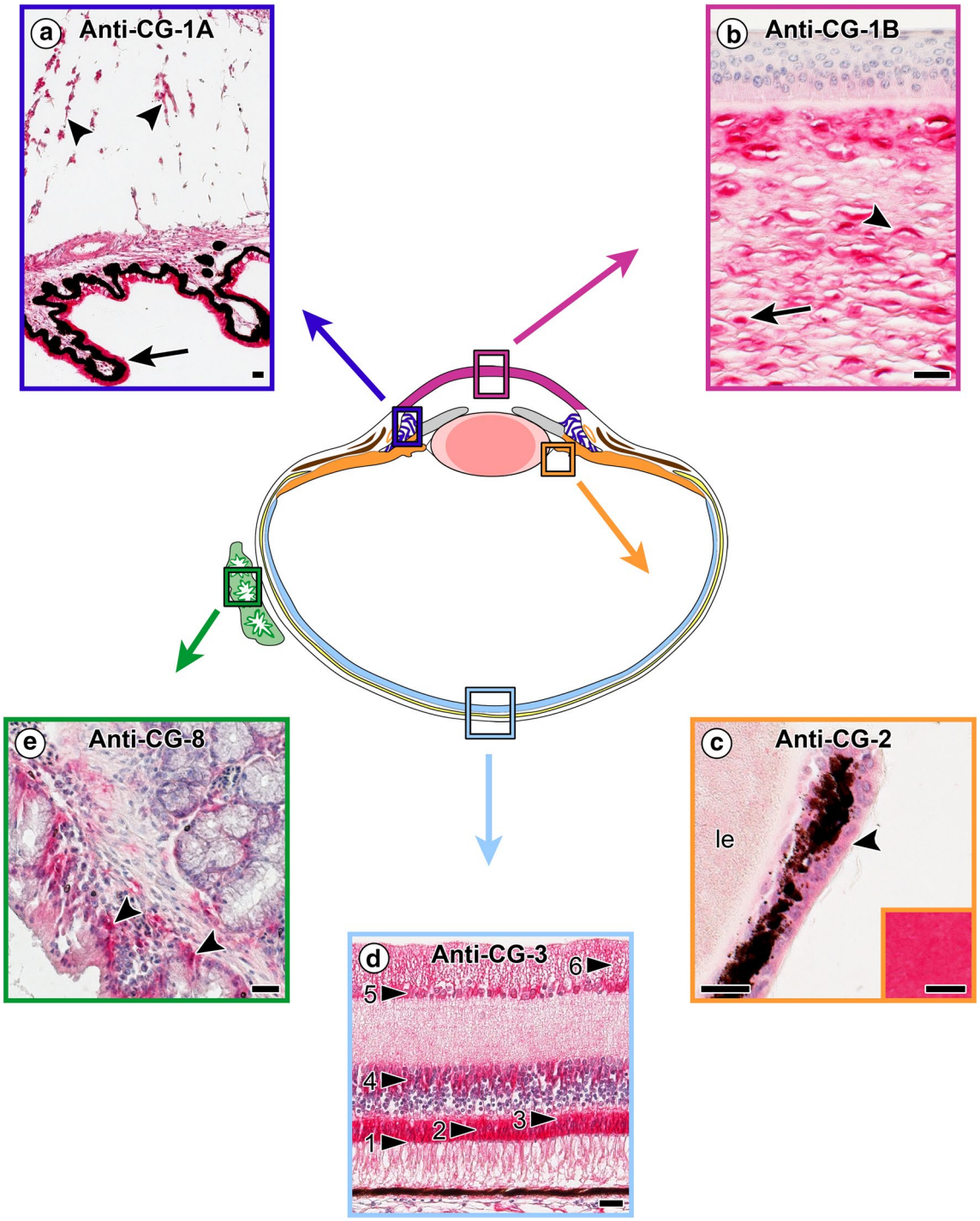


Fig. 6 Illustrations of immunohistochemical localization of the five canonical chicken galectins (CGs) and of C-GRIFIN in distinct regions of eyes of three-month-old chickens, as given by color coding in the scheme (center). **a** CG-1A presence in cytoplasm of non-pigmented epithelial cells of the ciliary body (arrow) and the trabecular reticulum (arrowheads). **b** Strong signals for CG-1B in fibrocytes (arrowhead) and fibroblasts (arrow) of the corneal stroma. **c** Moderate intensity of staining by anti-CG-2 in the non-pigmented epithelium of the ciliary body (arrowhead) and, comparatively weaker, in lens fiber cells (le), which show a strong signal for C-GRIFIN (inset to **c**). **d** Graded extent of presence of CG-3 in different layers of the retina defined by numbering and an arrowhead: the external limiting membrane (1), the outer nuclear layer (2), outer plexiform layer (3), amacrine cell layer (4), ganglion cell layer (5) and nerve fiber layer (6). **e** CG-8 presence in the Harderian gland, mainly in single cells disseminated throughout the gland's stroma (arrowheads). Scale bars 20 µm. For details, please see Manning et al. 2017a, 2018

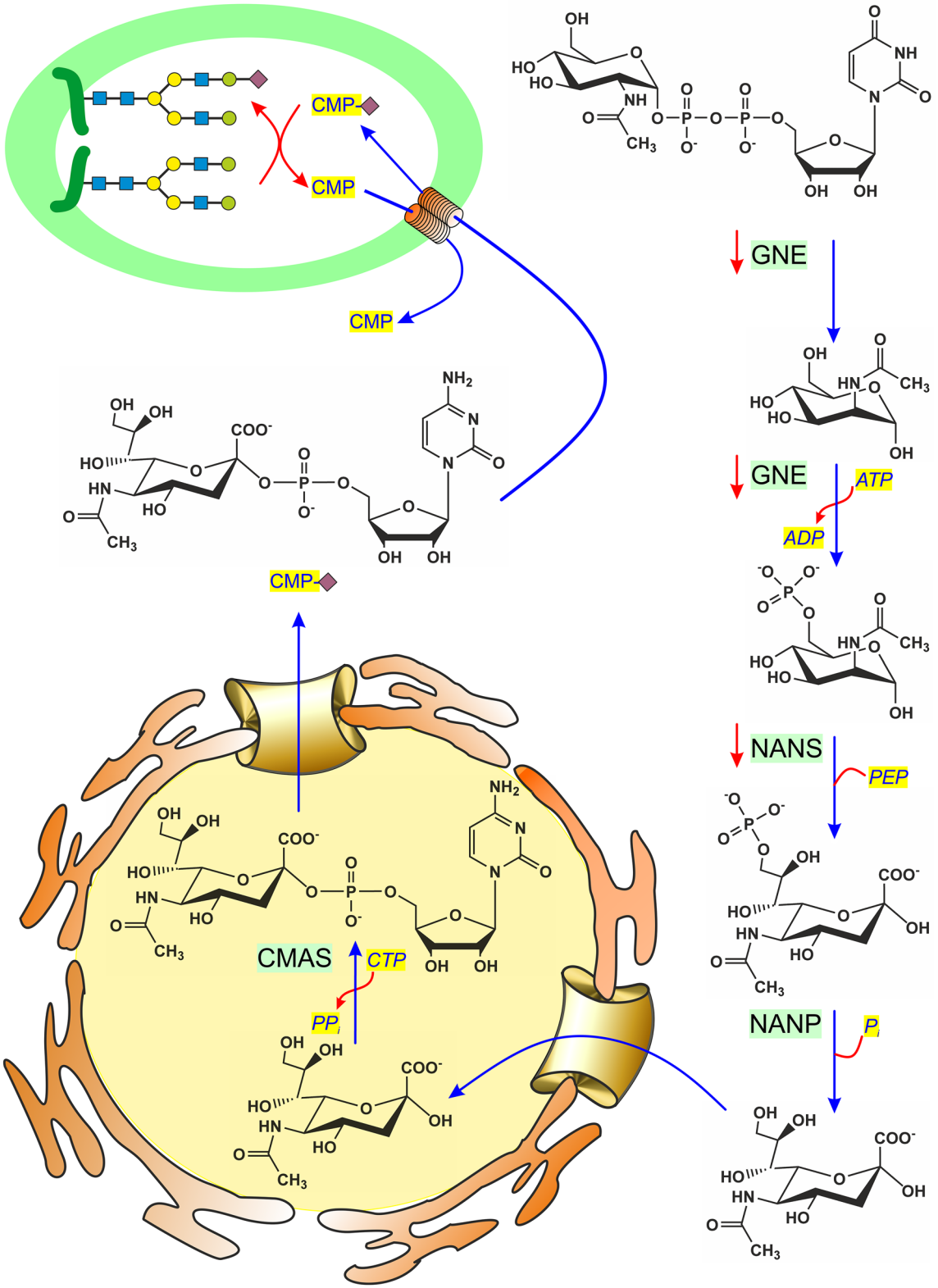
developed individual characteristics of their modular display. The way the CRD is presented goes beyond a lectin being constituted solely by a single site. In functional terms, bringing together bioactive modules (from homodimerization to a molecular puzzle) gives natural design of lectins a wide range of possibilities. The emerging principle of coordinated activities, for example, seen for the slime mold lectin discoidin I in first exporting the lectin and then second in mediating lectin-dependent cell–matrix contact formation (Gabius et al. 1985), explains why studying the modular display has become a focus of lectin research. A survey on this feature for mammalian lectins, presenting examples for the natural multitude of protein design, is given in Fig. 5. What starts with self-assembly of lectin domains into defined aggregates as seen for C-type lectins and galectins can even reach the level of joining modules of various origin in proteins like a puzzle to produce new combinations of often not yet fully understood physiological significance.

As was the case for plant lectins, the availability of endogenous lectins facilitated thorough determination of their specificity (for a survey on methods of specificity determination, please see Table 1 in Solís et al. 2015). Like the plant agglutinins, endogenous lectins exhibit preferences for distinct glycan determinants, as listed for three representatives in Table 1. They can equally conveniently be employed in lectin histochemistry, as done for the human galectin-1 (Gabius et al. 1991). Figure 2b, d, f presents examples of respective application for the three human proteins listed in Table 1. Beyond standard glycophenotyping, this application enables to take a first step to a functional interpretation when the result of monitoring for binding sites for a tissue lectin is seen as a measure of responsiveness of a cell to the lectin.

The realization of the phylogenetic diversification that leads to the current composition of lectin families poses a number of questions. Whether expression profiles are similar or distinct can be answered by immunohistochemistry, if non-cross-reactive antibody preparations are available. To address this issue with model character, organisms with complete representation of all the lectin subtypes but comparatively small overall number of proteins are suited. In the case of the galectins, the detection and purification of the first protein of this family by Teichberg et al. (1975) mentioned above, three modes of CRD presentation shown in Fig. 5k–m are known in vertebrates. They are all present in chicken with its total of (only) seven family members, what qualifies this animal for complete galectin network analysis (Kaltner et al. 2015, 2016, 2017; García Caballero et al. 2016a, b, 2018). Respective immunohistochemical studies on the chicken galectins have taught the lesson of individual distribution profiles, which can overlap in certain cases to some extent, as exemplarily shown in Fig. 6. Of potential relevance for diagnostic or prognostic assessments, biochemical and immunohistochemical network monitoring has also been introduced to clinical specimen (Gabius et al. 1984, 1986; Dawson et al. 2013; Katzenmaier et al. 2014; Toegel et al. 2014; Zivicová et al. 2017, 2018). Like glycan profiles, lectin presence is spatially ordered, and these two matching sides of a recognition system appear to be orchestrated.

The concept of the sugar code postulates translation of glycan-encoded messages into activity. Within the cell, this process already is a part of the quality control of nascent glycoproteins and their transport from the endoplasmic reticulum to Golgi cisternae or lysosomes, where glycans act as postal codes (Dahms and Hancock 2002; Roth 2002; Roth and Zuber 2017). As monitoring of cell binding of mammalian lectins attests, they appear to engage in very selective contacts with counterreceptors such as suitably glycosylated glycoproteins or presented glycosphingolipids and complexes thereof (Gabius et al. 2015, 2016). What is truly intriguing is the emerging evidence for fine-tuned co-regulation of presence of both sides to make functional pairing possible. A switch in glycan display can thus be an indicator for altering the cell's responsiveness to a tissue lectin, an assumption already presented above. At the same time, lectin presence is upregulated to turn this switch into bioactivity by glycan–lectin pairing. To give an instructive example, the increase of extent of surface presentation of ganglioside GM1 with cell differentiation (in neuroblastoma cells) or activation (in effector T cells) prompted the search for a suitable coregulatory event on the level of a receptor for this ganglioside's glycan. It concerned lectin presence on the cell surface. Galectin-1 was identified as the binding partner for this ganglioside's pentasaccharide, study of extent of its presence on the cell surface unveiled a tightly orchestrated process with similar upregulation, and the ensuing pairing by conformer selection was found to trigger growth inhibition (for details, please see Kopitz et al. 1998, 2001; Siebert et al. 2003; Wang et al. 2009; Ledeen et al. 2012, 2018). The same teaming-up within the sugar code applies to the regulatory activity of the tumor suppressor $p16^{INK4a}$.

In this case, the glycoprotein counterreceptor, i.e., $\alpha_5\beta_1$-integrin, is upregulated and its glycosylation tailored for galectin binding (André et al. 2007). In molecular detail, the tumor suppressor throttles sialic acid biosynthesis at two sites within the chain of enzymatic processes shown in Fig. 7. Hereby, substrate availability for sialyltransferases (for information on enzymatic sialylation, please see Bhide and Colley 2017) is reduced, what lowers the extent of α2,6-sialylation of *N*-glycans (Amano et al. 2012). Since presence of α2,6-sialylation will mask *N*-glycan termini for galectin-1 binding, cells now become responsive to this galectin acting as bridging factor between cell surface counterreceptors and hereby as elicitor of signaling. Fittingly, the



presence of the cognate receptor for the $\alpha_5\beta_1$-integrin on the cell surface, i.e., galectin-1, is enhanced via transcriptional activation, the lectin binds to its glycoprotein counterreceptor and the formation of the integrin–galectin aggregates leads to caspase-8-induced anoikis (Amano et al. 2012), and there is even more: expression of a physiologic galectin-1 antagonist, i.e., anti-apoptotic galectin-3, is attenuated in parallel to optimize the overall extent of pro-anoikis activity

◂**Fig. 7** Illustration of the route to enzymatic production of activated *N*-acetylneuraminic acid and the position of the two enzymes downregulated by the tumor suppressor $p16^{INK4a}$. UDP-*N*-acetylglucosamine (UDP-GlcNAc) is the substrate for the bifunctional enzyme UDP-GlcNAc 2-epimerase (generating *N*-acetylmannosamine (ManNAc) via release of UDP)/ManNAc kinase (generating ManNAc-6-phosphate), termed GNE. By adding phosphoenolpyruvate (PEP), *N*-acetylneuraminic acid 9-phosphate synthase (NANS) converts this substrate to a sialic acid derivative. *N*-Acetylneuraminic acid 9-phosphate phosphatase (NANP) removes the phosphate group, *N*-acetylneuraminic acid (Neu5Ac) is transported into the nucleus to become activated with CTP to form CMP-Neu5Ac by CMP-Neu5Ac synthase (CMAS). Export from the nucleus and import into the Golgi let CMP-Neu5Ac become substrate for sialyltransferases. GNE and NANS are downregulated in transfected cells relative to mock controls at a level of statistical significance below a *p* value of 0.001 (for details, please see Amano et al. 2012)

of galectin-1 (Sanchez-Ruderisch et al. 2010). In sum, the interplay of these regulatory events establishes a concept of paradigmatic character: observations on glycan profiles from glycophenotyping can be taken to the functional level via considering recognition of distinct glycans by a tissue lectin.

This conclusion stimulates efforts toward detection of functional pairing. Of note, a tissue lectin such as galectin-1 can target various structural glycan motifs depending on the cellular context, among them besides ganglioside GM1 and *N*-glycan termini also core 2 *O*-glycans, this for inducing apoptosis of leukemia/lymphoma and prostate cancer cells (Nguyen et al. 2001; Cabrera et al. 2006; Valenzuela et al. 2007; Petrosyan et al. 2014), and sequence divergence facilitated to generate marked differences between members of the galectin family, for example, establishing the unique specificity of bivalent galectin-4 to sulfatide that enables apical or axonal transport of glycoprotein cargo (Delacour et al. 2005; Díez-Revuelta et al. 2017).

Strategically, the emerging evidence for efficient teamwork between presenting glycan-encoded messages and their 'readers' correctly in space and time calls for detailed analysis in model systems using appropriate experimental approaches. On the glycan side, it would be ideal to adapt the cell part of the robust haemagglutination assay by surface programming of custom-made sensors for lectin activity. Theoretically, glycan presentation can be stepwisely refined from starting out either from a structurally simple level (bottom-up) on synthetic particles or by lowering the complexity of glycans on natural cells (top-down). The self-assembly of natural sphingolipids inspired the generation of such fully programmable test 'cells', as natural modulation of the glycome did to introduce changes at certain sites of the assembly line by engineering. Explicitly, chemical bottom-up design of surfaces of cell/vesicle-like models such as glycodendrimersomes (Percec et al. 2013; Xiao et al. 2016, 2018) or top-down approaches by targeted engineering of the glycan machinery, e.g., in mutant cells (Patnaik and Stanley 2006), together with an engraftment of neo-epitopes into cell membranes (Huang and Godula 2016), enable to program surface properties structurally and topologically. In essence, presence of a suited binding partner defined by its glycan structure in the appropriate spatial context is the prerequisite for productive binding (Roy et al. 2016). Besides aggregation as read-out, histochemical assays exploiting lectin binding to natural glycoconjugates in sections and inhibition with synthetic glycans or glycoclusters are becoming means to probe into parameters for specificity (André et al. 2016; Roy et al. 2017).

On the side of the protein, rational engineering is the means to provide tools to answer questions on structure–activity relationships, for example, why a non-covalently associated homodimer such as galectin-1 has become the physiological form in mammals rather than a covalently linked complex or high-level oligomers. Conceptually, protein tailoring exploits the same modes of change as routes in natural diversification attain, from single-site mutations that affect glycan fine-specificity to changing modular display by domain shuffling. The aims of the engineering are to resolve the problem how lectin structure governs target (counter-receptor) selection and the nature of post-binding effects.

Altering structural aspects of galectins on both levels is gaining access, too, to new tools for detecting certain glycans by mutational adaptations, besides enabling initial insights into functional aspects of protein display, both warranting further efforts (Kopitz et al. 2014, 2017; Hu et al. 2015; Swanson et al. 2015; Zhang et al. 2015). For example, non-covalent homodimer formation of galectin-1 is most suitable for transient *trans*-contacts (Dettmann et al. 2000) and *cis*-binding to read the switch in surface display from ganglioside GD1a to GM1 as signal for differentiation highly sensitively (Kopitz et al. 2017; Ledeen et al. 2018). To give direction to further work, Fig. 8 shows instructive examples of structural permutations between the three types of mammalian galectin design that are presented in Fig. 5. This engineering can also help generate hybrid galectins by heterodimerization, a process recently discovered for galectin-1 and -3 (Miller et al. 2018), hereby extending functional network analysis in mixtures that mimic the (patho)physiological situation. Initial work with galectins-1 and -3 points to potential for functional antagonism or cooperation depending on the context (Kopitz et al. 2001; Weinmann et al. 2016). The testing of such variants along with the natural proteins is a promising approach to enable us to reach the aim to understand the physiological efficiency and precision of glycan–lectin recognition (for compilation of functions of animal and human lectins, please see Table 5 in Manning et al. 2017b).

Although many questions on glycan–lectin recognition remain to be answered, it is essential to point out that glycans are also endowed with capacity for carbohydrate–carbohydrate interactions, for example, in the cases of ganglioside GM1 and the tyrosine kinase-type nerve growth factor receptor TrkA/glial cell line-derived neurotrophic

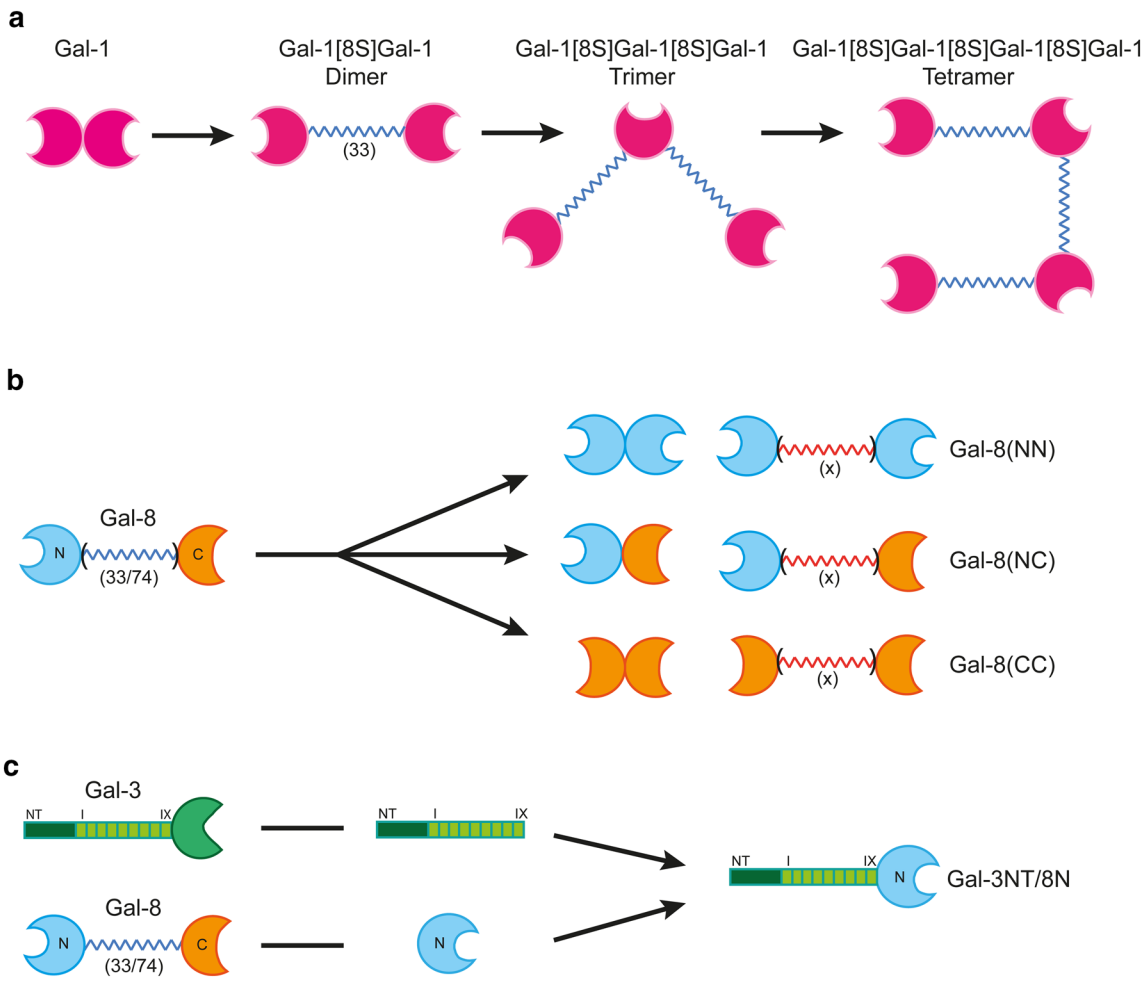


Fig. 8 Illustration of examples of lectin engineering by turning a non-covalently associated homodimer into covalently conjugated homo-oligomers (**a**) as well as by domain shuffling in a tandem-repeat-type lectin (**b**) and a chimera-type lectin (**c**). **a** The two CRDs of galectin-1 (Gal-1) are bridged by the linker of tandem-repeat-type galectin-8 (Gal-8), and the number of CRDs per protein is increased to produce tri- and tetramers (Vértesy et al. 2015; Kopitz et al. 2017). **b** Permutations of CRD arrangement of the tandem-repeat-type Gal-8 with its N- and C-terminal CRDs, connected either by the short (S; 33 amino acids) or the long linker (L; 74 amino acids), and linker length can be altered (Ludwig et al. 2017). **c** Domain shuffling between galectin-3 (Gal-3) and Gal-8 generates a new molecular hybrid (Ludwig et al. 2016)

factor receptor complex or inter-ganglioside binding (Mutoh et al. 1995; Bucior et al. 2009; Hadaczek et al. 2015). Likewise, lectins can be engaged in protein–protein association. It underlies the noted hybrid formation or association of proteins as counterreceptors such as the human pre-B cell receptor or anti-apoptotic bcl-2 (please see Table 2 in Kaltner et al. 2017 for further information).

Conclusions

The search for blood group-specific agglutinins in extracts from plant seeds and invertebrates has been a driving force for work with such antibody-like activities that turned out to 'read' carbohydrate signals on cell surfaces. Detected nearly 60 years ago, they were not only found to bridge cells to form aggregates but also to influence cell behavior, first in 1960 to stimulate resting leukocytes. This observation clearly illustrated the functional dimension glycan–lectin binding can have. With the growing realization that glycans are most suited for high-density information coding, lectins became versatile tools to map distribution profiles of sugar epitopes, from the assembly line to the glycocalyx, and endogenous lectins were detected.

When combined, the coding capacity of glycans and the ability to 'read' and translate these signals by lectins define a fundamental route of flow of biological information. Emerging evidence on (1) exquisite specificity of the functional pairing between tissue lectins and distinct cellular glycoconjugates such as an integrin or a ganglioside,

(2) fine-tuned co-regulation on both sides and (3) post-binding signaling attest broad versatility and physiological importance of glycan–lectin recognition. Cracking the sugar code is thus a major challenge.

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