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REVIEW

Lectins: a primer for histochemists and cell biologists

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Abstract An experimental observation on se*lecting* binding partners underlies the introduction of the term 'lectin'. Agglutination of erythrocytes depending on their blood-group status revealed the presence of activities in plant extracts that act in an epitope-specific manner like antibodies. As it turned out, their binding partners on the cell surface are carbohydrates of glycoconjugates. By definition, lectins are glycan-specific (mono- or oligosaccharides presented by glycoconjugates or polysaccharides) receptors, distinguished from antibodies, from enzymes using carbohydrates as substrates and from transporters of free saccharides. They are ubiquitous in Nature and structurally widely diversified. More than a dozen types of folding pattern have evolved for proteins that bind glycans. Used as tool, this capacity facilitates versatile mapping of glycan presence so that plant/fungal and also animal/human lectins have found a broad spectrum of biomedical applications. The functional pairing with physiological counterreceptors is involved in a wide range of cellular activities from cell adhesion, glycoconjugate trafficking to growth regulation and lets lectins act as sensors/effectors in host defense.

Keywords Agglutinin · Glycoprotein · Glycosylation · Protein fold · Sialylation

Introduction

Proteins are classified according to a specific activity. Similar to enzymes, lectins represent a superfamily of (glyco) proteins that all have a common characteristic. In retrospect, the pioneering work on measuring such a property had been done with concanavalin A (ConA), the hemagglutinin from the jack bean. The observation that ConA "unites with starch, glycogen, mucins" [experimental evidence for the glycoprotein character of snail mucin had first been provided by Eichwald (1865)] led Sumner and Howell (1936) to conclude that "ConA, like other haemagglutinins, unites within some chemical compounds present in the surfaces of such types of erythrocytes as it agglutinates" (p. 234 in Sumner and Howell 1936) and that "it is possible that this may be a carbohydrate group in a protein" (p. 236 in Sumner and Howell 1936). Fittingly, "negative results (in the activity assay) were possibly due to an inhibiting action of the sucrose solution employed to dissolve the ConA" (Sumner and Howell 1935), the sugar blocking the carbohydrate-binding site.

This specific carbohydrate (glucose/mannose)-dependent binding of ConA was considered "both as a tool for investigating the fine structure of polysaccharides and as a possible model for the antibody-antigen system" (Agrawal and Goldstein 1965). These project lines led Agrawal and Goldstein to investigate "the possibility of employing" Sephadexes (cross-linked dextrans) "as a means for

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purification." Obviously, they aimed at exploiting this lectin's inherent affinity to the matrix to illustrate the principle for a simplification of often tedious protocols to purify lectins. Of note, isolation and fractionation of human anti-dextran antibodies were possible in the same way (Schlossman and Kabat 1962), as noted by Agrawal and Goldstein (1965). Interestingly, this seminal report introducing affinity chromatography to lectin purification that was later adapted to wide applicability by using resin-immobilized glycoproteins and glycans (Lis and Sharon 1981; please see also Table 1) was judged to "represent a modest advance in an obscure area" at its first submission (Sharon 1998).[1]

With the perspective in mind to understand the flow of biological information at the level of glycosylation and glycan recognition (as outlined above in this special issue in Fig. 1 in Gabius and Roth 2017), we provide an introduction to the protein side of translating sugar-encoded information into functions. It starts with a survey of historical and structural aspects and then turns to applications of lectins. The following summary of lectin functions finally underscores that predicting "theoretical and practical importance" more than 70 years ago had been prophetic.

Lectins: history

The scientific description of lectin activity can be traced back to 1860 (Table 1). It was detected by the agglutination of erythrocytes: first by snake (*Crotalus durissus*) venom, when a drop of venom on a slide and "a drop of blood from a pigeon's wounded wing allowed to fall upon it. They were instantly mixed. Within three minutes the mass had coagulated firmly" and red blood corpuscles "fuse together into irregular masses acting like soft elastic material" (Mitchell 1860; Mitchell and Reichert 1886), and later by extracts of *Ricinus communis* beans (Stillmark 1888). In this case, the active principle, called ricin, "bewirkt in defibriniertem serumhaltigem Blute eine Zusammenballung der rothen Blutkörperchen unter Bildung einer fibrinähnlichen Substanz" (Stillmark 1888). The intimate connection to research on blood groups and serum antibodies against them (Kilpatrick and Green 1992; Hughes-Jones and Gardner 2002; Gabius et al. 2016; please see also Table 1) stood behind coining terms for this activity such as hemagglutinin (Elfstrand 1898) or phyt(ohaem)agglutinins when from plants (Krüpe 1956). It also inspired to put the noted selectivity for the blood-group status that resembles the equivalent potency of antibodies into one word, formulating the term lectin "from Latin *lectus*, the past principle of *legere* meaning to pick, choose or select" (Boyd 1954, 1963). W. C. Boyd, the inventor of this term, later stated "that he would like to use the word to mean a protein that had a more or less specific action and that there is no reason to think it is an antibody" (Bird 1989). This distinction from carbohydrate-binding antibodies was later extended to enzymes processing carbohydrate substrates such as glycosyltransferases (Barondes 1988), also to transport proteins for free sugars such as the bacterial periplasmic receptors (Quiocho 1986) and carbohydrate-binding modules grouped into 67 families, characteristically linked to glycoside hydrolases (Gilbert et al. 2013). Together with the specific property to bind carbohydrates, these four exclusion criteria established the currently accepted definition of a lectin (Gabius et al. 2011; please see also Table 1).

Equipped with the simple and robust hemagglutination assay and affinity chromatography, screening for activity and purification of lectins were readily feasible, as demonstrated above for ConA and below for galectins (Kaltner et al. 2017). Nowadays, aggregation assays no longer rely on erythrocytes but can be performed with custom-made reagents. For example, chemical synthesis facilitates to program the ligand display of vesicles such as glycodendrimersomes to trace binding activity of any specificity (Percec et al. 2013; Zhang et al. 2015a, b). Erythrocytes can thus be replaced by such versatile, chemically defined tools, and surface headgroups proven to be highly active can then be used as ligands in affinity chromatography. This work has taken the structural analysis of lectins from the sequence to the level of defining the protein fold. In fact, the ensuing structural characterization of lectins, again with ConA as starting point (Edelman et al. 1972; Hardman and Ainsworth 1972; please see also Table 1), provided the answer to the question whether or not binding a glycan is a unique capacity of a single fold.

Lectins: structures

The concept of the sugar code implies a broad physiological significance of glycan recognition (Gabius and Roth 2017). To be able to reach such a versatility, a large variety

[1] Such an apparent lack of enthusiasm in an early phase of study allows to draw an analogy to the course of development similarly encountered in the case of nuclein (first isolated in 1869, renamed to nucleic acid in 1889) (Chargaff 1970; Dahm 2005). Following nuclein's biochemical characterization, "then the long road began—in this case nearly 80 years—which every biologically important, complicated chemical substance must travel: first its structure, then its function" (Chargaff 1977). Accompanied by encouraging statements such as that lectins "promise to have theoretical and practical importance" (Boyd and Shapleigh 1954), the topic of an "obscure" research field eventually made it into the "limelight" (Sharon 1998). Breaking the sugar code and "identifying the receptors," then analyzing the functional pairing of lectin and its counterreceptor(s) "are of prime importance" (Sharon 1998), long-range aims that sparked vigorous research activity, for example by using ConA as research tool (Bittiger and Schnebli 1976).

Table 1 Brief historical account of lectinology

1860	Observation of blood "coagulation" by rattlesnake (*Crotalus durissus*) venom (S. W. Mitchell)
1888	Detection of erythrocyte agglutination by a toxic protein fraction from castor beans (termed ricin) and seeds of related plants (H. Stillmark)
1890	Detection of a toxic lectin in the bark of black locust (*Robinia pseudoacacia*, O. Power, O. Cambier)
1891	Toxic plant agglutinins applied as model antigens (P. Ehrlich)
1898	Introduction of the term "haemagglutinin" or "phytohaemagglutinin" for plant proteins that agglutinate red blood cells (M. Elfstrand)
1902	Detection of bacterial agglutinins (R. Kraus, S. Ludwig) and demonstration that blood "coagulation" by snake venom (seven to nine decades later shown to depend on the presence of a C-type lectin) observed in 1860 was due to cell agglutination but not to blood clotting (S. Flexner, H. Noguchi)
1906	Detection of an agglutinin in bovine serum (later characterized as the C-type lectin conglutinin) by use of activated complement-coated erythrocytes (J. Bordet, F. P. Gay); detection of a hemagglutinin in mushrooms (*Amanita* sp., W. W. Ford)
1907/1909	Detection of non-toxic agglutinins in plants, of their nature as proteins and of "deagglutination" of erythrocytes by hog gastric mucin (K. Landsteiner, H. Raubitschek)
1913	Use of intact cells for the purification of the agglutinin ricin (R. Kobert)
1919	Crystallization of a globulin from jack bean, concanavalin A, which was later defined as lectin and used in pioneering studies (please see below) (J. B. Sumner)
1935/1936	Concanavalin A identified as jack bean hemagglutinin; precipitation of starch, glycogen and mucins by concanavalin A defines carbohydrate as ligand and points to "a carbohydrate group in a protein" as binding partner on erythrocytes (J. B. Sumner, S. F. Howell)
1941	Detection of viral agglutinins (G. K. Hirst; L. McClelland, R. Hare)
1944	Description of anti-0(H) hemagglutinating activity in serum of *Anguilla anguilla* (B. Jonsson), following earlier work on a similar activity in *Anguilla japonica* (S. Sugishita; 1935)
1947/1948	Detection of plant agglutinins specific for the human histo-blood group A (W. C. Boyd; K. O. Renkonen), "good keeping qualities" and low "cost of producing them" are emphasized as advantageous properties
1952	Carbohydrate nature of histo-blood group H(0) determinant proven by eel-serum-mediated agglutination of respective erythrocytes and its L-fucose-dependent inhibition (W. M. Watkins, W. T. J. Morgan)
1954	Introduction of the term "lectin" for plant (antibody-like) agglutinins, primarily for those which are specific for a distinct histo-blood group (W. C. Boyd)
1956	Detection of an agglutinin specific for the human blood group B in the seeds of the African shrub *Griffonia* (*Bandeiraea*) *simplicifolia* (O. and P. Mäkelä)
1960	Detection of the mitogenic potency of lectins toward lymphocytes (P. C. Nowell)
1963	Introduction of affinity chromatography for the isolation of lectins, published in 1965 (I. J. Goldstein, B. B. L. Agrawal)
1968–1974	Detection of rapid serum clearance of asialoceruloplasmin in rabbits and isolation of a Gal-/Gal*N*Ac-specific lectin (asialoglycoprotein receptor) from liver, the first mammalian lectin (G. Ashwell, A. G. Morell and colleagues)
1971	Introduction of labeled lectins as ultrastructural marker (G. L. Nicolson, S. J. Singer)
1972	Determination of the amino acid sequence and the three-dimensional structure of a lectin, concanavalin A (G. M. Edelman, K. O. Hardman, C. F. Ainsworth et al.)
1972–1977	Detection of impaired synthesis of a marker for glycoprotein (lysosomal enzymes) routing as cause for a human disease (mucolipidosis II) and its identification as Man-6-phosphate, the ligand for P-type lectins (E. F. Neufeld and colleagues; W. S. Sly and colleagues)
1978	First conference focusing on lectins and glycoconjugates, termed Interlec (T. C. Bøg-Hansen)
1979	Detection of endogenous ligands for plant lectins (H. Rüdiger)
1981–1988	Further refinements of the definition of the term "lectin" as carbohydrate-binding protein, separated from antibodies and carbohydrate-processing enzymes/sensor or transport proteins for free sugars (S. H. Barondes, I. J. Goldstein, J. Kocourek et al.)
1982	Introduction of serial lectin affinity chromatography as analytical tool for structural analysis of glycans from glycoproteins (R. D. Cummings, S. Kornfeld)
1983	Detection of the insecticidal action of a plant lectin (L. L. Murdock)
1984	Isolation of lectins from tumors (H.-J. Gabius; R. Lotan, A. Raz)
1985	Immobilized glycoproteins as pan-affinity adsorbents for lectins (H. Rüdiger)
1989	Detection of the fungicidal action of a plant lectin (W. J. Peumans)
1992/1993	Detection of impaired synthesis of lectin (selectin) ligands by defective fucosylation as cause for leukocyte adhesion deficiency type II, a congenital disorder of glycosylation (CDG IIc) (A. Etzioni and colleagues)
1995	Structural analysis of a lectin–ligand complex in solution by NMR spectroscopy (J. Jiménez-Barbero and colleagues)
1996–2003	Detection of differential conformer selection by plant, bacterial and animal lectins (H.-J. Gabius and colleagues; L. Poppe and colleagues)
2001–2005	Development of glycan/lectin microarrays for specificity analysis of lectins/structural analysis of glycans and glycoproteomics (various laboratories worldwide)
2001–2017	Advances in lectinology and glycosciences honored by devoting theme issues to this topic in journals of diverse fields such as *Advanced Drug Delivery Reviews*, *Biochimica et Biophysica Acta*, *Biochimie*, *Biological Chemistry*, *Cells Tissues Organs*, *Chemical Reviews*, *Current Opinion in Structural Biology*, *Glycoconjugate Journal*, *Histochemistry and Cell Biology*, *Journal of Agricultural and Food Chemistry* ("Liener symposium"), *Nature*, *Science* and *Trends in Biochemical Sciences* to these topics

From Rüdiger and Gabius (2009a), extended, updated and modified, with permission

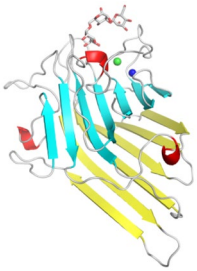
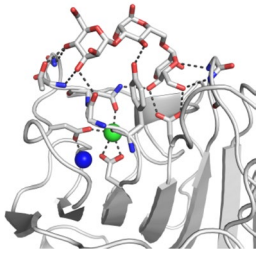
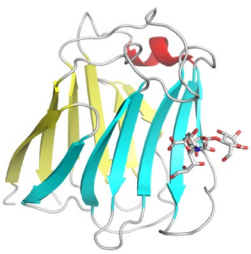
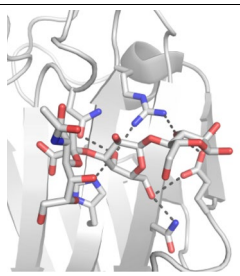
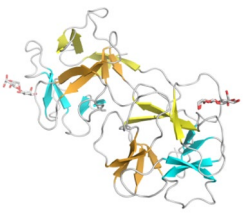
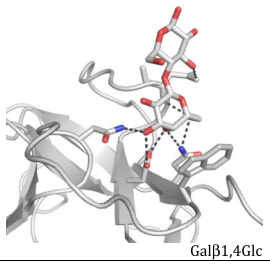
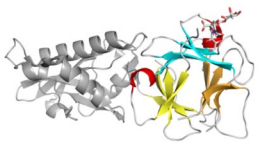
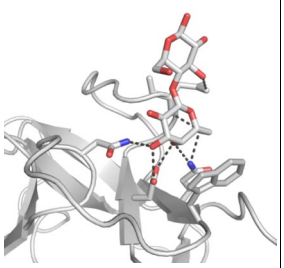
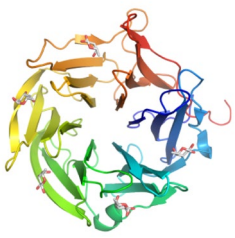
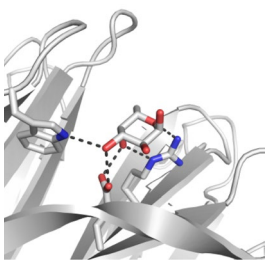
Type of fold	Overall fold	Carbohydrate-binding site	Examples for lectin/specificity	Examples for PDB entries*
β-sandwich (leguminous lectins)		 Manα1,3Manα1,6Man	Legume lectins / Man/Glc, Gal/Gal*N*Ac, $(GlcNAc)_n$, Fuc, Siaα2,3Galβ1,6Glc*N*Ac, complex-type *N*-glycan with β1,6-branching or bisecting Glc*N*Ac	*Arachis hypogaea*: **1CIW**, **2DVA**, **2DV9**, **2DVG**, **2PEL**, **2TEP**; *Canavalia ensiformis*: **1CVN***, **1ONA**, **1QDC**, **1QDO**, **1TEI**, **3D4K**, **4CNA**, **5CNA**; *Dioclea grandiflora*: **1DGL**; *Glycine max*: **1G9F**, **2SBA**; *Griffonia simplicifolia*: **1LEC**, **1LED**; *Lens culinaris*: **1LES**; *Phaseolus vulgaris*: **3WCS**, **3WOG**; *Pisum sativum*: **1OFS**, **1RIN**, **2BQP**; *Psophocarpus tetragonolobus*: **2D3S**, **2DU0**, **2E53**, **2ZML**; *Ulex europaeus*: **1JXN**; *Vicia villosa*: **1N47**
β-sandwich[#,&] (galectin fold)		 Siaα2,3Galβ1,4Glc	*Agrocybe* lectin / Siaα2,3Galβ1,4Glc; *Coprinus cinereus* galectin-2 (CGL2) / Galα1,3Galβ1,4Glc*N*Ac, Galβ1,4Fucα1,6Glc*N*Ac; *Coprinus cinereus* galectin-3 (CGL3) / $(GlcNAc)_4$, Gal*N*Acβ1,4Glc*N*Ac	*Agrocybe aegerita*: **2ZGK**, **2ZGL**, **2ZGM**, **2ZGN**, **2ZGO**, **2ZGP**, **2ZGQ**, **2ZGR**, **2ZGS**, **2ZGT**, **2ZGU**, **3AFK**, **3M3C**, **3M3E**, **3M3O**, **3M3Q**, **3WG1**, **3WG2**, **3WG3**, **3WG4**, **2ZGK**; *Agrocybe cylindracea*: **1WW4***, **1WW5**, **1WW6**, **1WW7**; *Coprinopsis cinerea (Coprinus cinereus)*: **1UL9**, **1ULC**, **1ULD**, **1ULE**, **1ULF**, **1ULG**, **2WKK**, **2R0F**, **2R0H**
β-trefoil (plants)		 Galβ1,4Glc	Ricin-B and amaranthin family / Gal, Gal*N*Ac, Siaα2,3Gal	*Abrus precatorius*: **1ABR**, **2QN3**, **2ZR1**; *Amaranthus caudatus*: **1JLX**, **1JLY**; *Ricinus communis*: **1RZO**, **2AAI***; *Sambucus ebulus*: **1HWM**, **1HWN**, **1HWO**, **1HWP**; *Sambucus nigra*: **3C9Z**, **3CA0**, **3CA1**, **3CA3**, **3CA4**, **3CA5**, **3CA6**, **3CAH**; *Trichosantes kirilowii*: **1GGP**; *Viscum album*: **1ONK**, **1PC8**, **1SZ6**, **2R9K**, **2RG9**, **1M2T**, **1OQL**, **1PUM**, **1PUU**, **1YF8**, **3D7W**
β-trefoil (mushroom)		 Siaα2,6Galβ1,4Glc*N*Ac	*Polyporus* lectin / Lac, Lac*N*Ac, Gal*N*Ac, Galα1,3Galβ1,4Glc*N*Ac, Siaα2,6Galβ1,4Glc*N*Ac	*Boletus edulis*: **4I4O**, **4I4P**, **4I4Q**, **4I4U**, **4I4S**, **4I4R**, **4I4V**, **4I4X**, **4I4Y**; *Clitocybe nebularis*: **3NBC**, **3NBD**, **3NBE**; *Coprinopsis cinerea (Coprinus cinereus)*: **2LIE**, **2LIQ**; *Laetiporous sulphereus*: **1W3A**, **1W3F**, **1W3G**, **2Y9F**, **2Y9G**; *Macrolepiota procera*: **4ION**, **4IYB**, **4IZX**, **4J2S**; *Marasmius oreades*: **2IHO**, **3EF2**; *Polyporus squamosus*: **3PHZ***; *Sclerotinia sclerotiorum*: **2X2S**, **2X2T**
β-propeller (6-bladed)		 Fuc	*Aleuria* and *Aspergillus* lectins / Fuc	*Aleuria aurantia*: **1OFZ***, **1IUB**, **1IUC**; *Aspergillus fumigatus*: **4UOU**, **4D52**, **4D4U**, **4C1Y**, **4AH4**, **4AGT**, **4AGI**

Fig. 1 Gallery of fungal and plant lectins. *Selected PDB files used for graphical representation. [#]β-Sandwich (chaperone): calnexin homologs: AtCnx1, AtCnx2, AtCnx3 (*Arabidopsis thaliana*); OsCnx (*Oryza sativa*); calreticulin homologs: Crt1a, Crt1b, Crt3 (*Arabidopsis thaliana*). [&]β-Sandwich (*N*-glycanase): Png1 (*Neurospora crassa*; *Aspergillus tubingensis*); AtPng1 (*Arabidopsis thaliana*); PNGase R (*Raphanus sativus*); PNGase J (*Canavalia ensiformis*)

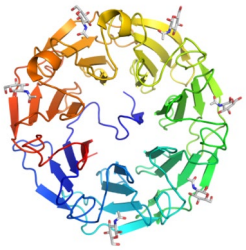
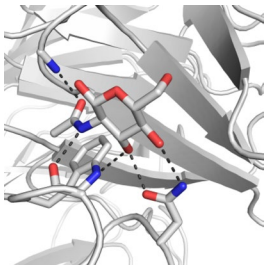
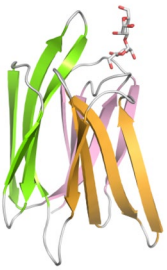
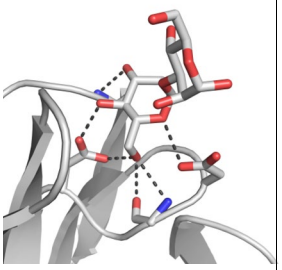
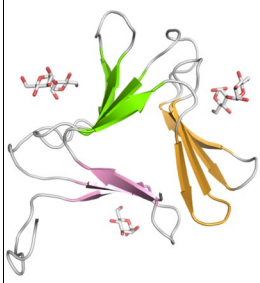
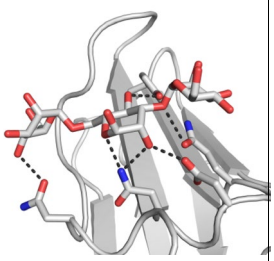
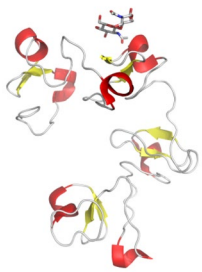
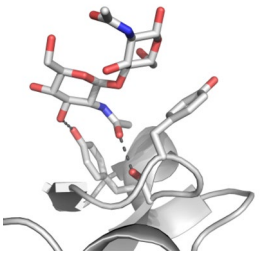
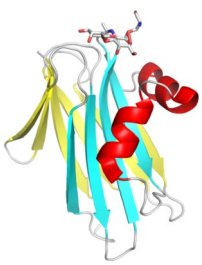
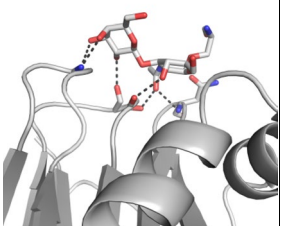
β-propeller (7-bladed)		 GlcNAc	*Agrocybe* and *Psathyrella* lectins / GlcNAc	*Agrocybe aegerita*: **4TQJ**, **4TQK***, **4TQM**; *Lacrymaria velutina* (*Psathyrella velutina*): **2BWM**, **2BWR**, **2C25**, **2C4D**, **4UP4**
β-prism I		 Manα1,3Man	Jacalin-related lectin family / Gal, Man	*Artocarpus integer*: **4AK4**, **1WS4**, **1WS5**, **4AKC**; *Artocarpus integrifolia*: **1KU8**, **1JAC**, **1KUJ**, **1M26**, **1UGW**, **1UGY**, **1UH0**, **1UH1**, **1J4S**, **1J4U**, **1VBO**, **1VBP**; *Calystegia sepium*: **1OUW**; *Helianthus tuberosus*: **1C3K**, **1C3M**, **1C3N**; *Morus nigra*: **1XXQ**, **1XXR**; *Musa acuminata*: **2BMY**, **3MIV**, **4PIF**, **4PIU**, **1X1V**, **2BMZ**, **3MIT**, **3MIU***, **4PIK**, **4PIT**
β-prism II		 Manα1,6Manα1,3Man	Monocot lectins / Man	*Allium sativum*: **1BWU**, **1KJ1**; *Galanthus nivalis*: **1JPC***, **1MSA**, **1NIV**; *Gastrodia elata*: **1XD5**, **1XD6**; *Narcissus pseudonarcissus*: **1NPL**, **3DZW**; *Polygonatum cyrtonema*: **3A0C**, **3A0D**, **3A0E**; *Scilla campanulata*: **1DLP**, **1B2P**
hevein fold		 $(GlcNAc)_2$	Hevein; *Urtica dioica* / $(GlcNAc)_n$	*Hevea brasiliensis*: **1HEV**, **1Q9B**, **1T0W**; *Phytolacca americana*: **1UHA**, **1ULK**, **1ULN**, **1ULM**; *Triticum aestivum*: **4AML**; **1WGC**, **2UVO**, **2CWG**, **2UWZ**, **2X3T***, **7WGA**, **2WGC**, **9WGA**, **1K7T**, **1K7U**, **1K7V**, **1WGT**; *Urtica dioica*: **1EHD**, **1EIS**, **1IQB**, **1EHH**, **1EN2**, **1ENM**
actinoporin-like fold		 Galα1,3GalNAc-Ser	*Agaricus*, *Boletus* and *Xerocomus* lectins / GalNAc, Galβ1,3GalNAc (TF antigen)	*Agaricus bisporous*: **1Y2T**, **1Y2U**, **1Y2V***, **1Y2W**, **1Y2X**; *Boletus edulis*: **3QDS**, **3QDT**, **3QDU**, **3QDV**, **3QDW**, **3QDX**, **3QDY**; *Athelia rolfsii* (*Sclerotium rolfsii*): **2OFC**, **2OFD**, **2OFE**, **4YLD**, **4Z2F**, **4Z2Q**, **4Z2S**; *Xerocomellus chrysenteron* (*Xerocomus chrysenteron*): **1X99**, **1XI0**

Fig. 1 continued

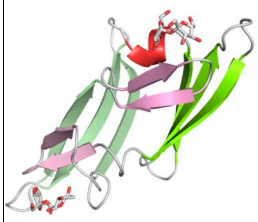
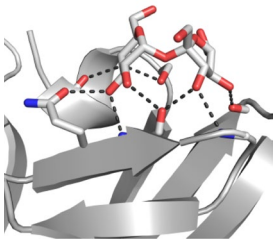
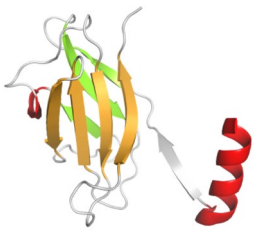
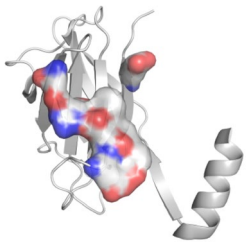
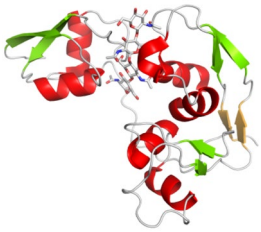
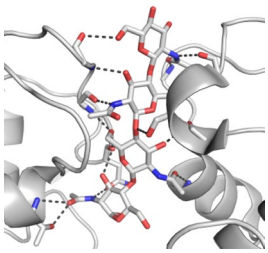
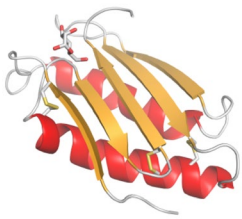
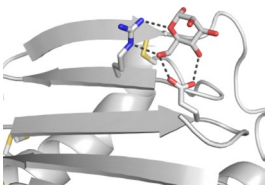
cyanovirin-*N*-like fold		 Glcβ1,2Fru	Cyanovirin-*N*-related lectins / Manα1,2Man, sucrose	*Ceratopteris richardii*: **2JZJ**; *Fusarium graminearum (Gibberella zeae)*: **2L2F**; *Magnaporthe oryzae*: **2L9Y**; *Neurospora crassa*: **2JZL**; *Tuber borchii*: **2JZK**, **2KJL**, **3HNU**, **3HNX**, **3HP8***
Ig-like fold		 region given as surface depicts putative site for ligand contact	*Flammulina velutipes* lectin/ sialylated glycoconjugates	*Flammulina velutipes*: **1OSY***
LysM fold		 $(GlcNAc)_4$	*Cladosporium fulvum* lectin / chitin	*Passalora fulva (Cladosporium fulvum)*: **4B9H***, **4B8V**
α+β fold		 Man	Ginkbilobin-2 / Man ; *Lyophyllum decastes* lectin / Galα1,4Gal	*Ginkgo biloba*: **3A2E, 4XRE***; *Lyophyllum decastes:* **4NDS**, **4NDT**, **4NDU**, **4NDV**

Fig. 1 continued

Table 2 Examples for source of lectins and their specificities

Species	Abbreviation	Monosaccharide specificity	Potent glycan ligands
Canavalia ensiformis (jack bean)	ConA	Man/Glc	Glc*N*Acβ2Manα6(Glc*N*Acβ2Manα3)Manβ4 Glc*N*Ac
Triticum vulgare (wheat)	WGA	Glc*N*Ac	Stem region of *N*-glycans, $(Glc\mathit{N}Ac)_n$
Griffonia simplicifolia isolectin-II	GSA-II	Glc*N*Ac	Glc*N*Acα3/4Gal, $(Glc\mathit{N}Ac)_2$
Phaseolus vulgaris erythroagglutinin (kidney bean)	PHA-E	[a]	Bisected complex-type *N*-glycans: Galβ4Glc*N*Acβ2Manα6(Glc*N*Acβ2-Manα3)(Glc*N*Acβ4) Manβ4Glc*N*Ac
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)Glc*N*Ac without/with α2,6-sialylation, Fucα2Gal
Limax flavus (slug)	LFA	Neu5Ac > Neu5Gc	Sialylated glycoconjugates
Maackia amurensis-I (leuko)agglutinin (Amur maackia)	MAA-I	[a]	Neu5Acα3Galβ4Glc*N*Ac/Glc; 3′-sulfation tolerated
Sambucus nigra-I (elderberry)	SNA-I	Gal/Gal*N*Ac	Neu5Acα6Gal/Gal*N*Ac; clustered T_n antigen
Polyporus squamosus (polypore mushroom)	PSL	[a]	Neu5Acα6Galβ4Glc(*N*Ac) (over 300-fold more active than Lac*N*Ac, not reactive with free Neu5Ac); 6′-sulfation tolerated; 6′-sialyl T_n not reactive
Homo sapiens	CD22/Siglec-2	[a]	Neu5Acα6Galβ3(4)Glc*N*Ac; 9′-*O*-acetylation blocks binding
Arachis hypogaea (peanut)	PNA	Gal	Galβ3Gal*N*Acα/β
Artocarpus integrifolia (jack fruit)	Jacalin (JAC)	Gal/Gal*N*Ac	Galβ3Gal*N*Acα; sialylation of T/T_n antigens tolerated

Histochemical applications are documented in Figs. 2, 3, 4, 5, 6 and 7

[a] No monosaccharide known as ligand

Table 3 Versatility of plant lectins as research tools

Biochemistry	Detection of defined carbohydrate epitopes of glycoconjugates in blots or on thin-layer chromatography plates
	Purification of lectin-reactive glycoconjugates by (serial) affinity chromatography
	Glycan characterization by serial lectin affinity chromatography (lectin affinity capture)
	Glycome analysis by lectin microarrays (glycomics)
	Quantification of lectin-reactive glycoconjugates in enzyme-linked lectin-binding assays (ELLA)
	Quantification of activities of glycosyltransferases/glycosidases by lectin-based detection of products of enzymatic reaction
	Model reagents to assess ligand functionality of carbohydrate-presenting scaffolds (for example glycodendrimers)
	Detection of glycomimetic peptides or aptamers
Cell biology	Characterization of intracellular assembly, routing and cell surface presentation of glycoconjugates in normal and genetically engineered cells (glycomic profiling, spatially defined by cyto- or histochemistry)
	Competitive assays with cell extracts on lectin microarrays to define differences in relative glycan abundance between cell populations
	Selection of cell variants (mutants, transfectants) with altered lectin-binding properties as models for dissecting the glycosylation machinery and glycan functionality (glycomic profiling, functionally defined)
	Fractionation of cell populations by sorting or bead binding
	Modulation of proliferation or activation status of cells and dissecting the involved signal pathways
	Model substratum for study of cell adhesion, aggregation and migration
Medicine	Analysis of glycosylation in recombinant glycoproteins
	Detection of disease-related alterations of glycan synthesis by lectin cyto- and histochemistry
	Histo-blood group typing and definition of secretor status
	Quantification of disease-associated aberrations of glycan presentation (serum glycoproteins, cell surfaces)
	Cell marker for diagnostic purposes incl. infectious agents (viruses, bacteria, fungi, parasites)
	Cell marker for functional assays to pinpoint defects in cell activities such as mediator release
	Marker for functional assays to block viral docking and entry

From Rüdiger and Gabius (2009b), extended and modified, with permission

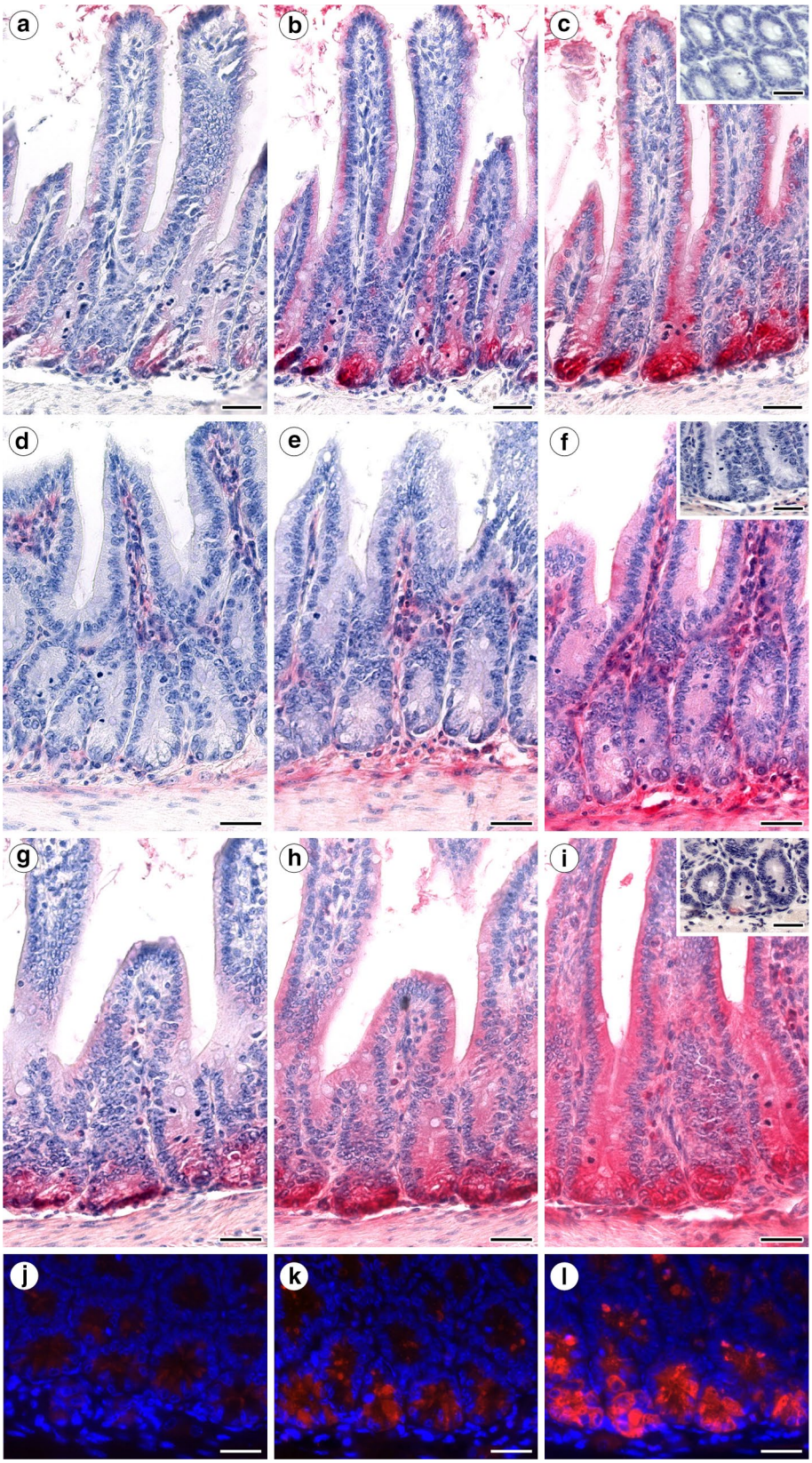


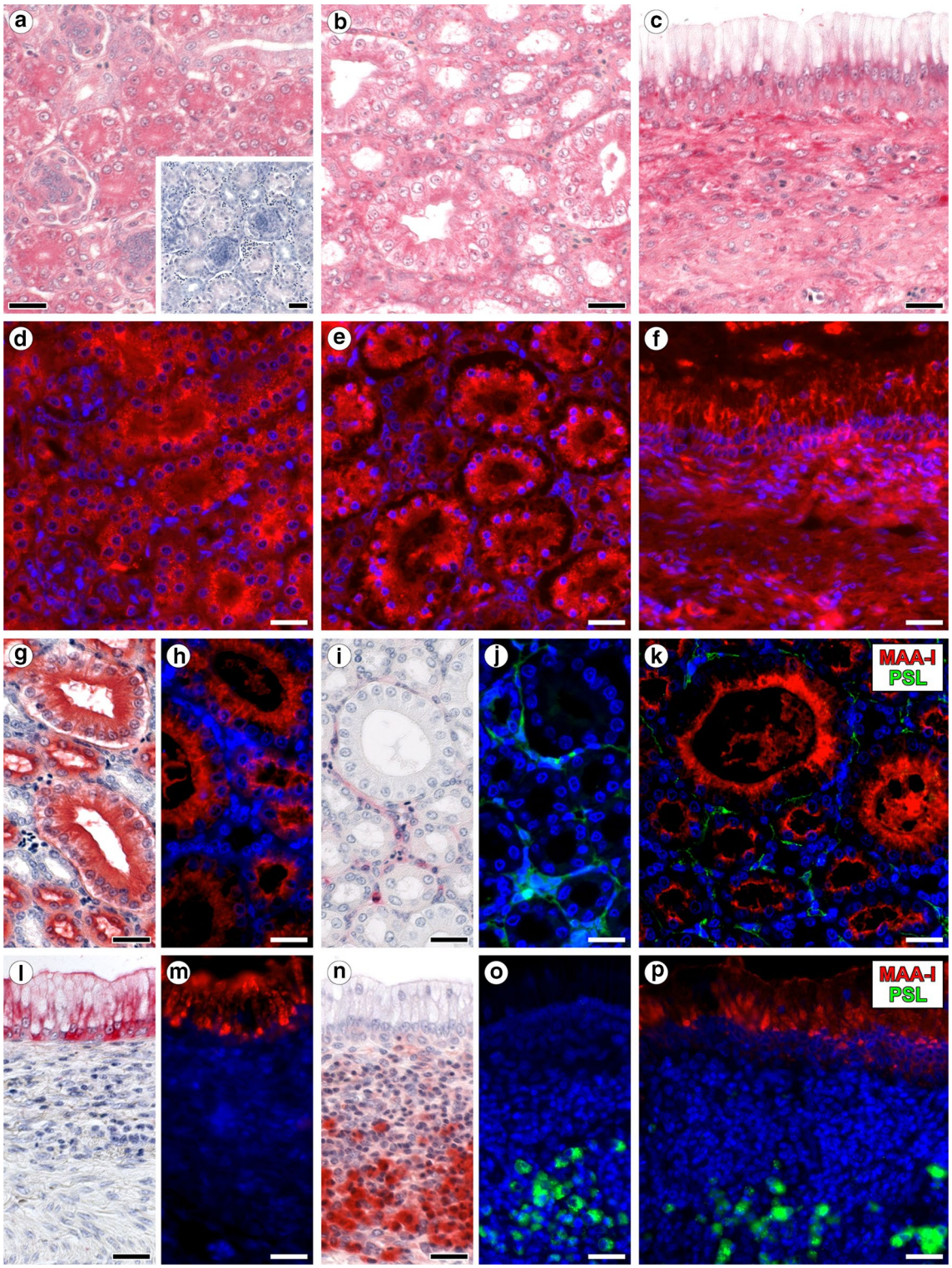
Fig. 2 Histochemical staining profiles of three labeled plant lectins, i.e., biotinylated GSA-II, VAA and WGA, tested at three concentrations in sections of murine jejunum. The second-step reagents avidin conjugated either with alkaline phosphatase (**a**–**i** *red* product after reaction with Vector® Red AP substrate) or with Texas Red (**j**–**l** *red*) were used for generation of the signal. Incubation with the lowest concentration of GSA-II led to weak staining in the glandulae intestinales (**a** 0.25 µg/ml). The intensity at this site increased at higher lectin concentrations, which also caused appearance of staining in the epithelial lining of villi intestinales and, to a lesser extent, in the lamina propria mucosae (**b** 0.5 µg/ml; **c** 1 µg/ml). Control with the inhibitory sugar Glc*N*Ac (250 mM) leads to pronounced reduction in staining (*inset* to **c**). Reactivity for VAA (*second row*) is detected in the lamina propria mucosae at the lowest concentration of lectin (**d** 0.25 µg/ml). Increase in this concentration led to enhanced intensity as well as appearance of positivity in the lamina muscularis mucosae and also in the epithelial lining of villi and glandulae intestinales (**e** 0.5 µg/ml; **f** 1 µg/ml). Inhibition of VAA binding with 300 mM lactose reduced staining (*inset* to **f**). WGA reactivity was detected in the glandulae intestinales both in light and in fluorescence microscopy (**g**–**l**). Weak signals with Vector® Red were detected at a low WGA concentration (**g** 0.0625 µg/ml) in glandulae intestinales. Lamina propria mucosae, epithelial lining of villi intestinales and lamina muscularis mucosae were labeled at higher concentrations (**h** 0.125 µg/ml; **i** 0.25 µg/ml). The control with 400 mM Glc*N*Ac ascertains inhibition of lectin binding by its cognate sugar (*inset* to **i**). *Scale bars* 20 µm

of structural epitopes with their special 'meaning' will be required. As glycome analyses for cells and secreted glycoproteins from different organisms attest, this prerequisite is fulfilled within an organism and can also be traced within phylogenesis (Wilson et al. 2009; Antonopoulos et al. 2011; Corfield and Berry 2015; Clerc et al. 2016; Corfield 2017). If structural diversity is also found on the protein side of glycan-based recognition, then the essential components for information transfer would be available, building an operative sugar coding/decoding system (Gabius 2009a, 2015). A guided tour into this realm is provided in the introduction of this special issue (Gabius and Roth 2017). As structural trait for diversity, the basic fold of a protein is a reliable marker. Indeed, binding a carbohydrate is not confined to a single protein fold, and the respective proteins appear to have developed by divergence or by convergence (Loris 2002; Taylor and Drickamer 2014; Gabius et al. 2015; Solís et al. 2015).

With focus on plant and fungal lectins, the capacity to associate with a glycan is present in 12 different types of protein fold, as compiled in Fig. 1 (for the Gallery of Animal and Human Lectins, please see Solís et al. 2015). As the case of the β-sandwich-type lectins attests, sugar specificity can vary within a group defined by a certain fold. Lectin specificity is usually given in terms of the mono- or disaccharide(s), which has the highest inhibitory effect in the activity assay (for a compilation of methods to analyze carbohydrate–lectin interactions, please see Table 1 in Solís et al. 2015). To give an example, mannose and glucose, and also sucrose as suspected by Sumner and Howell (1935) (please see above), inhibit ConA in hemagglutination assays. Such measurements and the results of crystallographic analysis of lectin–glycan complexes (Fig. 1) revealed that the binding site of a lectin is often extended to accommodate oligosaccharides, thereby increasing its specificity (for a detailed account on the development of the status of knowledge on ConA's specificity, please see Goldstein and Poretz 1986). Table 2 presents examples of lectins together with listing potent inhibitory glycans, and it is not surprising that these informations have inspired many applications in cell biology as well as cyto- and histochemistry. Instead of proving the presence of a glycan biochemically, it can alternatively be detected by its reactivity with a labeled lectin, when presented on the surface of microtiter plate wells, on blots or on/in cells. An overview of experimental approaches using (plant and fungal) lectins for glycan monitoring and profiling is given in Table 3. For the localization of glycans in cells and tissues, lectin histochemistry has become an invaluable technique, as recently reviewed by Roth (2011). In the following, applications of lectin histochemistry for light and electron microscopy including confocal laser scanning microscopy are presented.

Lectins: histochemistry

In the first step of the histochemical localization of glycans by lectins, a titration of the concentration of the probe is usually carried out. Performed on sections of fixed and paraffin-embedded specimen of murine jejunum for the plant lectins *Griffonia simplicifolia* isolectin-II (GSA-II), *Viscum album* agglutinin (VAA) and wheat germ agglutinin (WGA) (for glycan specificities, please see Table 2), the series of microphotographs in Fig. 2a–l documents staining patterns characteristic both for the lectin and for the tissue constituents. Insets in these illustrations document that even strong signals can be (nearly) completely precluded by incubation of lectin with the cognate sugar. Careful comparison of signal distributions


a
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PSL

◂**Fig. 3** Histochemical staining profiles of labeled plant/fungal lectins in chicken kidney (for carbohydrate specificities, please see Table 2). ConA staining in cortex (**a**, **d**), medulla (**b**, **e**) and ureter (**c**, **f**) visualized by light (**a**–**c**) and fluorescence microscopy (**d**–**f**). Signal was developed using the second-step reagent avidin conjugated either with alkaline phosphatase (AP) (**a**–**c** chromogenic reaction with Vector® Red AP substrate) or with Texas Red (**d**–**f**). Apical parts of the proximal tubules in the cortex (**a**, **d**) and of the ductuli colligentes in the medulla (**b**, **e**) were strongly positive. In the ureter (**c**, **f**), both the basal subnuclear portion of the epithelium and its lamina propria mucosae were stained. Blocking glycan binding by incubation with 200 mM D-mannose reduced the lectin-dependent signal (*inset* of **a**). MAA-I binding in the medulla (**g**, **h**, **k**) and the ureter (**l**, **m**, **p**) monitored by light (**g**, **l**) and fluorescence microscopy (*red* **h**, **k**, **m**, **p**) concerned the apical portion of the ductuli colligentes and the loops of Henle of the medulla (**g**, **h**, **k**) as well as the epithelium of the ureter (**l**, **m**, **p**). Binding of PSL in the medulla (**i**, **j**, **k**) and the ureter (**n**, **o**, **p**) was seen in light (**i**, **n**) and fluorescence microscopy (*green* **j**, **k**, **o**, **p**) in connective tissue between the ductuli colligentes and loops of Henle of the medulla (**i**–**k**) and the lamina propria mucosae of the ureter (**n**–**p**). Double staining [*green* (PSL) and *red* (MAA-I)] illustrates regional differences in medulla (**k**) and ureter (**p**). Labeled lectins were used at the following concentrations: ConA, 1 µg/ml (**a**–**c**) or 8 µg/ml (**d**–**f**); MAA-I, 0.5 µg/ml (**g**, **i**) or 5 µg/ml (**h**, **k**, **m**, **p**); PSL, 0.06 µg/ml (**i**, **n**) or 5 µg/ml (**j**, **k**, **o**, **p**). *Scale bars* 20 µm

obtained by an enzymatic detection reaction or by fluorescence resulted in equivalent data (Fig. 2g–i/j–l). These three aspects, i.e., characteristic profiles of lectin staining, their drastic reduction by the presence of the cognate sugar and independence from the method of making lectin binding visible, are further underscored in Fig. 3a–f. These illustrations show ConA-dependent staining in sections of fixed and paraffin-embedded specimen of adult chicken kidney by the two labeling techniques, as also presented in Fig. 2. In this case, a general feature of *N*-glycosylation is monitored, i.e., the trimannoside of the core pentasaccharide. Table 2 also lists cases of glycan determinants at branch ends. Here, the type of linkage of the sialic acid to the penultimate sugar is of physiological relevance (Reuter and Gabius 1996, 1999; Bhide and Colley 2017).

Using two lectins that interact with α2,3- or α2,6-sialylated *N*-glycans, i.e., *Maackia amurensis* agglutinin (MAA-I) and *Polyporus squamosus* lectin (PSL) (please see Table 2 for details), staining profiles were recorded in sections of medulla of kidney (Fig. 3g, h, k for MAA-I, Fig. 3i–k for PSL) and ureter (Fig. 3l, m, p for MAA-I, Fig. 3n, o, p for PSL). Of course, sections of an organ containing multiple cell populations are favorable for spotting differences in staining profiles. Direct comparison by two-color staining reveals impressively that distinct patterns of distribution of lectin binding can occur (Fig. 3k, p). Evidently, sialylated *N*-glycans are not just uniformly distributed in the chicken kidney, as also previously reported for mammalian kidney (Zuber et al. 2003). Thus, combined lectin application yields distinct patterns in the tissue depending on the nature of the linkage, a sign for functional significance, as Bhide and Colley explain in this issue. Obviously, it is therefore

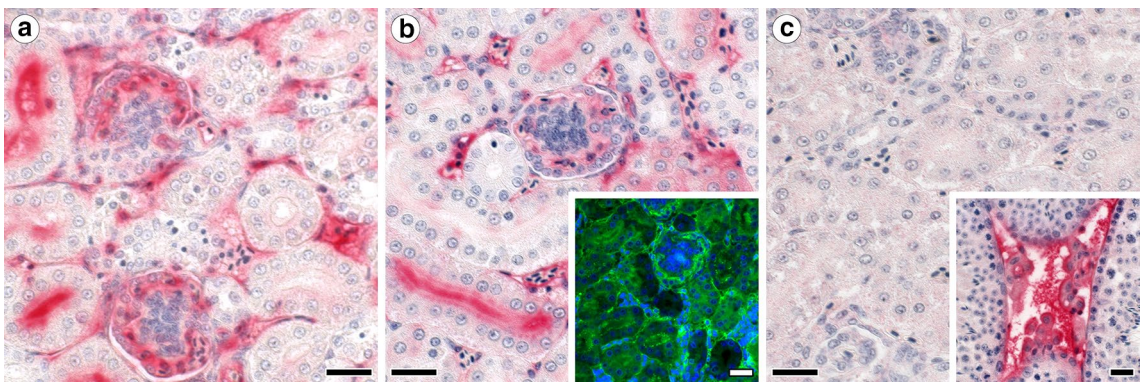


Fig. 4 Histochemical staining profiles of three labeled plant, fungal and human lectins reactive with α2,6-sialylated *N*-glycans. SNA binds to glomeruli, the apical part of epithelial cells of proximal tubuli, to connective tissue and blood vessels of chicken kidney cortex (**a**). A similar pattern is seen after applying biotinylated (**b**) or FITC-labeled PSL (*inset* of **b**) (please see Fig. 3i, j, n, o for PSL staining profiles in the medulla and ureter). CD22 led to a similar, albeit weak staining of glomeruli and epithelial cells of proximal tubuli (**c**), the strong reactivity to Leydig cells in mouse testis included as positive control (*inset* of **c**). Lectins were used at the following concentrations: SNA, 2 µg/ml (**a**); PSL, 0.06 µg/ml (**b**) or 5 µg/ml (*inset* of **b**); CD22 (fusion protein with F_c portion of human immunoglobulin G_1; kindly provided by R. Schwartz-Albiez, Heidelberg, Germany), 0.2 mg/ml (**c**, *inset* of **c**). *Scale bars* 20 µm

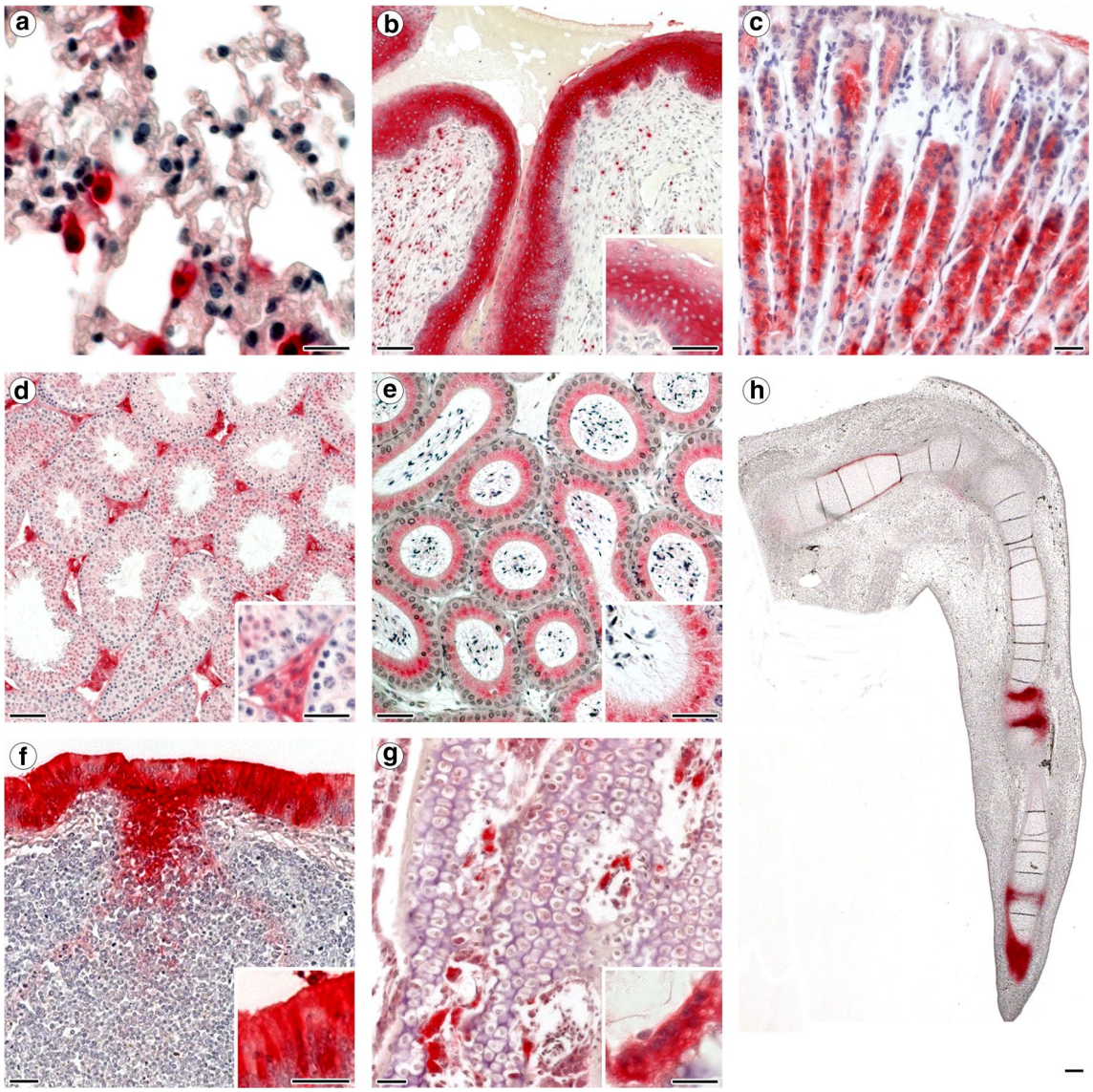


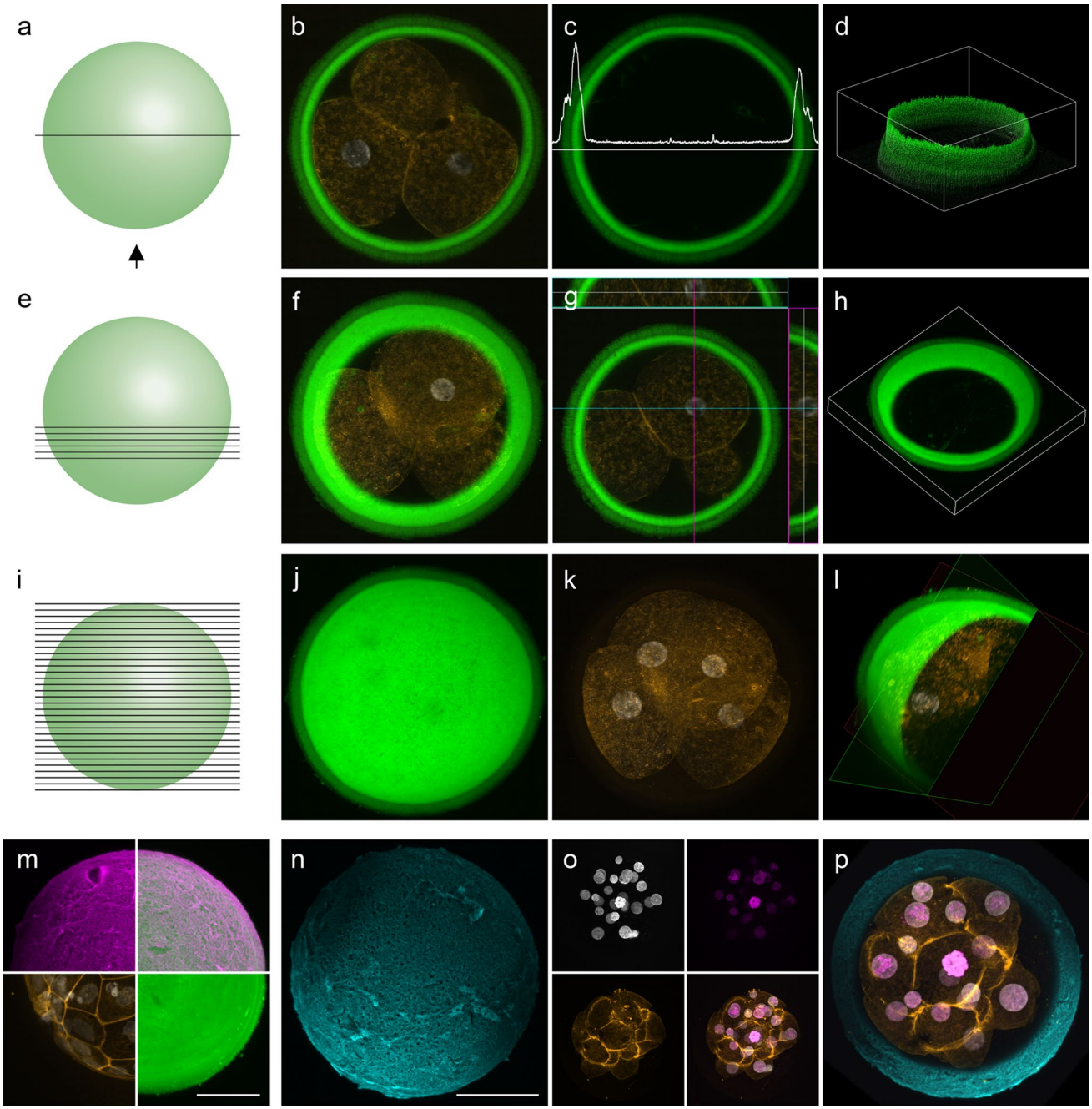
Fig. 5 Histochemical staining profiles of eight plant lectins to illustrate regional selectivity (for carbohydrate specificity, please see Table 2) in sections of murine and chicken tissues. **a** Staining by labeled ConA of alveolar macrophages located at the luminal surface of alveoli in a murine lung. **b** WGA reactivity of transitional epithelium (urothelium) and immune cells in the lamina propria mucosae of murine bladder, with strong signal of deeper layers of the epithelium revealed at increased level of magnification (*inset*). **c** GSA-II binding to surface mucous cells and mucous neck cells of gastric glands in the corpus. **d** Positivity of interstitial Leydig cells located in murine testis between tubuli seminiferi contorti after application of biotinylated VAA and signal generation. **e** Staining of the Golgi zone of principal cells in caput segments IV/V of murine epididymis by labeled Jacalin. **f** Strong signal intensity in the cytoplasm of follicle-associated and interfollicular epithelial cells in sections through follicles of chicken bursa of Fabricius after processing with labeled MAA-I. **g** Chondroclasts' reactivity for PHA-E in the calcification zone of a maturing femur of 14-day-old chicken. **h** PNA binding in regions of pre-chondrogenic condensations in a transversal section of a developing chicken hindlimb (7-day-old chicken embryo). Concentrations of lectins used were 4 µg/ml for MAA-I and JAC, 1 µg/ml for VAA, 0.5 µg/ml for PHA-E, PNA, ConA, and GSA-II, and 0.25 µg/ml for WGA. *Scale bars* 10 µm (**a**, *insets* to **b**, **d**–**g**), 20 µm (**c**, **e**, **g**), 50 µm (**b**, **d**, **f**) or 100 µm (**h**)

advisable to examine the information on the fine specificity of each lectin thoroughly to exclude incorrect conclusions. As listed in Table 2, three lectins bind to α2,6-sialylated glycans, but their individual reactivity profiles may not be identical.

The lectin from elderberry bark *Sambucus nigra* agglutinin-I (SNA-I) can also associate with sialylated T_n and clustered T_n antigens, two common forms of mucin-type *O*-glycosylation (Patsos and Corfield 2009; Ju et al. 2011; Corfield 2015), so that reactivity to these epitopes can contribute to the obtained staining (Taatjes et al. 1988; Toma et al. 2001). It is thus mandatory to run experimental series with lectins that share nominal specificity under the same conditions. Figure 4 demonstrates that glomeruli, the apical part of epithelial cells of proximal tubuli, connective tissue and blood vessels of chicken kidney are stained by biotinylated SNA (Fig. 4a), PSL (Fig. 4b; inset: fluorescent PSL used as probe) and CD22 [Fig. 4c; inset: positive control with murine testis showing positivity of Leydig cells as reported (Lohr et al. 2010)], albeit reaching different levels of signal intensity. The low-level staining with the human lectin may be due to *O*-acetylation of sialic acids, which impairs reactivity to CD22 (Kelm et al. 1994; Sjoberg et al. 1994; Brinkman-Van der Linden et al. 2002). This example, too, highlights the importance to determine binding properties of a lectin biochemically in great detail (for a compilation of methods to do so, please see Table 1 in Solís et al. 2015).

The principle that the cell/tissue distribution of lectin-binding sites is non-random although the ubiquitous nature of glycosylation is further emphasized in Fig. 5. In this figure, eight examples for prominent staining by a lectin at particular sites are put together as follows: alveolar macrophages in mouse lung stained by ConA (a), urothelium and immune cells in the lamina propria mucosae of mouse bladder by WGA (b), surface mucous cells and mucous neck cells in mouse stomach by GSA-II (c) also reactive with WGA in the cat (Knospe 1984), Leydig and endothelial cells of interstitial blood vessels by VAA (d; inset: cytoplasmic staining at increased level of magnification), the Golgi zone of principal cells of murine ductus epididymis by jacalin (e; inset at increased level of magnification), follicle-associated epithelium of bursa of Fabricius of a four-week-old chicken by MAA-I (f), chondroclasts of the calcification zone of the maturing femur of a 14-day-old chicken by phytohemagglutinin-E (PHA-E) (g; multinucleated chondroclast at increased level of magnification) and pre-chondrogenic condensations in a transversal section through an embryonic chicken hindlimb (at day 7) by peanut agglutinin (PNA) (h). In fact, this reactivity, signaling presence of the core 1 disaccharide of mucin-type *O*-glycans (Table 2), is characteristic of embryonic pre-cartilage blastemas (Zimmermann and Thies 1984; Aulthouse and Solursh 1987).

Moving from conventional light microscopy to confocal laser scanning microscopy, Fig. 6 gives an example how serial optical sections can be integrated to build a three-dimensional image. Presented are early bovine embryos, together with intensity profiles in the zona pellucida for staining with labeled WGA and SNA (Fig. 6). Lectin binding to the outer layers of the superficial filamentous network of the zona pellucida can be mapped in great detail and directly correlated with the developmental status of the embryo in three dimensions, and this even viewed from different angles (Fig. 6, bottom row; Online Resource 1). In order to obtain ultrastructural data, processing of semi- and ultrathin sections and electron microscopy are applied. The three microphotographs in Fig. 7 offer an insight into the distribution of sialic acid-containing glycoconjugates in rat colon. Gold particles used as label in



a two-step procedure with *Limax flavus* agglutinin (LFA) (Roth et al. 1984; please see also Table 2 for information on the carbohydrate specificity of this lectin) are densely present in mucous droplets. Combining the profiling of carbohydrate epitopes by lectin with the localization of glycosyltransferases that perform the enzymatic conjugation of

◂**Fig. 6** Three-dimensional (3D) glycophenotyping of early bovine embryos and the zona pellucida (ZP) with labeled plant lectins. **a–l** A 4-cell embryo (48 h post-insemination) surrounded by the ZP was stained with FITC-labeled WGA (*green*), DAPI (DNA, *white*) and TRITC-phalloidin (F-actin filaments, *orange*). One hundred and twenty-five serial optical sections (pixel size = 125 × 125 nm, z-step size = 1 µm) covering the ZP in its entirety (see scheme **i**) were recorded by confocal laser scanning microscopy. **a–d** Analysis of a single equatorial section of the embryo and the ZP. **a** Scheme for the perspective of the viewer and the position of the section. **b** WGA bound primarily to the main inner part and to a lesser extent to the outer margin of the compact layer of the ZP. **c** WGA staining and its fluorescence intensity profile along a cross-sectional line. **d** 3D plot of the WGA staining intensity in the equatorial plane given in (**c**). **e–h** 3D reconstruction and analysis of a spherical segment of the embryo and the ZP. **e** Scheme for the position of the sections. **f** Maximum intensity projection (MIP) of 20 confocal sections for three-color staining, as given in (**b**). **g** Midplane section and two virtual orthogonal sections cut along the *cyan* horizontal line and the *magenta* vertical line. **h** Oblique view of a 3D reconstruction of WGA binding in the ZP segment computed by MIP rendering. **i–l** 3D visualization of an entire embryo including the ZP. **i** Scheme for the position of the sections. **j**, **k** MIPs showing WGA staining of the ZP (**j**) and an overlay of staining of the cell nuclei and the F-actin cytoskeleton of the embryo (**k**). **l** Quarter-sphere of the embryo and the ZP visualized by MIP rendering. **m** MIPs showing quarter-spheres of an expanded blastocyst (day 8 post-insemination) stained with biotinylated SNA and Cy5-streptavidin (*magenta*), FITC-labeled WGA (*green*), DAPI (*white*) and phalloidin (*orange*). Clockwise from *top left* to *bottom left* are shown: SNA staining, an overlay of the SNA- and WGA-dependent staining patterns, WGA staining and an overlay of the cell nuclei and the F-actin cytoskeletons of the embryo. In contrast to WGA, SNA particularly delineated the outer layers of the superficial filamentous network (SFM) of the ZP. **n**, **o** Compacted morula (day 5 post-insemination) stained with biotinylated SNA and fluorescent streptavidin (*cyan*), DAPI (*white*), phalloidin (*orange*) and an antibody against the Ki-67 protein (*magenta*), which is an indicator for the proliferative stage of the embryonic cells. **n**, **o** MIPs encompassing the entire embryo and the ZP (see scheme **i**). **n** The texture of the SFM of the ZP as obtained by imaging with labeled SNA (see also **m**). **o** Individual MIPs of the cell nuclei (*top left*), Ki-67 antigen (*top right*) and F-actin cytoskeleton (*bottom left*) as well as the merged image (*bottom right*). **p** 3D view of the embryo inside the ZP computed by MIP rendering. Rotation and viewing from different angles are given in the animation (Online Resource 1). Labeled WGA and SNA were used at a concentration of 10 µg/ml. MIPs (**l**, **p**) were calculated with arivis Vision4D software (arivis AG, Unterschleißheim, Germany). *Scale bar* 50 µm

the lectin-reactive determinant to the glycan chain/protein, in this case sialyltransferases (for a review on sialylation of *N*-glycans, please see Bhide and Colley 2017), helped to shape the concept of functional subdivision of the Golgi apparatus-based glycosylation machinery (Roth et al. 1984, 1994). These methods, too, were invaluable to visualize the steps of glycan processing in the endoplasmatic reticulum associated with quality control of glycoprotein folding (Roth 1996; Zuber and Roth 2009; Roth and Zuber 2017). Alternatively, labeling can be performed in a one-step protocol with lectins adsorbed to particles of colloidal gold granules. These probes are readily prepared at the diameter of choice by reducing a solution of $HAuCl_4$ (1% in distilled water) with reagents such as white phosphor in ethyl ether, sodium citrate or tannic acid (Roth 1983a, b, 2011).

Letting lectin and glycan switch roles, that is using a (neo)glycoprotein as probe, sites with specificity for binding glycans can be detected in cells and tissues (Straus 1981; Gabius et al. 1988, 1993; Kayser et al. 1994) to proceed to test endogenous lectins in histochemistry. Such an application is illustrated for visualizing lectin reactivity in the glycocalyx. This term refers to the extracellular presence of glycans, the "sugary coating." Translated into Greek, the "sweet husk" became the glycocalyx. "Thus, the cell wall, the zona pellucida, the antennulae microvillares, the basement membrane and the type-specific-red-cell antigenic polysaccharide would all be special examples of a more general extracellular structure common to very many types of cells—the glycocalyx" (Bennett 1963).

The mentioned zona pellucida is thus a suited test case, as already illustrated in Fig. 6, and a panel of labeled human lectins, i.e., adhesion/growth-regulatory galectins (for details, please see Kaltner et al. 2017), has been applied for signal generation (Habermann et al. 2011). Figure 8 presents examples of staining profiles for different members of this lectin family in the zona pellucida and bovine germinal vesicle oocytes. Incubation with structurally closely related galectins results in the

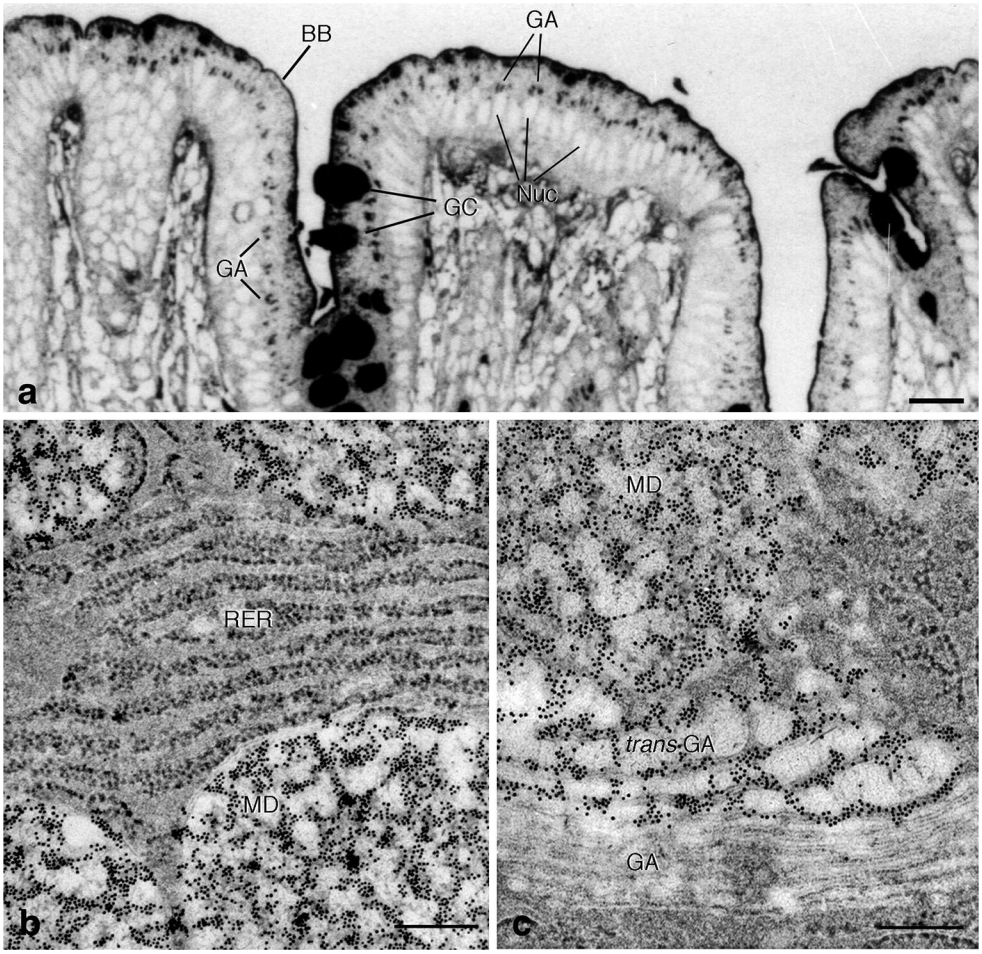


Fig. 7 Post-embedding light and electron microscopic lectin-gold labeling performed on semithin (0.5 µm; Lucocq and Roth 1984) and ultrathin (80 nm) (Roth 1983a) sections prepared from the same block of formaldehyde-/glutaraldehyde-immersion fixed and low-temperature Lowicryl K4 M-embedded rat colon. Both types of sections were incubated with the sialic acid-specific *Limax flavus* lectin (Roth et al. 1984) followed by fetuin–gold complex (8-nm gold particles, diluted to give an $OD_{525\ nm}$ of 0.35) and silver amplification (semithin section only; Taatjes et al. 1987). **a** In the semithin section, the mucus of goblet cells (GC) and the brush border (BB), as well as Golgi apparatus (GA) of columnar epithelia, is intensely stained in black due to silver intensification (**b**, **c**). In ultrathin sections, gold particle label can be seen over mucus droplets (MD) and *trans* cisternae of the Golgi apparatus (GA). No label is present over rough endoplasmic reticulum (RER) as well as *cis* and middle cisternae of the Golgi apparatus. Nuc: nuclei of columnar epithelia. Figure by courtesy of D. J. Taatjes and J. Roth; with permission. *Scale bar* 10 µm (**a**), 0.1 µm (**b**, **c**)

visualization of distinct textures of peripheral zona pellucida layers (Fig. 8i–p). This application, as also done with human CD22 (siglec-2) (Fig. 4; please see also Table 2), signifies reactivity of tissue glycoconjugates to endogenous lectins, a prerequisite for a functional pairing in situ. As is the case for the technique of lectin

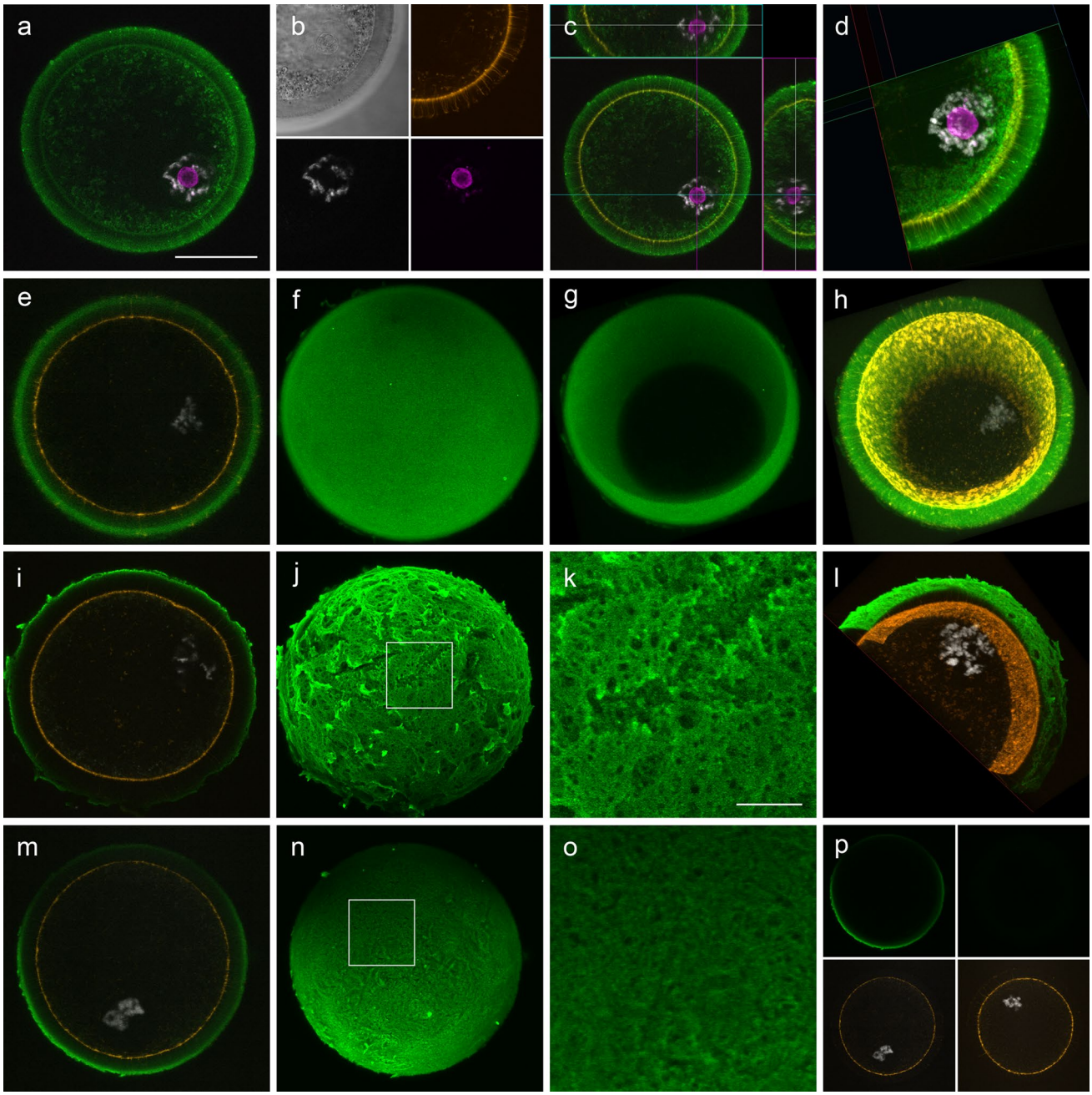
histochemistry moving from plant to endogenous proteins, the door to study lectin functionality was opened by work with a plant lectin.

In the historical context, cell-type specificity of agglutination of cells had led to the discovery that a lectin can trigger post-binding reactions (Table 1). Phytohemagglutinin from red kidney beans (*Phaseolus vulgaris*; PHA, a mixture of PHA-E and PHA-L, please see Table 2) "was originally employed for its erythrocyte-agglutinating ability in obtaining leukocytes from whole blood" and turned out to have "the ability to initiate mitosis among these leukocytes" (Nowell 1960). This finding was the starting point to identify a broad panel of lectins acting as mitogens (Borrebaeck and Carlsson 1989). Lectin binding to the cell surface is evidently a means to alter cell behavior depending on the actual counterreceptor(s). Thus, bridging cells in aggregations assays and eliciting signaling are both aspects of lectin functionality. Specificity to glycans and topological features of their presentation cooperate to ensure that the recognition process brings the suited binding partners (glycoproteins, glycolipids and proteoglycans; please see Buddecke 2009; Corfield 2017; Kopitz 2017 for informations on glycoconjugates) in the physiological context together (Gabius et al. 2015, 2016). Because synthetic chemistry delivers glycoclusters with diverse architecture of presenting the sugar headgroup (Roy et al. 2016), measuring staining intensity and profile as a function of glycocluster design is a way to collect information on the importance of spatial factors for lectin binding in the tissue context (André et al. 2016; Roy et al. 2017). What can happen after binding is summarized in the next section.

Lectins: functions

The aim to learn more about the enormous toxicity of seeds of *Ricinus communis* led to the pioneering work on ricin at the Pharmacological Institute in Dorpat (Tartu/Estonia) by Stillmark (1888) as summarized in his thesis. This protein, an AB toxin with 28S rRNA *N*-glycosidase activity (Endo 1989), was the cause of unintended and intended intoxications (Pita 2009; Worbs et al. 2011). The rather broad reactivity of its B-subunit to β-galactosides guarantees that ricin's lectin part can deliver the toxic A-chain to cells, thereby protecting the plant from herbivorous animals (Barbieri et al. 1993; Hartley and Lord 2004). This activity and further functions of plant lectins are summarized in Table 4. Compiling relevant information for animal and human lectins leads to Table 5 (please see also Higuero et al. 2017; Kaltner et al. 2017; Mayer et al. 2017 for information on human lectins).

In both cases, it should be noted that the quaternary structure and the modular design of lectins are key features for bioactivity. Bringing lectin domains into spatial vicinity, by non-covalent association or the tandem-repeat modus, will increase avidity to bind certain targets. Also, different contact sites in a lectin can cooperate to make a function possible, as sugar and protein binding reactions do for slime mold discoidin I in ordered cell migration (Gabius et al. 1985) or for leguminous lectins in organizing routing and packaging of storage proteins (Einhoff et al. 1986; Schecher and Rüdiger 1994) (please see also Table 1). Intriguingly, carbohydrate recognition domains even have the versatility to acquire new specificities. Bindings of peptide motifs to C-type lectin-like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◂**Fig. 8** 3D glycophenotyping of bovine germinal vesicle (GV) oocytes and the ZP with labeled galectins. The oocytes surrounded by their ZP were stained with biotinylated galectins using FITC-labeled streptavidin as second-step reagent. The chromosomes and the F-actin cytoskeleton were labeled with DAPI (*white*) and TRITC-phalloidin (*orange*), respectively, as counterstain (for schematic illustration of confocal optical sectioning, please see Fig. 6). **a–d** Proto-type galectin-1 bound to granulo-reticular structures in the oocyte periphery, also delineating the GV membrane and the oolemma. Additionally, this lectin diffusely stained the compact layer (CL) of the ZP with increasing intensity toward the outer margin of the CL. **a**, **b** Single optical section through the center of the GV. **a** Overlay showing galectin-1 binding (*green*), DAPI-labeled chromosomes (*white*) and the nucleolus, which was intensely stained by an antibody against the Ki-67 antigen (*magenta*). **b** Individual channel images of the sector containing the GV: transmission scan image (*top left*), F-actin (*top right*), DAPI-labeled chromosomes (*bottom left*) and Ki-67 protein (*bottom right*). **c** Midplane section and two virtual orthogonal sections cut along the *cyan* horizontal line and the *magenta* vertical line. **d** Oblique view of a 3D reconstruction of a quarter segment of the oocyte with the GV computed by MIP rendering. **e–h** The phosphorylated form of galectin-3 (pGal-3) bound in a diffuse pattern to the CL of the ZP with the highest signal intensities in the outer margin of the CL. **e** Single optical section through the GV. **f** MIP of the pGal-3-binding pattern in the ZP. **g**, **h** MIP renderings of a spherical section visualizing pGal-3-dependent staining of the ZP alone (**g**) and together with the F-actin filament network of the oocyte cortex and the chromosomes in the GV (**h**). **i–l** Tandem-repeat-type galectin-8 distinctly delineated the outer coarse layers of the superficial filamentous network (SFM) of the ZP. An equatorial section (**i**) and a MIP revealing the intricate structure of the galectin-8-labeled outer SFM (**j**). **k** Magnified image of the boxed area in **j**. **l** Oblique view of a 3D reconstruction of a hemisphere segment of the oocyte with the GV. **m–p** Compared to galectin-8, another member of the group of tandem-repeat-type galectins, i.e., galectin-9, stained the fine-structured inner layers of the SFM of the ZP. **m** Equatorial section showing the selective binding of this galectin to the ZP periphery. **n** MIP displaying the texture of the peripheral ZP layers, as visualized by binding of galectin-9. **o** Magnified image of the boxed area in **n**. **p** Equatorial sections demonstrating that binding of galectin-9 to the SFM of the ZP (*top left*) can be inhibited by pre- and co-incubation of the lectin-containing solution with a mixture of the glycoprotein asialofetuin (1 mg/ml) and 100 mM lactose (*top right*). Respective counterstain images (DAPI and phalloidin) are presented *below*. The galectins were used at a concentration of 20 µg/ml. MIPs (**d**, **g**, **h**, **l**) were computed with arivis Vision4D software (arivis AG, Unterschleißheim, Germany). *Scale bars* 50 µm (overview images) or 10 µm (enlarged area)

domains or to galectins are instructive cases for the structural plasticity of a protein fold by diversification that can involve positions of the signature sequence (Gready and Zelensky 2009; Kaltner and Gabius 2012; Nagae and Yamaguchi 2015; García Caballero et al. 2016a, b; Mayer et al. 2017).

Hereby, the range of functions is extended so that a lectin can be active extracellularly and within the cell by a molecular rendezvous with the suited ligand at each site (for further information on intracellular presence of tissue lectins, please see Kaltner et al. 2017). Since the glycan display is dynamically regulated by neosynthesis and enzymatic remodeling, reactivity to a lectin can be modulated, even switched off or on in a spatiotemporally distinct manner (for examples on galectin reactivity and ensuing consequences for cell growth regulation, please see Kaltner et al. 2017).

Conclusions

The development of reactivity to glycans in diverse protein folds is compelling evidence for the physiological importance of this type of recognition. Used as tool, lectins have been instrumental to delineate sites of glycosylation. In general, they enable to map distinct aspects of the glycome. The aim to add new probes for certain biorelevant structures warrants further systematic investigations of extracts (for a recent example on a fungal lectin-binding core-fucosylated *N*-glycans, please see Inamdar et al. 2016) and also engineering of already known proteins, to rationally alter lectin properties toward an objective (Swanson et al. 2015). Of note, natural polymorphisms also give rise to variants of lectins. Their analysis can be conducive for laboratory applications and can provide insights into structure–activity relationships and functionality of lectins in situ (Feinberg et al. 2013; Ruiz et al. 2014; Zhang et al. 2015a). In addition, the work with peptides derived from lectins (Moise et al. 2011) and chemical design of synthetic sugar receptors (Solís et al. 2015) have potential to deliver custom-made probes for particular purposes including glycan detection in fluids. The ease to obtain recombinant proteins has paved the way from working with seed agglutinins (with quantities of more than 1 g per 100 g starting material) to adding mammalian lectins to the panel of probes for glycan profiling and functional assays. That clues will increasingly emerge on how distinct aspects of the glycome lead to cellular activities via lectin binding, a central aspect of the concept of the sugar code (Gabius 2009a), can thus be confidently expected.

Table 4 Functions of plant lectins

Activity	Example of lectin
External activities	
Protection from fungal attack	*Hevea brasiliensis* (rubber tree), *Urtica dioica* (stinging nettle), *Solanum tuberosum* (potato)
Protection from herbivorous animals	*Phaseolus vulgaris* (French bean), *Ricinus communis* (castor bean), *Galanthus nivalis* (snowdrop), *Triticum vulgare* (wheat)
Association of hydrolytic enzyme activity with lectin-like domain	Strawberry β-galactosidase, tomato endo-β1,4-glucanase (cellulase) SICel9C1
Involvement in establishing the symbiosis between plants and bacteria	*Pisum sativum* (common pea), *Lotononis bainesii* (miles lotononis), *Arachis hypogaea* (peanut), *Triticum vulgare* (wheat), *Oryza sativa* (rice)
Biosignaling by a receptor kinase with an extracellular lectin-like domain	LysM domain in Nod-factor perception (*Lotus japonicus*, *Medicago truncatula*) and leguminous lectin-like domain (*Arabidopsis thaliana*, *Populus nigra* var. *italica*)
Internal activities	
Storage proteins	Valid for all seed lectins
Ordered deposition of storage proteins and enzymes in protein bodies and mediation of contact between storage proteins and protein body membranes	*Pisum sativum* (common pea), *Lens culinaris* (lentil), *Glycine max* (soybean), *Oryza sativa* (rice)
Modulation of enzymatic activities such as phosphatases	*Secale cereale* (rye), *Solanum tuberosum* (potato), *Pleurotus ostreatus* (oyster mushroom), *Glycine max* (soybean), *Dolichos biflorus* (horse gram)
Participation in growth regulation	*Medicago sativa* (alfalfa), *Cicer arietinum* (chick pea)
Adjustment to altered environment conditions	*Triticum aestivum* (winter wheat)

For further information on carbohydrate specificities of selected lectins, please see Table 2; from Rüdiger and Gabius (2009b), with permission

Table 5 Functions of animal and human lectins

Activity	Example of lectin
Recognition of stem region of *N*-glycans, a signal for ubiquitin conjugation when accessible in incorrectly folded glycoproteins	F-box proteins Fbs1 and Fbs2, which comprise the ligand-specific part of SCF[a] ubiquitin ligase complexes
Molecular chaperones with dual specificity for $Glc_2/Glc_1Man_9GlcNAc_2$ and protein part of nascent glycoproteins in the ER	Malectin/ribophorin I complex, calnexin, calreticulin
Targeting of misfolded glycoproteins with $Man_{8\text{-}5}GlcNAc_2$ as carbohydrate ligand to ER-associated degradation (ERAD)	EDEM1,2[b]/Mnl1 (Htm1) (α1,2-mannosidases; in mammals active as lectins), Yos9p (MRH[c] domain) in yeast, erlectin (XTP3-B[d]) and OS-9[e] in mammals
Intracellular routing of glycoproteins and vesicles and apical delivery	Comitin, ERGIC53[f] and VIP36[g] (probably also ERGL[h] and VIPL[i]), galectins-3, galectins-4 and galectins-9, P-type lectins
Intracellular transport and extracellular assembly	Non-integrin 67-kDa elastin/laminin-binding protein
Enamel formation and biomineralization	Amelogenin
Inducer of membrane superimposition and zippering (formation of Birbeck granules)	Langerin (CD207)
Cell-type-specific endocytosis	Cysteine-rich domain (β-trefoil) of the dimeric form of mannose receptor for Gal*N*Ac-4-SO_4-bearing glycoprotein hormones in hepatic endothelial cells, dendritic cell and macrophage C-type lectins (mannose receptor family members (tandem-repeat type) and single-CRD[j] lectins such as trimeric langerin/CD207 or tetrameric DC-SIGN[k]/CD209), hepatic and macrophage asialoglycoprotein receptors, HARE[l], P-type lectins
Recognition of foreign glycans (β1,3-glucans, β1,2-mannosides, cell wall peptidoglycan, LOS[m] and LPS[n]), mycobacterial glycolipid or host-like epitopes	CR3[o] (CD11b/CD18, Mac-1 antigen), C-type lectins such as collectins, DC-SIGN, dectin-1, Mincle and RegIIIγ (murine)[p] or HIP/PAP (human), ficolins, galectins, immulectins, intelectins, *Limulus* coagulation factors C and G, siglecs, tachylectins

Table 5 continued

Activity	Example of lectin
Recognition of foreign or aberrant glycosignatures on cells (including endocytosis or initiation of opsonization or complement activation) and of apoptotic/necrotic cells (glycans or peptide motifs)	Collectins, C-type macrophage and dendritic cell lectins, CR3 (CD11b/CD18, Mac-1 antigen), α/Θ defensins, ficolins, galectins, pentraxins (CRP, limulin), RegIIIγ (HIP/PAP), siglecs, tachylectins
Targeting of enzymatic activity in multimodular proteins	Acrosin, *Limulus* coagulation factor C, laforin, β-trefoil fold ($(QxW)_3$ domain) of Gal*N*Ac-Ts[q] involved in mucin-type *O*-glycosylation—frequent in microbial glycosyl hydrolases for plant cell wall polysaccharides termed carbohydrate-binding modules
Bridging of molecules	Cerebellar soluble lectin, cytokines (e.g., IL-2[r]–IL-2R and CD3 of TCR), galectins
Induction or suppression of effector release (H_2O_2, cytokines, etc.)	Chitinase-like YKL-40, galectins, I-type lectins (e.g., CD33 (siglec-3), siglec-7 and siglec-9), selectins and other C-type lectins such as CD23, BDCA2 and dectin-1, Toll-like receptor 4
Modulation of enzymatic activities in modular proteins/receptor endocytosis via lattice formation	Mannan-binding lectin (acting on meprins); galectins
Cell growth control, induction of apoptosis/anoikis and axonal regeneration, anti- and pro-inflammatory regulation with/without modulating gene expression	Amphoterin and other heparin-binding proteins, cerebellar soluble lectin, chitinase-like lectins, C-type lectins, galectins, hyaluronic acid-binding proteins, siglecs (e.g., CD22 and CD33)
Cell migration and routing	Galectins, hyaluronic acid-binding proteins (CD44, hyalectans/lecticans, RHAMM[s]), I-type lectins, selectins and other C-type lectins
Cell–cell interactions	Galectins, gliolectin, I-type lectins (e.g., siglecs, N-CAM[t], P_0 or L1), selectins and other C-type lectins such as DC-SIGN or macrophage mannose receptor
Cell–matrix interactions	Calreticulin, discoidin I, galectins, heparin- and hyaluronic acid-binding lectins including hyalectans/lecticans
Matrix network assembly	Galectins (e.g., galectin-3/hensin), non-integrin 67 kDa elastin/laminin-binding protein, proteoglycan core proteins (C-type CRD and G1 domain of hyalectans/lecticans)

Adapted from Gabius (2009b) and Solís et al. (2015), extended and modified

[a] Skp-1-Cul1-F-box protein complex

[b] ER degradation enhancing α-mannosidase-like protein

[c] Mannose-6-phosphate receptor homology

[d] XTP3-transactivated gene B precursor

[e] Osteosarcoma 9

[f] ER-Golgi intermediate compartment protein (lectin) (MW: 53 kDa)

[g] Vesicular-integral (membrane) protein (lectin) (MW: 36 kDa)

[h] ERGIC-53-like protein

[i] VIP-36-like protein

[j] Carbohydrate recognition domain

[k] Dendritic cell-specific ICAM-3-grabbing non-integrin

[l] Hyaluronan receptor for endocytosis

[m] Lipooligosaccharide

[n] Lipopolysaccharide

[o] Complement receptor type 3

[p] Member of regenerating (reg) gene family of secreted proteins

[q] UDP-Gal*N*Ac: polypeptide *N*-acetylgalactosaminyltransferases

[r] Interleukin-2

[s] Receptor for hyaluronan-mediated motility

[t] Neural cell adhesion molecule

For further details on lectin families, please see Gabius 1997, 2002; Kilpatrick 2000; Bhide and Colley 2017; Kaltner et al. 2017; Mayer et al. 2017; Roth and Zuber 2017

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