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Review

A toolbox of lectins for translating the sugar code: the galectin network in phylogenesis and tumors

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Summary. Lectin histochemistry has revealed cell-type-selective glycosylation. It is under dynamic and spatially controlled regulation. Since their chemical properties allow carbohydrates to reach unsurpassed structural diversity in oligomers, they are ideal for high density information coding. Consequently, the concept of the sugar code assigns a functional dimension to the glycans of cellular glycoconjugates. Indeed, multifarious cell processes depend on specific recognition of glycans by their receptors (lectins), which translate the sugar-encoded information into effects. Duplication of ancestral genes and the following divergence of sequences account for the evolutionary dynamics in lectin families. Differences in gene number can even appear among closely related species. The adhesion/growth-regulatory galectins are selected as an instructive example to trace the phylogenetic diversification in several animals, most of them popular models in developmental and tumor biology. Chicken galectins are identified as a low-level-complexity set, thus singled out for further detailed analysis. The various operative means for establishing protein diversity among the chicken galectins are delineated, and individual characteristics in expression profiles discerned. To apply this galectin-fingerprinting approach in histopathology has potential for refining differential diagnosis and for obtaining prognostic assessments. On the grounds of *in vitro* work with tumor cells a strategically orchestrated co-regulation of galectin expression with presentation of cognate glycans is detected. This coordination epitomizes the far-reaching physiological significance of sugar coding.

Key words: Adhesion, Galectin, Glycosylation, Phylogenesis, Prognosis, Tumor Suppressor

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Background

The well-known paradigm for the flow of genetic information, shown in Figure 1 (top panel), implies that the study of proteins is sufficient to understand the molecular basis of cellular activities. This notion is supported by the apparent complexity of regulatory events that act on protein expression by mRNA and product processing. In fact, the generation of more than one product from a single gene by alternative splicing and proteolytic processing exemplifies the means to increase genomically encoded structural diversity, and there is even more: distinct amino acids serve as acceptors for a wide panel of co- and posttranslational protein modifications. Textbook knowledge on the impacts of phosphorylation tells us that it can profoundly affect protein activity and structure (up to the level of oligomerization) as well as introduce new sites for intermolecular contacts in the kinase substrates. In other words, genomic information, by developing mechanisms to attach site-specific substitutions into side chains of certain amino acids, leads to installing biochemical signals. They facilitate novel routes of biochemical communication. This processing of proteins is not covered by the illustration given in the top panel of Figure 1. The growing awareness of the role of substitutions deserves to be accounted for by a proper amendment of this figure.

In terms of structural complexity and frequency of occurrence the covalent attachment of carbohydrates (glycosylation) stands out from the crowd of protein modifications. In detail, at least eight amino acids are acceptors for glycans and 41 types of bond for the sugar-protein linkage are known (Spiro, 1973, 2002; Montreuil, 1975; Reuter and Gabius, 1999; Patsos and Corfield, 2009; Zuber and Roth, 2009). That the N-glycosylation pathways present in all three kingdoms "share a great deal in common", as recently emphasized (Larkin and Imperiali, 2011), attests to the fundamental

nature of principles of glycosylation. The size of glycans ranges from just one sugar residue in O-GlcNAcylation of serine/threonine or N/C-mannosylation of tryptophan to the long glycosaminoglycan chains of proteoglycans or multi-antennary complexes with varying branch lengths, characteristic especially of complex-type N-glycosylation (Buddecke, 2009; Wilson et al., 2009; Schachter, 2010; Ihara and Ikezaki, 2011). In biomedical terms the diversification of glycan structures in phylogenesis and their emerging association to diseases (Hennet, 2009; Honke and Taniguchi, 2009) give ample reason to ascribe functional relevance to the glycan part of proteins.

That considerably more than 1% of the coding genome is devoted to glycogenes, e.g. enzymes involved in glycan assembly and processing, strengthens this functional implication, and these enzymes are even known to work together in intimately coordinated and regulated manners (Roth, 1996; Brockhausen and Schachter, 1997; Pavelka, 1997; Nilsson et al., 2009; Zuber and Roth, 2009; Tu and Banfield, 2010; Maccioni et al., 2011). Their exquisite cooperation invokes the analogy with an assembly line. Together with products from dynamic remodelling as a further source for diversity, the list of known glycan structures has already surpassed the amazing number of 20 000 entries, and still counting (Cummings, 2009; Parker and Kohler, 2010). That sphingolipids also serve as carriers of glycans, on the cell surface as well as intracellularly, underlines the functionally oriented reasoning (Kopitz, 2009; Ledeen and Wu, 2009, 2011). Viewed together, three lines of evidence guide to the required amendment of the scheme depicting the flow of genetic information (Fig. 1, bottom). Including glycosylation, though, means much more than just listing one example for a modification. Like nucleotides and amino acids, carbohydrates qualify for information coding, as the third alphabet of life (Gabius et al., 2011). Often not yet fully realized, the glycans are unsurpassed among biomolecules in their capacity for coding information in oligomers, the basis for the concept of the sugar code (Gabius, 2009). This exceptional talent becomes apparent when looking at the special chemical features of the monomers (letters) from this angle.

Carbohydrates: chemical basis of the sugar code

A common feature of biopolymers (code words) is the occurrence of the same type of linkage between different building blocks (letters). Diversity (vocabulary of code words) is generated by sequence permutation. What the phosphodiester or peptide bonds are for nucleic acids or proteins is the glycosidic linkage for glycans. While its chemistry remains the same, an extent of structural variability not seen in nucleic acids and proteins is attained. The underlying reason is that carbohydrates offer a total of four additional parameters, when compared to the other two mentioned code systems, to achieve the noted high degree of structural complexity. Each of the four parameters has its share in increasing the coding capacity: a.) anomeric status (α or ß) of the glycosidic linkage, b.) positions of the glycosidic linkage by variation of involvement of hydroxyl groups (known linkage types, for instance, for adding L-fucose to a glycan in α-position are 1,2; 1,3; 1,4 or 1,6; Becker and Lowe, 2003; Ma et al., 2006; Zuber and Roth, 2009; Moriwaki and Miyoshi, 2010), c.) size of the ring (pyranose or furanose) and d.) branching (a single pyranose such as mannose can be connected to four sugar moieties, e.g. in a bisected N-glycan).

Similar to the acceptor properties of certain amino acids in proteins (e.g. serine/threonine or tyrosine for phosphorylation) substitutions such as acetylation, phosphorylation or sulfation can then be enzymatically introduced to the glycan at specific sites. The various types of modification equip glycans to access a further

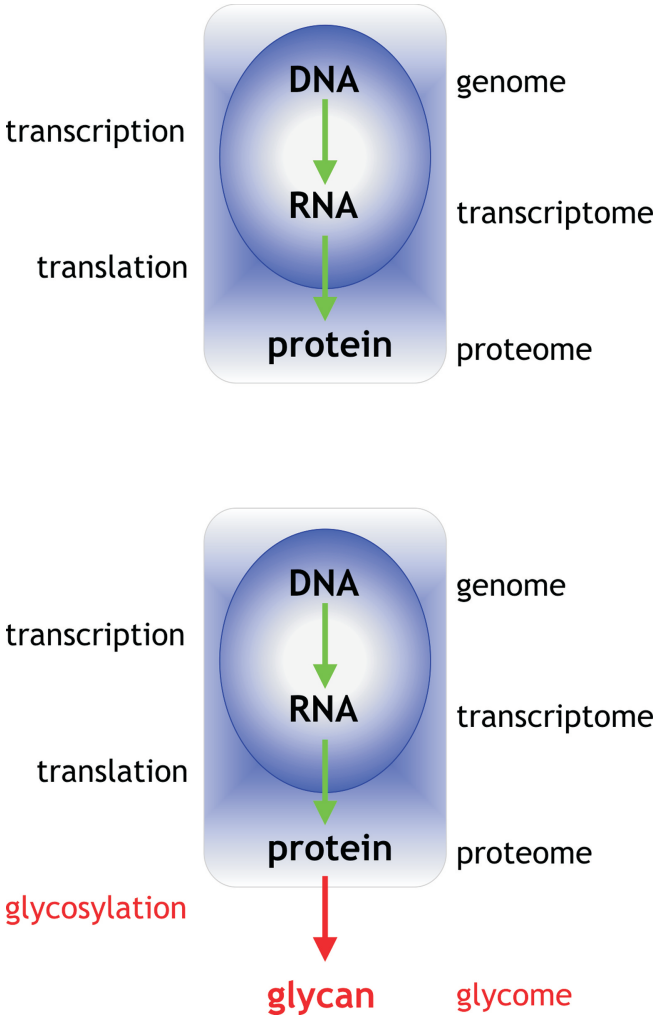


Fig. 1. Schematic illustration of the change in our view on the flow of genetic information. Historically, it was restricted to proteins as direct products (top). With evidence for glycans serving in information coding accumulating, the paradigm has been revised accordingly (bottom).

level of structural sophistication, especially known from glycosaminoglycans (Habuchi et al., 1998; Buddecke, 2009). Here, N- and 3/6-O-sulfation of D-glucosamine and 2-O-sulfation/epimerization to L-iduronic acid of D-glucuronic acid are possible for the repeating disaccharide unit in heparan sulfate. Totals of 32 (2^5) different disaccharides and 32^4=1 048 576 different octasaccharides are theoretically feasible, if the combinatorial potential is fully exploited. On the level of the involved enzyme, a single mode for sulfation such as the 6-O-modification in N-acetylglucosamine is assigned to more than one enzyme. The presence of isoenzymes not only guarantees development of fine-specificities for substrate selection. Of importance beyond this particular case, the presence of four genes in mice is a prerequisite for developing distinct organ expression profiles (Kawashima, 2010). Besides fine-tuning enzyme production the synthesis of a glycotope can further be modulated by other mechanisms, e.g. formation of enzyme complexes in assembly lines responsible for synthesis of glycosaminoglycans or the sulfated HNK-1 trisaccharide (Buddecke, 2009; Kizuka and Oka, 2010). In aggregate, the given structural features of the carbohydrates, together with the enzymatic machinery for oligomer synthesis and subsequent modification, establish the first and second dimensions of the sugar code (for third dimension, please see below).

Obviously, the use of all given possibilities at hand on the way to turn the letters of the sugar alphabet into natural glycans yields "a potential of information content several orders of magnitude higher in a short sequence than any other biological oligomer", a statement which is backed by detailed calculations (Laine, 1997). It thus appears driven by necessity that a large repertoire of signals on the cell surface is written with carbohydrate letters. The high-density coding capacity yet poses an enormous challenge for the aim of analytical chemistry to completely and unequivocally determine all structural parameters of a glycan. It is being mastered by the strategic combination of different methods, prominently by mass spectrometry and comparison of signal profiles to data bank entries (Lee, 2009; Nakagawa, 2009; Yagi and Kato, 2009; Higgins, 2010; Mariño et al., 2010). That these efforts took more than two decades to come to fruition explains why analytical work on sugar sequences tended to lag considerably behind respective work on proteins and nucleic acids. In the words of S. Roseman (2001) it should be kept in mind that "glycoconjugates are much more complex, variegated, and difficult to study than proteins or nucleic acids." Inevitably, the presence of protein glycosylation was therefore first assumed to exert passive effects on protein features (Spiro, 1973; Sharon and Lis, 1997; Solá et al., 2007; Skropeta, 2009).

The interpretation of the glycan presence shifted from being viewed as a rather inert appendage to considering active roles, and these depending on structural details. Three lines of evidence converged to shape the new concept: a.) the realization that carbohydrates are "ideal for generating compact units with explicit informational properties" (Winterburn and Phelps, 1972), b.) the growing number of studies which tied defects in glycan assembly to diseases in animal models or at the clinical level (Hennet, 2009; Honke and Taniguchi, 2009) and c.) the detection of dynamic non-random changes in glycan display measured cyto- and histochemically, e.g. for different cell types and in the course of their differentiation or as response to genetic or microenvironmental factors (Schrével et al., 1981; Spicer and Schulte, 1992; Danguy et al., 1994; Madrid et al., 1997; André et al., 2007a; Patsos et al., 2009; Lohr et al., 2010; Roth, 2011; van de Wouwer et al., 2011). With a focus on N-glycans, their first job starts already right after cotranslational transfer of the oligosaccharide from the dolichol pyrophosphate donor to the acceptor asparagine residues in the nascent protein. It is to assist molecular chaperones (calnexin, calreticulin) in quality control in the endoplasmic reticulum (Roth et al., 2008; Aebi et al., 2010). The stepwise glycan processing then uncovers routing signals for lectin-mediated transport to the Golgi stacks, an instructive lesson on structure-function correlations within the sugar code. Along this route, already a single moiety as signal can make a significant difference. The same applies to N-glycan core substitutions. They can be added later in the Golgi such as the bisecting N-acetylglucosamine moiety, which was noted above for being emblematic for the branching capacity of sugars. Their presence has looked like an accidental addition for a long time. Instead, these monosaccharide additions have turned out to be molecular switches for the conformation (shape) of the entire glycan. The shape changes are relevant for *cis/trans*-interactions, e.g. exerting a sizeable impact on the rate of hepatic clearance of the respective glycoprotein from serum, with relevance for rationally engineering glycosylated pharmaproteins for prolonged circulation (André et al., 2007b, 2009). A single moiety is thus capable of producing spatial long-range effects, making it likely that filling such positions is under tight control. Indeed, application of array technology for transcriptional mapping of glycogenes has verified this assumption. Distinct transcriptional patterns reflecting orchestrated gene activities definitely argue against an idea of random glycan synthesis (André et al., 2007a; Julien et al., 2007; Nairn et al., 2008; Willhauck-Fleckenstein et al., 2010). Of course, the introduction of the core substitutions will also decide on the route of further assembly of the glycan chain. Once turned into a bisected N-glycan, certain reactions in glycan maturation (α-mannosidase-II, N-acetyl-glucosaminyl-transferases-II, -IV and -V) are precluded, thereby connecting glycogene expression with product generation (Brockhausen and Schachter, 1997). A facile detection of distinct glyco-epitopes, for example the bisecting N-acetylglucosamine moiety, is possible with carbohydrate-specific tools (lectins or antibodies), in this case the plant lectin PHA-E (Cummings and Kornfeld, 1982). The experimental ease to detect changes in the

glycophenotype (glycome) by lectin histochemistry has inspired the following notion: endogenous lectins may act as functional sensors for presence of certain glycan epitopes (structure and topological presentation) within the glycome.

The idea of *in situ* protein-glycan recognition can be turned into a synthetic probe to track down tissue lectins. The resulting neoglycoconjugates (a chemically glycosylated scaffold for multivalent presentation of bioactive carbohydrate epitopes; Stowell and Lee, 1980; Lee and Lee, 1997; Chabre and Roy, 2009) rendered mapping the presence of such glycan-binding activities possible, by light and electron microscopy (Gabius et al., 1988, 1993; Kuchler et al., 1992; Danguy et al., 1994, 1995). Already mono- or disaccharides are sufficient to obtain signals in cell preparations or tissue sections and increasing the structural complexity of the sugar part enhances the selectivity of neoglycoconjugate binding. Thus, the carbohydrate-specific binding seen in cyto- and histochemistry is attributed to the presence of endogenous sugar receptors. They are the physiological tools to translate sugar-encoded messages into cellular activity. Taken to the structural level of the detected sites, the phylogenetic emergence of at least 14 different folds in animal proteins with the ability to bind glycans appears to reflect the far-reaching significance of this route of molecular information transfer (Gabius et al., 2011). The capacity of proteins to interact with glycans has thus developed independently fairly often. Complementarity in matching hydrogen bonding and coordination (with Ca^{2+}; for recent review on this aspect, please see Gabius, 2011) and the stacking for C-H/π-interaction, if an aromatic amino acid is optimally positioned (Solís et al., 2009), contribute to reach the required precision of the molecular rendezvous. These structural parameters pave the way to complex formation. They act in concert with a further, so far not mentioned property of glycans that is beneficial for biorecognition. Its description takes us to the third dimension of the sugar code.

The initial recognition process is favored by the naturally low extent of intramolecular dynamics of glycans: in contrast to highly flexible peptides, di- and oligosaccharides are conformationally restricted. In other words, they often adopt only few, energetically privileged conformations (von der Lieth et al., 1998; Solís et al., 2001). By drawing on E. Fischer's famous lock-and-key analogy (Fischer, 1894), these insights on the third dimension of the sugar code were succinctly summarized as follows: "the carbohydrate moves in solution through a bunch of shapes ("keys") each of which can be selected by a receptor" (Hardy, 1997). This selection of one from the few low-energy conformations minimizes the entropic penalty incurred upon binding. Having herewith outlined the chemical basis of the sugar code in its three dimensions, together with including information on lectin diversity and their detection *in situ*, we can proceed to examine the range of post-binding effects.

Lectins: translating sugar coding into bioactivities

Lectins (carbohydrate-binding proteins but not enzymes acting on the sugar ligand, immunoglobulins and transport proteins for free mono- to oligosaccharides) are commonly classified by their specificity to free sugars. An activity assay, classically by detecting haemagglutination (Mitchell, 1860; Stillmark, 1888), is set up for this purpose. Systematic study of inhibition of lectin-dependent haemagglutination by sugars will then deliver this information. However, it would be a complete misconception to assume that a binding specificity of a lectin to a common sugar such as D-galactose will automatically entail homing in on this epitope indiscriminately, thus forming complexes with all galactose-containing glycoconjugates. Whereas a rather non-selective binding mode to allow cell entry is characteristic of toxic lectins such as ricin, thus protecting plants against predators (Barbieri et al., 1993; Roberts and Lord, 2004), most lectins show preference to a restricted set of cellular glycoconjugates. This selection, guided by the structure (shape) of the ligand and the density/topology of its presentation, is at the heart of the physiological functions following binding (Fig. 2).

As illustrated in the general scheme of Fig. 2, endogenous lectins, having made their ligand selection, become potent effectors in diverse processes. To give an example, human galectin-1 (a homodimeric representative of a lectin family with specificity to galactosides, the ß-sandwich folding and a sequence signature with a conserved tryptophan residue that is engaged in C-H/π-interaction mentioned above to the galactose core (please see Fig. 3 for structural details; Kasai and Hirabayashi, 1996; Gabius, 1997, 2006; López-Lucendo et al., 2004; Barondes, 2008; Schwartz-Albiez, 2009)) can apparently "read" distinct

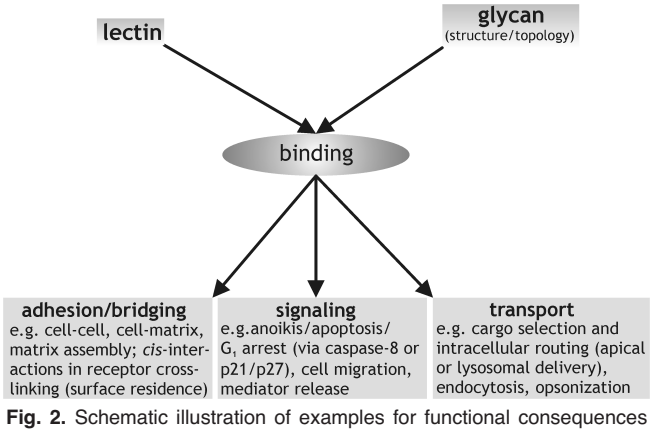


Fig. 2. Schematic illustration of examples for functional consequences of lectin-glycan recognition. The binding of certain galectins to distinct glycoconjugates presented in suited topology leads to *cis/trans*-interactions on the cell surface, elicitation of signaling as well as intracellular routing and uptake relevant for diverse aspects of cellular physiology.

determinants on the level of the saccharide part of glycoproteins and glycolipids. By identifying the counterreceptors for galectin-1 in different cell types, it was disclosed that high-affinity binding on cells is restricted to few types of glycoconjugates, a survey given in Table 1.

A conspicuous feature of galectin-1 is its bivalency, a prerequisite to act as cross-linker (Fig. 3). This design is graphically depicted in Fig. 4. Listed in Fig. 2 in general terms, adhesion and attachment can be brought about by binding of this bivalent module to cellular glycans such as matrix glycoproteins fibronectin and laminin, especially their poly-N-acetyllactosamine repeats (Merkle and Cummings, 1988; Zhou and Cummings, 1990; Ohannesian et al., 1995; André et al., 1999). Next in Fig. 2, galectin-1-dependent cross-linking of the fibronectin receptor ($\alpha_5\beta_1$-integrin) or T-cell-specific glycoproteins and also ganglioside GM1 initiates growth-regulatory downstream signaling. It can cause caspases-3 or -8 activation, TRPC5 (a cation channel of the transient receptor potential (canonical) group) channel opening for Ca^{2+}-influx or transcriptional activation of the genes for cyclin-dependent kinase inhibitors p21/p27 via reducing Sp1 phosphorylation for G_1 arrest (Fischer et al., 2005; Villalobo et al., 2006; Garner and Baum, 2008; Wang et al., 2009; Blaskó et al., 2011; Sanchez-Ruderisch et al., 2011; Wu et al., 2011). Conversely, galectin-1's interaction with tissue plasminogen activator in the tumor-stroma transition zone appears to trigger proliferation, along with tumor cell migration and invasion in pancreas tumor progression (Roda et al., 2009). These data illustrate a context-dependent activity profile for galectin-1. Clinically, the restriction of the $CD4^+CD7^+/CD4^+CD7^-$ T cell populations, clonally expanded in the early stage of cutaneous T cell lymphoma (Sézary syndrome), to predominantly $CD7^-$ cells later on can also be based on galectin-1, driving the $CD7^+$ T cells into apoptosis (Pace et al., 2000; Rappl et al., 2002). Lack of counterreceptor presence protects the T cells, as also noted in an animal model with respect to ganglioside GM1 in pathogenic effector T cells (Wu et al., 2011). This decrease makes these cells resistant to their suppression by regulating T cells. In addition to the signaling activities, galectins can also be a part of molecular transport.

To give an instructive example, the specific interplay of a galectin with certain glycoproteins and glycolipids accounts for a mechanism of cargo selection and routing in polarized cells (Fig. 2). In detail, galectin-4 is capable to target sulfatide with 2'-hydroxylated long-chain (C_{24}) fatty acids and also brush border-associated glycoproteins with their complex-type N-glycans (e.g. dipeptidyl peptidase IV or mucin-like membrane MUC1) within the raft-dependent apical routing of glycoproteins (Delacour et al., 2005; Stechly et al., 2009). A high density of N-acetyllactosamine termini is supposed to convey reactivity to galectin-4 (Wu et al., 2004; Morelle et al., 2009). The tandem-repeat design of galectin-4 with two different domains (please see Fig. 4 for a scheme) makes interactions possible with two different classes of ligand so that they become part of the fully loaded complex. In other words, a tandem-repeat-type galectin can be a heterobifunctional cross-linker. At the same time, galectin-4 can stabilize microdomains by its cross-linking capacity (Braccia et al., 2003; Danielsen

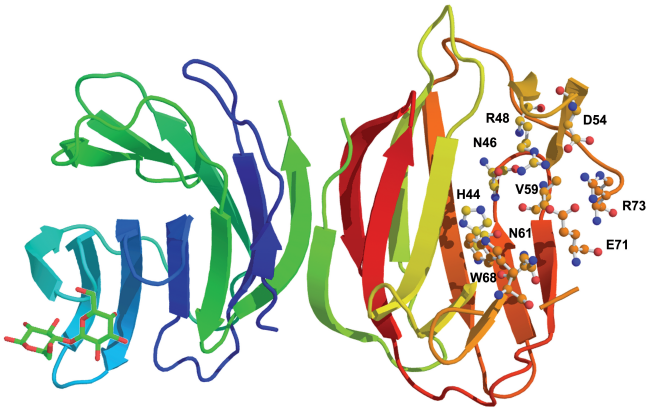


Fig. 3. Ribbon diagram of the crystallographic structure of homodimeric CG-1A (PDB entry: 1QMJ) drawn with PyMOL. The two carbohydrate-binding sites in the homodimer are depicted by either entering the ligand lactose in its low-energy conformation (left subunit) or the structures of the highly conserved amino acids involved in binding this disaccharide, hereby illustrating key characteristics of the sequence signature of galectins (right subunit).

Table 1. Cellular glycoconjugates and proteins as ligands for endogenous lectins: case study of galectin-1[a].

Type of ligand	galectin-1
glycoprotein	ovarian carcinoma antigen CA125, CD2, CD3, CD4, CD7, CD43, CD45, CD95(Fas), carcinoembryonic antigen (CEA), fibronectin (tissue), gastrointestinal mucin, hsp90-like glycoprotein, β_1-integrin (CD29), $\alpha_1/\alpha_4/\alpha_5/\alpha_7\beta_1$- and $\alpha_4\beta_7$-integrins, cell adhesion molecule L1, laminin, lamp-1, Mac-2-binding protein, nephrin, neuropilin-1, receptor protein-tyrosine phosphatase (RPTPß), thrombospondin, Thy-1, tissue plasminogen activator, chondroitin sulfate proteoglycan
glycolipid	distinct neutral glycolipids, ganglioside GM1
protein	Gemin4, oncogenic H-Ras, OCA-B, pre-B cell receptor (human, not murine system)

[a]: from Gabius, 2006, modified and updated

and Hansen, 2006).

In order to further substantiate the emerging clinical potential of these versatile cross-linking interactions, the role of an insect lectin with this type of design in vector competence for parasite (*Leishmania*) survival is outlined (Kamhawi et al., 2004). The galectin is abundantly expressed in the midgut of the sand fly (*Phlebotomus papatasi*), present on the luminal surface of epithelial cells. Its counterreceptor on the parasite are ß-galactosides of the lipophosphoglycan. This instance stands for a growing number of cases with lectin involvement in infection and inflammatory processes (for recent reviews, please see Holgersson et al., 2009; Osborn and Turkson, 2009; Gabius et al., 2011).

Beyond this combination of two sugar specificities in one protein lectins can even additionally be endowed with sites interacting with non-carbohydrate ligands. The potential for productive protein-protein (lipid) interactions besides their reactivity with sugars broadens the functional spectrum in the lectin toolbox. This extension of the range of binding partners is central e.g. for ordered cell migration in slime molds, a model for cell-matrix interactions (discoidin I; Gabius et al. 1985a), or for tumor growth regulation exerted by oncogenic H-ras (galectin-1; Rotblat et al., 2004; please see also Table 1).

In principle, tissue lectins are thus highly capable sensors for the presence of certain carbohydrate ligands. The resulting interaction can trigger post-binding events of functional significance (Fig. 2). A salient feature for effector functionality appears to be the cross-linking capacity of lectins (Fig. 3), and the next section will furnish more information on this aspect. On the level of the ligand, controlled changes in reactivity, e.g. by altering sialylation or degree of clustering in microdomains, both parameters effective for galectin-1 (André et al., 2007a; Bi and Baum, 2009; Kopitz et al., 2010), will matter. These observations turn attention to answer the question how lectins attain their specificity in terms of targeting their cognate counterreceptors in a cell-specific manner and triggering post-binding events. This question thus gives our review the direction to more closely look at galectins as an instructive model system.

Galectins: structural design and phylogenesis

Their presence was first described in extracts of electric organ tissue of *Electrophorus electricus* by monitoring agglutination of trypsinized rabbit erythrocytes. This assay disclosed high inhibitory preference of lactose and thiodigalactoside, which prompted the purification of electrolectin on acid-treated agarose (Teichberg et al., 1975). Detection of a lactose-dependent blue-shift of tryptophan fluorescence is a sign of its direct contact to the sugar (Teichberg et al., 1975). This lectin is clearly different from the asialo-glycoprotein receptor of rabbit hepatocytes, a galactoside-binding C-type lectin responsible for glycoprotein clearance from serum after desialylation, as initially tested with ceruloplasmin (Morell et al., 1966; Hudgin et al., 1974). Considering information on tissue distribution as a means to "shed some light on their possible functions", Teichberg et al. (1975) went on to report a rather wide distribution of the ß-galactoside-specific haemagglutinin activity. It was most pronounced in extracts of chick embryo pectoral muscle and also detectable in rat lung. The protein thereafter isolated from extracts of calf heart and lung proved similar in properties to the electrolectin (De Waard et al., 1976). It is now known as galectin-1, which we introduced above. The structure of the human protein is shown in Fig. 3 (further information on what followed with respect to the activity in chicken is given in the next section).

Essential for maintaining the protein's activity was

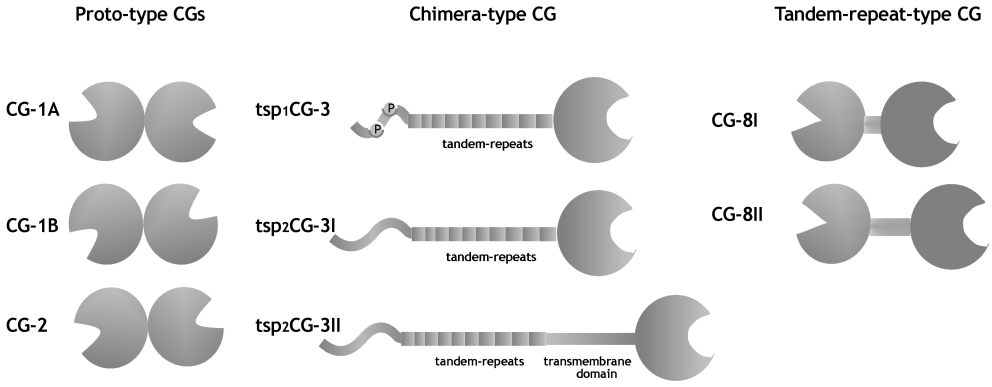


Fig. 4. Schematic illustration of the three types of modular arrangement characteristic for galectins. The five CGs including natural derivatives are depicted. Three proto-type CGs (CG-1A, CG-1B, CG-2; representation of relative orientation of the two binding sites follows the structural information in the PDB entries 1QMJ (CG-1A), 3DUI (CG-1B), and 1HLC (human galectin-2); please see Fig. 3 for a ribbon diagram). The use of two transcription start points (tsp1, tsp2) combined with alternative splicing generates isoforms for the chimera-type CG-3 (for respective details, please see also Fig. 7). The molecular design of this galectin combines a C-terminal CRD with a collagenase-sensitive section of ten Gly/Pro-rich sequence repeats consisting of either five (one repeat), seven (five repeats) or eight (four repeats) amino acids. Serine phosphorylation sites first identified in mammalian galectin-3 are present only in tsp1CG-3 (Ser^5, Ser^7), tsp2CG-3II contains a putative transmembrane domain of 70 amino acids. The tandem-repeat-type structure with two non-identical CRDs is represented by CG-8, i.e. its two isoforms CG-8I and CG-8II arising from alternative splicing. The two CRDs are connected by a linker peptide of either nine (CG-8I) or, in the extended version, 28 amino acids (lengths of linkers are drawn to scale).

its protection by a reducing agent (ß-mercaptoethanol). This requirement explains its initial designation as S-type lectin, which was abandoned in the light of evidence that other members of this lectin family did not share this sensitivity to oxidation (Barondes, 2008). What they all, however, appear to have in common is the selection of distinct, energetically preferred ligand conformations. As noted above, the property of oligosaccharides to adopt only a few shapes is thermodynamically advantageous. It was first demonstrated crystallographically for lactose, as shown in Fig. 3, and then in solution by NMR-spectroscopical methods for mammalian galectin-1 and extended/flexible ligands (Liao et al., 1994; von der Lieth et al., 1998; Asensio et al., 1999; Siebert et al., 2003; García-Aparicio et al., 2007). Applying these techniques recently helped in the discovery that galectin-1 binding is not limited to ß-galactosides (Miller et al., 2011). The α1,3-linked digalactoside is also a ligand for galectin-1. The non-reducing-end galactose unit topologically resides in the central place upon binding as it does for ß-galactosides (Miller et al., 2011). The galectin, often referred to as specific exclusively for ß-galactosides, is thus able to tolerate the α-anomeric linkage. Detailed comparison of the structures of complexes formed with a natural pentasaccharide (the glycan of ganglioside GM1) by computational chemistry disclosed fine-structural differences in the shaping of binding sites beyond the galactose core for galectins (André et al., 2005). Elucidation of this structural fine-tuning among galectins has been taken to the level of fully flexible ligand docking (Krzeminski et al., 2011). This procedure provides statistically weighted data on ligand contact and, more generally, ligand-selection profiles by mapping the degree of contact complementarity, at the level of the carbohydrate recognition domain (CRD). Synthesis of respective compounds and bioassays quantitating relative potency of binding will figure out whether a selectivity level suited for applications is attained (André et al., 2010; Giguère et al., 2011). The combination of crystallography with docking analysis and spectroscopy has further proven its value as a powerful means to pinpoint binding-site-specific events, such as a structural reorganization upon ligand binding (Solís et al., 2010). In addition to a sequence divergence at the contact site for the ligand the use of the term "cross-linking" implies another factor for functional variability. Comparison to other lectin families, e.g. C-type lectins (Gready and Zelensky, 2009), teaches the salient lesson that the topology of CRD presentation can vary widely, as a result of adapting to the topology of counterreceptor presentation. It is thus mandatory to review which modes in this respect are established for galectins. This issue has already been touched upon above but not yet dealt with comprehensively.

Three modes of CRD display are known (Fig. 4). In addition to proto-type proteins (monomeric or homodimeric such as galectin-1; please see also Fig. 3) the CRD can either be integrated into a context with other domains, establishing a structural chimera, or two different CRDs can be covalently connected by a linker peptide in a tandem-like display (Fig. 4). This group, to which galectin-4 (the organizer of super-rafts; Braccia et al., 2003) mentioned above belongs, combines the ability of cross-linking of proto-type proteins with the presence of two domains of non-identical specificity. The tandem-repeat-type motif has even been found extended to four CRDs. They are presented in tandem in galectins of bivalve mollusks, first seen in the Eastern oyster (Tasumi and Vasta, 2007; Zhang et al., 2011). The chimera-type design applies to galectin-3 (please see Fig. 4 for details). Recently, a 28 kDa protein in the phylum Cnidaria was described which harbors a second structural motif. It is constituted by 30 N-terminal collagen-like tripeptide (GlyXY including two hydrophobic amino acids) repeats and, unique for galectins, a signal peptide is present (Hwang et al., 2010). Having now introduced the common structural themes of CRD organization in galectins, the question arises whether this separation in three groups is a phylogenetically conserved feature. It is answered by putting the genomes of organisms from different branches of the evolutionary tree under scrutiny.

This work of mining data bases resulted in a graphical illustration, which documents the following: the occurrence of the three types of CRD display is mostly conserved, except for absence of a gene for a chimera-type-like galectin in *Drosophila* (Fig. 5). The actual number of genes/proteins is shown to vary even between rather closely related species, e.g. mouse and rat. When examining this pair of rodents more closely, it is found that the murine genome encodes a unique tandem-repeat-type protein which is produced in the gastrointestinal tract (galectin-6; Gitt et al., 1998a,b). Several laboratory strains (Balb/c, NOD, NMRI and others), however, lack the gene (Houzelstein et al., 2008). The presence/absence polymorphism also occurs in wild mice. This situation explains the asterisk marking tandem-repeat-type proteins in Fig. 5 for mouse.

On the level of proto-type proteins, too, mouse and rat are separated by an interesting difference. Proto-type galectin-5 appears to be unique for rat (Gitt et al., 1995). It is present in erythrocytes and involved in exosomal sorting during reticulocyte maturation (Gitt et al., 1995; Lensch et al., 2006; Barrès et al., 2010). Sequence analysis has uncovered sequence identity of galectin-5 with the C-domain of galectin-9 of 86% on the level of the protein (Wada and Kanwar, 1997; Lensch et al., 2006). These two cases, explaining occurrence of a certain difference/marking by asterisk in Fig. 5, let us draw the conclusion that galectin gene display is subject to a certain degree of fluidity, even among strains of a species. Such a variation in gene number is observed in other lectin families. For instance, CD33-related siglecs show species- and lineage-specific deletions (Angata et al., 2004). The macrophage galactose-specific C-type lectin, to name a second example, is represented by a

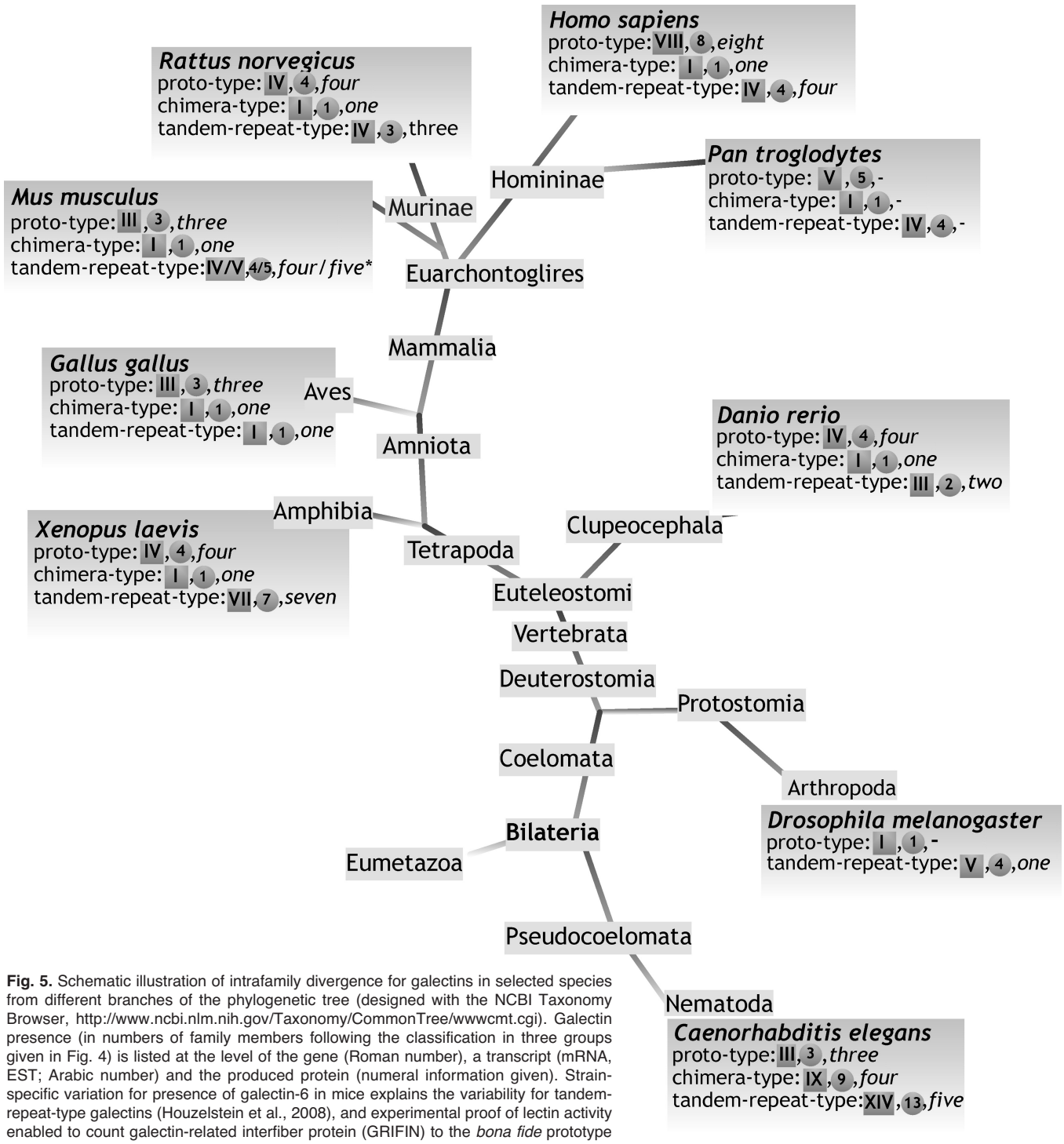


Fig. 5. Schematic illustration of intrafamily divergence for galectins in selected species from different branches of the phylogenetic tree (designed with the NCBI Taxonomy Browser, http://www.ncbi.nlm.nih.gov/Taxonomy/CommonTree/wwwcmt.cgi). Galectin presence (in numbers of family members following the classification in three groups given in Fig. 4) is listed at the level of the gene (Roman number), a transcript (mRNA, EST; Arabic number) and the produced protein (numeral information given). Strain-specific variation for presence of galectin-6 in mice explains the variability for tandem-repeat-type galectins (Houzelstein et al., 2008), and experimental proof of lectin activity enabled to count galectin-related interfiber protein (GRIFIN) to the *bona fide* prototype galectins in *Danio rerio* (Ahmed and Vasta, 2008). Numbers of galectins in each group and species were obtained by using both BLAST/PSI-BLAST search algorithms as well as the available entries in the Protein Knowledgebase (UniProtKB, Expasy Proteomics Server, www.expasy.ch), the UCSC Genome Browser Gateway (http://genome.ucsc.edu/cgi-bin/hgGateway), the Ensemble Genome Browser (http://www.ensembl.org/index.html), the NCBI Web Viewer (http://www.ncbi.nlm.nih.gov/mapview), the NCBI Unigene (www.ncbi.nlm.nih.gov/unigene), the NCBI EST (www.ncbi.nlm.nih.gov/nucest) and the NCBI conserved domains (www.ncbi.nlm.nih.gov/unigene) database.

single protein in the human genome but by two genes for the mouse (Suzuki et al., 1996; Tsuiji et al., 2002). Clearly, these differences advise caution when trying to extrapolate and adopt functional interpretations in cross-species comparison. Problems with this apparent variability notwithstanding, understanding lectin expression and function in a certain organism will help to derive testable hypotheses and design respective experiments. In this sense, the presented survey diagram (Fig. 5) directs special interest to those organisms with a comparatively low degree of complexity of galectin presence, ascertained on the level of the protein. At the same time, they should not be too distant from mammals evolutionarily. Evidently, the chicken galectins (CGs), by meeting these criteria (Fig. 5), are an attractive study object. In detail, the three groups of proto-, chimera- and tandem-repeat-type galectins are represented, with a total of five members (Fig. 5). Thus, this situation gives reason to focus on the CGs in order a.) to delineate rules of structural divergence, b.) to define expression profiles and c.) to elucidate the functional significance of each member within the galectin network.

Chicken galectins: tracing rules of divergence

Historically, Teichberg et al. (1975) first pointed to the presence of galectins in chicken by reporting high haemagglutination titers for extracts of embryonic pectoral muscle. Starting purification with extracts from embryonic thigh/pectoral muscle, the first CG was isolated with yields of more than 30% (Den and Malinzak, 1977; Nowak et al., 1977), cross-linking activity for glycoproteins typical for a homodimeric proto-type galectin proven (Gupta et al., 1996) and its crystal structure reported (Varela et al., 1999). Besides muscle as source for this protein (termed CG-1A to signal its relationship to mammalian galectin-1), embryonic skin was found to be rich in lectin activity, a second proto-type CG (CG-1B; Oda and Kasai, 1983). Moreover, the processing of adult intestine yielded the third member of this group (CG-2; Beyer et al., 1980; Kaltner et al., 2008). Corroborating results of previous calculations to depict phylogenetic trees (Cooper, 2002; Houzelstein et al., 2004), these three proteins have their distinct routes of divergence: first, the genes for galectins-1 and -2 arose by duplication of an ancestral gene, then - around the time of separation of lineages for birds and mammals about 300 million years ago - a second duplication led to the genes for CG-1A/B (Fig. 6; Sakakura et al., 1990). Their structural organizations maintain exon/intron characteristics typical for all known proto-type galectins (Fig. 7). Spectroscopic visualization of involvement of tryptophan in binding and chemical mapping with ligand derivatives, determination of glycan and glycoprotein specificities and crystallography reflect the expected homology, with clear evidence for development of individual properties (Solís et al., 1996; Siebert et al., 1997; Varela et al., 1999; Wu et al., 2001, 2007; López-Lucendo et al., 2009). Subsequently, the chimera-type CG-3 and the tandem-repeat-type CG-8 were purified, thereby completing the full set of CGs (for details on spatial arrangements of the CRDs and other domains/linker peptide, please see Fig. 4; Kaltner et al., 2009, 2011). This status of investigation is the basis for the comprehensive comparative analysis.

The selection of CGs as model system implies that important traits are conserved when related to mammalian galectins. This turns out to be already true when examining all gene structures (Fig. 7). The high-level accordance of CGs to respective mammalian galectins is further substantiated by similarities in chromosomal environment, exon/intron display, presence of two promoters for production of CG-3, of which one form can be phosphorylated in the N-terminal section like mammalian galectin-3, and alternative splicing to yield the linker-length variation in CG-8 (Fig. 7; Kaltner et al., 2008, 2009, 2011). Thus, knowledge obtained by studying CG expression can be assumed to be relevant for mammalian orthologs and beyond. Considering the rather small number of proteins in the CG family a complete expression analysis can be performed much more easily than for mammals. This work has been done for adult organs. Protein presence was systematically studied immunohistochemically using non-cross-reactive antibodies (Kaltner et al., 2008, 2009, 2011). The resulting data provide the first case of a complete documentation of galectin localization in an animal.

The inspection of the proximal promoter regions for all five CG genes had identified conspicuous differences (Kaltner et al., 2008, 2009, 2011). These sequence-based data have raised the expectation for rather individual expression patterns. As exemplarily shown by mi-

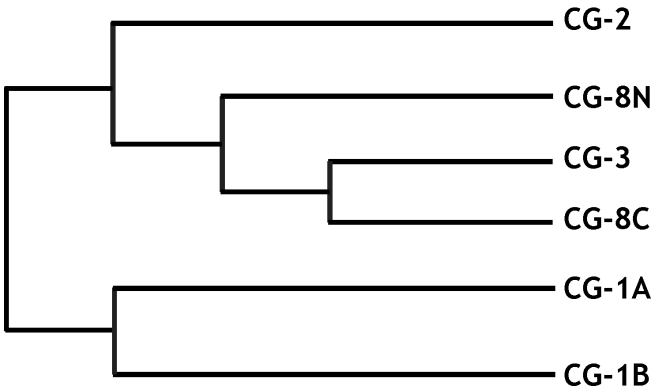


Fig. 6. Schematic illustration of phylogenetic relationships between the CRDs of the CGs in a family-tree diagram (www.phylogeny.fr). CG sequences were processed by the multialign program ClustalW, the alignment curation software Gblock as well as the programs PhyML (maximum likelihood) and ProtDist/FastDist + BioNJ (distance) for construction of evolutionary relationships prior to using the visualization software TreeDyn. Note that branch lengths in both diagrams were modified in order to place emphasis on routes of divergence.

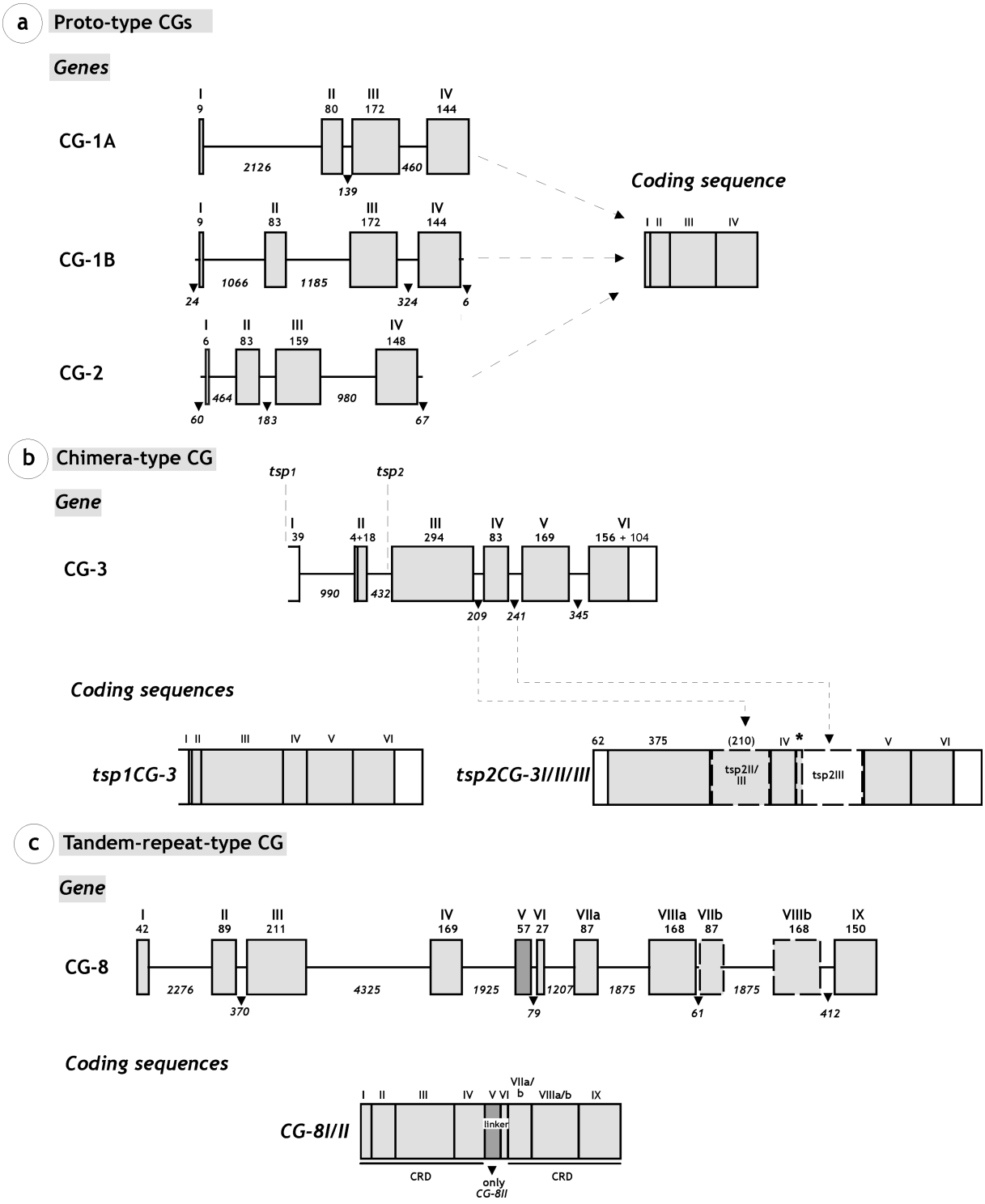
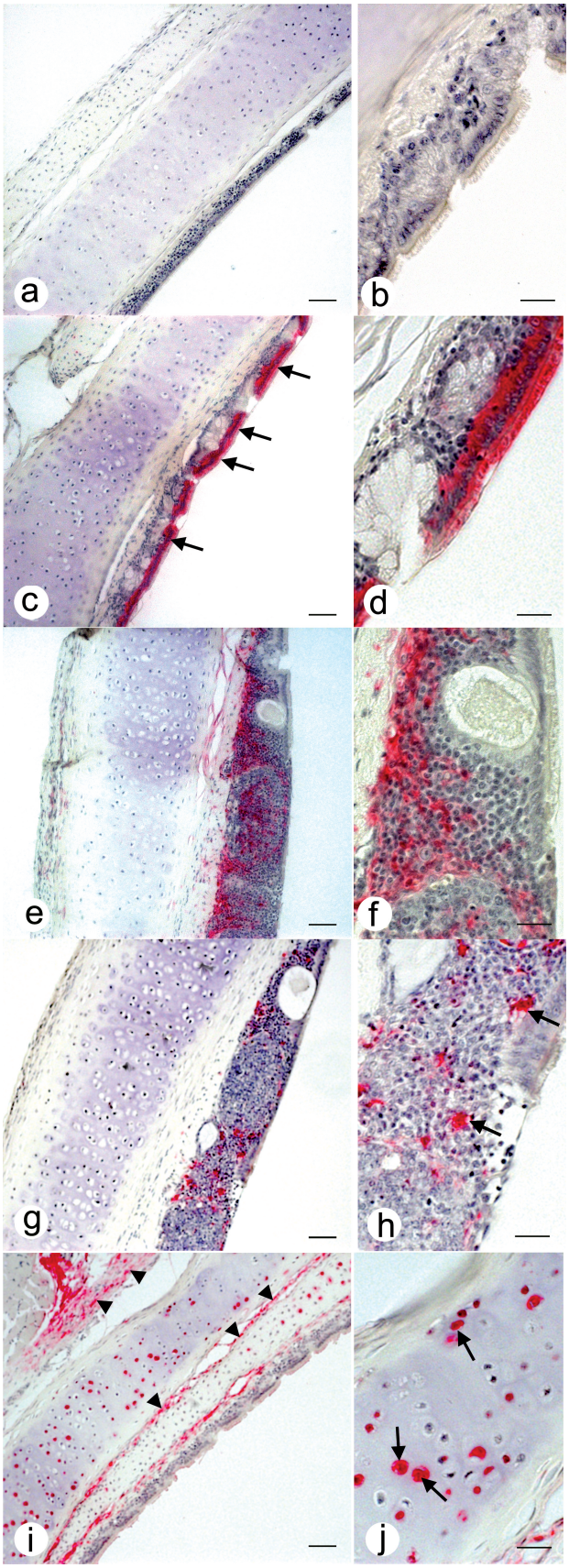


Fig. 7. Schematic illustration of the organization of CG genes and the resulting mRNAs. Exons are given as boxes (coding exons in grey, untranslated exon parts as open boxes), introns and 5'/3'-untranslated areas as lines (size proportional to exon and intron lengths). Roman numbers reflect the order of exons, Arabic numbers above exon boxes and below intron lines (partially combined with arrowheads) the length of each exon/intron in base pairs. The coding region in CG-1A, CG-1B and CG-2 is constituted by four exons in each case **(a).** Two transcription start points, i.e. tsp1 and tsp2 (indicated with dashed lines), controlled by independent promoters result in two mRNA forms, i.e. tsp1CG-3 or tsp2CG-3I **(b)**. Alternative splicing of the latter product, when a 209 bp (tsp2II/III) or a 241 bp long intron (tsp2III), respectively, are turned into coding sequences, leads to further product diversity. Translation of tsp2CG-3III mRNA is expected to terminate prematurely within the new exon after coding for ten amino acids (asterisk) due to presence of a stop codon **(b)**. Alternative splicing is also operative in the case of CG-8, with exon V (dark grey box) being the source of the second CG-8 protein from the single gene **(c).** Of note, the genomic region between exons VI and IX contains an almost perfect duplication of exons VII and VIII and the connecting intron. These duplicated exons are given in the illustration as hatched boxes and numbered VIIa/b and VIIIa/b, respectively. The mature mRNAs with eight (CG-8I) or nine exons (CG-8II) can be generated by alternative splicing either with exon VI (CG-8I) or exons V and VI (CG-8II), establishing the different linker lengths of nine and 28 amino acids.



crophotographs presented in Fig. 8 for trachea, actual regulation of presence of CGs is strict. Qualitative differences are apparent. Proto-type CG-2 was not detectable (Fig. 8a,b), thus serving as negative control. In contrast, strong signals for CG-1A were seen in the epithelium, exclusively in the cytoplasm (Fig. 8c,d). The most closely related CG-1B is present in connective tissue and stroma adjacent to the epithelium (Fig. 8e,f). Within the *lamina propria mucosae* a second lectin (CG-8) was found. The staining profiles of CG-1B and CG-8 differed markedly. CG-8 positivity was specific for macrophages, in the cytoplasm and in nuclei (Fig. 8g,h), a labeling pattern characteristic for galectin-3 (Mac-2 antigen) in lungs of adult mammals (Flotte et al., 1983; Kaltner et al., 2002; Maldonaldo et al., 2011). Besides the tracheal epithelium, chondrocytes express galectin-3 in mammals (Colnot et al., 1999; Boileau et al., 2008), and those cells were the site of CG-3 expression in adult chicken trachea (Fig. 8i,j). The complete survey for the processed adult organs is presented in Table 2.

In overview, regional and cell-type selectivity are characteristic for CG expression. This holds true even for closely related proteins as based on their positions in the phylogenetic tree presented in Fig. 6 (Table 2). As quality control, the immunohistochemical data are in full accord with previous reports on preferential galectin presence by purifying a CG from organs, e.g. for CG-1A (liver)/CG-2 (intestine) (Beyer et al., 1980). These results argue against a pronounced extent of redundancy in the group of proto-type CGs. The illustrated evidence for individual patterns is flanked by functional data. Selective inhibitory activity of CG-1A on stimulated T cells as well as effector potency in axon guidance and in cell self-organizing dynamics into condensations within limb skeletal morphogenesis is known (Schneller et al., 1995; Kopitz et al., 2004; Bhat et al., 2011). Formation of such condensations and the processes to let cartilage mature are promoted by CG-1A, which is produced in

Fig. 8. Immunohistochemical localization of CGs in cross sections through trachea of a six-month-old chicken. Microphotographs of serial sections after processing with antibody preparations specific for CG-2 **(a,b)**, CG-1A **(c,d)**, CG-1B **(e,f)**, CG-8 **(g,h)** and tsp1CG-3 **(i,j),** respectively, and reagents for signal generation. The lack of reactivity for the CG-2-specific immunoglobulin G preparation **(a,b)** serves as inherent negative control, thus excluding any antigen-independent staining. Intense staining for CG-1A was seen in ciliated pseudostratified columnar epithelium (**c**, arrows). On the cellular level, reactivity is confined to the cytoplasm; nuclei and cilia are negative (**d**). CG-1B presence was detected in connective tissue fibers of the *lamina propria mucosae* (**e**) and the stroma of embedded lymphatic tissue adjacent to the epithelium (**e,f**). Distinct from this staining pattern of CG-1B positivity for CG-8 was limited to macrophages in this region (**g**), with strong staining intensity both in the cytoplasm and in nuclei (**h**, arrows). Staining for CG-3 was restricted to the cartilage layer of the trachea with strong intensity in the perichondrium (arrowheads) and in chondrocytes scattered throughout the cartilage layer (**i**). In reactive chondrocytes, walled off in small lacunae and grouped in small clusters of isogenous cells (arrows), CG-3-specific immunoreactivity resides both in nuclei and in the cytoplasm (**j**). Scale bars: a, c, e, g, i, 20 μm; b, d, f, h, j, 50 μm.

the zeugopod region of 5-day leg buds, together with CG-8 (Bhat et al., 2011). That staining by labeled CG-1A was detectable in cartilage primordia and functional antagonism between the two CGs could be delineated (Bhat et al., 2011) is in line with the concepts of a.) an orchestrated regulation of galectin expression and presence of counterreceptors (glycans) as well as b.) a galectin network. This staining could be due to the presence of bi- or trianntennary N-glycans. Much like mammalian glycoprotein glycans, they can be α2,3/6-sialylated but are devoid of N-acetyllactosamine repeats, as comparative glycomic profiling of the N-glycans of chicken and human erythrocyte surfaces revealed (Aich et al., 2011). Taking this emerging information in the avian model to the level of galectins in human tumor cells, such a cell-type selectivity of expression could aid diagnosis. It may even allow prognostic assessments. Of course, in view of the concern of invalid extrapolation given above, cell-type selectivity needs to be ascertained. In a stepwise manner, the given hypotheses have been tested, starting with biochemical galectin purification using murine and human tumor specimen (Gabius et al., 1984, 1985b) and moving from initial galectin immunohistochemistry, here on breast cancer (Gabius et al., 1986), to galectin fingerprinting.

Galectin fingerprinting in tumors

The systematic mapping of the presence of a certain galectin in tumors, e.g. performed on galectins-2 and -8 (Danguy et al., 2001; Saal et al., 2005), reached a similar conclusion, as drawn above for the individual CGs: galectin presence is tightly regulated and not a common ("house-keeping") function of all cell types. This documented, galectin fingerprinting (i.e. the determination of expression profiles of different members of this family) becomes a promising approach. Building a solid foundation for immunohistochemistry, the monitoring of gene expression of seven galectins in 61 human cancer cell lines using RT-PCR disclosed marked profile differences (Lahm et al., 2001). They can be likened to a galectin signature. Its occurrence in head and neck as well as skin tumors was revealed by applying non-cross-reactive antibodies (Saussez et al., 2006, 2007, 2008a, 2010; Čada et al., 2009a,b; Cludts et al., 2009).

Of note, besides detecting the presence, the pattern of localization of a lectin has also emerged as informative parameter, with particular changes for instance characterized during tumor progression in laryngeal and hypopharyngeal tumors and cell models (Dvoránková et al., 2008; Saussez et al., 2008b). The presence (in quantitative terms) and site of localization combined can harbor diagnostic potential. This is exemplified in the class of salivary gland tumors by distinguishing mucoepidermoid from adenoid cystic carcinomas and even subtypes of the latter tumor (Remmelink et al., 2011). Details of syntactic structure analysis such as cluster radius of galectin-expressing cells or distance between tumor and inflammatory cells likewise add salient information, in testicular cancer providing prognostic correlations (Kayser et al., 2003). Disease mortality is also reflected in galectin detection obtained by fingerprinting in bladder cancer (Langbein et al., 2007). In colon cancer, the positivity for galectins-1 and -4 in Dukes A/B tumors or the labeling index for galectin-8 in Dukes C/D tumors have prognostic value (Nagy et al., 2002, 2003). To resolve the pertinent question on the underlying molecular mechanisms *in vivo* as a means to envision new therapeutic strategies, likely routes of action derived from *in vitro* experiments on galectin functionality, as compiled for colon cancer recently (Barrow et al., 2011), will be helpful. Toward this end, the identification of operative counterreceptors is of foremost importance.

Along this line, the capacity of tumor suppressors to enhance galectin expression *in vitro* (p53 in DLD-1 colon cancer cells (Polyak et al., 1997) and $p16^{INK4a}$ in Capan-1 pancreatic carcinoma cells (André et al., 2007a)) inspires further histopathological studies, together with testing for any concomitant change in galectin reactivity. This histochemical application of labeled galectins for the purpose of visualizing reactive sites presents no technical problems (Gabius et al., 1991; Brinck et al., 1996). The detection of disparate staining profiles by comparison of several galectins used as probe is a means to make fine-specificity differences visible on the cellular level (Habermann et al., 2011). Since fine-specificity and CRD presentation of the galectins are not identical to those of plant agglutinins, the tissue lectins are the proper tools to infer changes in the glycomic profile with functional relevance. Physiologically, an alteration in glycoprotein glycosylation has the potential to act like a switch for functionality of the protein part, akin to the role of core substitutions for the glycan given above. This assumed process has been proven for the fibronectin receptor and its reactivity with galectin-1, the molecular trigger to start the cascade toward caspase-8-dependent anoikis or, in more general terms, attenuate cell cycle progression in carcinoma cells (Fischer et al.,

from [1] Kaltner et al., 2008 extended [2] Kaltner et al., 2011 [3] Kaltner et al., 2009. Abbreviations: l, lamina; gldd, glandulae. Signal intensity was semiquantitatively grouped into the categories: - negative, + weak but significant, ++ medium, +++ strong. [a]smooth muscle layers are negative; [b]duodenum, jejunum, ileum, caecum, rectum; [c]exclusively cytoplasmic positivity; [d]infundibulum, isthmus, magum, vagina; [e]perikarya of neurons in the outer cortex; [f]perikarya of Purkinje cells; [g]cytoplasm of oocytes; [h]connective tissue; [i]cytoplasmic and nuclear; [j]exclusively apical cytoplasma and cilia; [k]exclusively alveolar macrophages; [l]single cells scattered in the parenchyma; [m]jejunum only tips of microvilli; [n]jejunum, ileum; [o]duodenum; [p]caecum, rectum; [q]renal cortex only; [r]medullary cones only; [s]staining membrane-associated; [t]flat layer of closely apposed cells covering the subjacent layer of loosely associated arachnoid trabecular cells; [u]subepithelial; [v]subepithelial surrounding glands localized in the *l. propria mucosae*; [w]connective tissue between tubules and surrounding glomeruli; [≠]only single cells positive; [¶]clusters of cells positive; [§]>50 % of cell population positive

Table 2. Immunohistochemical profiling of the five CGs in various organs of adult animals.

Type of organ				CG-1A[1)]	CG-1B[1)]	CG-2[1)]	tsp1CG-3[2)]	CG-8[3)]
Cerebrum				++[e]	-	-	-	++[t]
Cerebellum				+++[f]	-	-	-	++[t,≠]
Thymus	thymocytes, macrophages			-	++[c,§]	-	-	+/++[§]
	epithelio-reticular cells			-	++[c,§]	+[c]	++[¶]	-
Larynx[a]	respiratory epithelium			++[c]	-	-	+[c,j]	-
	l. propria mucosae			-	+/++[§]	‘-	-	++[c,u,≠]
Trachea[a]	respiratory epithelium			++[c]	-	-	-/+[c,j]	-
	l. propria mucosae			-	++[§]	-	-	++[c,u,¶]
	cartilage layer	perichondrium		-	-	-	++	-
		chondrocytes		-	-	-	+++[¶]	-
Lung[a]	parabronchial wall			-	+[h]	-	+++[k,¶]	+++[¶]
	atria	respiratory epithelium		-	+++[c]	-	-	-
		interatrial septa		-	-/+[h]	-	+++[k,¶]	+++[≠]
Heart	epicardium			-	-	-	-	+/++[≠]
	myocardium			++	-	-	-	+[≠]
	endocardium			-	-	-	-	-
Liver	hepatocytes			+++[c]	-	-	-	-
	Kupffer cells			-	-	-	+++[l]	++[¶]
Esophagus[a]	epithelium			-	+	-	++/+++[c]	-
	l. propria mucosae			-	++[c,≠]	-	-	++/+++[v,§]
Proventriculus[a]	*gldd. proventriculares superficiales*	epithelium		-	-	-	-/+[c]	-
		l. propria mucosae		-	++[c]	-	-	+/++[≠]
	gldd. proventriculares profundae	epithelium		++	-	-	-	-
		l. propria mucosae		-	-	-	-	+/++[≠]
Gizzard[a]	*gldd. ventriculares*	epithelium		-/+	++[c]	+[c]	+[c]	-
		l. propria mucosae		-	-	-	-	+/++[≠]
	stratum compactum			-	-	-	-	-
Gut[a,b]	epithelial lining[c]	villi		-	-	+++	+++[m]	-
		crypts		-	-	+++	-[n],+[o],+++ [p]	-
		goblet cells		-	-	-	-	-
	l. propria mucosae			-	+	-	-	+++[§]
Kidney	glomeruli			-	-	-	-	-
	epithelial lining of tubules	proximal convolution		+++[c]	-	-	-	-
		distal convolution		+++[c]	-	-	+++[c,q]	-
		loops of Henle		+++[c]	-	-	++[c,r]	-
		collecting ducts		-	-	++[i]	-	-
	connective tissue			-	-	-	-	++[w,¶]
Ovary	prehierarchical follicles	healthy		+++[c,g]	-	-	-	-
		early atretic	granulosa cell layer	-	-	-	-	-
			theca cell layer	-	-	-	-	+/++[¶]
		late atretic	granulosa cell layer	-	-	-	-	+[≠]
			intercalating cells	-	-	-	-	+++[§]
			theca cell layer	-	-	-	-	++[≠]
	hierarchical follicles			-	-	-	-	-
	interstitium			-	+	-	-	-
Oviduct[d]	surface epithelium			-	-	-	+[c]	-
	glandular epithelial lining			+	-	-	-	-
Uterus (shell gland)[a]	surface epithelium			+[c]	-	-	+++[c,j]	-
	glandular epithelial lining			-	-	-	++[s]	-
	l. propria mucosae			-	-	-	-	+[≠]
Skin[a]	epidermis	*stratum corneum*		-	-	-	-	-
		stratum intermedium		-	+++[i]	-	+++	-
		stratum basalis		-	+[c]	-	-	-
	dermis			-	++	-	-	+→+++[¶]
	subcutis			-	+	-	-	+/++[≠]

2005; André et al., 2007a; Sanchez-Ruderisch et al., 2011). That the underlying control mechanisms by master regulators can be intricate is illustrated for the tumor suppressor $p16^{INK4a}$. At the same time, it increases susceptibility to galectin-1-dependent anoikis induction in the Capan-1 cell model by a) upregulating galectin-1 expression and cell surface presence, b) enhancing cell reactivity for galectin-1 mostly by altering α2,6-sialylation and c) reducing availability of the competitive inhibitor for galectin-1, i.e. galectin-3 (André et al., 2007a; Sanchez-Ruderisch et al., 2010). As noted for CG-1A/CG-8 above, human galectins-1 and -3 thus constitute a system of functional competition in these tumor cells. This type of inter-galectin competition is also seen in action for human neuroblastoma *in vitro* (Kopitz et al., 2001). Evidently, cross-linking engaging ganglioside GM1 by the chimera-type galectin, which is capable of forming pentamers in the presence of multivalent ligands (Ahmad et al., 2004), is topologically different from the lattice formation of homodimeric galectin-1. In essence, these *in vitro* data definitely encourage to pursue galectin fingerprinting. Keeping in mind the broad range of functionality given in Fig. 2 and therefore the essential part played by the counterreceptors, this work is advisedly tied to the concomitant visualization of cellular galectin reactivity. That the given examples for functional competition have inspired the design of a selective glycocluster-based inhibitor, integrating glycan tailoring with valency (André et al., 2003, 2008, 2011), charts a route how to turn our growing understanding of the galectin network into experiments to devise innovative therapeutic approaches.

Conclusions

Lectin histochemistry is a potent means to unveil the complexity of glycosylation. It is under the control of an astounding degree of regulation, which underlies the dynamic non-random changes and cell-type selectivity. This glycophenotyping, with the detection of marker epitopes, intimates functional relevance. Therefore, the set of natural saccharides can rightfully be called the third alphabet of life. A brief look at the basic chemical properties of carbohydrates reveals a genuine suitability for high-density coding in oligomers. Fittingly, the generation of a wide array of glycan determinants is accompanied by presence of a wealth of endogenous lectins (for recent reviews, please see Gabius, 2009). They translate the sugar-encoded structural information into cellular responses as shown in Fig. 2. Carbohydrate recognition is coupled with diverse ways of spatially reacting with polyvalent ligands (please see Figs. 3, 4). An apparently complex diversity of lectins is generated by evolution. The first step to give physiological meaning to the evolutionary divergence is to take stock of the inventory. This is done here for the family of adhesion/growth-regulatory galectins. Whereas the presence of three groups of galectins (please see Fig. 4) is conserved among invertebrates and vertebrates, the number of genes can vary markedly even between closely related species. Owing to the comparatively low level of complexity for galectin genes, chicken with its total of five genes (Fig. 5) is selected as a model system. Its study is unravelling a.) the sites to explain molecular diversity on the level of transcription and splicing, b.) the individual, tightly regulated expression profiles, c.) the details of the structures and, last but not least, d.) the functions, together shaping the concept of a galectin network.

Similarities between avian and mammalian glycosylation underscore the promising perspective of this choice for helping to resolve the listed issues for human galectins. Collectively considered, a medical application of this idea through galectin fingerprinting in tumors is proving its merit for diagnostic and prognostic evaluations. Cases of functional competition and orchestrated lectin/glycan presentation reinforce the validity of the network idea in the realm of the sugar code. These discoveries signify that protein (lectin) - carbohydrate recognition is a key route of biological information transfer, as depicted in Fig. 1 (bottom part).

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