Glycoconjugate Journal 19, 433–440, 2004

Introduction to galectins

Hakon Leffler[1], Susanne Carlsson[1], Maria Hedlund[1], Yuning Qian[1] and Francoise Poirier[2]

[1]*Section MIG (Microbiology, Immunology, Glycobiology), Inst. Laboratory Medicine, Lund University, Sweden,* [2]*Institut Jacques Monod, Paris, France*

Good evidence suggest roles of galectins in cancer, immunity and inflammation, and development, but a unifying picture of their biological function is lacking. Instead galectins appear to have a particularly diverse, bewildering but intriguing array of activities both inside and outside cells—"clear truths and mysteries are inextricably twined". Fortunately this has not discouraged but rather enthused a large number of good galectin researchers, some of which have contributed to this special issue of Glycoconjugate Journal to provide a personal, critical status of the field. Here we will give a brief introduction to the galectins as a protein family with some comments on nomenclature.
Published in 2004.

Keywords: **galectin, lectin, carbohydrate, non-classical secretion**

Abbreviations: **CRD, carbohydrate recognition domain.**

Good evidence suggest roles of galectins in cancer, immunity and inflammation, and development, but a unifying picture of their biological function is lacking. Instead galectins appear to have a particularly diverse and bewildering, yet intriguing, array of activities both inside and outside cells—one is tempted to say that "clear truths and mysteries are inextricably twined" [1]. Fortunately this has not discouraged but rather enthused a large number of good galectin researchers. Some, but far from all, of these have contributed to this special issue of Glycoconjugate Journal to provide a personal, critical status of the field. Other recent reviews are [2–9].

The galectin family

The term galectin was introduced in 1994 [10] as follows: "Membership in the galectin family requires fulfillment of two criteria: affinity for β-galactosides and significant sequence similarity in the carbohydrate-binding site, the relevant amino acid residues of which have been identified by X-ray crystallography (Lobsanov *et al.*, 1993)". This served the need to bring together under one nomenclature proteins that had been given a wide variety of names depending on the circumstances of their discovery.

To whom correspondence should be addressed: Hakon Leffler, Professor of Molecular Medicine, Sect. MIG (Microbiology, Immunology, Glycobiology), Inst. Laboratory Medicine, Lund University, Sölvegatan 23, SE 22362 Lund, Sweden. Tel.: 46 46 173270; Fax: 46 46 137468; E-mail: hakon.leffler@mig.lu.se

The galactoside binding activity was included in the definition, after some discussion, because it was feared that carbohydrate binding properties could not be inferred by sequence alone. Moreover, defining the family by overall sequence similarity in the 135 amino acid CRD, not just the seven amino acid motif in the carbohydrate binding site, might have given ambiguous cases. Now we know more because of the wealth of new sequence information, both whole genomes, and many cDNAs from a wide range of species [2]. At least in mammals it is fairly easy to delimit a family of proteins by overall sequence similarity to the canonical CRD that contains all the known galectins and a few galectin like proteins (lacking β-galactoside binding activity); there are no real straddlers where it is hard to decide if they are in or out. Going to non-mammalian species, there would be problems in defining galectins based on sequence only. For example, the sequences of mushroom galectins are only recognizable as similar to mammalian galectins after a motif based comparison, but are not significantly similar by a standard BLAST-comparison. Therefore, at present it is best to keep both the sequence motif and β-galactoside binding criteria in the definition of galectins, and use the term galectin like proteins for the proteins that are similar enough by overall sequence but lack proven β-galactoside binding activity and might lack some of the residues in the defining motif.

The family name galectin was also intended to replace the previously used S-type lectin designation [11]. The S was meant to indicate dependence on thiols (reducing conditions) for activity, a property of galectin-1, the first galectin studied, but, as

later found, not a property of many other galectins. However, S-type fits so well with C-type, I-type etc. so it has its own staying power. It is sometimes used to designate a larger protein family to which galectins belong or a subgroup of galectins, but either is inappropriate, as no such family/group has been defined. It is sometimes used as a synonym of galectins, but this is unnecessary. So the term "S-type lectin", unless redefined, can safely be considered obsolete and be replaced by "galectin" as done by its originator [12].

Galectin early discoveries

Galectins were discovered based on the hypotheses that cell surface carbohydrates take part in cell adhesion [13]. Thus, tissue extracts were analyzed for their ability to agglutinate erythrocytes and/or fractionated on affinity columns with immobilized β-galactosides, a common cell surface carbohydrate that at the same time was reasonably accessible in quantities enough for biochemical experiments (*e.g.* in lactose, and asialofetuin) [14,15]. Bound proteins were eluted with lactose and analyzed further. A key technical discovery at this time was that the first "galectin", now known as galectin-1, required reducing conditions to preserve activity. As mentioned above this is, however, not true for most galectins found later.

These lactose binding lectins were given names, depending on their source and discoverer, such as electrolectin, CLL-I and -II, L-14, L-29, L-31, CBP35 and galaptin. When cloning began, sequence comparisons demonstrated that a number of proteins, discovered and named based on other properties (such as εBP, and Mac-2), were identical or similar to the lactose binding lectins. The galectin term then unified the nomenclature.

Using "galectin" to search PubMed gives over 1150 hits. So the field is alive and well and rapidly growing. However, many important and still valuable early papers are not found, as they have not been indexed with "galectin" as a keyword yet. Included are papers describing: (i) the first galectin discovered (in electric organ of the electric eel) [15], (ii) galectins in chick tissues with analysis of expression in different tissues, during development, and subcellular compartments [16–19], (iii) galectins in bovine heart and lung with specificity studies [20,21], (iv) galectins in blood cells [22], (v) galectins in cancer cells [23–26], (vi) galectins in cultured cells [27,28], (vii) multiple galectins in rat lung and intestine with detailed specificity studies [29–31], and an early review [32].

Galectin domain organisation and subgroups [2]

The galectins known so far have either one or two CRDs within a single polypeptide chain (Figure 1). The galectin CRD is (with a few invertebrate exceptions) not associated with other types of well defined protein domains, but only short or long (galectin-3) relatively flexible peptides. Thus the galectin CRD acts mainly by itself or together with another galectin CRD. This is in striking contrast to many other types of protein domains,

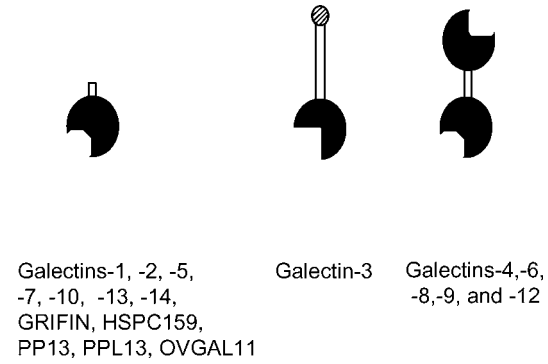

Figure 1. Galectin subunit types [2]. The CRDs are shown filled and other parts of the peptide open or hatched. The mono CRD galectins can occur as monomers or dimers (Figure 2), or in some cases (*e.g.* galectin-3) as higher order oligomers.

e.g. C-type lectin domains, which often occur together with other domain types in the same peptide chain [12].

The mono CRD galectins can occur as monomers, dimers or higher order oligomers depending on specific case and conditions (concentration, presence of ligand). Two different dimer interfaces have been defined, one as found in galectin-1, and another as found in galectin-7 and suggested in galectin-3 (Figure 2). Galectin-3 has a C-terminal CRD associated with

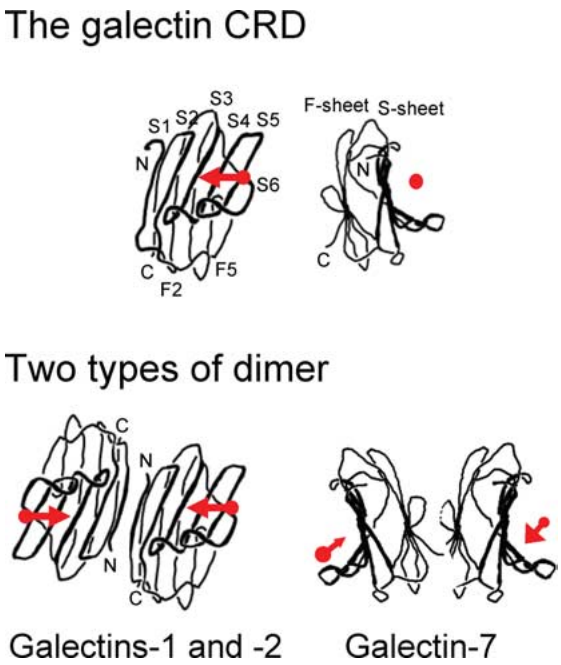

Figure 2. The galectin CRD. The top part shows an artist's view of the β-sandwich based on the structure of the galectin-3 CRD [43]. Two orthogonal views illustrate the relationship between the S- (thicker lines) and F-beta-sheets. Some strands and the N and C-termini are labelled. The bottom. shows two types of dimers represented by the structures of galectins 1 and -2 (left) and -7 (right) [8,42]. Bound disaccharides are shown as gray (online pdf version color: red) arrows pointing from the reducing end to the non-reducing end.

an N-terminal part consisting of 18 conserved amino acids followed by 7-14 repeats, each having 8-11 amino acids that include one aromatic and multiple Pro and Gly. The bi-CRD galectins are bivalent as monomers and might also associate into higher oligomers.

In 1993 Hirabayashi and Kasai proposed the designation of galectin subfamilies as proto-, chimera and tandem repeat types based on their domain organization [33]. These are still valid and often used, but as they were defined based on very few proteins, they should be applied with some caution in light of what is now known. The prototype was defined as similar to dimeric lectins with 14 kDa subunits known at the time, now known as galectin-1, but in current literature all vertebrate mono-CRD galectins (except galectin-3) tend to be included in this group [3]. These are, however, not homogenous regarding mode of oligomerization as the group includes monomers and at least two different types of dimers (Figure 2). They do not segregate as a subfamily from other galectins based on sequence or fine specificity [2,3,34], and they are not prototype in an evolutionary sense [35]. The chimera type was defined as galectins having a CRD and another type of domain, which at the time included proteins with a variety of names all now known to be galectin-3 (identical or orthologues from different species). In vertebrates no other chimera type galectins have been found, and so this is a subgroup with one member and, moreover, it is a matter of definition whether the N-terminal part of galectin-3 is a defined protein domain or a long peptide extension, *e.g*. similar as found in annexin VII [36]. In non-vertebrate species there are a few chimera type galectins, but they can have either one CRD or two CRDs, the latter thus being tandem repeat type also. For the tandem repeat type it is important to recognize that in all cases the two CRDs are as different from each other as they are from other galectins and can have very different fine specificity. Thus the genetic "tandem repeat" occurred a long time ago. In conclusion, the proto-, chimera-, and tandem repeat nomenclature may still be useful with the precautions mentioned above. However, one can with equal convenience and clarity describe galectins as having one or two CRDs (alternatively describe as mono-CRD or bi-CRD) and specify other properties as needed.

Individual galectins

In the galectin nomenclature, mammalian galectins were numbered 1, 2, 3 etc. since it was considered likely that the orthologues in different mammals should be easy to identify and distinguish from paralogues. This has largely turned out to be the case. With the completion of the human, mouse and rat genome sequences the extent of the mammalian galectin family and its variation between species is becoming apparent [2,35,37]. There are currently 13 numbered mammalian galectins (galectins-1-10 and 12-14), and 5 galectin-like proteins (GRIFIN, HSPC159, PP13, PPL13, and OvGal11). The name galectin-11 has been used both for GRIFIN [38] and OvGal11 [39], even if neither has been shown to have carbohydrate binding activity. So the galectin-11 "slot" will have to remain reserved .

Galectins-1, -2, -3, -4, -7, -8, -9 and -12, GRIFIN, and HSPC159 have orthologues in human, mouse, rat and other mammalian species. They are also different enough from each other to indicate that they diverged well before mammals, and can thus safely be regarded as separate mammalian galectins and galectin-like proteins. However, the remaining galectins appear only in a few mammalian species. Galectin-5, a mono-CRD galectin, has been found only in rat and is almost identical to the C-terminal CRD of rat galectin-9; galectin-6, a bi-CRD galectin, is only found in mouse and is almost identical to mouse galectin-4. Human has two additional galectin-9-like genes, one possibly encoding a second galectin-9, and the other the C-terminal CRD alone. Galectin-10, -13, PP13, PPL13 and additional related putative genes have been clearly identified only in human, with no apparent orthologues in mouse and rat. These are instead about 40% identical to OvGal11, a galectin-like protein in sheep stomach [39] and related bovine sequences as discussed in detail by Cooper [2].

Non-mammalian galectins were not numbered in the original galectin nomenclature, as it was too difficult to tell if they were orthologues of a mammalian galectin or not. This is still true for invertebrate galectins, and other numbering systems such as LEC-1, -2. in *C. elegans* [40] have emerged. For vertebrates the identification of orthologues to some mammalian galectins is beginning to be possible with the increasing availability of sequences; galectin-3 in birds, amphibians, and fish [2,35], for example.

How ancient, large and conserved is the galectin family?

With the discovery of more and more galectins in a wider and wider range of species, the galectin family has often been described as large and highly conserved. However, the completed genome sequences now permit a more accurate assessment of its size and conservation. Compared to other protein families, the galectins and galectin like proteins, with about 15 members in one mammalian species, is a moderate size family. There are many much larger families and also smaller ones. The galectin family is ancient as members, or putative members, are present in all animal kingdoms and even in plants and fungi. The degree of conservation can be roughly estimated by comparing the amino acid sequence of a human galectin with its orthologues, if found, in other mammalian, and sometimes in non-mammalian, species. Based on such comparisons most galectin CRDs are, on average, as conserved as a functional protein like hemoglobin (about 80% identity between mouse and man). Galectins-2 and -9 are slightly more variable (about 70% identity, mouse-man), galectins-1 and -3 slightly more conserved (about 87% identity), and HSPC159, as an exception, remarkably conserved (99% identity, as high as for tubulin). A more sophisticated analysis of galectin conservation and evolution is presented by Ogawa *et al*. in this issue [41], and in [35].

Galectin structure and specificity [3,8,42–44]

The galectin CRD is a beta-sandwich of about 135 aa (Figure 2). The two sheets are slightly bent with 6 strands forming the concave side (S1–S6) and 5 strands forming the convex side (F1-F5). The concave side forms a groove in which carbohydrate is bound, and which is long enough to hold about a linear tetrasaccharide. Thus, one can schematically describe the galectin carbohydrate binding site as having four subsites (A-D) as is supported by specificity for small saccharides and has been formally proposed [3,30,45]. It is also useful for the discussion to add a fifth site E even if it is less defined on the structure. In this model subsite C is the defining β-galactoside binding site of the galectins, and subsite D contributes the second part of the conserved core disaccharide-binding site (Figure 3). Here we will give a general overview of galectin binding specificity in terms of these sites, but a detailed description of specificity of different galectins is beyond the scope of this introduction. In this volume papers by Ogawa *et al*. [41], and by Brewer [46] cover interesting cases of galectin structure and specificity.

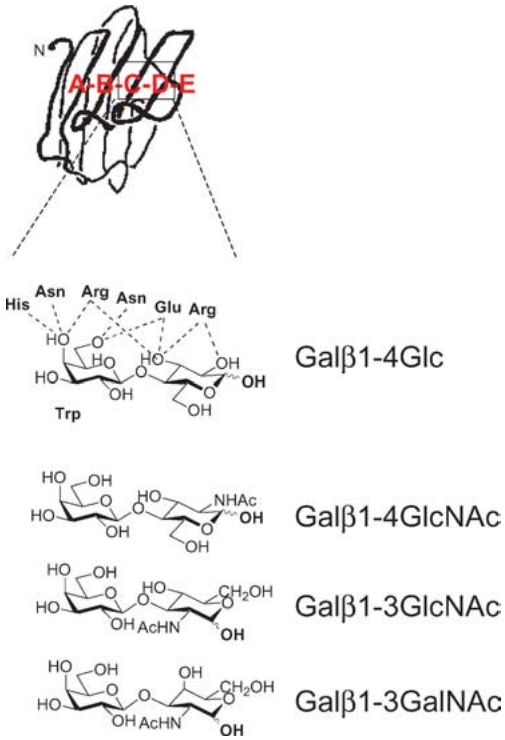

Figure 3. The galectin carbohydrate-binding site. At top is an artist's view of the CRD as in Figure 2 with the four established subsites indicated as A, B, C and D and a more loosely defined proposed site as E (see text). The core binding site C–D is boxed and a close up view with bound lactose (Galβ1-4Glc) and named interacting amino acid side chains is shown in the middle. At the bottom are three common natural disaccharides, which may also bind in the core site (C–D). They present similar structures towards the protein, but with different preference for different galectins. Note that the reducing OH (bold) of the 1–3 disaccharides point to the E-site in a somewhat different direction compared to those of the 1–4 disaccharides (see text).

The binding of a galactose residue in site C is the most conserved feature of galectin binding activities. Six of the seven "motif aa" interact with the Gal (Figure 3). However, binding may be weaker in some galectins (*e.g*. galectin-10) or lacking in galectin-like proteins, having some of the the amino acids in the galectin signature motif replaced.

The binding of a saccharide in site D is the second most conserved feature, but here the structure requirements for interaction are much less, and can thus be fulfilled by different saccharides (Figure 3). Preference for one or the other of these is one source of the variation in specificity between galectins. The pyranose ring is probably required in most cases as saccharides with an open ring in site D binds about 10 fold weaker to galectin-1 [47] or -3 [48].

A second, and major, source for variation in specificity between galectins is their different ability to accommodate saccharides (GlcNAc, Gal, GalNAc, NeuAc) or other groups (*e.g*. sulphate) in subsite B (and further extensions into subsite A). These would be linked to the 3-OH of the Gal in subsite C and may strongly increase or decrease affinity. Fuc at of 2-OH of Gal may also affect binding but less so.

The structurally less defined Site E is included to summarize possible interactions with moieties linked at the reducing end of the saccharide in site D. In cells these would be another saccharide, protein or lipid. Even if the glycosidic bond from D points out into solution, large enough moieties linked here could interact with the galectin CRD outside the binding groove proper. It is also of interest that the type of disaccharide (1-3 or 1-4 linked) in site C–D determines the direction of the glycosidic bond leading into site E (Figure 3), and, thereby, may be an indirect specifier of interactions there, even in cases when the two disaccharide types by themselves bind with similar affinity.

Interactions in site E might help explain some proposed galectin binding activities that are not easily explained based on the properties of the well-defined sites (A-D). Polylactosamines with linearly repeated LacNAc residues have been suggested to be preferred high affinity ligands for galectin-1 [49], but at the same time other studies suggest that galectin-1 binds preferentially terminal LacNAc residues in sites C and D with A and B empty [46,50]. Then the next LacNAc residue would be in site E, which might explain the higher affinity. In another case, the GM1-ganglioside saccharide (Galβ1-3GalNAcβ1-4(NeuAcα2-3)Galβ1-4Glc) has been proposed as a high affinity ligand for galectins-1, -3 and -7 [51]. However, only the terminal Galβ1-3GalNAc of this saccharide would be able to bind, albeit weakly, to the core site C-D, as the GalNAcβ1-4 would block binding of the internal Galβ1-4Glc there [30]. The latter could instead, then, project into site E where additional interactions with it and the NeuAc might occur (see also note added in proof). In both cases differences in techniques used in the affinity measurements may also be a confounding factor.

A few reports have suggested that some galectins bind mannose [52,53]. Although further studies are needed, this opens the possibility that galectins with weak galactoside binding activity (*e.g.* galectin-10) and galectin-like proteins may in fact have other specificities.

The relationships between CRD fine specificity and galectin biological activities remain largely undefined (however see [54]). Moreover, although various modes of oligomerization and consequent ligand cross linking abilities appear to be an essential part of some galectin activities, the details remain largely undefined [5]. The interplay of the two domains of a bi-CRD galectin, each of which possess a different specificity, is beginning to be explored [55]. Galectins most likely bind a range of different receptors at a cell surface at the same time, yet they induce seemingly specific signaling effects. How specific are galectins at the cell surface? How many ligands do they bind at the same time? What is the relationship between receptor subset bound, and their fine specificity and/or quartenary structure? What are the biological consequences? What is the role of the galectin carbohydrate binding site intracellularly?

Other binding sites and structures

The amino acids in the C and D subsites of the galectin CRD (Figure 3), are the only residues higly conserved among the family as a whole. However, other parts of the CRD may be conserved between species for one particular galectin and have other interesting binding activities, *e.g.* act as dimer interfaces (Figure 2), or induce non-carbohydrate dependent regulatory effects on cells [56].

There is also evidence that under certain circumstances the galectin CRD may adopt alternative folds with other activities [57] and even, surprisingly, act as a membrane transporter and perhaps refold into a transmembrane protein [58].

Intra- and extracellular activities of galectins and non-classical secretion

Galectins have features typical of cytosolic proteins. They are synthesized on cytosolic ribosomes, have no-signal peptide, and have acetylated N-termini. Galectin-3 can be phosphorylated, but no other post-translational modifications have been established with certainty for galectins. From the cytosol, galectins can be targeted to the nucleus or other subcellular sites, as well as secreted by non-classical (non-ER-Golgi) pathways.

Galectins display an intriguing combination of intra- and extracellular activities [59–62]. Even the same galectin, *e.g.* galectin-3, can have activities ranging from regulation of RNA-splicing in the nucleus [60] to regulation of cell adhesion and signalling outside cells [59]. This striking and surprising finding is one of the major galectin mysteries. Are these activities connected or separate?

Non-classical secretion, which is a possible link between the intra- and extracellular activities, is a characteristic feature of galectins not shared by most other lectins. The mechanism remains unknown but possibilities have been reviewed for galectins [63] and for other proteins also secreted this way [64]. The realization that certain proteins, traditionally thought of as nuclear factors, can both enter and exit cells by non-classical mechanisms and mediate a novel way of intercellular communication is an exciting emerging area [65].

Galectin function *in vivo*

Based on activities in tissue culture, various biological roles of galectins in the whole organism have been proposed, for example in regulation of immunity and inflammation [55,66–69], progression of cancer [70–73], and in specific developmental processes [74,75]. Experiments in animals have supported some of these.

Mice deficient in galectins-1 and -3 have been generated and analyzed in some detail, and a review has recently been published [76]. In summary, galectin-1 and -3 are dispensable for survival and fertilization under animal house conditions. They do not substitute for each other, as the double-null mutant mice are equally viable. However, clear but more subtle and transient defects have been found after careful analysis, and more will probably be found.

The galectin-1 null mice are deficient in the development of a subset of olfactory neurons and transiently in muscle development [75]. Notably defects in the immune system as expected from the effects of galectin-1 on immune cells in culture, *e.g.* induction of apoptosis [38,67,68], were not found in the galectin-1 null mice [76].

The galectin-3 null mutant mice show a defect in bone development, some effects on survival or maintenance of neutrophils and macrophages at an inflammatory site [76], and delayed phagocytosis [77].

So far, there is no indication for functional redundancy amongst galectins *in vivo* (at least galectin 1 and galectin 3, as mentioned above). This is consistent with their specificity in tissue distribution and in subcellular localization, as well as with their different biochemical properties. That double galectins-1 and -3 mutants are alive suggests that these galectins may be "optimising molecules" (not required for absolute function but for most efficient function, [76]) rather than key players in developmental processes. Thereby they could fulfil functions that are clearly dispensable for an individual in animal house conditions, but may well be detrimental for the population in the long run.

The above does not rule out the possibility, worth exploring further, that galectins can have decisive rate limiting functions in pathological conditions, *e.g.* cancer and inflammation as indicated in many studies, and, thus, galectin inhibitors or galectins themselves may find therapeutic use in the future [67,72,78–81].

Acknowledgments

The research was funded by the Swedish Research Council (grant 12165) and Swedish Funds for Health-care system—Academic Science integration (ALF). We thank Professor James Rini, University of Toronto, for valuable comments and input.

Note added in proof

Recently an NMR and modelling analysis supporting the proposed type of interaction between galectin-1 and GM1 ganglioside was published [82].

References

1 Heaney S, The golden bough (translation from Virgil's Aeneid). In *Seeing Things* (Faber and Faber, London, 1991), p. 1.
2 Cooper DN, Galectinomics: Finding themes in complexity, *Biochim Biophys Acta* **1572**, 209–31 (2002).
3 Hirabayashi J, Hashidate T, Arata Y, Nishi N, Nakamura T, Hirashima M, Urashima T, Oka T, Futai M, Muller WE, Yagi F, Kasai K, Oligosaccharide specificity of galectins: A search by frontal affinity chromatography, *Biochim Biophys Acta* **1572**, 232–54 (2002).
4 Danguy A, Camby I, Kiss R, Galectins and cancer, *Biochim Biophys Acta* **1572**, 285–93 (2002).
5 Fred Brewer C, Binding and cross-linking properties of galectins, *Biochim Biophys Acta* **1572**, 255–62 (2002).
6 Kilpatrick DC, Animal lectins: A historical introduction and overview, *Biochim Biophys Acta* **1572**, 187–97 (2002).
7 Liu FT, Patterson RJ, Wang JL, Intracellular functions of galectins, *Biochim Biophys Acta* **1572**, 263–73 (2002).
8 Loris R, Principles of structures of animal and plant lectins, *Biochim Biophys Acta* **1572**, 198–208 (2002).
9 Rabinovich GA, Rubinstein N, Toscano MA, Role of galectins in inflammatory and immunomodulatory processes, *Biochim Biophys Acta* **1572**, 274–84 (2002).
10 Barondes SH, Castronovo V, Cooper DN, Cummings RD, Drickamer K, Feizi T, Gitt MA, Hirabayashi J, Hughes C, Kasai K, *et al*., Galectins: A family of animal beta-galactoside-binding lectins, *Cell* **76**, 597–8 (1994).
11 Drickamer K, Two distinct classes of carbohydrate-recognition domains in animal lectins, *J Biol Chem* **263**, 9557–60 (1988).
12 Dodd RB, Drickamer K, Lectin-like proteins in model organisms: Implications for evolution of carbohydrate-binding activity, *Glycobiology* **11**, 71R-9R (2001).
13 Barondes SH, Galectins, a personal overview, *Trends Glycosci Glycotech* **9**, 1–7 (1997).
14 Nowak TP, Haywood PL, Barondes SH, Developmentally regulated lectin in embryonic chick muscle and a myogenic cell line, *Biochem Biophys Res Commun* **68**, 650–7 (1976).
15 Teichberg VI, Silman I, Beitsch DD, Resheff G, A beta-D-galactoside binding protein from electric organ tissue of Electrophorus electricus, *Proc Natl Acad Sci USA* **72**, 1383–7 (1975).
16 Beyer EC, Zweig SE, Barondes SH, Two lactose binding lectins from chicken tissues. Purified lectin from intestine is different from those in liver and muscle, *J Biol Chem* **255**, 4236–9 (1980).
17 Beyer EC, Barondes SH, Quantitation of two endogenous lactose-inhibitable lectins in embryonic and adult chicken tissues, *J Cell Biol* **92**, 23–7 (1982).
18 Beyer EC, Barondes SH, Secretion of endogenous lectin by chicken intestinal goblet cells, *J Cell Biol* **92**, 28–33 (1982).
19 Oda Y, Kasai K, Purification and characterization of beta-galactoside-binding lectin from chick embryonic skin, *Biochim Biophys Acta* **761**, 237–45 (1983).
20 Briles EB, Gregory W, Fletcher P, Kornfeld S, Vertebrate lectins comparison of properties of beta-galactoside-binding lectins from tissues of calf and chicken, *J Cell Biol* **81**, 528–37 (1979).
21 de Waard A, Hickman S, Kornfeld S, Isolation and properties of beta-galactoside binding lectins of calf heart and lung, *J Biol Chem* **251**, 7581–7 (1976).
22 Harrison FL, Beswick TM, Chesterton CJ, Separation of haemopoietic cells for biochemical investigation. Preparation of erythroid and myeloid cells from human and laboratory-animal bone marrow and the separation of erythroblasts according to their state of maturation, *Biochem J* **194**, 789–96 (1981).
23 Raz A, Lotan R, Lectin-like activities associated with human and murine neoplastic cells, *Cancer Res* **41**, 3642–7 (1981).
24 Raz A, Lotan R, Endogenous galactoside-binding lectins: A new class of functional tumor cell surface molecules related to metastasis, *Cancer Metastasis Rev* **6**, 433–52 (1987).
25 Gabius HJ, Vehmeyer K, Engelhardt R, Nagel GA, Cramer F, Carbohydrate—binding proteins of tumor lines with different growth properties. I. Differences in their pattern for three clones of rat fibroblasts transformed with a myeloproliferative sarcoma virus, *Cell Tissue Res* **241**, 9–15 (1985).
26 Gabius HJ, Engelhardt R, Cramer F, Batge R, Nagel GA, Pattern of endogenous lectins in a human epithelial tumor, *Cancer Res* **45**, 253–7 (1985).
27 Moutsatsos IK, Davis JM, Wang JL, Endogenous lectins from cultured cells: Subcellular localization of carbohydrate-binding protein 35 in 3T3 fibroblasts, *J Cell Biol* **102**, 477–83 (1986).
28 Roff CF, Wang JL, Endogenous lectins from cultured cells. Isolation and characterization of carbohydrate-binding proteins from 3T3 fibroblasts, *J Biol Chem* **258**, 10657–63 (1983).
29 Leffler H, Masiarz FR, Barondes SH, Soluble lactose-binding vertebrate lectins: A growing family, *Biochemistry* **28**, 9222–9 (1989).
30 Leffler H, Barondes SH, Specificity of binding of three soluble rat lung lectins to substituted and unsubstituted mammalian beta-galactosides, *J Biol Chem* **261**, 10119–26 (1986).
31 Cerra RF, Gitt MA, Barondes SH, Three soluble rat beta-galactoside-binding lectins, *J Biol Chem* **260**, 10474–7 (1985).
32 Barondes SH, Soluble lectins: A new class of extracellular proteins, *Science* **223**, 1259–64 (1984).
33 Hirabayashi J, Kasai K, The family of metazoan metal-independent beta-galactoside-binding lectins: Structure, function and molecular evolution, *Glycobiology* **3**, 297–304 (1993).
34 Oda Y, Herrmann J, Gitt MA, Turck CW, Burlingame AL, Barondes SH, Leffler H, Soluble lactose-binding lectin from rat intestine with two different carbohydrate-binding domains in the same peptide chain, *J Biol Chem* **268**, 5929–39 (1993).
35 Houzelstein D, Goncalves I, Sidhu SS, Drickamer K, Cooper D, Leffler H, Poirier, F, Phylogenetic analysis of the galectin family, *Mol Biol Evol*, in press (2004).

36 Satoh H, Nakano Y, Shibata H, Maki M, The penta-EF-hand domain of ALG-2 interacts with amino-terminal domains of both annexin VII and annexin XI in a Ca^{2+}-dependent manner, *Biochim Biophys Acta* **1600**, 61–7 (2002).

37 Chiariotti L, Salvatore P, Frunzio R, Bruni CB, Galectin genes: Regulation of expression, *Glycoconjugate Journal* **19**, 441–449 (2004).

38 Rabinovich GA, Baum LG, Tinari N, Paganelli R, Natoli C, Liu FT, Iacobelli S, Galectins and their ligands: Amplifiers, silencers or tuners of the inflammatory response?, *Trends Immunol* **23**, 313–20 (2002).

39 Young AR, Meeusen EN, Galectins in parasite infection and allergic inflammation, *Glycoconjugate Journal* **19**, 601–606 (2004).

40 Hirabayashi J, Arata Y, Kasai K, Glycome project: Concept, strategy and preliminary application to Caenorhabditis elegans, *Proteomics* **1**, 295–303 (2001).

41 Ogawa T, Shirai T, Shionyu-Mitsuyama C, Yamane T, Kamiya H, Muramoto K, The speciation of conger eel galectins by rapid adaptive evolution, *Glycoconjugate Journal* **19**, 451–458 (2004).

42 Rini JM, Lobsanov YD, New animal lectin structures, *Curr Opin Struct Biol* **9**, 578–84 (1999).

43 Seetharaman J, Kanigsberg A, Slaaby R, Leffler H, Barondes SH, Rini JM, X-ray crystal structure of the human galectin-3 carbohydrate recognition domain at 2.1-A resolution, *J Biol Chem* **273**, 13047–52 (1998).

44 Barondes SH, Cooper DN, Gitt MA, Leffler H, Galectins. Structure and function of a large family of animal lectins, *J Biol Chem* **269**, 20807–10 (1994).

45 Knibbs RN, Agrwal N, Wang JL, Goldstein IJ, Carbohydrate-binding protein 35. II. Analysis of the interaction of the recombinant polypeptide with saccharides, *J Biol Chem* **268**, 14940–7 (1993).

46 Brewer CF, Thermodynamic binding studies of galectin-1, -3, and -7, *Glycoconjugate Journal* **19**, 459–465 (2004).

47 Lee RT, Ichikawa Y, Allen HJ, Lee YC, Binding characteristics of galactoside-binding lectin (galaptin) from human spleen, *J Biol Chem* **265**, 7864–71 (1990).

48 Öberg CT, Carlsson S, Fillion E, Leffler H, Nilsson UJ, Efficient and expedient two-step pyranose-retaing fluoerescein-conjugation of complex reducing oligosaccahrides: Galectin oligosaccahride specificity studies in a fluorescnce polarization assay, *Bioconjugate Chemistry* **14**, 1289–1297 (2003).

49 Zhou Q, Cummings RD, L-14 lectin recognition of laminin and its promotion of in vitro cell adhesion, *Arch Biochem Biophys* **300**, 6–17 (1993).

50 Di Virgilio S, Glushka J, Moremen K, Pierce M, Enzymatic synthesis of natural and 13C enriched linear poly-N-acetyllactosamines as ligands for galectin-1, *Glycobiology* **9**, 353–64 (1999).

51 Kopitz J, Andre S, von Reitzenstein C, Versluis K, Kaltner H, Pieters RJ, Wasano K, Kuwabara I, Liu FT, Cantz M, Heck AJ, Gabius HJ, Homodimeric galectin-7 (p53-induced gene 1) is a negative growth regulator for human neuroblastoma cells, *Oncogene* **22**, 6277–88 (2003).

52 Swaminathan GJ, Leonidas DD, Savage MP, Ackerman SJ, Acharya KR, Selective recognition of mannose by the human eosinophil Charcot-Leyden crystal protein (galectin-10): A crystallographic study at 1.8 A resolution, *Biochemistry* **38**, 13837–43 (1999).

53 Fradin C, Poulain D, Jouault T, Beta-1,2-linked oligomannosides from Candida albicans bind to a 32-kilodalton macrophage membrane protein homologous to the mammalian lectin galectin-3, *Infect Immun* **68**, 4391–8 (2000).

54 Amano M, Galvan M, He J, Baum LG, The ST6Gal I sialyltransferase selectively modifies N-glycans on CD45 to negatively regulate galectin-1-induced CD45 clustering, phosphatase modulation, and T cell death, *J Biol Chem* **278**, 7469–75 (2003).

55 Hirashima M, Kashio Y, Nishi N, Yamauchi A, Imaizumi T-a, Kageshita T, Saita N, Nakamura T, Galectin-9 in physiological and pathological conditions, *Glycoconjugate Journal* **19**, 593–600 (2004).

56 Scott K, Weinberg C, Galectin -1: A bifunctional regulator of cellular proliferation, *Glycoconjugate Journal* **19**, 467–477 (2004).

57 Horie H, Kadoya T, Galectin-1 plays essential roles in adult mammalian nervous tissues. Roles of oxidized galectin-1., *Glycoconjugate Journal* **19**, 479–489 (2004).

58 Lipkowitz M, Leal-Pinto E, Cohen BE, Abramson RG, Galectin 9 is the sugar-regulated urate transporter/channel UAT, *Glycoconjugate Journal* **19**, 491–498 (2004).

59 Ochieng J, Furtak V, Lukyanov P, Extracellular functions of galectin-3, *Glycoconjugate Journal* **19**, 527–535 (2004).

60 Patterson RJ, Wang W, Wang JL, Understanding the biochemical activities of galectin-1 and galectin-3 in the nucleus, *Glycoconjugate Journal* **19**, 499–506 (2004).

61 Zick Y, Eisenstein M, Goren RA, Hadari YR, Levy Y, Ronen D, Role of galectin-8 as a modulator of cell adhesion and cell growth, *Glycoconjugate Journal* **19**, 517–526 (2004).

62 Hsu DK, Liu FT, Regulation of cellular homeostasis by galectins, *Glycoconjugate Journal* **19**, 507–515 (2004).

63 Hughes RC, Secretion of the galectin family of mammalian carbohydrate-binding proteins, *Biochim Biophys Acta* **1473**, 172–85 (1999).

64 Nickel W, The mystery of nonclassical protein secretion. A current view on cargo proteins and potential export routes, *Eur J Biochem* **270**, 2109–19 (2003).

65 Maizel A, Tassetto M, Filhol O, Cochet C, Prochiantz A, Joliot A, Engrailed homeoprotein secretion is a regulated process, *Development* **129**, 3545–53 (2002).

66 Sato S, Nieminen J, Seeing strangers or announcing "danger": Galectin-3 in two models of innate immunity, *Glycoconjugate Journal* **19**, 583–591 (2004).

67 Rabinovitch G, Toscano MA, Ilarregui JM, Rubinstein N, Shedding light on the immunomodulatory properties of galectins:novel regulators of innate and adaptive immune responses, *Glycoconjugate Journal* **19**, 565–573 (2004).

68 Pace KE, Baum LG, Insect galectins: Roles in immunity and development, *Glycoconjugate Journal* **19**, 607–614 (2004).

69 Almkvist J, Karlsson A, Galectins as inflammatory mediators, *Glycoconjugate Journal* **19**, 575–581 (2004).

70 Grassadonia A, Tinari N, Iurisci I, Piccolo E, Cumashi A, Innominato P, D'Egidio M, Natoli C, Piantelli M, Iacobelli S, 90K (Mac-2 BP) and galectins in tumor progression and metastasis, *Glycoconjugate Journal* **19**, 551–556 (2004).

71 Bidon-Wagner N, Le Pennec, J-P, Human galectin-8 isoforms and cancer, *Glycoconjugate Journal* **19**, 557–563 (2004).

72 Takenaka Y, Fukumori T, Raz A, Galectin-3 and metastasis, *Glycoconjugate Journal* **19**, 543–549 (2004).

73 van den Brule F, Califice S, Castronovo V, Expression of galectins in cancer: A critical review, *Glycoconjugate Journal* **19**, 537–542 (2004).
74 Hughes RC, Galectins in kidney development, *Glycoconjugate Journal* **19**, 621–629 (2004).
75 Watt D, Jones GE, Goldring K, The involvement of galectin-1 in skeletal muscle determination, differentiation and regeneration, *Glycoconjugate Journal* **19**, 615–619 (2004).
76 Poirier F, Roles of galectins *in vivo*, *Biochem Soc Symp* 95–103 (2002).
77 Sano H, Hsu DK, Apgar JR, Yu L, Sharma BB, Kuwabara I, Izui S, Liu FT, Critical role of galectin-3 in phagocytosis by macrophages, *J Clin Invest* **112**, 389–97 (2003).
78 John CM, Leffler H, Kahl-Knutsson B, Svensson I, Jarvis GA, Truncated galectin-3 inhibits tumor growth and metastasis in orthotopic nude mouse model of human breast cancer, *Clin Cancer Res* **9**, 2374–83 (2003).
79 Andre S, Pieters RJ, Vrasidas I, Kaltner H, Kuwabara I, Liu FT, Liskamp RM, Gabius HJ, Wedgelike glycodendrimers as inhibitors of binding of mammalian galectins to glycoproteins, lactose maxiclusters, and cell surface glycoconjugates, *Chembiochem* **2**, 822–30 (2001).
80 Glinsky VV, Glinsky GV, Rittenhouse-Olson K, Huflejt ME, Glinskii OV, Deutscher SL, Quinn TP, The role of Thomsen-Friedenreich antigen in adhesion of human breast and prostate cancer cells to the endothelium, *Cancer Res* **61**, 4851–7 (2001).
81 Sorme P, Qian Y, Nyholm PG, Leffler H, Nilsson UJ, Low micromolar inhibitors of galectin-3 based on 3'-derivatization of N-acetyllactosamine, *Chembiochem* **3**, 183–9 (2002).
82 Siebert HC, Andre S, Lu S-J, Frank M, Kaltner H, van Kuik JA, Korchagina EY, Bovin N, Tajkhorshid E, Kaptein R, Vliegenthart JFG, von der Lieth CW, Jiménez-Barbero J, Kopitz J and Gabius H-J, Unique conformer selection of human growth-regulatory lectin galectin-1 for ganglioside GM1 *versus* bacterial toxins, *Biochemistry* **42**, 14762–73 (2003).