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Background

The term lectin was derived from a Latin word 'legere' means 'to choose' to describe a class of proteins of plant origin which agglutinates cells and exhibit antibody like sugar binding specificity (Boyd and Shapleigh, 1954). The saga began in 1886 with Weir Mitchell’s observation of agglutinating and lytic activity of snake venoms (Kilpatrick, 2002). Two years later, Peter Hermann Stillmark isolated a highly toxic hemagglutinin (ricin) from seeds of *Ricinus communis* (Boyd and Shapleigh, 1954). Flexner and Noguchi (1902) discovered first animal lectin when they reported the agglutinating and lytic activity of snake venoms. An early plant lectin to be isolated was ‘abrin’ (Tschrich, 1925). The first mammalian lectin was reported from rabbit liver. This discovery was a major milestone in the history of lectin research in animal kingdom. Since then, lectins have been isolated and characterized from a variety of novel animal sources expressed in a wide array of different tissues including, heart and lungs (Waard et al., 1976), muscle (Nowak et al., 1977), brain (Ola et al., 2001; Shahwan et al., 2004), pancreas (Beyer et al., 1979), electric organ of electric eel (Levi and Teichberg, 1981), colon (Schoeppner, 1995), intestines (Tardy et al., 1995), retina (Uehara et al., 2001), skin (Marshal et al., 1992), serum (Colley et al., 1988), oocytes (Perillo et al., 1998), placenta (Than et al., 2004), uterus (Gray et al., 2004) etc. These lectins are dimers with subunit molecular weights of 14 kDa, requires reducing agents for their activity and bear specificity for β-galactosides.

Classification of animal lectins

Based on the primary structure of animal lectins, Drickamer (1988) classified them into two structural families: the C-type lectins (requiring Ca\(^{2+}\) for activity) and S-type lectins (sulfydryl-dependent or β-galactoside binding). Although, C-type and S-type domains are completely unrelated to each other, both families possess a conserved CRD of approximately 120 amino acid residues. The few exceptions known are the members of a heterogeneous group referred to as N-type lectins (Kilpatrick, 2002). Gradual advancements in the research have vastly enhanced the structural and functional information on lectins, thus making it possible to list at least fifteen more families of animal lectins (Table 1). Of these, five major classes include C-type lectins, P-type lectins, I-type lectins, pentraxins and S-type lectins.
### Table 1
Different families of animal lectin with known three dimensional structures

<table>
<thead>
<tr>
<th>Family</th>
<th>Carbohydrate specificity</th>
<th>Fold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galectins</td>
<td>Strict galactose/lactose</td>
<td>β-sandwich</td>
</tr>
<tr>
<td>Pentraxins</td>
<td>Variable, often non-carbohydrates</td>
<td>β-sandwich</td>
</tr>
<tr>
<td>C-type lectins</td>
<td>Highly variable</td>
<td>C-type lectin</td>
</tr>
<tr>
<td>I-type lectins</td>
<td>Sialic acid</td>
<td>Immunoglobulin</td>
</tr>
<tr>
<td>P-type lectins</td>
<td>Mannose-6-phosphate</td>
<td>M6P-β-sandwich</td>
</tr>
<tr>
<td>Cys-MR and FGF2</td>
<td>Sulfated carbohydrates</td>
<td>β-trefoil</td>
</tr>
<tr>
<td>ERGIC-53</td>
<td>Mannose</td>
<td>β-sandwich</td>
</tr>
<tr>
<td>Ym1</td>
<td>Heparin/heparin sulfate</td>
<td>Chitinase</td>
</tr>
<tr>
<td>HGF/SF(NK1)</td>
<td>Heparin/heparin sulfate</td>
<td>Unique</td>
</tr>
<tr>
<td>Lectin from spider toxin</td>
<td>Heparin</td>
<td>Unique</td>
</tr>
<tr>
<td>Cobra venom cardiotoxin</td>
<td>Mannosamine</td>
<td>Hevein</td>
</tr>
<tr>
<td>Spermadesins</td>
<td>Heparin, often non-carbohydrate</td>
<td>CUB</td>
</tr>
<tr>
<td>TNF</td>
<td>Chitobiose</td>
<td>TNF-β-sandwich</td>
</tr>
<tr>
<td>Calnexins</td>
<td>Glucose</td>
<td>β-sandwich</td>
</tr>
<tr>
<td>Tachylectin</td>
<td>GlcNAc/GalNAc</td>
<td>Five-bladed-β-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>propeller</td>
</tr>
</tbody>
</table>
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C-type lectins

C-type lectins are the most diverse members of animal lectins and are generally multi-domain proteins, in which C-type homologous CRDs provide Ca\(^{2+}\) dependent sugar recognition activity. A variety of other modules initiates a broad range of biological activities such as cell adhesion, endocytosis and pathogen neutralization. The sugar binding sites in vertebrate C-type CRDs are formed in part by a bound Ca\(^{2+}\), which must be present for sugar binding (Dodd and Drickamer, 2001). The majority of C-type lectins bind either to D-mannose, D-glucose and related sugars (mannose type ligands) or to D-galactose and its derivatives (galactose type ligands) (Kolatkar and Weis, 1996). C-type lectins with high affinity for glycoconjugates bearing terminal galactose residues have been identified on the surfaces of Kupffer cells and peritoneal macrophages and appear to mediate tumor cell recognition (Sato et al., 1992). However, C-type lectins with lower affinity for galactose type ligands are found in proteoglycan core proteins of cartilage and other tissues and are presumed to contribute to the organization of the extracellular matrix (Drickamer and Taylor, 1993). The genuine C-type lectins are more varied than it has been thought to be, and depending upon gene structure and the nature of additional non-lectin domain they have been classified into various subgroups like hyalectans, collectins, selectins, asialoglycoprotein receptors, transmembrane receptors, macrophage mannose receptor and single domain lectins (Kilpatrick, 2002). A wide range of functions are performed by these lectins, including catabolism of partially degraded glycoproteins containing terminal galactose or N-acetylgalactosamine residues (Schwartz, 1984), clearance of galactose terminal glycoproteins, desialyted erythrocytes and other blood cells (Sharon and Lis, 1989), chronic rejection of transplanted tissues (Russell et al., 1994) and cell-cell interaction during spermatogenesis and fertilization (Goluboff et al., 1995; Crottet et al., 1996).

P-type lectins (phospho-mannosyl receptors)

P-type lectins are mannose-6-phosphate (M-6-P) recognizing, calcium independent group of lectins which have unique repeating motifs in their CRD (Dahms, 1996). These lectins play an essential role in the generation of functional lysosomes within higher eukaryotic cells by directing newly synthesized lysozomal enzymes bearing the M-6-P signal to lysosomes (Dahms and Hancock, 2002).
The members of the P-type lectin family, the cation-dependent M-6-P receptor (CD-MPR) and the insulin-like growth factor II (IGF-II)/MPR are distinguished from all other lectins by their ability to recognize phosphorylated mannose residues (Drickamer and Taylor, 1993). The reported sub-cellular distribution of P-type lectins varies among cell types with about 10% of the MPRs present at the cell surface while the remainder are found predominantly in late endosomal compartments and trans Golgi network (Waguri et al., 2001). The ability of the IGF-II/MPR to recognize functionally distinct ligands illustrates the multifunctional nature of this receptor, its involvement in a myriad of important physiological pathways and its crucial role in mammalian growth and heart development by influencing fetal cell division and differentiation (O’dell and Day, 1998). Over-expression of IGF-II is observed in various human cancers (Ellis et al., 1998), overgrowth syndromes (Sperandeo et al., 2000) and development of atherosclerosis (Zaina et al., 2002), thus suggesting its role in various pathological conditions.

I-type lectins

I-type lectin is a collective term introduced by Powell and Varki (1995) to describe carbohydrate recognizing proteins that belong to the immunoglobulin (Ig) super family. I-type proteins recognize sialic acids, glycosaminoglycans and other sugars. According to their specificity and binding affinities, these lectins have been classified into many subclasses, like Siglecs, CD-83 (Scholler et al., 2001) and cell adhesion molecule L1 (Kleene et al., 2001).

Siglecs (sialic acid binding immunoglobulin super family lectins) are I-type lectins recognizing sialic acids. Each Siglec has a distinct expression pattern in different cell types, indicating that they perform highly specific functions including critical roles in neural development, such as neural cell adhesion, positive and/or negative regulation of neurite outgrowth and myelin sheath formation (Filbin, 1995). CD83 is a 45 kDa glycoprotein expressed on mature dendritic cells, thus suggesting its involvement in the interaction between dendritic cells and circulating monocytes as well as activated and/or stressed T cells (Zhou and Tedder, 1995). Cell adhesion molecule L1 is a 200 kDa homophilic and heterophilic adhesion molecule expressed in the nervous system, CD4+ T cells, monocytes and B cells, where it plays an important role in axon guidance, cell migration and neurogenesis ((Ebeling et al., 1996; Angata et al., 2002).
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I-type lectins bearing specificity towards sugars other than sialic acids includes neural cell adhesion molecules (NCAM), myelin protein zero (MP0), intracellular adhesion molecules (ICAM-1/CD54), CD2 and hemolin. They are expressed in a wide array of tissues including nervous system, vascular endothelium, and certain lymphocytes and monocytes, where they are involved in T cell development and myelination of peripheral neurons (Warner et al., 1996), inflammation and wound healing (Angata et al., 2002). I-type lectins interacting with sulfated glycosaminoglycans (GAGs) shows selectivity towards the type of GAGs they interact favorably with heparin/heparan sulfate. This category includes vertebrate fibroblast growth factor receptors (FGFR), perlecan (heparan sulfate proteoglycan-2/HSPG2) and CD48, and are essential for mammalian embryonic development (Arikawa et al., 2001) and CD4+ T cell activation (Hopf et al., 2001).

Pentraxins

The term ‘pentraxins’ was applied to C-reactive proteins (CRP) and its homologue, serum amyloid P component (SAP) to reflect their unusual quaternary structure in which five identical polypeptide subunits combine to form a ring with a central hole, loosely resembling a doughnut. These proteins are complement activating molecules with high affinity for phosphorylcholine. However, CRP has also been found to bind galactans and galactose phosphate through a different binding site. Pentraxins are expressed in vertebrate species, where the division into CRP or SAP is not made on the basis of primary structural homology to the human examples, but rather on a preference for phosphorylcholine (CRP) or phosphaethanolamine (SAP) (Kilpatrick, 2002).

S-type lectins

S-type lectins refer to soluble vertebrate tissue lectins which require thiol reducing agents to maintain their activity. However, this property is not shared by S-type lectins, but specificity towards β-galactosides is a common characteristic among this class of lectins. Therefore, a consensus was reached that the term ‘β galactoside binding lectin’ or ‘galectin’ would be more apt for the nomenclature of these specific proteins.
Galectins

Galectin or β-galactoside binding lectin was first discovered in a popular slime mould species *Dictyostelium discoideum* during the study of eukaryotic development and differentiation in 1973. This agglutinin, first named as discoidin and then discoidin-1, was inhibited by lactose, galactose and other related saccharides and exhibited a 400-fold increase in specific activity during aggregation of amoebae, and was originally thought to mediate the adhesive contact between the cells. Within few years, several rather similar lectins were discovered in related species (Barondes, 1986).

All the isolated lectins were readily inhibited by lactose, but differed in fine specificity, including relative affinities for galactose and N-acetylgalactosamine. An apparently similar lactose specific lectin was then reported from electric organ of the electric eel (Teichberg et al., 1975). The discovery of electrolectin prompted the researchers to survey various tissues of higher animals for lactose specific agglutinins. Such activity was found to be very widely distributed among numerous rat organs, but the highest specific activity was found in pectoral muscle of chicken embryos. All these lectins were dimers exhibiting subunit molecular weights around 15 kDa with the exception for intestine lectin (14 kDa) which behaved as a monomer and had a somewhat different carbohydrate binding site.

Later, soluble lectins were isolated from several mammalian tissues including human heart and muscle (Child and Feizi, 1979) as well as rabbit bone marrow (Harrison et al., 1984). Thus, it was apparent that these ‘soluble lectins’, ‘endogenous lectins’, ‘galaptins’ or ‘β-galactoside binding proteins’ occurred universally in vertebrate tissue (Harrison, 1991). Since, many of the vertebrate lectins are structurally homologous; the term ‘galectin’ is now preferred to denote members of this family (Kilpatrick, 2002).

The research work on galectins is now no more limited to purification and characterization, but has rather spread to wide arrays like artificial synthesis of galectins using recombinant DNA techniques (Leffler et al., 2004; Ahmed and Vasta, 2008; Nagae et al., 2008). Studies on galectins are mainly related to their functional roles and their structural changes upon binding to sugar residues (Patnaik et al., 2006; Mok et al., 2007).
Analysis of Gene Bank databases has led to the identification of more galectin like proteins in mammals, invertebrates, plants and microorganisms, confirming that these carbohydrate binding proteins are highly conserved throughout the evolution (Cooper, 2002; Leffler et al., 2004). Almost all of them appear in human genomic DNA and their mRNA is also expressed, suggesting that they are not pseudogenes (Cooper and Barondes, 1999; Leffler et al., 2004). As sequence databases continue to explode, many more galectin relatives will be discovered and fill the gaps in the galectin family tree. In fact, soon, genomics and proteomics may advance to the point that specific interactions of galectin subunits with each other, carbohydrate ligands, or other proteins could be accurately predicted from sequence alone. Most galectins have been proposed to exert discrete biologic effects, according to their sub-cellular compartmentalization, developmentally regulated expression and cell activation status (Zuniga et al., 2001). The wealth of new information promises a future scenario in which galectins or their antagonists will be targeted in respect to their fine structure and detailed mechanism of crucial roles played by them in every aspect of living system can be understood.

Classification of galectins

All galectins contain conserved CRDs that are responsible for carbohydrate binding. Based on differences in the sequence homology of CRDs, 15 different mammalian galectins have been identified till date (Ahmad et al., 2004; Ahmed and Vasta, 2008; Nagae et al., 2008). The characteristics and functions of all the 15 mammalian galectins are shown in Table 2. A schematic representation of the overall structures of Gal-1, -2, -3 and -4 is shown in Fig. 2.

Galectin-1

Galectin-1 (Gal-1) is a homodimer protein composed of two non-covalently linked 14 kDa subunits with one CRD of 134 amino acids (Kiss et al., 2007). Since each dimeric molecule possesses two galactoside binding sites, Gal-1 can mediate either intra-molecular or inter-molecular cross-linking by binding to more than one sugar residue (Gabius et al., 2002; Kilpatrick, 2002; Imbe et al., 2003). This protein is expressed on the cell surface, in extracellular matrix, in the cytoplasm and the nucleus of the cells in various tissues including skin, muscle, lymph node, dorsal root ganglion, thymus, lung, spleen and placenta (Imbe et al., 2003; Leffler et al., 2004).
### Table 2

**Characteristics and functions of galectins**

<table>
<thead>
<tr>
<th>Galectins (Mol. wt) Structural type</th>
<th>Localization</th>
<th>Biochemical and functional properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gal-1 (14 kDa) Proto type</td>
<td>Ubiquitous expression</td>
<td>Apoptosis induction in activated T cells and activated thymocytes, induction a polarized Th2 immune response, cell growth, mRNA splicing, regeneration of nerve axon (oxidized Gal-1), aberrant neurite outgrowth of the olfactory neuron, inhibition of acute inflammation.</td>
</tr>
<tr>
<td>Gal-2 (14 kDa) Proto type</td>
<td>Small intestine, stomach epithelial cells</td>
<td>Expressed at minor level in tumor cells, risk factor of myocardial infarction.</td>
</tr>
<tr>
<td>Gal-3 (29-35 kDa) Chimera type</td>
<td>Ubiquitous expression</td>
<td>Cell adhesion, mRNA splicing, inhibition of T cell apoptosis, macrophage chemotactic factor AGE receptor, reduced intra-peritoneal inflammatory response, accelerated progression of diabetic nephropathy, anti-apoptotic and pro-inflammatory functions, induces chemotaxis of monocytes, down regulates IL-5 gene transcription, inhibition of anoikis.</td>
</tr>
<tr>
<td>Gal-4 (36 kDa) Tandem repeat type</td>
<td>Gastrointestinal tract</td>
<td>Activation of intestinal CD4(^+) T cells, expressed at sites of tumor cell adhesion.</td>
</tr>
<tr>
<td>Gal-5 (17 kDa) Proto type</td>
<td>Erythrocytes</td>
<td>No function assigned.</td>
</tr>
<tr>
<td>Gal-6 (34 kDa) Tandem repeat type</td>
<td>Gastrointestinal tract</td>
<td>High homology to Gal-4.</td>
</tr>
<tr>
<td>Gal-7 (14.5 kDa) Proto type</td>
<td>Keratinocytes</td>
<td>Marker of all subtypes of stratified epithelium, increases susceptibility of keratinocytes to UBV induced apoptosis.</td>
</tr>
<tr>
<td>Gal-8 (34 kDa) Tandem repeat type</td>
<td>Ubiquitous expression</td>
<td>Cell adhesion, regulation of neutrophil function, modulation of integrin interaction with the extracellular matrix.</td>
</tr>
<tr>
<td>Gal-9 (35 kDa) Tandem repeat type</td>
<td>Immune cells, gastrointestinal tract, lung</td>
<td>Apoptosis induction in activated T cells, eosinophil chemotactic factor, apoptosis induction in cancer cells, cell adhesion.</td>
</tr>
<tr>
<td>Gal-10 (17 kDa) Proto type</td>
<td>Eosinophils, basophils</td>
<td>Charcot-Leyden crystal, affinity for mannose.</td>
</tr>
<tr>
<td>Gal-11 (10 kDa) Proto type</td>
<td>Eye lens</td>
<td>Represents a new lens crystalline, lacks affinity for β-galactosides.</td>
</tr>
<tr>
<td>Gal-12 (37 kDa) Tandem repeat type</td>
<td>Adipose tissue</td>
<td>Apoptosis induction in adipocytes, causes cell cycle arrest.</td>
</tr>
<tr>
<td>Gal-13 (16 kDa) Proto type</td>
<td>Placenta</td>
<td>Pregnancy-related protein.</td>
</tr>
<tr>
<td>Gal-14 (18 kDa) Proto type</td>
<td>Eosinophils (sheep)</td>
<td>Possible involvement in allergic reaction</td>
</tr>
<tr>
<td>Gal-15 (14 kDa) Proto type</td>
<td>Sheep stomach with parasitic infection, uterus</td>
<td>Endometrial interactions, uterine immune and inflammatory responses, placental morphogenesis.</td>
</tr>
</tbody>
</table>
Figure 1. Schematic representation of the overall structures of Gal-1, -2, -3 & -4:
The galectins are shown schematically as linear diagrams corresponding to single peptide chains (top) and as assembled proteins (bottom). The CRDs of about 130 amino acid residues are indicated in blue, linker peptide are shown in orange and the N-terminal domain of Gal-3 (about 30 residues) is indicated in black (Barondes et al., 1994).
Gal-1 expression has also been reported in immune privileged sites such as placenta and cornea, suggesting an important role in generating and maintaining immune tolerance (Ilarregui et al., 2005). In vitro, Gal-1 induces cell cycle arrest and apoptosis of activated T cells or T cell lines (Ion et al., 2006). However, the in vivo functions of Gal-1 are currently unclear because targeted disruption of the Gal-1 gene in null mutant mice resulted in the absence of major phenotypic abnormalities, probably because of functional compensation by other family members (Kiss et al., 2007). Moreover, it has also been shown that Gal-1 inhibits chemotaxis and trans-endothelial migration of polymorphonuclear leukocytes in vitro (La et al., 2003).

Expression of Gal-1 in bone marrow derived mesenchymal (stromal) cells has been implicated in bone marrow cell differentiation (Kadri et al., 2005). Although many of these effects were found to depend on the carbohydrate binding activity of galectins, some were mediated exclusively by protein interactions. Gal-1 has also been shown to be a positive growth regulator towards other cell types, such as vascular endothelial cells. It has been speculated that growth inhibitory or stimulatory properties of Gal-1 is highly dependent on the cell types, cell activation status and concomitant environmental signals. In addition, these effects might be regulated alternatively by the relative levels of Gal-1 in the extracellular milieu and the equilibrium between its monomeric and dimeric forms, because dimeric Gal-1 is required to induce some, but not all of the biological effects mediated by this carbohydrate binding protein (Rabinovich et al., 2002).

**Galectin-2**

Gal-2 is closely related to Gal-1 (non-covalent dimer with subunits of about 14 kDa), but has a distinct and restricted expression profile (Rabinovich et al., 2007). Structurally, Gal-2 shares 43% amino acid sequence identity with Gal-1, and the analysis of expression in rat tissues and human tumor cell lines had revealed its presence to be confined to the gastrointestinal tract (Ahmed and Vasta, 2008). Thus, Gal-2 is likely to encounter T cells, especially in inflammatory bowel disease, shedding light on its immuno-modulatory capacity. In contrast to Gal-1, it lacks reactivity towards CD3 and CD7. However, it is a potent inducer of apoptosis in activated T cells, probably mediated by binding to β1 integrin (a closely associated glycoprotein) (Lahm et al., 2004).
**Galectin-3**

Gal-3 is a 29-35 kDa member of the galectin family which promotes cell growth and proliferation and acts as a mitogenic signal (Ahmed and Vasta, 2008). Gal-3 consists of an N-terminal domain (about 130 amino acids) made of tandem repeats of short stretches of amino acids connected to a C-terminal CRD (Dumic et al., 2006). It is ubiquitously expressed in a wide range of tissues and in inflammatory cells like neutrophils, dendritic cells, macrophages and monocytes (Acosta et al., 2004). In the peripheral and central nervous system, Gal-3 has been reported to be expressed in neurons, Schwann cells and astrocytes including gliomas and microglia (Yang et al., 2006).

Gene expression profiling and immuno-histochemical analysis has shown Gal-3 expression to be highly up-regulated in prion-infected nervous tissues at the mRNA and protein level, thus suggesting its role in chronic neuro-degeneration (Mok et al. 2006, 2007). Moreover, Gal-3 has been implicated in a variety of inflammatory conditions mostly involving innate immune reactions (Bernandes et al., 2006). Extracellular Gal-3 can also function as an inducer of T-cell apoptosis through binding to CD7 and CD29 on the T cell surface (Fukumori et al., 2004), as well as a positive or a negative growth factor, depending on the target cells (Marer, 2000).

In addition, Gal-3 plays a critical role in host protection against infection (Vray et al., 2004), for example, it displays a direct fungicidal activity by inducing death of Candida species containing specific β-1→2 linked oligomannans and also binds to wide variety of mammalian pathogens (Levroney et al., 2005). Gal-3 is also strongly expressed in wide variety of cancers, thus implicating its role in prognosis and malignancy of tumors and also inhibits apoptosis in response to chemotherapeutic drugs (Plazk et al., 2004).

**Galectin-4**

Gal-4 was the first mammalian lectin reported with a single polypeptide chain having two distinct but homologous CRDs separated by an un-conserved linker sequence of up to 70 amino acids (Dumic et al., 2006) and can bind to two individual carbohydrate epitopes. This galectin is a monomer with molecular weight of 36 kDa and is abundantly present in intestinal epithelium (Barondes et al., 1994).
Galectin-5

Gal-5 was first discovered as a lactose binding protein isolated from rat lung (Cerra et al., 1985) and erythrocytes (Rabinovich et al., 2007). It consists of one CRD with little additional sequence, and has up to 40% identity with Gal-3 and -4 (Leffler et al., 2004; Ahmed and Vasta, 2008).

Galectin-6

Gal-6 has two CRDs that are about 80% identical with those of Gal-4, but the linker peptide joining them is 24 amino acids shorter than Gal-4 (Gitt et al., 1998). Gal-4 and -6 are so similar that they cannot be distinguished in most localization assays such as immuno-histochemical and northern blots, but their combined expression can be easily detected and distinguished from other proteins. Possibly, Gal-6 is also expressed in small intestine of several mammalian species (Leffler et al., 2004).

Galectin-7

Gal-7 is a 14 kDa member of the lectin family expressed in stratified epithelia, inter-follicular epidermis and the outer root sheath of the hair follicle (Leonidas et al., 1998). Thus, Gal-7 is considered as a marker of all subtypes of keratinocytes and its expression does not seem to be influenced by the stages of differentiation (Magnaldo and Darmon, 1997). In addition, Gal-7 is also reported to increase the efficacy of chemotherapeutic drugs in urothelial cancer mediated via intracellular reactive oxygen species (Matsui et al., 2007).

Galectin-8

Gal-8, also known as prostate carcinoma tumor antigen-1 (PCTA-1) is a 35 kDa protein made up of tandem repeat CRDs joined by a linker peptide (Bidon-Wagner and Le Pennec, 2004), and structurally related to Gal-4 (34% identity). However, unlike Gal-4, which is confined to the intestine and stomach, Gal-8 is expressed in liver, kidney, cardiac muscle, lung and brain (Hadari et al., 1995, 2000). Native Gal-8 exists as a monomer and its two CRDs are structurally different with different specificities for sugar residues which do not require complex N-glycans for binding (Patnaik et al., 2006).
Galectin-9

Gal-9 is a 40 kDa protein consisting of 353 amino acids and having 35% sequence identity between the N- and C-terminal CRDs. Gal-9 is a lactose binding protein reported to be expressed in a sub-population of cells present in peripheral blood leukocytes, lymphoid tissues (Tureci et al., 1997), kidney, intestine and thymus (Wada et al., 1997). It controls wide range of activities like T-cell development (Wada and Kanwar, 1997), chemo-attraction of eosinophils, induction of apoptosis and down regulation of effector TH1 responses (Nagae et al., 2008).

Galectin-10

Gal-10 has originally been isolated as Charcot-Leyden crystal because of its unusual solubility properties and spontaneous crystallization in eosinophil mediated inflammatory tissues (Charcot and Robin, 1853; Leyden, 1872; Kilpatrick, 2002). It constitutes of less than 7% of the total protein content of a typical eosinophils (Ackerman et al., 1993) and displays weak lactose and mannose binding specificity in soluble and crystalline state, respectively (Kilpatrick, 2002).

Galectin-11

Very little information is available on Gal-11, except that they contain one CRD (Kilpatrick, 2002). Gal-11 (also called GRIFIN for ‘galectin related inter-fiber protein’) was found to be expressed in eye lens, suggesting a role for this protein in the maintenance of immune privilege in this vulnerable tissue (Rabinovich et al., 2002, 2007).

Galectin-12

Gal-12, a recently identified member of galectin sub-family, contains two CRDs and possesses growth inhibitory properties (Hotta, 2001). Expression of Gal-12 is high in peripheral blood leukocytes and adipocytes, but very low or undetectable expression has been reported in many tissue and cell lines, except in those of myeloid origin with the potential to undergo terminal differentiation (Yang and Liu, 2003).

Galectin-13

Except that they contain one CRD (Kilpatrick, 2002), very little information is available on Gal-13.
Galectin-14

Galectin-14 is a novel sheep galectin specifically expressed in eosinophils, and is similar in sequence to the first CRD of Gal-9. It has been shown to possess galactoside binding activity and reported to be released into the airway lumen in response to allergen challenge suggesting its possible role in immune defense (Dunphy et al., 2002).

Galectin-15

Gal-15 (also known as OVGAL11) a previously uncharacterized member of the galectin family containing a conserved CRD and a separate putative integrin binding domain, was discovered in sheep uterus. It is concentrated near and on the apical surface of the endometrial luminal epithelia and localized within discrete cytoplasmic crystalline structures of conceptus trophectoderm (Tr).

Gal-15 possess affinity for lactose and mannose sugars and is immunologically identical to an unnamed 14 kDa protein isolated from the ovine uterus, that forms crystalline inclusion bodies in endometrial epithelia and conceptus Tr (Gray et al., 2004).

Architectural types of galectins

Based on the protein architecture and its ability to function as a cross linker, the galectin family has been divided into three sub-groups as shown in Fig. 2 (Vasta et al., 2004).

Prototype

The proto-type galectins (Gal-1, -2, -5, -7, -10, -11, -13, -14 and -15) consist of a single CRD per subunit with a short N-terminal sequence and a single core protein domain (Rabinovich et al., 2007; Nagae et al., 2008). Most of these galectins are homodimers or multimers but some of them, such as Gal-5, -7 and -10 exists in monomeric form (Kopitz et al., 2003). They also show distinct tissue specificity and developmentally regulated expression in different sources (Cooper, 2002). Dimerization of prototype galectins involves self association of monomer subunits at the sides opposite to their CRDs through non-covalent interactions (Rabinovich et al., 2007).
The CRD pockets and bound ligand face away from each other, an arrangement unlikely to allow multivalent binding to closely clustered ligands (Brewer, 2002; Dam et al., 2005). However, these dimers can very effectively cross-link separated ligands and can even form chains or crystalline networks of the divalent lectins bound to multivalent ligands (Liu and Rabinovich, 2005).

**Chimera type**

Chimera type galectins are composed of a protein domain joined to a distinct N- or C-terminal domain (Rabinovich et al., 2007), which is responsible for interaction between subunits, thus facilitating its oligomerization (Liu and Rabinovich, 2005). In vertebrates, the only chimeric galectin discovered till date is Gal-3, but several other chimeric galectins are apparent in invertebrates.

Gal-3 is composed of an N-terminal domain with several repeats of a peptide sequence rich in proline, glycine and tyrosine residues, followed by a C-terminal galectin domain (Dumic et al., 2006). The activity of C-terminal domain remains intact, but loses much of its propensity to multimerize (Kuklinski and Probstmeier, 1998).

Gal-3 monomers are in equilibrium with higher order oligomers in solution, and precipitates as a pentamer with multivalent oligosaccharides. This lectin binds to multi-glycosylated proteins with positive cooperativity, suggesting that Gal-3 monomers, after ligand binding, recruit additional lectin molecules to form a complex of multivalent interactions (Ahmed et al., 2004). The biological functions attributed to Gal-3 are thus, likely to depend upon both ligand cross-linking and oligomerization (Stillman et al., 2005; Toscano et al., 2007).

**Tandem repeat type**

Tandem repeat type galectins (Gal-4, -6, -8, -9 and -12) are composed of two non identical core galectin domains joined either directly or via a linker peptide of variable length (Toscano et al., 2007). Their CRDs are positioned in such a way that they can simultaneously bind to multivalent ligands, greatly enhancing the binding avidity (Hirabayashi et al., 2002) as opposed to homo-functional cross-linking by dimeric prototype galectins (Cooper, 2002).
Figure 2. Architectural types of galectins: The first group consists of prototype galectins having one CRD. The second group consists of chimera type galectins that have a CRD and a non-CRD domain linked together. The third group consists of tandem repeat type galectins with two CRDs. Although prototype and chimera type galectins have only one CRD, two molecules bind to each CRD (dimerization) so that they can actually bind to two carbohydrate chains, like tandem repeat type galectins (Stillman et al., 2005).
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Secretion and biosynthesis of galectins

Although galectins are often reported to be expressed on cell surfaces or in extracellular matrix, they lack recognizable secretion signal sequences and do not pass through the standard ER/Golgi pathway (Cooper, 2002), with the possible exception of a sponge galectin (Miarons and Fresno, 2000). Instead, most galectins have characteristics typical of cytoplasmic proteins, such as an acetylated N-terminus, free sulfhydryl groups and lack of glycosylation. Nevertheless, there is strong experimental evidence that at least some galectins are indeed secreted, albeit by novel non classical mechanisms (Hughes, 1999; Leffler et al., 2004). Galectin secretion from cells is tightly controlled during development, and several unrelated factors including cytokines as well as adhesive and membrane fusion can modulate secretion (Hughes, 1999). Pulse chase experiments also demonstrated that lectins are actively secreted from cells, rather than leaked from damaged cells (Cooper, 2002; Hughes, 2001). Upon secretion, galectins typically bind to and stay associated with glycoproteins and glycolipids on the cell surface, or within the surrounding extracellular matrix to complete its folding and stability (Stillman et al., 2005). Thus, while galectins are abundant in tissues where they are synthesized, only low levels are found in serum, which do not reflect the rates of synthesis in tissues (He and Baum, 2004).

Detection and identification of galectins

The detection and identification of galectins has come a long way from the time when their ability to bind β-galactosides and their cross-reaction with other galectins were exploited (Hirabayashi and Kasai, 1993). Hemagglutination of trypsin treated erythrocytes was also widely used as an indicator of their presence but suffered from the problem of hemolysis of the cells, even under isotonic conditions (Nowak et al., 1977). This drawback was overcome when glutaraldehyde was used to strengthen the cells before they were used for galectin detection (Turner and Liener, 1975).

With advances in techniques of molecular biology, the methods of detection of galectins were also revolutionized. Immunoscreening of cDNA was advancement over these primitive methods and resulted in the discovery of Gal-5 and -8 (Hadari et al., 1995). Screening the tumor cDNA libraries from sera of afflicted patients identified another molecule, Gal-9 (Tureci et al., 1997).
RT-PCR was another technique that was used to detect, with much success, the differential expression of galectins (Wolff et al., 2005) and its results match well with Western blot data (Hittelet et al., 2003). For all its popularity, the method still provides only an indirect estimate of galectin levels.

Recently, search algorithms have been developed to search for sequences that encode structures similar to the known galectin domains. The screening of the GenBank databases identified seven new putative galectins genes. Similar approaches have been applied to other organisms with the result that there has been a massive increase in the number of possible galectins.

Amongst a total of 20,000 genes in C. elegans, 26 have been identified as candidate galectin genes. The ubiquitous distribution of galectins is evident from the identification of candidate genes in the Mastadenovirus (U25120) (Perillo et al., 1998) and lymphocystis disease virus (L63545, 26549-27313=053R), Drosophila (LP06039), Zebrafish (AI384777 and G47571) and Arabidopsis (AC000348, T7N9.14), with the report in Arabidopsis being the first in any plant (Cooper, 1999).

While all these methods are useful in the research laboratory, and have yielded 15 mammalian galectins till date (Pieters, 2006), advances made in clinical studies of galectins and their implication in tumorigenesis has made the need to develop rapid and accurate protocols for their accurate detection and estimation very pressing.

Western blotting using anti-galectin antibodies has been one of the biggest success stories as far as detection is concerned and has been used to confirm the increased expression of Gal-1 in pancreatic tumors (Shen et al., 2004).

Labeled antibodies have also been used in situ to study expression patterns of Gal-1 and -3 in lung cancer (Szcke et al., 2005). Membrane based methods have utilized the use of a LacNAc conjugated biotinylated polyacrylamide probe to demonstrate the increased expression of Gal-3 in Escherichia coli. The detection system was based on enzyme-streptavidin conjugates (Kamemura et al., 1998).

Quantification of galectins was not possible until the advent of ELISA, which provided information about the amounts of different galectins (Pieters, 2006). Commercially available detection and quantification systems can detect Gal-3 at as low a concentration as 0.2 ng/ml. With the emphasis on cancer based research in galectins, flow cytometry based on the use of Glyc-PAA-fluo probes has been used to detect total galectin activity in cancer cells (Moiseeva et al., 2005). The technique has proved effectual for Gal-3 with the use of LacNAc and asialoGM-1 (Pieters, 2006).
The problem with most of the methods described so far is their dependence on specific anti-galactin antibodies. Recently, efforts have been made to devise strategies based on chemical approaches. Photoaffinity based probes are being synthesized (Pieters, 2006). In one case, benzophenone was attached on galactose-C3 and irradiated to link the galectin captured by the sugar from a mixture of proteins and the complex was visualized, in gel, by the use of fluorescent label attached to the other end of the probe. While this method annuls the need for antibodies, it is still not proven for its efficacy as a diagnostic and prognostic tool.

**Purification strategies of galectins**

Over the years galectins have been isolated and purified to homogeneity by various chromatographic methods. The fresh animal tissue is solubilized with suitable buffer containing reducing agents and competing sugars in order to dissociate galectins from insoluble substances of starting tissues (Kasai and Hirabayashi, 1996). Multiple forms of galectin have also been isolated from human and rat lung by employing ion exchange chromatography on DEAE-cellulose or sephadex column followed by chromatography on CM-sephadex column (Cerra et al., 1985) in the presence of protease inhibitors to minimize the proteolytic degradation of lectin. Galectins have also been isolated from crude extract by ammonium sulphate fractionation followed by affinity chromatography on asialofetuin or lactosyl-Sepharose 4B columns (Ola et al., 2001) or gel filtration chromatography on Sephadex columns (Rizvi et al., 2007) and bound protein eluted with a suitable ligand. Galectin activity is measured by hemagglutination assay using fresh rabbit and human erythrocytes treated with trypsin or neuraminidase (Muramoto et al., 1999).

**Physico-chemical properties of galectins**

Galectins are soluble proteins bearing an affinity for β-galactoside containing moieties, besides possessing a CRD with conserved sequence elements that require a reducing environment for action but no divalent ions (Hasan et al., 2007). They are distinguishable from all other lectins by their low molecular weights (ranging from 14-36 kDa), dimeric nature and their variable sub-cellular location. By virtue of their multi-valency, galectins are able to cross-link cell surface glycoconjugates and initiates cell biological response (Rabinovich et al., 2007).
These lectins binds to N-acetyllactosamine with relatively low affinity (dissociation constant, $K_d$ in the range of 90-100 $\mu$M), but they bind to glycoproteins containing polylactosamine sequences with a high affinity ($K_d$-1$\mu$M) (Dam et al., 2005). While CRDs of all galectins share affinity for N-acetyllactosamine found on many cellular glycoproteins, individual galectins can also recognize different modifications of this sugar, thus demonstrating the fine specificity of certain galectins for tissue specific ligands (Ahmed et al., 2004).

In the isoelectric focusing experiment, 90% of rat lung galectin moved with the isoelectric point (pI) of pH 5.5 (Clerch et al., 1988). Amino acid composition of galectins isolated from different mammalian sources has been found to be considerably similar, if not identical (Shahwan et al., 2004). The total number of acidic amino acid residues is found to be significantly higher than the total number of basic amino acid residues. All the galectins studied so far have blocked N-termini, and an acetyl group has been demonstrated to be the blocking group (Leffler et al., 2004; Rabinovich et al., 2007). Galectins occasionally contain cysteine residues, but no disulfide bond is formed and all -SH groups are in a free state. Cloning of cDNAs revealed that galectins are synthesized without a signal sequence (Kasai and Hirabayashi, 1999), strongly suggesting that galectins are designed as intracellular proteins (Leffler et al., 2004).

**Ligands for galectins**

Despite the large number of $\beta$-galactoside containing glycoconjugates present in the cellular milieu, only few glyco-proteins from cell extracts bind to particular galectins *in vitro* (Barondes et al., 1999), suggesting that these may be the interactions that are physiologically significant. Among naturally occurring glyco-conjugates, glycoproteins that contain polylactosamines are especially good ligands for galectins (Liu, 2000; Rabinovich et al., 2007). Of these, laminin, a glycoprotein with many polylactosamine chains, has been implicated as a natural ligand for Gal-1 (Hughes, 2001) and is also bound by Gal-3 (Liu, 2000).

Gal-1 has also been shown to bind other glycoconjugates, including polylactosamine rich lysosome associated membrane proteins that are sometimes found on the cell surface (Imbe et al., 2003), a lactosamine containing glycolipid on olfactory neurons (Horie and Kadoya, 2004), and integrin $\alpha_7\beta_1$ on skeletal muscle cells (Hadari et al., 2000).
Carbohydrate binding domain and specificity

Galectins have in common a highly conserved CRD with affinity for lactose and N-acetyllactosamine (Barondes et al., 1999; Vasta et al., 2004; Ahmed and Vasta, 2008), formed by part of the six stranded anti-parallel \( \beta \)-pleated sheets that form an extended sandwich (Fig. 3) with a typical jellyroll topology and is around 134 amino acid long (Liao et al., 1994). The core sequence of this domain lies between the 30\(^{\text{th}}\) and the 90\(^{\text{th}}\) residues and is encoded by a single exon (Cooper and Barondes, 1999). The number and arrangement of the CRDs may vary and has been used as a basis of their classification. The galectin CRD has a concave side forming a groove long enough to hold a linear oligosaccharide made of up to four monosaccharide units (Leffler et al., 2004). The fine specificity of the two CRDs may differ (Hirabayashi et al., 2002; Ideo et al., 2003) displaying tightest interaction with galactose residues, but interaction with glucose is also significant, making the affinity for lactose 50-150 folds higher compared to galactose for most galectins (Toscano et al., 2007).

However, the carbohydrate binding cleft can accommodate an additional one to three saccharide residues justifying polylactosaminoglycans as good ligands for galectins (Sorme et al., 2004). In fact, in addition to the primary site which accommodates lactose/ N-acetyllactosamine (Toscano et al., 2007), polylactosaminoglycans are bound by the galectins more tightly than lactose/N-acetyllactosamine due to the presence of secondary sites for interaction with more extended oligosaccharides (Leppanen et al., 2005). Each galectin has a unique specificity, for example, the affinity of Gal-1 for blood group A tetrasaccharide is about 100 fold lower than that of Gal-3 and certain complex mucin derived saccharide that bind Gal-3 well, do not bind Gal-1 at all. Gal-1, -3, and -5 also differ in their affinity for certain disaccharides such as Gal-\( \beta \)-1-\( \beta \) GalNAc (Brewer, 2002). The two chicken galectins, C14 and C16, were shown to differ in their fine specificity for a panel of synthetic lactose derivative (Barboni et al., 2000).

Multivalency of galectins

Most galectins are divalent, either by self-association or by including two CRDs in one protein (Gabius et al., 2002). All the galectins identified till date can be structurally classified into three basic types (Vasta et al., 2004), which achieve multivalency by distinct mechanisms.
Multimerization is a common feature across various families of carbohydrate binding proteins, perhaps because binding interactions of individual CRDs with even their most favored carbohydrate ligands are of relatively low affinity, and multimerization can greatly increase binding avidity for multivalent or clustered ligands (Rabinovich et al., 2007). However, in studies of a range of multivalent galectins, only some show enhanced avidity for multivalent ligands (Hirabayashi et al., 2002). This may mean that for some galectins, the significance of di- or multivalency is to provide them the ability to cross-link ligands (Cooper, 2002). Indeed, there is considerable evidence that some galectins regulate in association with their glycoconjugate ligands on cell surfaces, in extracellular matrices, or both (Brewer, 2002; Rabinovich et al., 2007). Monovalent galectins might function to competitively block such cross-linking activity (Cooper, 2002).

Crystal structure of galectins

The refined X-ray model of S-lectin revealed 133 amino acid residues in one monomer and 132 amino acid residues in the other monomer, two N-acetyllactosamine molecules and 154 water molecules. S-lectin dimer forms a 22-strand anti-parallel \( \beta \)-sandwich, with the N and C termini of each monomer at the dimer interface (Fig. 4). The two molecules are related to each other by a non-crystallographic two-fold rotation perpendicular to the \( \beta \)-sheets displaying a jelly roll topology containing a \( \beta \)-hairpin insertion with the first and second parallel \( \beta \)-strands omitted. The structure reveals that there is one carbohydrate-binding site per monomer, which is located on the same side of the \( \beta \)-sandwich and on the far ends of the dimer 46 Å apart. The integrity of the dimer is maintained by the \( \beta \)-sheet interaction across the monomers and by the formation of a hydrophobic core common to both. In S-lectin, the two \( \beta \)-sheets of the monomers extend continuously across the dimer interface and all direct protein carbohydrate interactions involve side chains located on \( \beta \)-strands (Liao et al., 1994).

The well-defined electron density of both carbohydrate molecules clearly indicates the \( \alpha \)-anomeric form for the GlcNAc unit (Liao et al., 1994). Most amino acid residues involved in sugar binding are invariant in all sequences of S-lectins. The binding-site depression is shaped to complement the galactose moiety with extensive van Der Waals contacts and a network of electrostatic interactions (Fig. 5).
In addition, a water molecule mediates the interaction of the side chain of Asn-46 with the 3-OH and 4-OH of galactose. The aromatic side chain of the conserved Trp-68 stacks adjacent to the galactose ring. Such van der Waals interactions between sugar and aromatic side chains are quite common in protein-carbohydrate complexes (Vyas, 1991). The axial 4-OH of galactose is the main determinant of S-lectin specificity which forms two key electrostatic interactions i.e., one with N-atom of Arg-48 and the other with N-atom of His-44. The interactions of the GlcNAc moiety with the protein are less extensive than those of H-44 of galactose, whereas the binding of N-acetyllactosamine to S-lectin is five-fold tighter than the binding of lactose which may be attributed to van der Waals interactions between the N-acetyl group and the side chains of Arg-73 and Glu-71 (Ahmed et al., 1990).

The carbohydrate-protein interactions are supported by exquisite protein-protein electrostatic interactions, assuring optimal side-chain conformations. Most striking is the spatial disposition of the charged residues (Arg-48, Asp-54, Arg-73 and Glu-71) which together form a network of three salt bridges unprecedented in other sugar binding proteins of known structure. Of these, Asn-46, Glu-71 and Arg-73 have been suggested as essential for binding by site-directed mutagenesis (Hirabayashi and Kasai, 1991). These structural data explain why those amino acid residues that are directly and indirectly associated with the carbohydrate binding are conserved.

**Thiol group environment of galectins**

The crucial role that the oxidation states of S-lectin thiol groups play in functional regulation *in vivo* is still a mystery. According to the refined structure, the thiol groups of Cys-42 and Cys-60 are reduced and buried, whereas that of Cys-2 is disordered, and those of Cys-16, Cys-88 and Cys-130 are oxidized and solvated. No thiol group is directly involved in sugar binding, and all thiol groups bind heavy atoms without impairing sugar binding. Cys-60 is the only cysteine residue close to the binding site. Its main chain atoms are involved in the formation of the active site depression, and its side chain is buried and surrounded by hydrophobic residues (Liao et al., 1994). Site-directed mutagenesis studies of the 14 kDa S-lectins by replacing Cys-60 and Cys-2 by serine residues did not inhibit sugar binding, consistent with earlier reported structural informations (Hirabayashi and Kasai, 1991).
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Figure 3. Fine structure of interaction between Gal-2 and lactose: The sugar residues are shown in green and oxygen atoms are represented in red along with their position numbers. The amino acid side chains interacting with the saccharides are shown in light blue. The principal hydrogen bonds between amino acid side chains and the sugar residues are shown as yellow dotted lines (Barondes et al., 1999).
Figure 4. Three dimensional structure of S-lectin: β-strands are shown as ribbons and the N-acetyllactosamine molecules are shown as yellow stick models. The model was generated by the computer program RASTER 3D written by David Bacon (University of Alberta, Canada) (Barondes, et al., 1994).
Figure 5. Stereoscopic representation of S-lectin carbohydrate binding site: Bonds between carbohydrate atoms are solid and those between protein atoms are open. The electrostatic interactions between protein and sugar atoms are shown in thin N-46 lines. For clarity, protein-protein electrostatic interactions of residues involved in sugar binding are not indicated; these include salt bridges between Arg-48 and Asp-54, Arg-73 and Asp-54, and Arg-73 and Glu-7 (Liao et al., 1994).
Hemolytic and membrane perturbing action of galectins

The ability of galectins to act as humoral factors in the defense mechanism against various pathogenic agents suggests important applications for these proteins. Gal-1 preferentially binds to ganglioside GM1 on neuroblastoma cells to exert growth control (Kopitz et al., 2003) and harbors a site to interact with hydrophobic tails of oncogenic H-Ras (Rotblat et al., 2004). The underlying mechanism to carry out this role is its glycan binding property present on the cell membranes, thereby causing lysis of the cells.

Lytic action of some galectins have been ascribed to enzymatic activity (Hittelet et al., 2003), perturbation of the activities of membrane associated enzymes (Lowe and Marth, 2003), and pore formation in the membranes (Yu et al., 2002). The galectin dependent association of glycans may affect membrane features such as fluidity, permeability and osmofragility. In addition, lectin might also interact with hydrophobic membrane patches adding to its impact on membrane characteristics (Gupta et al., 2006).

Role of galectins in cell-cell and cell-matrix interactions

Galectins plays a pivotal role in the adhesion and migration of cell mediated by cross-linking a mosaic of extracellular matrix glycoproteins like laminin, fibronectin, lysosome associated membrane proteins and CD45. Despite specific binding of galectins to these glycoconjugates, its anti or pro-adhesive role still remains obscure. For example, Gal-1 promotes the adhesion of ovarian carcinoma cells to extracellular matrix (Rabinovich et al., 2004), whereas it inhibits the adhesion of myoblast to laminin by blocking the laminin receptor integrin α7β1 from recognizing laminin (Matarrese et al., 2000).

Similarly, Gal-3 preferably mediates adhesion of the neutrophils to laminin in comparison to melanoma (Sato et al., 2002). While, Gal-3 contributes to interaction between dendritic cells and naive T-lymphocytes in lymph nodes (Rabinovich et al., 2002, Ilarregui et al., 2005), a recent study revealed that Gal-3 even disrupts thymocyte interaction within the thymic microenvironment, thus acting as a de-adhesion molecule (Villa et al., 2002).
Role of galectins in pre-mRNA splicing

The process of deletion and reconstitution of splicing activity, assayed in cell free system suggested that galectins are major factors involved in pre-mRNA splicing (Park et al., 2001). Other techniques such as co-localization studies (Wang et al., 2007) and immuno-fluorescence microscopy (Liu et al., 2002) also revealed nuclear speckled structures containing galectins and known splicing factors, thus providing additional evidence confirming the role of galectins in mRNA splicing. The domain structure for Gal-1 and Gal-3 suggested that homologous CRD is necessary and sufficient for splicing activity, which thereby interacts with various functional proteins constituting the spliceosome assembly (Park et al., 2001).

Role of galectins in cell growth and apoptosis

Several studies provide correlative evidence for an association between galectin expression and cell proliferation. Gal-3 is expressed at high levels in a wide range of neoplasms, including spontaneous, viral, ultraviolet and chemically induced tumors, thus suggesting the possibility of galectins having a role in cell growth regulation (Plazk et al., 2004; Dumic et al., 2006). Some pro-apoptotic galectins, such as Gal-1 and Gal-9 directly initiate apoptosis by cross-linking cell surface receptors, whereas intracellular expression of other galectins, such as Gal-7 potentiates other death signals (Rabinovich et al., 2004). Galectins affect cell fate decisions in a variety of tissues and cell types. For example, Gal-1 kills T-cells, B-cells and prostate and breast cancer cell lines, suggesting that Gal-1 may recognize a common carbohydrate ligand on diverse cell surface receptors to initiate a common intracellular death pathway (Stillman et al., 2005).

Glycol chlorophytes have also been shown to influence viability in a single cell type. Complex expression patterns of galectins and their carbohydrate ligands may allow temporal regulation of cell viability during development, tissue remodeling and inflammation (Leffler et al., 2004). In addition, some galectins can act extracellularly to induce apoptosis in concert with other stimuli. For example, addition of exogenous Gal-8 induced apoptosis of serum-starved carcinoma cells in a carbohydrate dependent manner (Hadari et al., 2000). Furthermore, Gal-1 also induces apoptosis of breast and prostate cancer cell lines (Yang and Liu, 2003) and melanoma cells (Stillman et al., 2005).
Role of galectins in cell cycle regulation

Galectins play important role in cell growth and apoptosis, and also contribute significantly in cell cycle regulation. For example, Gal-1 has been shown to inhibit growth of mouse embryonic fibroblast at relatively low concentrations and induce cell cycle arrest during S to G2 transition of mammary cell lines (Yang and Liu, 2003). It also inhibits IL-2 induced proliferation of phytohemagglutinin activated T lymphocytes, IL-independent proliferation of T lymphoma cells, Con-A stimulated rat T-cells (Rabinovich, et al. 2004), human neuroblastoma cells (Rubinstein et al., 2004), human leukemia T cells and murine fibroblasts (Nakahara et al., 2005).

Role of galectins in cancer and metastasis

The myriad critical roles played by galectins ranging from cell signaling to apoptosis make them potent tumorigenic molecules. Galectins are often overexpressed in cancerous cells and cancer associated stromal cells (Lahm et al., 2004). In general, this altered expression correlates with the aggressiveness of tumors and the acquisition of metastatic phenotype, indicating that galectins might modulate tumor progression and influence disease outcome (Greco et al., 2004). There is increasing evidence that galectins have important functions in several aspects of cancer biology (Rabinovich et al., 2005; Liu and Rabinovich, 2005), including tumor transformation (Paz et al., 2001), apoptosis (Rabinovich et al., 2002) and cell growth regulation (Yamaoka et al., 2000).

In addition, galectins are also involved in various steps of tumor metastasis, including tumor cell adhesion (Levy et al., 2001), homotypic cell aggregation (Glinsky et al., 2003), invasiveness (Hittelet et al., 2002) and angiogenesis (Nangia-Makker et al., 2000). Gal-3 could be used as a prognostic marker for thyroid cancer, colon cancer and cancers of pancreas, bladders, stomach, kidneys, and head and neck squamous epithelial cells (Hasan et al., 2007). Gal-1 is also involved in cancer development as it anchors the molecule Ras, which is involved in cellular transformation (Paz et al., 2001). Increased malignant potential of human thyroid tumors (Xu et al., 1995), glioma (Rorive et al., 2001,) and prostate adeno-carcinoma (Rabinovich et al., 2004) has been correlated with enhanced expression of Gal-1, both on the surface of tumor cells and the stromal tissue surrounding the tumors (He and Baum, 2004; Stillman et al., 2005).
Role of galectins in inflammatory and immunomodulatory responses

Among the various functions of galectins, its role in modulation of the immune response has been very well documented. Recent research indicates that Gal-1 ameliorates phospholipase A2 induced edema (Rabinovich et al., 2002), regulates the release of soluble mediators from lipopolysaccharide stimulated macrophages, blocks neutrophil extravasation (Elola et al., 2005), induces mast cell degranulation (Rubenstein et al., 2004) and stimulates nitric oxide synthesis (Rabinovich et al., 2004). Moreover, presence of Gal-1 in bone marrow derived mesenchymal (stromal) cells suggests its implications in bone marrow derived cell differentiation and mobilization (Kadri et al., 2005; Kiss et al., 2007). In addition, Gal-1 displays pleiotropic immunomodulatory functions, including regulation of lymphocyte survival and cytokine secretion in autoimmune system, transplant diseases, and parasitic and viral infection models (Santucci et al., 2000, 2003; Rabinovich et al., 2004; Levroney et al., 2005).

Therapeutic applications of galectins

Research over the past decade indicated that the local administration of Gal-1 prevented clinical and histo-pathological manifestations of many well known diseases such as Mysthania Gravis, experimental encephalomyelitis, (Santucci et al., 2000; Wada and Makino, 2001; Rabinovich et al., 2004), autoimmune encephalitis (Kilpatrick, 2002), collagen induced arthritis (Wada and Makino, 2001), colitis (Santucci et al., 2000), hepatitis (Santucci et al., 2003), autoimmune uveitis (Toscano et al., 2007) and anti-glomerular basement membrane (anti-GBM) glomerulonephritis (Tsuchiyama et al., 2000). In anti-GBM glomerulonephritis in experimental rats, the administration of Gal-9 induced apoptosis of activated CD 8+ cells and ameliorated proteinuria and renal tissue injuries (Tsuchiyama et al., 2000). The ability of Gal-1 to suppress the allogeneic T-cell response through apoptotic and non-apoptotic mechanisms suggests its potential use for immuno-suppression in organ transplantation and graft versus host diseases (Rabinovich et al., 2002; Stillman et al., 2005). For example, Gal-1 treatment significantly improved reconstitution of normal splenic architecture following hematopoietic stem cell transplantation and, similar to its effects on autoimmune settings, this β-galactoside binding protein reduced the production of T1-type cytokines (Toscano et al., 2007).
Possible usage of galectins in the therapy of atherosclerosis and diabetic vascular complications by modulating macrophage function and advanced end glycation product clearance is suggested by several studies (Stillman et al., 2005). Gal-7 is also reported to increase the chemotherapeutic efficacy of cisplatin in treatment of urothelial cancer through the accumulation of intracellular reactive oxygen species (Matsui et al., 2007). Furthermore, galectins display antimicrobial activity by acting as immunosuppressant during various infections caused by Trypanosoma cruzi (Zuniga et al., 2001), Leismania major (Pelletier et al., 2003), HIV (Lanteri et al., 2003) and Nipah virus (Levroney et al., 2005).

Interestingly, the discovery of anti-galectin antibodies in pathological sera and autoimmune disorders like Chagas’ cardiomiopathy (Giordanengo et al., 2001), non-endemic loiasis (Rabinovich et al., 2002, 2004) and Hodgkin’s disease (Kilpatrick, 2002) opens new diagnostic and potentially therapeutic avenues for researchers in application of galectins as suitable target for novel drugs and pharmacological molecules. Detection of altered galectin expression also serves as excellent diagnostic markers in various carcinomas (Stillman et al., 2005; Hasan et al., 2007) and inflammatory cells (Rabinovich et al., 2005). Direct administration of oxidized galectin has implication in axonal regeneration and functional recovery after peripheral nerve injury (Kadoya et al., 2005).

Role of galectins in heart tissue and cardiovascular diseases

Gal-1 has nearly ubiquitous distribution in animal tissues including heart of mammals. In mammalian heart, Gal-1 is localized mainly in endocardial tissue, myocardial cell constituents, connective-tissue elements and vascular structures. In the heart of healthy individuals, Gal-1 is involved in basic physiological functions like adhesion, transportation and apoptosis (Bardosi et al., 1990). Though galectin activity in mammalian heart was first reported from calf heart almost four decades ago (Waard et al., 1976), only few mammalian heart galectins, like those from porcine heart and bovine heart (Southan et al., 1987) have been thoroughly characterized till date. Till the end of the last century, functional aspect of mammalian heart galectins was limited to basic physiological functions like adhesion, transportation and apoptosis in the heart of healthy individuals. However, some breakthrough studies in the last decade have implicated significant Gal-1 involvement in various cardiovascular disorders.
Giordanengo et al. (2001) demonstrated the occurrence of anti-Gal-1 autoantibodies in sera from patients in the acute and chronic stages of Chaga’s disease, caused by *Trypanosoma cruzi* infection of heart and other tissues. A marked increase in the level and frequency of IgE anti-Gal-1 antibodies was found in the sera from patients of acute Chaga’s disease, but a low frequency of IgM anti-Gal-1 immunoreactivity was observed. Moreover, Ig G immunoreactivity to Gal-1 was found to be correlated with the severity of cardiac damage in chronic Chaga’s disease. A couple of years later, Gal-1 was shown be involved in the vascular extracellular matrix (ECM) assembly by affecting the incorporation of some ECM components (Moiseeva et al., 2003). Over-expression of the Gal-1 gene, parallel to the other genes encoding ECM proteins and genes involved in ECM turnover has been reported in aneurysmal dilation of brain blood vessels. These data also indicate the role of Gal-1 in the ECM assembly during wound healing and tissue matrix remodeling, and in the developmental stages of atherosclerosis and restenosis. Further studies suggested remarkable involvement of Gal-1 in hypoxia-induced pulmonary hypertension (Case et al., 2007) and atherogenesis (Chellan et al., 2007).

The involvement of Gal-1 in such potentially life threatening cardiovascular diseases strongly demand detailed exploration of mammalian heart galectins. Thus, we decided to carry out purification and extensive characterization of buffalo heart galectin, so as to provide an impetus regarding the easy availability of mammalian heart galectins to help understand the basic molecular mechanism behind these deadly diseases.

**Role of glycosylation in galectin functioning**

Glycosylation is an event known to be of paramount importance to cellular functioning and interactions. Its aberrations have been found in all types of cancers and several glycosyl epitopes function as tumor associated antigens (Hakomori, 2002). Yet, the information available concerning its role in carcinogenesis is quite nebulous, primarily because of the lack of attention given to this field of investigation in comparison with more attractive and rewarding avenues like genetic studies. However, the implications of abnormal glycosylation in cancer development are being recognized. The specific steps involved in the development of cancer because of incorrect glycosylation are not known.
One molecule that has been studied in much detail is cell surface GM3 (Kojima et al., 1991). Its interaction with CD9 and CD82 bestows anti-metastatic potential on the cell (Miura et al., 2004). GM3 and CD9 have even been found to be co-expressed in several colorectal (Ono et al., 2001) and bladder cell lines (Satoh et al., 2001). A reduction in the expression of this ganglioside may correlate with increased chances of metastasis (Hakomori, 2002).

Though there is a shortage of unambiguous data, but the close analogy between the expression patterns of glycoconjugates and their binding to galectins cannot be ruled out. GM3 has been found to be a ligand for Gal-8 (Ideo et al., 2003). This galectin has two CRDs (Hadari et al., 1995) and is involved in cross-linking of its ligands. Extracellularly, it can organize cell adhesion molecules on the same cell as well as on different cells and the matrix. A change in the ligands of such a cellular anchor may be very important, if not tantamount, to metastasis. This could also be the missing link in the elucidation of galectin functioning and further studies to explore similar interactions between other galectins and their ligands should direct the course of research in the future.

Despite such a significance of galectin glycosylation, only few galectins like sponge galectin (Miarons and Fresno, 2000) have been found to be glycosylated. This can be attributed to the lack of attention given to this aspect of galectin biology, as compared with more attractive and rewarding avenues like genetic studies. In an attempt to elucidate the role of glycosylation in galectin functioning, we carried out the glycosylation and deglycosylation studies of purified buffalo heart galectin.

Role of galectins in activation of oxidative burst and degranulation of neutrophils by neutrophil activators

Various functions have been demonstrated for galectins that appear to be multifunctional proteins with particular relevance in the physiology of the immune response. Gal-3 has been found to activate mast cells, neutrophils and eosinophils in an IgE-mediated fashion, playing a role in allergic inflammation and host defense against bacterial and parasitic infections (Truong et al., 1994). In relation to superoxide production, human Gal-3 induces the formation of oxygen radical in the presence of cytochalasin B (CB) by human neutrophils and monocytes in a dose-dependent manner (Yamaoka et al., 1995).
Karlsson et al. (1998) and Almkvist et al. (2002) demonstrated that recombinant human Gal-3 induced NADPH-oxidase activity in exudated but not in peripheral blood PMN. Gal-1 from human placenta also induces superoxide release by human PMN of patients with certain tumors (Timoshenko et al., 1993) and Gal-1 from human spleen induces superoxide formation by homologous neutrophils in the presence of CB (Elola and Fink, 1999). More recently it has been also shown that recombinant Gal-1 had the same effect (Almkvist et al., 2002). During phagocytosis, phagocytes destroy invading microorganisms through the generation of oxidizing radicals derived from partial reduction of oxygen to superoxide anion (O$_2^-$). The enzyme responsible for O$_2^-$ production is NADPH-oxidase which uses NADPH as substrate, and consists of a membrane flavocytochrome b and three cytosolic proteins—p47phox, p67phox and p40phox (Babior, 1999).

Bacterial killing also involves another event called degranulation, the process of fusion between the primary phagosome and the granules present in the phagocyte cytoplasm. Some of the enzymes contained inside these granules are elastase, myeloperoxidase and lysozyme. A chicken galectin (16 kDa) produces a significant release of lysozyme from human neutrophils in the presence of CB (Timoshenko et al., 1995). In the present work, we compared the level of activation of NADPH-oxidase in buffalo PMNs under the stimulation with N-formyl-l-methionyl-l-leucyl-l-phenylalanine (fMLP), phorbol 12-myristate 13-acetate (PMA) and Gal-1 purified from buffalo (*Bubalus bubalis*) heart. For PMN degranulation studies, we evaluated lysozyme release under similar experimental procedures.