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Galectin genes: Regulation of expression

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In this review we have summarized the more recent studies on the expression of mammalian galectins. One interesting observation that can be made is that in most of microarrays and/or differential display analysis performed in recent years one or more galectins have been picked up. From a critical evaluation of the pertinent studies the main conclusion that can be drawn is that, although it is not yet clear whether the 14 galectins identified so far have functions in common, a striking common feature of all galectins is the strong modulation of their expression during development, differentiation stages and under different physiological or pathological conditions. This suggests that the expression of different galectins is finely tuned and possibly coordinated. In spite of these observations it is rather unexpected that very few studies have been performed on the molecular mechanisms governing the activity of galectin genes.
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Abbreviations: **CRD: carbohydrate recognition domains; TSH: thyroid stimulating hormone; RA: retinoic acid; butyrate: sodium butyrate; MM-LDL: minimally oxidized low-density lipoprotein; SIE: sis-inducible elements; PMA: phorbol 12-myristate 13-acetate; C/EBP: CCAAT/enhancer-binding protein.**

Introduction

Galectins belong to a family of proteins, which contain one or more carbohydrate recognition domains (CRD) with affinity for β-galactosides [1–3]. Another common characteristic of galectins is their cytosolic localization and their ability to be secreted from the cytosol by non-classical pathways or to be translocated to the nucleus or to other cellular compartments. All galectins lack a typical signal peptide. These proteins are evolutionary conserved from fungi to man. To date fourteen galectins have been identified in mammals [3,4]. According to their structural organization [5,6] mammalian galectins can be subdivided in three groups: prototype, consisting of a peptide chain containing a single CRD and found either as monomers (galectins-5, -7, -8, -10, -13 and -14) or as monomers/dimers (galectins-1, -2 and -11); tandem repeat, characterized by the presence of two CRD on the same chain connected by a short link peptide (galectins-4, -6, -8, -9 and -12); and chimera, composed of a single CRD attached to a domain possessing different functions (galectin-3). There are six known galectin-8 isoforms, of which three belong to the prototype group and three to the tandem repeat group [7].

The function of galectins in the extracellular compartment has been thoroughly investigated; they may modulate cell-cell and cell-matrix interactions, cell adhesion and cell signaling by cross-linking to glycoconjugates that contain β-galactosides [1–3,8]. Each member of the galectin family possesses additional specific functions. Galectins-1 and -3 have been traditionally involved in the process of carcinogenesis, tumor progression and metastatic potential [9,10]. Recently, galectin-1 has been identified as a selective binding partner of oncogenic H-Ras (12V) and mediates membrane anchorage of this oncogene and cell transformation [11,12]. The mechanisms through which galectin-3 favors the metastatic process have been further investigated and several other galectins have been associated to tumor progression [13,14]. Some galectins do play an important role as pro-apoptotic or anti-apoptotic factors in normal and tumor cells and in cells of the immune system [15–25]. Recent studies have established a role of galectins-1 and -3, in T cell homeostasis and survival and in T cell-mediated immune disorders; of galectins-9, -10 and -14 in the context of allergic processes [4,17,26–28]; of galectins-1 and -3 in acute

To whom correspondence should be addressed: Lorenzo Chiariotti, Dipartimento di Biologia e Patologia Cellulare e Molecolare, Via S. Pansini 5, 80131 Naples, Italy. Tel.: +39-81-7462056; Fax: +39-81-7703285; E-mail: chiariot@unina.it

inflammation and in microbial infections [27]. Some galectins do participate in the development and differentiation programs [29]. For instance, high galectin-1 expression has been detected in sensory, motor and olfactory neurons and it has been demonstrated to participate in the regulation of olfactory axon fasciculation and targeting [30,31]; galectin-1 also regulates myoblast and fibroblast differentiation [32]. Galectin-7 might play a role in the modulation of the proliferation/differentiation program of epithelia [33,34]. Galectins-1, -3 and -5 are expressed in the trophoectoderm, but a putative role in embryonic implantation has not been confirmed by the analysis of knock-out mice [35]. Additional intracellular functions of galectins-1 and -3 include pre-mRNA splicing [36,37]. Galectin-9 in addition to its other function as an eosinophil chemoattractant (ecalectin) [26] has been identified as the urate transporter/channel [38] a transmembrane protein. The finding that galectin-9 gene gives rise to different mRNA isoforms sustain the idea of the involvement of galectin-9 both in immune/inflammation processes and in potential-sensitive uric acid translocation [39]. Galectin-10 has been identified as the Charcot-Leiden crystal protein of eosinophils and basophils [40].

Because most if not all galectins are functionally multivalent exerting their wide range of biological activities both during development and in adult tissues, it is clear that each galectin must have a specific role, either intra- or extracellularly, depending on the tissue context or on a given differentiation or developmental stage. In other words each galectin should act in a given tissue at a given time. Hence, a precise modulation of their expression and activity is required. Such modulation can be exerted mainly at the transcriptional level or by changing the subcellular localization or, possibly, by the modulation of the expression or glycosilation of specific ligands.

Tissue distribution of galectins

The expression pattern of galectins varies among different adult tissues, changes during development and it is dramatically altered upon neoplastic transformation or in other pathological conditions. Most of these data are summarized in Table 1. Galectin-1, -3, -8 and in part -5 and -9 are expressed in a wide range of tissues. The analysis of galectin-5 and -9 distribution was first complicated by the high degree of similarity. The only certain location for galectin-5 is in rat reticulocytes, and their precursors. Using more specific probes, galectin-9 was found especially in thymus, liver, intestine, spleen, and, at lower levels, in reticulocytes [41,42]. The expression of galectin-9 is also developmentally regulated and is detected at high levels in developing thymus and liver [42].

The expression pattern of the remaining galectins is much more restricted (Table 1). As an example of restricted expression related to a specific function, galectin-14 expression in eosinophils appears to have an important role in allergic inflammation [28]. Another galectin expressed in a specific organ, the lens, is GRIFIN (galectin-related inter-fiber protein) [43] or galectin-11 [4].

Most galectins are also expressed during embryogenesis [29]. The spatiotemporal distribution of galectin-1 and -3 in human and mouse embryogenesis has been thoroughly investigated [29]. Galectin-1 and -3 are first expressed in the trophoectoderm cells of the implanting embryo and have been implicated in the process of implantation [29]. In mouse embryo a prominent expression of galectin-1 in the myotomes of the somites and a specific detection of galectin-3 in the notochord, cartilage and skin has been reported [29]. In human embryogenesis galectin-1 is found prevalently in connective tissues and some epithelia such as the basal layers of the skin and epithelial cells of the gonads whereas galectin-3 is detected mainly in epithelia but also in preossifying condrocytes, notochord, liver and myocardial cells. Because the expression of cell surface lactosaminoglycans undergoes stage- and tissue-specific changes during embryogenesis, it is possible that galectins participate to the complex process of tissue differentiation.

Modulation of galectins expression

Although it is not yet clear whether the 14 galectins identified so far have functions in common, a striking feature of all galectins is the strong modulation of their expression during development, differentiation stages and under different physiological or pathological conditions. The best studied conditions in which galectins expression is modulated are presented in Table 1. However, it is expected that much more information will be available in a short time considering the number of studies currently in progress.

Waiting for further elucidation of the physiological activities of galectins, one of the most exciting fields in the galectin research is the potential biomedical use of galectins or galectin inhibitors as diagnostic tools or therapeutic agents in neoplastic and autoimmune diseases. Carbohydrate structures have been for a long time implicated in cell adhesion, immunomodulation and cancer cell invasion and metastasis, but only in the past few years many investigators have focused attention on the endogenous molecules that bind carbohydrates, *i.e.* galectins. Because galectins are involved in the modulation of cell adhesion, cell growth, immune response, apoptosis and angiogenesis, it is clear that changes of their expression might have a critical role in tumor progression.

In the last decade the role and expression pattern of galectin-1 and -3 in carcinogenesis and tumor progression have been extensively investigated [9,10]. In more recent years it has been found that the expression of other galectins also changes in tumors (Table 1). A particularly attractive system for this kind of studies has been thyroid carcinogenesis. Galectin-1 expression correlates with the degree of malignancy in rat thyroid cell lines transformed with several cellular or viral oncogenes [44]. Galectin-1 mRNA levels increase 20-fold in low tumorigenic and up to 100-fold in high tumorigenic cells. Galectin-1 mRNA

Table 1. Distribution of galectins and relevant changes of expression in different conditions

		Regulation in systems		
Galectin	*Prevalent distribution*	*Up-regulation*	*Down-regulation*	*Regulators*
1	Most tissues: skeletal muscle, heart, placenta, lymphatic tissues, etc.	Most transformed cell lines and tumors. Chronic pancreatitis. Parasites infection	Tumors: head and neck	Azacytidine, butyrate, RA, budesonide, TSH, MM-LDL (up)
2	Gastro- intestinal tract			
3	Macrophages, epithelia	Tumors: thyroid, colon late stages, gastric, CNS, bladder, large cell lymphomas. Renal failure, chronic pancreatitis, brain ischemia. Streptococcal pneumonia	Tumors: colon initial stages, breast, ovarian, prostate, head and neck, basal cell. HTLV-I infection	RA, Heat-shock, trypsine (down). alkylating agents, UV-C, HIV tat (up)
4	Gastro-intestinal tract	Tumors: liver, gastric	Tumors: colon	
5	Blastocyst at implantation, reticulocytes			
6	Gastro-intestinal tract			
7	Epithelia	Tumors: breast	Tumors: bladder, skin	RA (down). UV-B (up)
8	Liver, kidney, cardiac muscle, lung, brain, colon	Tumors: pancreas, liver, skin, larynx	Tumors: Colon	
9 (ecalectin)	Eosinophils, monocytes, macrophages, gastro-intestinal tract, melanocytic nevi	Hodgkin's lymphomas. Allergic and parasitic response.	Tumors: melanoma	
9 (urate transporter /channel)	Kidney, gastro-intestinal tract, melanocytic nevi	Hodgkin's lymphomas	Tumors: melanoma	
10	Eosinophils, basophils			Butyrate (up)
11 (GRIFIN)	Lens			
12	Adipocytes	Cell cycle: G1 phase		PPARγ ligand troglitazone, food restriction (up)
13	Placenta			
14	Eosinophils	Allergic response		

levels are very low in normal thyroid, non-neoplastic goiters, adenomas and follicular carcinomas, while are increased (5-20-fold) in a large proportion of papillary and in almost all the aggressive anaplastic carcinoma [45]. By immunohistochemical studies, however, a higher galectin-1 content compared to normal thyroid (20-30-fold) has been reported in all follicular and also in some medullary carcinomas [46]. In these latter studies the percentage of papillary carcinomas positive for galectin-1 was higher than in Northern blot studies. The expression of galectin-3 in thyroid tumors shows a very similar behavior being very low or absent in non-malignant and high in all malignant tissue samples [46–48]. Thus, galectin-1 and -3 are potentially very useful diagnostic markers in the thyroid neoplastic pathology. Galectin-3 evaluation in thyroid specimens obtained by fine-needle aspiration biopsy can distinguish, in a pre-surgical phase, follicular, papillary and follicular variant of papillary carcinomas from the benign follicular adenomas allowing a better selection of the patients that really require surgery [48,49].

It is worth to draw the attention to the fact that specific galectins are modulated in different tumors and that in the same kind of tumor different galectins are up or down modulated (Table 1). An attractive system to illustrate this last point is colorectal cancer in which galectin-1 is upregulated, galectin-4 and -8 are downregulated and galectin-3 is downregulated in the initial stages and upregulated during tumor progression and metastasis [50–53].

Galectin expression is modulated in other pathological conditions such as immune response, inflammation and infection in which each galectin may play a specific role [4]. All these observations support the possible use of galectins or galectin inhibitors in therapy as immunosuppressors or as antimetastatic agents, respectively. Very interestingly, saccharides interacting with galectins inhibit metastasis of experimental cancer *in vivo* [14,54]. The biomedical use of galectin-1 inhibitors is particularly attractive since the galectin-1 and galectin-3 null mutant mice are relatively healthy [29].

In addition to changes under physiopathological conditions, galectins expression can be modulated by a variety of physicochemical agents (Table 1). For example, a transient increase in galectin-1 expression is achieved upon treatment of thyroid cells with the thyroid stimulating hormone (TSH) [55]. Treatment of oncogene transformed neural cells with retinoic acid (RA), a differentiating agent, leads to extinction of galectin-1 expression [55]. Conversely, it has been reported that in human head and neck carcinoma cells, galectin-1 expression is stimulated by sodium butyrate (butyrate) treatment [56]. Because galectin-1 expression is increased also upon treatment with other inhibitors of histone deacetylases, it has been proposed that galectin-1 expression be linked to the status of histone acetylation [56]. In human endothelial cells galectin-1 synthesis is upregulated by minimally oxidized low-density lipoprotein (MM-LDL) [15,57]. Activation of galectin-1 gene can be also achieved by fusing non-expressing cells with tumor cells or by treatment of non-expressing cells with the DNA demethylating agent azacytidine [58–60]. Galectin-3 expression is modulated in cultured cells by differentiation inducers [61], is induced by human T lymphotropic virus-I infection of human T lymphocytes [62], and by the HIV tat protein [63]. Finally, galectin-7 expression in keratinocytes is moderately repressed by RA, a behavior contrasting with other keratinocyte markers [33,64].

Transcriptional regulation of galectin genes

The gene structure and chromosomal localization of the available human galectin genes has been determined (Table 2) using the MapViewer program and Entrez genome database at the NCBI web site.

Based on what has been discussed in the previous paragraphs, the study of the transcriptional regulation of galectin genes is particularly worth of interest. Despite these considerations, in recent years still very few experimental data have appeared on this subject. The upstream regulatory regions of galectin-1, -2, -3, -4, -6 and -10 from different species [41,52,65–70], and more recently human galectin-9 and -12 [24,38] and rat galectin-11 (GRIFIN) [43] have been cloned. Only for few of them promoter function studies have been performed. The main functionally characterized or suggested as potential regulatory elements within the galectin genes are shown in Figure 1.

A small genomic region spanning the transcription start site (−63/+45) is sufficient for the transcriptional activity of the mouse galectin-1 gene [68]. Both an upstream and a downstream position-dependent cis-elements are necessary for efficient transcriptional activity [68]. Recently, an additional start site has been mapped at position −31. A Sp1 site (−57/−48) and a consensus initiator (Inr) element, which partially overlaps a non-canonical TATA box, direct RNA initiation from both start sites [71]. The upstream transcripts contribute to more than half of the galectin-1 mRNA population. The 5′ end of this transcript is extremely GC-rich and may fold into a stable hairpin structure, which could influence translation [71]. Lotan *et al.* have

Table 2. Chromosomal location and structure of human genes encoding galectins

Gene (Protein encoded)	*Human locus*	*No. of exons*	*Transcript length (kb)*
LGALS1 (galectin-1)	22q12	4	0.6
LGALS2 (galectin-2)	22q12	4	0.5
LGALS3 (galectin-3)	14q21-22	6	1.1
LGALS4 (galectin-4)	19q13.2	9	1.0
LGALS7 (galectin-7)	19q13.2	4	0.6
LGALS8 (galectin-8)	1q42-q43	11	4.3
LGALS9 (galectin-9)	17q11.1	11	1.7
CLC (galectin-10)	19q13.1	4	0.7
LGALS12 (galectin-12)	11q13	9	1.7
PP13 (galectin-13)	19q13.1	5	0.6

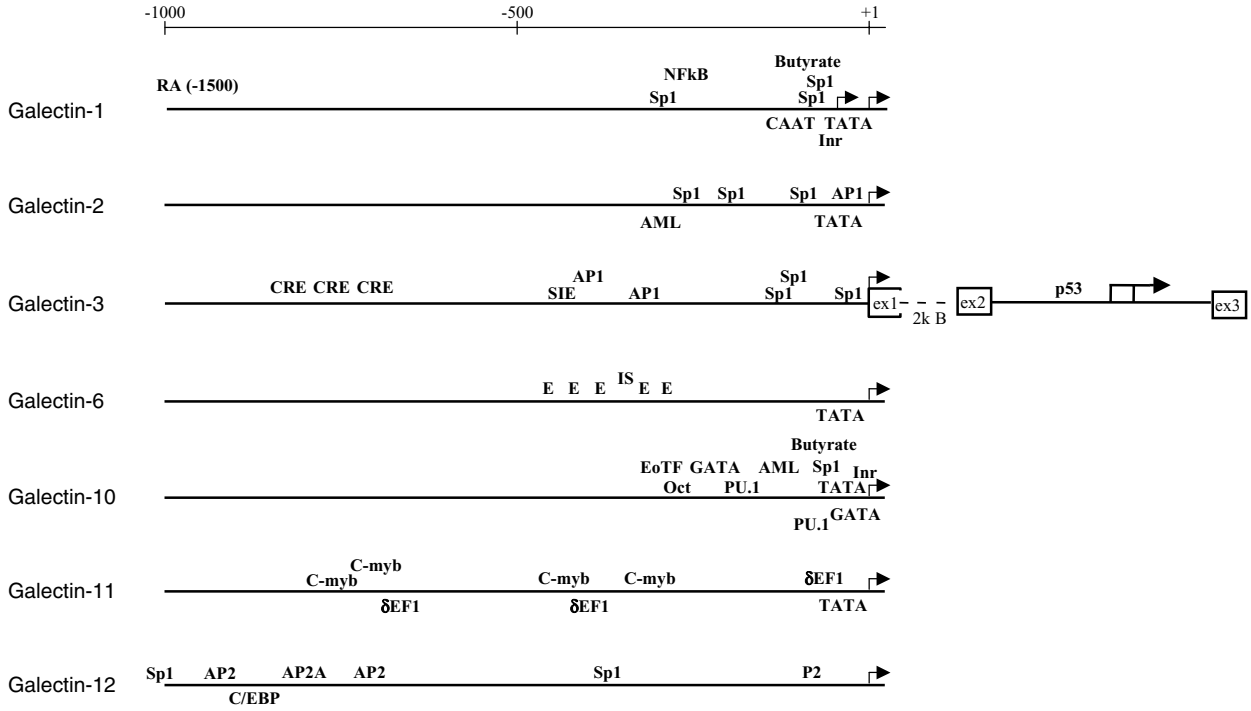

Figure 1. Promoter regions of some galectin genes. The approximate position of the putative and/or characterized regulatory element is indicated: Sp1, CAAT box, TATA box, C/EBP, nuclear factor kB binding site (NFkB), cAMP responsive element (CRE), sis inducible element (SIE), consensus binding site for p53 (p53), E box (E), intestine specific sequence (IS), myeloid specific sequence (MS), GATA factor binding site (GATA), purine rich sequence 1 (PU.1), AML binding site (AML), eosinophil transcription factor binding site (EoTF), sodium butyrate (butyrate) and retinoic acid (RA) responsive sequences, initiator (Inr), AP2 and AP1. For further details see text. Bent arrows indicate the transcription start sites. The second intron of the galectin-3 gene, 651 bp long, is not drawn to scale.

shown that the Sp1 site is also crucial for the butyrate-induced expression of galectin-1 while an RA responsive region was mapped within the region −1578/−1448 [70,72].

A few regulatory elements have been recognized in the *LGALS2* gene promoter region [65]. Among them an AML recognition site similar to that found in the *CLC* gene promoter has been identified (Figure 1). Because AMLfactors have been suggested to be master regulators of hematopoiesis [73], their role in the regulation of *LGALS2* gene, which has a restricted expression in the gastrointestinal tract, remains to be established.

The human and mouse galectin-3 promoters have been functionally characterized [69]. The genomic region from −339 to +141 has promoter activity. Galectin-3 gene can be considered an early immediate gene since its expression is rapidly increased upon serum stimulation. The serum responsive activation regions are located between −513 and −339 and between −339 and −229. However these regions lack a typical sequence motif, which forms the core-binding site of the serum response element. The authors suggest that serum activation may be accomplished via different elements such as SIE (sis-inducible elements) or CRE that are present in the galectin-3 promoter (Figure 1). Legrand *et al.* have demonstrated that the second intron of the galectin-3 gene has promoter activity, which is downmodulated by wild-type p53 but not by a mutated form of p53 [67] (Figure 1). The distal and the internal promoters are independent rather than exclusive. It has been shown that transcripts from the internal promoter define an internal gene embedded within *LGALS3* and named *GALIG* (galectin-3 internal gene). *GALIG* transcripts contain two overlapping open reading frames, which, upon translation, give rise to proteins unrelated to galectin-3 and localized to different subcellular compartments [74]. By BLAST analysis we could not identify transcripts homologous to GALIG in other species. The rabbit galectin-3 gene has been isolated and characterized. Its structure has an organization similar to that of the murine galectin-3 gene. The genomic sequences located upstream from its 5′ end, upon insertion upstream from a promoter-free reporter gene, exhibit a strong promoter activity. This activity is upregulated upon treatment of transfected smooth muscle cells with phorbol 12-myristate 13-acetate (PMA) as well as upon transfection with an EJ/ras encoding plasmid. Conversely, it is downmodulated upon transfection with wild-type p53 but not with mutated p53. The regulatory sequences involved in the positive regulation of the gene were located upon serial deletion experiments [75].

The mouse *Lgals6* gene promoter contains a sequence between bp −354 through bp −367, which is highly homologous to a 19 bp sequence within the apolipoprotein B upstream region that has been implicated in intestine-specific expression of this protein [76]. In addition the galectin-6 promoter contains several E boxes, DNA elements that have been implicated in the regulation of gene expression in proliferating and differentiating epithelial cells. In particular, in the galectin-6 promoter region, one E box resembles the MycMax binding site and others resemble the MyoD binding site [76].

Human galectin-10 promoter constructs have been functionally analyzed in the attempt to identify DNA elements that regulate gene expression during the commitment and differentiation of the eosinophil lineage [66]. The authors found that the −292 to −411 bp region of the galectin-10 promoter may confer some specificity for expression in the eosinophil lineage. The galectin-10 promoter contains two consensus GATA binding sites, a purine-rich sequence that presents potential binding sites for PU.1, a member of the ets family of genes, as well as sequences described in other myeloid-specific genes [66] (Figure 1). In a recent paper Dyer and Rosenberg have performed a functional study of the human galectin-10 promoter [77]. The integrity of the Sp1 site, of the Oct site and of the GATA and EoTF sites (Figure 1) is necessary for full promoter activity. The Sp1 and Oct factors bind the respective sites in *in vitro* assays. Similarly to galectin-1, galectin-10 expression is induced by butyrate and this effect requires the integrity of the Sp1 site. Moreover, AML3 and YY1 factors can bind their respective sites (AML and Inr) and repress promoter function.

The rat galectin-11 (GRIFIN) promoter region contains three sets of direct repeats [43]. Within or between these repeats several consensus sequences for C-myb and δEF1 have been identified [43] (Figure 1). The presence of several δEF1 sites in the promoter region and in exon 4 suggests a role in the lens specific expression of this gene. In fact, a similar distribution of binding sites for the zinc finger δEF1 transcription factor is found within the δ1-crystallin chicken gene, whose lens-specific expression has been shown to depend on this factor [43].

In the human galectin-12 promoter region, in addition to several Sp1 and AP2 sites, a potential binding site for the CCAAT/enhancer-binding protein (C/EBP) has been identified [24] (Figure 1). This suggests a role of adipose-specific enhancers such as C/EBP on galectin-12 induced expression and apoptotic potential.

Role of DNA methylation in the control of galectin genes expression

It is becoming evident that epigenetic changes are among the most common alterations observed in human cancer cells. The expression of several oncogenes and tumor suppressor genes is profoundly altered through changes in their methylation pattern in human tumors of different origins [78,79]. Because several galectins are strongly modulated and play a role in the neoplastic process, the study of DNA methylation in the control of galectin genes is particularly worth of interest. For several years we have studied the regulation of the galectin-1 gene expression. The expression of this gene is increased in oncogene transformed cell lines [44] and in a variety of human tumors [45,80]. These studies led us to identify the methylation status of the galectin-1 promoter as the main mechanism that controls the expression of the gene in transformed cells, in normal tissues and in tumors. The demethylating agent azacytidine is able to reactivate galectin-1 transcription in several cell systems [58–60,81]. Trans-acting factors that potentially could activate galectin-1 gene are produced by both normal and transformed cells [68] but in several cell hybrids obtained by the fusion of human osteosarcoma cells with normal rat liver cells, a constant reactivation of the previously silent galectin-1 alleles was observed [58]. We have established that the mechanism controlling the activation of galectin-1 gene in tumors and in cell hybrids is the transition from a methylated to an unmethylated state of 11 CpG sites lying in a small 90 bp region surrounding the transcription initiation site [81] (Figure 1). On the contrary, in the adjacent region no changes in the methylation profile were observed. Bisulfite genomic sequencing of the galectin-1 promoter in normal tissues showed that the methylation pattern in each cell was heterogeneous but some features were always conserved: (i) the density rather than site-specific methylation is responsible of the activity of the gene; (ii) a specific site (the last of the 11 CpG cluster) is always methylated in both normal expressing and nonexpressing tissues, in all cells and on both strands [10,82]. On these evidences we have developed a model in which specific CpG sites in their methylated state could be part of an in-cis signal for the recruitment of ubiquitous regulatory molecules (methylases, demethylase, methyl-binding proteins). Interestingly, we have found that what distinguishes the active alleles of a normal expressing tissue from that of a tumor is the loss of methylation of the specific site that is always methylated in normal expressing and nonexpressing tissues (L. Chiariotti, and C.B. Bruni, unpublished results).

To date there are no data on the role of DNA methylation in the control of expression of other galectin genes. In Figure 2 we present the distribution of CpG sites within the genomic region surrounding the transcription start site of the available human galectin genes. It is interesting to observe that most of the galectin genes that are strongly modulated in carcinogenesis exhibit a distribution of CpG sites around the transcription initiation site compatible with a role of DNA methylation in transcription control.

In conclusion, the identification of coordinate mechanisms governing the activity of the different galectin genes and the search for common biological activities of their products has been elusive so far. It is hoped that a growing number of studies in these two apparently unrelated fields could lead to a unifying picture of the biological functions of this family of proteins.

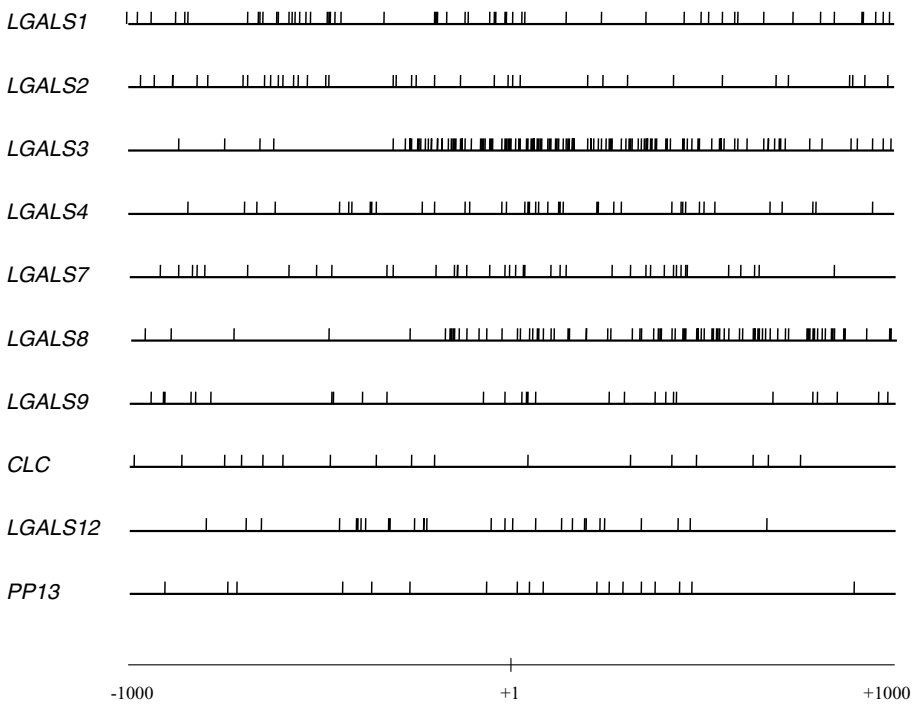

Figure 2. Distribution of CpG dinucleotides in the human galectin genes −1000/+1000 regions. Vertical lines indicate the position of each dinucleotide in the DNA sequences of the indicated human galectin genes. Sequences were retrieved using the MapViewer program and Entrez genome database at the NCBI web site and analyzed using the GeneWorks program produced by IntelliGenetics Inc.

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