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REVIEW

Mammalian Galectins: Structure, Carbohydrate Specificity, and Functions

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Abstract—Galectins are a family of β-galactoside binding lectins, homological by a sequence of the carbohydrate-binding site. In this review literature data about structure and carbohydrate specificity of galectins are discussed. The role of galectins in the regulation of cell adhesion in immune response, inflammation, and cancer progression is considered.

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Galectins are of interest because of the involvement of these proteins in numerous processes of cell vital activity, such as cell cycle regulation, cell–cell and cell–intercellular matrix adhesion, and transmission of intercellular signals. Moreover, some galectins are markers of cell transformation and mediators of inflammation [1-3]. Initially the term galectins was used for proteins that bound carbohydrates in the presence of thiols, and they were considered to be S-proteins [4]. But with discoveries of new galectins with thiol-independent specificity, the group was enlarged. Galectins now are β-galactoside-binding lectins defined by shared consensus amino acid sequence. By 2006, fourteen representatives of this family isolated from mammalian tissues have been described [5]. Galectins are usually small soluble proteins with molecular weight of 14-35 kD and having only a carbohydrate-recognition domain and no other functional domains.

GENERAL CHARACTERISTICS OF GALECTINS

Galectins are found both inside cells (in the cytoplasm and nucleus) and on the membrane surface [5].

Galectin-1 and galectin-3 were the first ones isolated from mammalian tissues [3]. Galectins-1-4 and -6-9 are expressed on epithelial, endothelial, and immune cells, and galectins-5, -10-14 have been detected on different cell populations (Table 1) [6]. No N- and O-chains have been found in galectins, although some galectins contain potential N-glycosylation sites [7].

Galectins have no signaling sequence and are secreted via a nonclassical pathway escaping the endoplasmic reticulum and Golgi apparatus [7, 8]. Galectins are exported onto the cell surface through vesicles (exosomes) produced by protruding of the membrane region in the place of galectin accumulation. Some factors usually associated with stress for the cell (inflammation, malignization) induce destruction of the vesicles with release of galectin into the intercellular space [7, 8]. The mechanism of galectin transfer to the cell membrane and of the subsequent destruction of the vesicles is unknown, although heat shock proteins, calcium ionophores, and monensin are shown to induce export of galectins towards the membrane surface [7]. Galectins with point mutations making them incapable of binding saccharides are not transferred onto the membrane surface and are not secreted into the intercellular space; therefore, glycolipids and glycoproteins of the cell membrane are suggested to act as chemoattractants for galectins [9, 10].

The majority of galectins are located in chromosomes, and the structure of genes encoding them has been established (Table 2). In some cases regulatory elements have been found represented by binding sites of different

Abbreviations: CRD) carbohydrate-recognition domain; LacNAc) lactosamine; $(LacNAc)_n$, where $n > 1$) oligolactosamine; LNnT) lactoneotetraose; Su) sulfate.

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Table 1. Galectin expression on cell surface

Galectin	Molecular weight	Cells, tissues, and organs
1	14-15	skeletal, muscle, and lymphoid tissues heart, placenta, kidneys, lungs neurons endothelium, dendritic cells, macrophages, bone marrow stroma cells
2	14	gastrointestinal tract (GIT)
3	27-36	activated macrophages, granulocytes, mast cells GIT and respiratory pathway epithelium neurons kidneys
4	36	GIT and oral cavity epithelium
5	17	rat erythrocytes and reticulocytes
6	33	intestinal epithelium
7	15	keratinocytes
8	34	liver, kidneys, heart, lungs, brain
9	36	liver, spleen, kidneys, small intestine, lungs lymphoid (thymus) and muscular tissues
10	16.5	eosinophils, basophils
11	14	eye lens
12	35	adipocytes
13	16	placenta
14	18	eosinophils

transcription factors (Sp1, NFκB, p53, retinoic acid, cAMP) required for expression of galectin genes [6, 11]. Some data suggest that the methylation degree of DNA CpG-sites is important for the regulation of galectin gene expression [6].

STRUCTURE AND DOMAIN ORGANIZATION

Mammalian galectins are subdivided into three groups depending on their structural organization [1-3, 5] (Fig. 1). The first group consists of prototype galectins with molecular weight of ~14 kD which have the only carbohydrate-recognition domain (CRD) and exist either as monomers (galectins-5, -10, and -14) or as noncovalent dimers (galectins-1, -2, -7, -11); galectin-13 homodimer stabilized by two disulfide bonds is an exception. The second group includes tandem type galectins-4, -6, -8, -9, and -12 characterized by the presence in the molecule of two CRDs, homologous but not identical in the amino acid sequences; these CRDs are connected by a short linker peptide. The third group is known to contain the only chimeric type galectin-3, which consists of one CRD and one regulatory domain with repeated collagen-like regions. Galectins of invertebrates have other structures as well, e.g. GC-1 and GC-2 prototype galectins isolated from the sponge *G. cydnium* form tetramers [12], and galectin from the oyster *Crassostrea virginica* has four CRDs in the same polypeptide chain [13].

There are identified mRNAs encoding six galectin-8 isoforms; three of them belong to prototype galectins, the other three being tandem type ones [3]; the corresponding proteins have not been isolated [14].

The spatial structure of the CRD is established for galectin-1 [15, 16], -2 [16, 17], -3 [18, 19], -7 [20, 21], -9 [22], and -10 [23, 24].

The globular CRD of galectins consists of two antiparallel β-sheets (β-sandwich), one of which is formed by six (S1-S6) and the other formed by five (F1-F5) chains (Fig. 2) [16, 21, 25, 26]. The topology of the galectin globule is β jelly-roll (Fig. 2a). The S3-S6 chains are disposed in the protein sequence immediately one after another and form two long fingers which are steeply

Table 2. Structure and chromosomal location of human genes encoding galectins [6]

Gene (protein encoding)	Chromosomal locus	Number 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

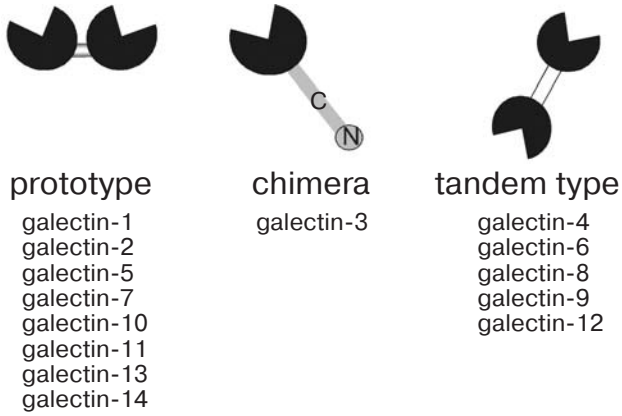

Fig. 1. Structural organization of galectins. CRDs are shown in black, the tandem type galectin linker is shown in white, and the galectin-3 regulatory domain is shown in gray. C and N are, respectively, collagen and N-terminal regions of the regulatory domain.

bent on the ends and form a long negatively charged groove where carbohydrate is bound (Fig. 2b). Leffler proposed subdividing the carbohydrate-binding site of galectins into five regions (A, B, C, D, and E; Fig. 3), according to the binding with a linear glycan [3]. The main role belongs to amino acids of the C and D sites, which bind with galactose residues of a lactosamine fragment, whereas the A and B regions recognize a fragment X and the region E recognizes a fragment Y in the lactosamine chain structure, which can be presented as 3-O-X-Galβ1-4GlcNAc-Y [3, 16, 26].

Structure of the CRD of prototype galectins. The sequence of prototype galectins includes several cysteine residues (e.g. Cys2, Cys16, Cys42, Cys60, Cys88, and Cys130 in galectin-1), but they all are located beyond the carbohydrate-binding site. During the isolation of galectins, SH-groups are oxidized with production of intramolecular disulfide bridges, e.g. between Cys16 and Cys88 (i.e. between chains F1-F5), which influences the spatial packing of the protein [16].

Prototype galectins dimerize due to interaction of hydrophobic regions of monomers located in the groove. Such a hydrophobic region in galectin-1 is formed by side chains of amino acids Leu4, Val5, Ala6, Leu9 (the S1-chain), and also by Ile128, Lys129, Val131, and Phe133 (the F1-chain) located on the N- and C-ends, respectively [15-17]. In solution, galectins-1 and -2 exist as noncovalent dimers; data on the subunit organization of galectin-7 are contradictory, some authors thinking it to be a monomer in solution but the others believing it to be a dimer [25].

Prototype galectin CRDs are located on the opposite ends of the dimer (at a distance of 40-50 Å in the galectin-1 molecule [26] on the same side of the β-sandwich) (Fig. 1). The N- and C-ends of the protein chain are at the place where two monomers contact [15].

Eight conservative amino acid residues of prototype galectin CRDs are responsible for the lectin binding with sugars: these are His44, Asn46, Arg48, Val59, Asn61, Trp68, Glu71, and Arg73 (Fig. 4) [15-17, 26]. Three hydroxyl groups, those at the C-4 and C-6 of the Gal residue and at the C-3 of the GlcNAc residue form

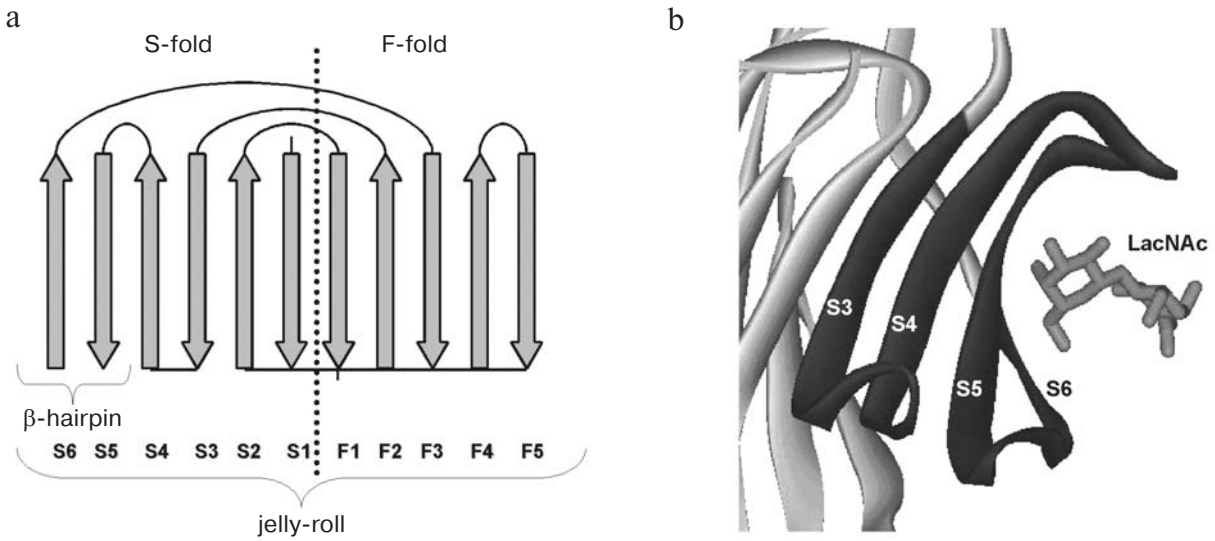

Fig. 2. Secondary and tertiary structures of galectin CRDs. In the topological scheme (a), a bilayer β-sandwich is unfolded and placed onto a plane; the dotted line separates two sheets of the β-sandwich (F- and S-folds). Amino acid residues inside the β-sheets are shown by vertical arrows. The galectin globule looks like a jelly-roll or a manifold curved β-hairpin. The galectin-1 complex with c Galβ1-4GlcNAc (b) was designed with the ViewerPro program based on a model proposed in [15] (PDB: 1W6P, http://www.rcsb.org). Black color indicates the chains S3-S6, which are arranged immediately one after another in the protein sequence and form two long fingers; the carbohydrate ligand binding occurs in the groove formed by the S3-S6 chains.

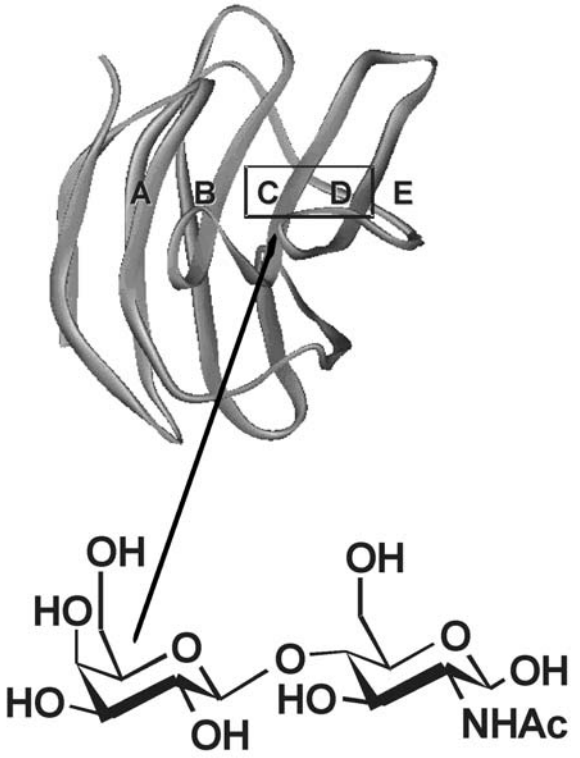

Fig. 3. The galectin CRD model proposed by H. Leffler consists of five regions: A, B, C, D, and E. Galactose residues in X-Galβ1-4GlcNAc-Y bind with regions C and D, and amino acids of regions A, B, and E recognize substituents X and Y [3].

hydrogen bonds with side chains of the amino acid residues His44, Asn46, Arg48, Asn61, Glu71, and Arg73. The OH-group at the Gal C-4 is crucial for carbohydrate binding with galectin. Hydrophobic amino acid residues Val59 and Trp68 form Van der Waals contacts with the carbohydrate (stacking interactions of Trp indole ring with C-3, C-4, C-5, and C-6 of the galactose residue). Some data suggest that galectins can bind more complicated oligosaccharides than Galβ1-4GlcNAc.

Side chains of the Arg48 and Asp54, Arg73 and Asp54, Arg73 and Glu71 residues form salt bridges with each other. These bridges, as well as electrostatic bonds between the residues Asn33 and His44, Arg111 and Asn61, Arg48 and Asn46, Asn46 and Ser29 determine the orientation of the protein functional groups which is required for binding the carbohydrate residue [15-17, 25].

Structure of galectin-3 (chimeric type). Galectin-3 consists of two functional domains: the N-terminal regulatory domain (100-150 amino acid residues) and the C-terminal CRD (135 amino acid residues) (Fig. 2). The regulatory domain contains repeated collagen-like sequences [18, 19, 27, 28] and has high mobility, which can cause difficulties for crystallization and studies on the native galectin-3 structure. Human galectin-3 CRD is formed by amino acids 118-250. Similarly to the carbohydrate-recognition domains of galectins-1 and -2, it consists of five (F1-F5) and six (S1-S6) β-folds packed in a β-sandwich. The CRD is formed by the S4-S6 chains and looks like a groove with two open ends (Fig. 2). The galactose residue in LacNAc is deeply submerged into the carbohydrate-binding groove: the protein covers 166 Å^2 of 230 Å^2 total area of galactose, whereas the whole GlcNAc residue is virtually completely available to the solvent (Fig. 5) [18]. The OH-group at C-4 of the Gal residue plays a key role in the binding because it forms hydrogen bonds with His158, Asn160, and Arg162. The OH-group at the C-6 of Gal interacts with Glu184 and Asn174. Atoms C-3, C-4, C-5, and C-6 of the galactose residue form Van der Waals contacts with Trp181. The OH-group at C-3 of the residue GlcNAc forms hydrogen bonds with Glu184 and Arg162. The N-acetyl group at C-2 of the GlcNAc residue forms additional bonds with Glu165 and Arg186, and this provides for the higher affinity of galectin-3 for lactosamine than for lactose. The active site of galectin-3 includes residue Arg144 capable of interacting with a carbohydrate residue X in 3-O-X-Galβ1-4GlcNAc (Fig. 5) [18]. Near the reducing end of the carbohydrate to be bound, the groove is enlarged, which promotes contacts with one or several residues (Y) in Galβ1-4GlcNAc-Y. This explains the ability of galectin to dis-

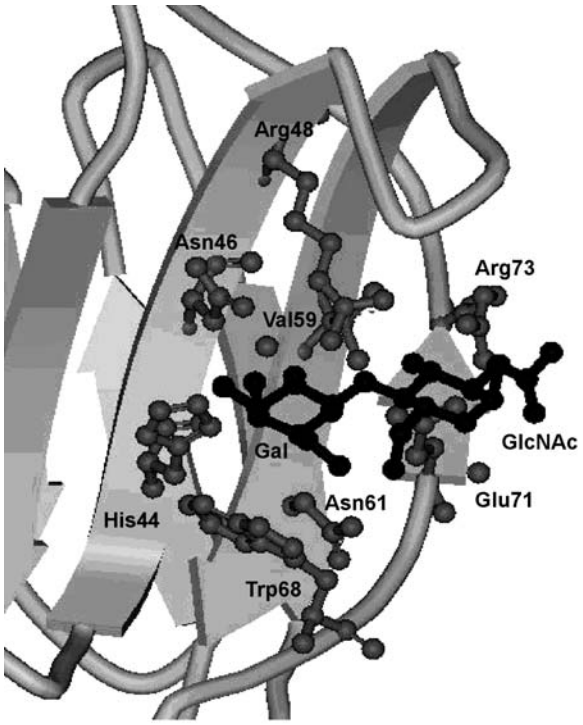

Fig. 4. Structure of galectin-1 CRD in the complex with disaccharide Galβ1-4GlcNAc (black). Amino acids involved in the complexing with the disaccharide are shown in gray. The complex was designed using the ViewerPro program based on the model proposed in [15] (PDB: 1W6P, http://www.rcsb.org).

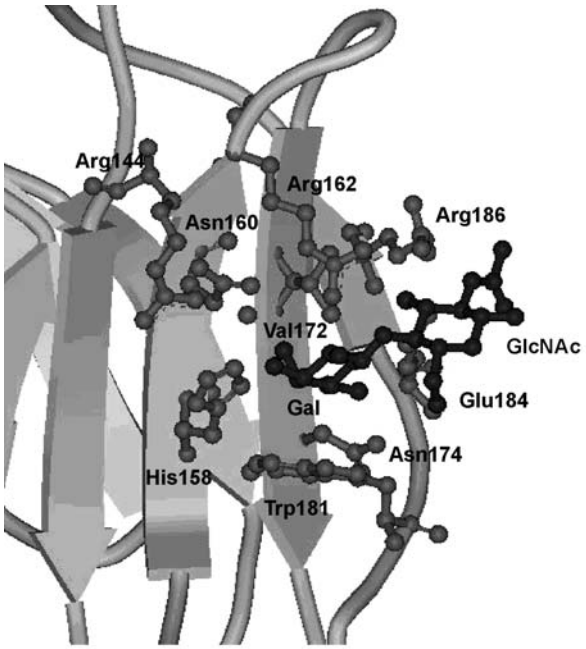

Fig. 5. Structure of galectin-3 CRD in the complex with disaccharide Galβ1-4GlcNAc (black). Amino acids involved in the complexing with the disaccharide are shown in gray. The complex was designed using the ViewerPro program based on the model proposed in [18] (PDB: 1KJL, http://www.rcsb.org).

criminate significantly larger oligosaccharide fragments than disaccharide Galβ1-4GlcNAc [18].

Molecular dynamics studies have revealed allosteric changes in galectins-1 and -3 on binding a carbohydrate ligand. These changes concern both the carbohydrate-binding site and the adjacent loop regions [29].

The galectin-3 regulatory domain contains 7-14 collagen-like regions with 8-11 amino acids in each, depending on the mammal species, and is encoded by the same exon [27, 30]. The terminal region of the regulatory domain (18 amino acids) has two phosphorylation sites by residues Ser6 and Ser12. It is not known what enzymes are involved in the *in vivo* phosphorylation of galectin, whereas the *in vitro* phosphorylation is performed under the influence of casein kinase-I and -II [27]. The collagenase-caused cleavage of galectin-3 at residue Ala62 increases the carbohydrate-binding activity of lectin, whereas galectin cleaved in the interval of Pro101-Pro106 loses the ability to bind oligosaccharides [30]. Most likely, the positive contribution of the regulatory domain is a result of interaction of the collagen-like region Pro101-Pro106 with the hydrophobic region of the CRD, which leads to conformational changes in the latter [30, 31].

It is generally believed that association in the regulatory domain region in the presence of multivalent carbohydrate ligands results in oligomerization of galectin-3 to pentamer [32]; however, these experiments have been performed *in vitro*, and there are no direct data on the *in vivo* production of multimers by galectin-3.

Structure of tandem type galectins. Tandem type galectins have two CRDs located near the N- and C-end of the protein and separated by a short (25-35 amino acid residues) peptide linker enriched with proline and glycine (Fig. 2). The peptide linker is homologous to the collagen-like domain of galectin-3 [3, 14, 22], and the homology between N-CRD and C-CRD is 35-40%.

The spatial organization of the galectin-9 N-CRD has been studied by X-ray crystallography, whereas the C-CRD cannot be crystallized because of a low solubility [22]. The spatial packing of the galectin-9 N-CRD is similar to that of the prototype and chimeric type galectins [22].

Specificity of galectins. To understand the functions of galectins and search for their natural ligands, it is necessary to know their carbohydrate specificity. The specificity of galectins was studied by such approaches as hemagglutination, equilibrium dialysis, and capillary affinity electrophoresis. The galectin specificity has been more completely characterized by solid-phase methods, frontal affinity chromatography, and fluorescence polarization [12, 33-36]. Note that data on LacNAc binding obtained by different groups and by different methods are similar but markedly different in details. The principal conclusion of these studies is that the binding is mainly determined by hydroxyl groups at galactose residues C-4 and C-6 and also at the GlcNAc residue C-3. Oligosaccharides of the mammalian cell manifesting an affinity for galectins are presented in Table 3.

Interactions of galectins with oligolactosamines. As shown by frontal affinity chromatography, oligolactosamines ($LacNAc_n$, where n = 2, 3, 5) bind to galectins-3, -8, and -9, but not to prototype galectins [12]. On the contrary, linear and branched oligogalactosamines in a solid-phase system displayed an affinity for prototype galectins, and the binding level increased with an increase of LacNAc units in oligolactosamine chain [35, 36]. However, oligolactosamines $LacNAc_2$ and $LacNAc_3$ manifested a high affinity to galectins-2, -3, -7, and -8 [34], whereas the binding level to galectins-1 and -4 was significantly lower (Table 3). Moreover, lactoneotetraose (LNnT) bound to galectin-3 and -4 considerably weaker than to galectins-2, -7, and -8, and no interaction with galectin-1 was observed [34]. But in [35], the binding of LNnT to galectin-1 was shown to be similar to the binding of $LacNAc_2$. The affinity of LNnT for galectins-1 and -3 was also revealed by calorimetry [37]. It should be noted that the high affinity of oligolactosamines for galectins is not due to interaction of galectins with internal residues of the oligolactosamine chain, because crystallographic studies on galectin complexes with saccharides have detected no such interaction, i.e. galectins bind the terminal Gal residue, and other units of the oligolactosamine chain only promote

this binding [38]. This was also supported by the finding that degalactosylation of the terminal LacNAc was associated with the loss by oligolactosamines of the ability to bind with galectins [35].

Interaction of galectins with sulfated derivatives of disaccharides Galβ1-4/3GlcNAc and Galβ1-3GalNAc. Negatively charged sulfate in β-galactosides can affect their interaction with galectins. Thus, interaction of 3-O-Su-Galβ1-4GlcNAc and 3-O-Su-Galβ1-3GlcNAc with galectin-1 is an order higher than with LacNAc and oligolactosamines (n = 2, 3) (Table 3). Note that isomers with sulfate in position 4 or 6 of the galactose residue do not bind to galectin-1 [34]. Other prototype galectins do not bind with sulfated LacNAc derivatives [34].

Table 3. Carbohydrate specificity of galectins (data from http://www.functionalglycomics.org)

Ligand	Galectin						
	1	2	3	4	5	7	8
Galβ1-4GlcNAc (LacNAc)	+/–	+	–	++	–	+	+
Galβ1-3GlcNAc (Le^c)	–	+	–	+		+	++++
Galβ1-4GlcNAcβ1-3Galβ1-4Glc (LNnT)	+++[1]	+++	+	++	+	++++	++++
Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAc ($LacNAc_2$)	++[1]	+++	+	+	+	++++	++
Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAc ($LacNAc_3$)	+++[1]	+	++	–		++++	+++
Galβ1-3GalNAcβ ($T_{ββ}$)	–	–	+/–	++++		–	++++
Galβ1-3GalNAcα (TF)	+	–	–	+++		–	++
3-O-Su-Galβ1-3GlcNAc	++++	–	+/–	+/–		–	++++
3-O-Su-Galβ1-3GalNAcα	–	–	–	+++		–	++++
3-O-Su-Galβ1-3GalNAcβ	–	–	–	+++		–	++++
Galα1-3Galβ1-4GlcNAc	–	+	+++	+++	++++[2]	+++	+
GalNAcα1-3Galβ	–	–	–	+++	++++[2]	–[3]	+++[3]
Fucα1-2Galβ1-4GlcNAc (H type 2)	++	++++	++	++		+++	++
Fucα1-2Galβ1-3GlcNAc (H type 1)		+	+++	+++		+++	++++
GalNAcα1-3 \ Galβ1-3GlcNAc (A type 1) / Fucα1-2	++	++++	–	–		–	++++
GalNAcα1-3 \ Galβ1-4GlcNAc (A type 2) / Fucα1-2	+	++++	++	++++		–	++
Galα1-3 \ Galβ1-3GlcNAc (B type 1) / Fucα1-2	++	++++	–	–		++++	++++
Galα1-3 \ Galβ1-4GlcNAc (B type 2) / Fucα1-2	–	++++	+++	++++		++++	+++
Neu5Acα2-3Galβ1-4GlcNAc (3′SiaLacNAc)	+++[1]	+	+++	++		++	++++

Note: Except the data for galectin-5, the data are presented for human galectins; –, no binding recorded; +/–, weak binding.

[1] Data from [36, 37].

[2] Data from [46].

[3] Data from [12].

Sulfate in the carbohydrate ligand most strongly influences the binding of tandem type galectins. Thus, 3-O-Su-Galβ1-3GalNAcβ and 3-O-Su-Galβ1-3GalNAcα display high affinities for galectins-4 and -8 [34, 39-41]. Unlike galectin-4, galectin-8 also interacts with 3-O-Su-Galβ1-4GlcNAc and its 1-3-isomer [34]. Derivatives with sulfate located in position 4 or 6 of the galactose residue bind neither to galectin-8 nor to galectin-1 (Table 3). High affinities of LacNAc, Galβ1-3GalNAcβ ($T_{\beta\beta}$), and Galβ1-3GalNAcα (TF) sulfated derivatives to galectin-8 seems to be caused by the presence in the CRD of the Gln47 residue, which enhances the polarity of the domain, increasing its interaction with negatively charged groups; note also that the CRDs of prototype galectins and galectin-3 do not contain the Gln47 residue [40, 41].

Interaction of galectins with sialylated lactosamines. Galectins can bind the sialylated chain 3′SiaLacNAc but not 6′SiaLacNAc (Table 3) [34, 36]. In solid phase systems, galectins-1, -3, and -8 were shown to bind oligolactosamines 3-sialylated at the terminal residue similarly to their non-sialylated analogs, whereas the sialylated oligolactosamines bound to galectins-2, -4, and -7 more weakly than non-sialylated ones.

Interaction of galectins with Galβ1-3GalNAc-containing oligosaccharides. Tandem type galectins bind Galβ1-3GalNAc-containing oligosaccharides. Affinities of Galβ1-3GalNAcβ ($T_{\beta\beta}$) and Galβ1-3GalNAcβ1-3Galβ1-4Glc (asialoGM1) to galectin-8 are comparable to that of oligolactosamines (n = 2, 3), whereas the affinity to galectin-4 is two-three-fold higher (Table 3) [34]. The binding of tandem type galectins to oligolactosamines and saccharides containing the fragment Galβ1-3GalNAc is determined by the N-CRD [22]. It should be remembered that the 3-OH group of the GlcNAc residue is involved in the binding with galectins. Although the disaccharide Galβ1-3GlcNAc does not have this group, its interaction with galectins may be easily explained because the 4-OH hydroxyl in a stable conformation occupies the same position as the 3-OH of the GlcNAc residue within LacNAc. As to disaccharides TF and $T_{\beta\beta}$, as well as GM1, the "classic" residue GlcNAc is substituted in them by GalNAc, which has the 4-OH group located axially (not equatorially as in GlcNAc). Therefore, it is concluded that the fragment Galβ1-3GalNAc is not equivalent to the fragment LacNAc with respect to mutual location of crucial hydroxyl groups. It is not surprising that the disaccharide Galβ1-3GlcNAc is recognized by galectins similarly to the disaccharide LacNAc, whereas Galβ1-3GalNAc (α or β) is recognized otherwise.

The literature presents different opinions concerning the interaction of prototype galectins with glycans containing the fragment Galβ1-3GalNAc. The GM1 ganglioside containing this fragment seems to be a major ligand of galectins-1 and -7 on the human neuroblastoma cell surface [42, 43]. Binding sites in the CRD of lectin and the GM1 conformation in the complex with protein were determined by NMR spectroscopy and molecular modeling [44, 45]. Amino acids of the C and D sites bind the terminal disaccharide Galβ1-3GalNAc, and amino acids of the site E bind the trisaccharide Neu5Acα2-3Galβ1-4Glc within GM1. According to other data, prototype galectins do not or poorly interact with the fragments $T_{\beta\beta}$ and TF (Table 3) [34].

Interaction of galectins with Galα/GalNAcα-terminated lactosamines. Oligosaccharides containing the residue Galα or GalNAcα as a substituent at the O-3′ in LacNAc were also shown to be ligands of galectins. By fluorescence polarization, it was shown that Galα1-3′LacNAc displays high affinity to galectin-3 [33]. This trisaccharide also binds to galectin-7 and galectin-4, and in the case of galectin-4 the binding is significantly higher than to oligolactosamines (n = 2, 3) (Table 3) [34]. In a solid-phase system, the affinity of Galα1-3′LacNAc to galectin-5 was found to be six-fold higher than to oligolactosamines (n = 2, 3) and LNnT [46].

Tandem type galectins recognize oligosaccharides containing terminal residues Galα and GalNAcα in polylactosamine chains (Table 3) [22]. Moreover, GalNAcα1-3GalNAcβ and GalNAcα1-3GalNAcβ1-3Galα1-4Galβ1-4Glc (Forssman glycolipid oligosaccharide) display high affinities to galectins-4 and -8 [12, 22, 34, 40, 41]. Although the binding sites of these oligosaccharides are not yet described, they are suggested to include S1 and S3 chains [22].

Interaction of galectins with fucosylated saccharides. Galectins bind the fucosylated oligosaccharides exemplified by blood group antigens with the fucose residue in a "permitted" position, i.e. as a substituent at 2-OH in the galactose residue. Thus, galectin-3 binds blood group antigens A (type 2) and B (type 2) (Table 3) [34], and this binding is explained by additional interactions of the fucose residue near the CRD inside the galectin regulatory domain [12, 22]. This explanation is also supported by the two-four-fold lower affinity of fucosylated saccharides to "truncated" galectin-3 which does not contain the regulatory domain (as a result of digestion by collagenase) than to its complete form [12].

It should be noted that blood group A (type 1) and B (type 1) antigens did not manifest affinity to galectin-3, thus, for galectins it is important whether the fucose residue is linked to the core Galβ1-4GlcNAc or Galβ1-3GlcNAc (Table 3) [34].

Tetrasaccharides A (type 2) and B (type 2) display affinities to galectins-4 and -8 (Table 3) [34]. High affinity to these oligosaccharides are due to the C-CBD [12, 22, 40]. Unlike galectin-4 galectin-8 binds trisaccharide H (type 1) and tetrasaccharides B (type 1) and A (type 1).

Blood group antigens A, B, and H have high affinity to galectin-2 (Table 3); galectin-7 selectively interacts with antigens of group B (types 1 and 2) and H (type 1). Unlike galectins-2 and -7, galectin-1 binds with tetrasac-

charides A (type 1) and B (type 1), and the binding level is two-fold lower than for galectin-2 (Table 3).

Thus, not only core regions Galβ1-4/3GlcNAc of oligosaccharides are involved in the interactions with galectins but also their corresponding derivatives at 2-OH and 3-OH galactose residues, i.e. sulfated, sialylated, fucosylated, etc. saccharides which contain typical terminal motifs of the cell surface glycoproteins and glycolipids, and substitutions can markedly increase the affinity. However, sialylation at 6-OH or sulfation at 4-OH or 6-OH abolish the binding; i.e. the change in the pattern of terminal glycosylation of cellular glycoconjugates seems to act as a regulatory mechanism of galectin-mediated interaction.

In case of carbohydrate–protein recognition, the affinity and specificity are realized due to polyvalent (i.e. multipoint) interaction of lectin with a ligand [47-50]. Galectins are not an exception; monovalent ligands are bound to galectins markedly lower than polyvalent ligands [49]; thus, the binding level of biantennary oligosaccharides containing terminal Galβ1-4GlcNAc to galectin-1 is four-fold higher than of the disaccharide terminal LacNAc [34]. However, it should be noted that in the case of galectins this effect is considerably less pronounced than for most other lectins; this may be because all (with the exception of galectin-3) other galectins are only bivalent.

Specificity of invertebrate, mammalian, and human galectins is now being studied systematically. The results allow carrying out a comparable analysis of carbohydrate specificity of galectins and revealing the principal differences in specificity of galectins from various species. The data available so far suggest the presence of such differences, e.g. disaccharides $T_{\beta\beta}$ and TF and oligogalactosamines ($n = 2, 3$) can bind to human galectin-4 but not to its mouse homolog [34].

Table 4. Galectin receptors

Receptor	Galectin
Intracellular	
H-Ras	galectin-1
K-Ras	galectin-3
Bcl-2	galectin-3
Cyclins	galectin-3, -12
Transcription factors	galectin-3, -7
Cytokeratins	galectin-3
Chrp protein	galectin-3
Extracellular	
Extracellular matrix proteins: laminin, fibronectin, vitronectin	galectin-1, -2, -3, -4, -8
Integrins	galectin-1, -2,-8
T-Lymphocyte antigens: CD45, CD43, CD7, CD3, CD2	galectin-1, -3
Cell membrane glycoproteins and glycolipids	
LAMP-1 and -2	galectin-1, -3
GM1	galectin-1, -7
3′-O-Su-Gal-terminated glycosphingolipids	galectin-4, -8
90K/MAC-2BP	galectin-1, -2, -3, -7
Oncoantigens: CA125 and CEA	galectin-1, -3, -4, -8
Glycosaminoglycans	galectin-1

BIOLOGICAL FUNCTIONS OF GALECTINS

At present, there are no direct data indicating how galectins bind *in vivo*. But studies on recombinant proteins or on cells that express galectins suggest that some molecules can be natural receptors of galectins. (Here the term "receptor" refers to the whole biomolecule that binds galectin inside or outside of the cell surface in the presence or absence of CRD, and the term "ligand" refers to glycan that binds CRD of galectin.)

Galectins are located in the cytoplasm but are also found on the cell surface if the cell is under stress conditions, such as inflammation or oncological transformation. Galectins interact with integrins, proteins, and glycosaminoglycans of the extracellular matrix [5]. Carbohydrate chains of glycolipids and glycoproteins of immune, epithelial, and endothelial cells act as ligands for galectins (Table 4).

Inside the cell, galectins are involved in protein–protein interactions. There are no direct data on the galectin binding inside the cell with carbohydrate chains of proteins, although some proteins, such as heat shock proteins and cytokeratins, are glycosylated. There are also indirect data, e.g. galectin-1 possessing two mutations in the CRD is not expressed on the surface of Chinese hamster ovary (CHO) cells transfected by the corresponding gene [10]. Another example is galectin interaction with cytokeratin glycans of MCF-7 cells (human breast carcinoma) *in vitro*; it was suggested that binding of cytokeratins to CRD of galectins mediate transfer of galectins into cell membrane.

Functions of intracellular galectins. Some studies on galectin co-immunoprecipitation with the cell nuclear extract [52-54] or cytoplasmic proteins [55-57] have revealed intracellular receptors of galectin-1 and galectin-3 (Table 4). Although Gemin4, Chrp, and proteins of the Ras and Bcl families are not glycosylated, CRD of

galectin-1 and CRD or the N-regulatory domain of galectin-3 are implicated in interaction with them [53-57].

As spliceosome components, galectins interact with small nuclear RNAs and the protein Gemin4 and are involved in pre-mRNA-splicing [53, 54]. Some data indicate the involvement of galectin-3 in the regulation of gene transcription through binding with factors Sp1 and CREB [27, 28].

Galectins regulate the cell cycle through binding with signaling proteins, which induce or suppress apoptosis [58-60]. Thus, galectins-1 and -3 bind directly with Ras proteins, which normally are involved in transmission of mitogenic signals; this interaction leads to regulation of cell proliferation, differentiation, and apoptosis [61]. The increased expression or mutation of these proteins has been detected in many tumors [62, 63]. Galectins are supposed to interact with the membrane-associated form Ras-GTP owing to production of a disulfide bond between cysteine residues of galectin and the oncoprotein [55]; in the case of galectin-3 regulatory N-domain is additionally involved in the interaction [56]. The binding of galectins-1 and -3 with Ras-GTP results in stimulation of effectors of Ras—serine-threonine kinase Raf and phosphoinositol-3-kinase P13K [55, 56].

Intracellular galectin-3 can act as both inducer and inhibitor of apoptosis. On one hand, galectin-3 binds with the protein Ras, which activates apoptosis, but on the other hand the mitochondrial protein Bcl-2, which is a suppressor of apoptosis, is a receptor of galectin-3 [27, 64, 65]. This protein is bound due to the sequence Asn-Trp-Gly-Arg within its BH1 domain, and this sequence is located in the galectin-3 regulatory domain [64]; the substitution of alanine for glycine in the galectin-3 regulatory domain abolishes the antiapoptotic activity of lectin. Although Bcl-2 is not glycosylated, the binding is inhibited by lactose, and this is explained by location of the Asn-Trp-Gly-Arg sequence nearby the carbohydrate-binding site of galectin-3 [64].

Intracellular galectins-1 and -7 are positive regulators of apoptosis. The mechanism of apoptosis activation is unclear, but *in vitro* induction of tumor cell apoptosis by galectin-7 seems to be associated with activation of caspase-8 [61]. It is also known that cultivation of ST4 cells (T-lymphocytes) in the presence of galectin-1 enhances the expression of IFN-γR on the T-lymphocyte surface and stimulates IFN-γ-dependent apoptosis [58].

Galectins have been shown to regulate the cell cycle through activation of cyclin-dependent kinases. The mechanism of this regulation is not known in detail, but the *in vitro* coprecipitation of galectins with activators of cyclin-dependent kinases from cell lysate has been shown [59, 60]. Galectins-3 and -12 inhibit activities of cyclins E, A, and D1 that results in arresting of the cell cycle in the G1-phase [27, 59, 60].

Information about receptors of intracellular galectins is very limited. The above-presented examples show that only galectins-1 and -3 can directly interact with receptors. It remains possible that the major function of galectins located in the cytoplasm is interaction with proteins involved in galectin transport and expression on the cell membrane surface where galectins are functioning as carbohydrate-binding proteins [55, 56].

FUNCTIONS OF GALECTINS ON THE CELL SURFACE

Unlike the above-described intracellular situation [53-65], on the cell surface galectins are implicated in protein–carbohydrate interaction [5, 27, 28, 66-78]. On the cell surface, galectins are involved in adhesion, apoptosis, and proliferation of cells and in immune system regulation during inflammation and oncological transformation.

Galectins as cell adhesion molecules. Galectins mediate cell–cell and cell–matrix adhesion via binding the polylactosamine chains of glycoproteins. On the cell surface, galectin receptors are represented by intercellular matrix proteins (laminin, fibronectin, vitronectin, thrombospondin), actin, integrins $\alpha1\beta1$, $\alpha3\beta1$, $\alpha6\beta1$, and $\alpha7\beta1$, integral proteins of lysosomal membranes LAMP-1 and -2, and also gangliosides and other glycosphingolipids (Table 4) [27, 28, 66]. Galectin-1 binds with glycosaminoglycans of the extracellular matrix [67]. The above-mentioned proteins are lactose/lactosamine-dependent coprecipitated with galectins [27, 28, 66-76]. Depending on the galectin concentration and on the type and degree of cell transformation, galectins are suggested to be both positive and negative regulators of cell adhesion [52]. Thus, galectin-1 stimulates adhesion of cancer cells to laminin [68, 69] but decreases the adhesion of activated T-lymphocytes to the intercellular matrix [70].

Galectin interactions with carbohydrate chains of integrins, intercellular matrix proteins, and membrane glycolipids induce a cascade of signaling reactions that control cell proliferation and apoptosis. Thus, the interaction of galectin-8 with integrins results in activation of tyrosine kinase FAK (focal adhesion kinase), which plays a key role in transmission of integrin-mediated signals [71-79]. On binding with integrins and intercellular matrix proteins, galectin-1 and -3 inhibit adhesion of tumor cells to one another or to cellular matrix, which leads to uncontrolled growth and invasion of tumor cells [73-75]. However, the interaction of galectin with extracellular matrix proteins and glycoprotein 90K/MAC-2BP leads to formation of conglomerates inaccessible for proapoptotic agents [76]. Galectin-1 and galectin-7 binding to neuroblastoma GM1 ganglioside mediates the cell–cell adhesion, and this results in inhibition of the tumor cell proliferation [42, 43].

Galectin-1 and -3 expressed on intestinal carcinoma cells bind to polylactosamine chains of the carcinoembryonic antigen and cause adhesion of the tumor cells to one

another or to the endothelium [77, 78]. The binding of galectin-1 and -3 to glycoconjugates of endothelial cells initiates the penetration of tumor cells into the blood and leads to metastases [80].

Galectins and the immune system. Galectin-1 binds to N-glycans of proteins CD45, CD43, and CD7 on the surface of activated T-cells, and this results in segregation of these proteins into membrane microdomains and triggering of apoptosis [81, 82]. Galectin-3 interacts with glycoproteins CD29, CD7, and CD95, and formation of the CD29/CD7 complex is a signal for initiating apoptosis [65, 83]. Galectin-2 activates apoptosis of T-lymphocytes due to its interaction with polylactosamine chains of β1-integrin [84]. The galectin-induced apoptosis of cytotoxic T-lymphocytes is believed to be associated with the resistance of tumor cells to effectors of innate and specific antitumor immunity: tumor growth was observed in mice with an increased expression of galectin-1, whereas in mice with a decreased secretion of galectin-1 the T-cell response was stimulated and the tumor was rejected [85, 86].

Galectins contribute to lectin-mediated phagocytosis. Thus, macrophagal galectin-3 binds with N-glycan LacdiNAc fragment of helminth cells and enhances their phagocytosis by macrophages [87]. In galectin-3 knockout mice, phagocytosis of apoptotic bodies [88] by macrophages is decreased. Galectin-1 binds with complement C3 component and thus promotes an increase of phosphatidylserine exposure in the tumor cell membrane without inducing their apoptosis; such cells are rapidly eliminated by peritoneal macrophages [89]. The THP-1 cells of macrophagal origin and macrophages isolated from mononuclear cells of patients with breast cancer galectin-dependently engulf apoptotic bodies obtained from the tumor cells [90, 91].

Galectin-3 and -9 act as chemoattractants [28, 92-94]. The mechanism of neutrophil and eosinophil attraction, respectively, by galectin-3 and galectin-9, into an inflammation focus is unclear, but some data indicate that galectins bind oligolactosamine chains of branched N-glycans [27, 92]; glycoproteins CD66a and CD66b are supposed to be receptors of galectin-3 on neutrophils [94]. Activation of eosinophils by galectin-9 is associated with an increase in the expression of intercellular adhesion molecules CD69 and VLA-4; in diseases of the upper respiratory pathways, galectin-9 expressed on pulmonary fibroblasts induces the adhesion of eosinophils to fibroblasts, which improves the entry of eosinophil-released inflammatory mediators into the inflammation focus [93]. Galectin-1 expressed on fibroblasts in chronic pancreatitis is shown to stimulate the production of chemokines by monocytes and neutrophils [95].

Cross-linking by galectin of carbohydrate ligands induces their aggregation and generation of glycoclusters [96, 81]. This is supposed to be a pathway for regulation of cell signal transmission by galectins [96]. It has been mentioned that the induction of apoptosis by galectin-1 is a result of clustering of CD45, CD7, and CD43 antigens caused by binding of their glycans with galectin-1 [81, 82]. The clustering (patching) is also induced by other lectins. Thus, adhesion of intestinal adenocarcinoma cells to one another is a result of SB1a, GM1, and of CEA clustering, and, in this case, the patching is induced by galectin-4 which binds to the 3′-O-Su-Galβ1-3GalNAc fragment of glygosphingolipid SB1a, Galβ1-3GalNAcβ fragment of ganglioside GM1, and Galβ1-3GlcNAcβ-terminated chains of the carcinofetal antigen [40]. Moreover, cross-linking of neutrophil and endothelium glycans by galectin-3 leads to stimulation of the neutrophil adhesion to endothelium in inflammation [97]. These and other data indicate that the galectin functional activity is realized via clustering.

Galectin-3 seems to be capable of producing protein complexes with intra- and extracellular receptors. Such a cross-linking mediated by galectin-3 can occur due to binding of its N-regulatory domain with cytoplasmic proteins and of CRD with cell glycans, respectively. This is supported by the finding that the binding of the Chrp protein containing a cysteine-histidine domain with galectin-3 does not prevent the interaction of the latter with oligolactosamine chains of laminin [57]. The formation of such a complex is supposed to be a mode for transporting galectin-3 from the nuclear membrane surface where it is collocated with the Chrp protein to the cytoplasmic membrane [57].

Numerous literature examples seem to make an impression that galectins exposed on the cell surface can bind to any complementary ligands, both β-Gal-terminated and "permitted" sialylated, fucosylated, α-galactosylated ones. Actually, it is not the case, it has been shown that galectins selectively bind *in vitro* only to certain ligands. Thus, galectin-4 mediates adhesion only of cells with sulfated glycolipids exposed on the surface [40]; induction by galectins of the cell–cell and cell–matrix adhesion is associated with the selective binding of galectins to polylactosamine chains [67-72, 98]. Another example: galectins are coprecipitated from the cell lysate not with all proteins of glycocalix, but only with particular ones [76, 78, 80, 99]. Based on these and many other examples, it was suggested that *in vivo* galectins should function similarly or even more selectively. The selectivity can be, in particular, provided by masking galectins with ligands exposed on the same cells (*cis*-ligands), i.e. the protein interaction with *cis*-ligands controls the binding of "external" ligands [100]. A similar mechanism of activity regulation was described earlier for another class of lectins, siglecs, capable of binding to exogenous glycoconjugates or glycans of other cells only upon desialylation of the cell surface [101, 102].

Masking with *cis*-ligands does not always prevent the binding of one cell lectins with ligands of another cell (*trans*-ligands). In some cases, *trans*-ligands, which have

a higher affinity, can displace *cis*-ligands [103]. From the entropy viewpoint, the *cis*-interaction of lectin with an adjacent glycan is more advantageous than the binding with the same external glycan, but if the affinity of the external glycan is higher, it can displace the *cis*-ligand, as it has been exemplified by the cellular siglec-2 [103, 104]. This seems to be an explanation of the narrow functional specificity of lectin during intercellular recognition, notwithstanding its wide *in vitro* specificity: the *cis*-binding prevents all interactions below a certain affinity threshold but does not interfere with a highly specific recognition. It is probable that the functional activity of galectins is regulated just in this manner. On one hand, *cis*-ligands mask galectins preventing their interaction with ligands of the adjacent cells, and on the other hand they prevent the interaction with low affinity ligands and promote the interaction with high affinity ligands.

Thus, not only the ligand affinity is important for interaction of galectins with their receptors but also the mutual topography of all biomolecules involved in the intercellular interaction, i.e. in some cases galectin can be completely masked and in other cases it can be ready for another interaction, in dependence on the structure and geometry of *cis*- and *trans*-ligands.

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