Functions of cell surface galectin-glycoprotein lattices

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Abstract

Programmed remodeling of cell surface glycans by the sequential action of specific glycosyltransferases, can control biological processes by generating or masking ligands for endogenous lectins. Galectins, a family of animal lectins with affinity for β-galactosides, can form multivalent complexes with cell surface glycoconjugates and deliver a variety of intracellular signals to modulate cell activation, differentiation, and survival. Recent efforts involving genetic or biochemical manipulation of *O*- and *N*-glycosylation pathways, as well as blockade of the synthesis of endogenous galectins, have illuminated essential roles for galectin-glycoprotein lattices in the control of biological processes including receptor turnover and endocytosis, host-pathogen interactions and immune cell activation and homeostasis.

Keywords

galectins; galectin-glycoprotein lattices; receptor turnover; immunity; inflammation

Introduction

For many systems, the clustering of protein receptors and ligands is required for optimal transmission of signals into a cell. The thermodynamically favorable assembly of ordered arrays of lectins and saccharides on the cell surface may thus be integral to cellular signaling and adhesion [1]. Lectin multivalency enables recognition of multiple binding partners, allowing these glycan-binding proteins to play leading roles in signal transduction in different biological processes as well as in cell-cell and cell-pathogen interactions [2–4].

Galectins are a family of soluble lectins that bind β-galactoside-containing glycans and are defined by a conserved carbohydrate recognition domain (CRD) and a common structural fold [2–4]. Among the various lectin types, galectins are probably the most conserved and ubiquitous family, with members identified in most animal taxa examined so far [4]. As many as fifteen galectins have been identified in mammals and proposed to mediate diverse biological processes involved in the regulation of innate and adaptive immune responses, such as cell

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activation, differentiation, cytokine secretion and apoptosis [5,6]. Here we discuss recent findings on the biochemistry of galectin-glycoprotein lattices and their functional relevance in the control of receptor endocytosis, host-pathogen interactions and activation and homeostasis of immune cells.

Biochemical aspects of galectin-glycoprotein lattices formation

Lectin-monosaccharide interactions are relatively weak (dissociation constants $\sim 10^{-4}$ M), with two to five hydrogen bonds complemented by hydrophobic and van der Waals interactions [4,5,7]. Galectins preferentially bind β-galactoside-containing glycans comprised of repeating units of *N*-acetyllactosamine (Galβ1,4GlcNAc; LacNAc), either as disaccharide units at the termini of complex *N*-glycans, or as repeating units in a poly-*N*-acetyllactosamine chain on *N*- or *O*-glycans [5,7,8]. Galectin binding affinities to complex *N*-glycans are proportional to their LacNAc content and to their GlcNAc branching [5,7–11].

Lattice formation by galectins requires multivalent oligomerization to attain stability and biologic functionality[5]. Based on structural features, galectins have been classified into three types: proto, chimera, and tandem-repeat [4]. Proto-type galectins contain one CRD per subunit and typically dimerize through noncovalent interactions to create functionally bivalent lectins. Proto-type galectin-1 is a dimer in solution and crystallizes as a dimer in cross-linked complexes with a divalent oligosaccharide[12]. The presence of more than one CRD in a galectin-1 homodimer makes it well-suited for mediating cell adhesion, eliciting signaling, and forming lattices [3,5]. The chimera-type galectin-3 has a C-terminal CRD similar to the proto-type, but exhibits an N-terminal domain that is responsible for interactions between subunits, facilitating its oligomerization [3]. Galectin-3 monomers are in equilibrium with higher order oligomers in solution, and galectin-3 precipitates as a pentamer with multivalent oligosaccharides [13]. This lectin binds to multi-glycosylated proteins with positive cooperativity, suggesting that galectin-3 monomers, after ligand binding, recruit additional lectin molecules to form a complex of multivalent interactions [5,13]. The biologic functions attributed to galectin-3 are thus likely to depend upon both ligand cross-linking and oligomerization [6,14–17]. The tandem-repeat type galectins have two CRDs connected by a linker peptide and, thus are bivalent, although the two CRDs may be able to recognize different saccharide ligands [6] (Figure 1).

Glycoproteins often bear multiple copies of the saccharide ligands that are recognized by galectins [18]. While galectin binding to a single saccharide ligand is typically a low-affinity interaction (association constants $\sim 10^4$ M^{-1}), the multivalent nature of galectin-saccharide interactions results in high overall avidity (association constants $\sim 10^6$ M^{-1}) [5,9]. This multivalency also allows the formation of lectin-carbohydrate lattices. Both in solution and on the cell surface, multivalent galectins selectively cross-link a single species of glycoprotein to form homogeneous lectin-carbohydrate lattices [5,17,19].

The ability of galectins to reorganize membrane glycoproteins into lipid raft microdomains suggests that multivalent lectin-saccharide interactions occur preferentially in these microdomains [5]. In this regard, the ganglioside GM$_1$ is able to organize microdomains into raft-like structures and is a prominent glycolipid headgroup in such rafts. Galectin-1 binds the GM$_1$ pentasaccharide glycan and these interactions may represent a mechanism by which galectins organize lipid rafts [20]. Since lipid rafts are considered essential for assembling signal transduction components at the plasma membrane [1], association of galectins with lipid rafts is likely to be important for galectin-mediated signaling events.

Biological aspects of galectin-glycoprotein lattices

Galectins and receptor turnover

Lattice formation following the binding of complex *N*-glycans to galectins effectively traps glycoprotein receptors at the cell surface, preventing their endocytosis. Thus, interactions between *N*-glycans and galectins can regulate the distribution of cell surface receptors as well as the cell's responsiveness to receptor agonists [11]. Recently, Dennis and colleagues described a link among *N*-glycan multiplicity, *N*-glycan branching kinetics, integration of nutrient metabolism and changes between cell growth and arrest [10]. The authors described that *N*-branching in the Golgi is sensitive to hexosamine flux for its production of complex *N*-glycans. Whereas arrest-promoting receptors (*e.g.*, *e.g.*, TGFβR and CTLA-4) have few *N*-glycosylation sites and show a 'switch-like' responses to hexosamine concentrations, growth-promoting receptors (*e.g.*, EGFR, IGFR, FGFR, and PDGFR) with high numbers of *N*-glycans exhibit hyperbolic responses to hexosamine [10]. Therefore, increased nutrient flux stimulated by growth-promoting receptors, will ultimately activate cellular arrest and differentiation programs by increasing surface levels of glycoreceptors with low numbers of *N*-glycans. The authors report that increasing UDP-GlcNAc leads to increased branching of *N*-glycans, increased receptor association with cell surface galectin-3 and enhanced signaling [10]. Thus, galectin-carbohydrate lattices can regulate the decision between cell growth and arrest by regulating receptor turnover (Figure 2).

A second biological example of galectin-mediated control over receptor endocytosis is the galectin-9-glucose transporter 2 (GLUT-2) system. All vertebrate glucose transporters have a single conserved *N*-glycan site. Cell-type and glycoprotein-specific *N*-glycans attached by *N*-acetylglucosaminyltransferase IVa (GlcNAcT-IVa) are needed to maintain the glucose transporter GLUT-2 on the surface of pancreatic β cells [21]. GlcNAcT-IVa-dependent glycosylation increases the cell-surface half-life of GLUT-2, suggesting that interactions involving the GLUT-2 *N*-glycan structure may suppress its endocytosis. Since GLUT-2 and galectin-9 normally co-localize in pancreatic β cells in a GlcNAcT-IVa-dependent manner [21], it is surmised that galectin-9 acts to retain glucose receptors on the cell surface.

Also by interfering with receptor endocytosis, *N*-acetylglucosaminyltransferase V (GlcNAcT-V) expression-dependent galectin lattices, such as galectin-3-TGFβR lattices, maintain growth-factor receptor densities at levels that promote invasive phenotypes in transformed cells [11, 22]. In addition, galectin-3 interactions with GlcNAcT-V-modified *N*-glycans stimulate $\alpha_5\beta_1$ integrin activation, focal adhesion remodeling and phosphatidylinositol 3-kinase activation, thus promoting tumor cell motility [23].

Galectin-carbohydrate lattices in host-pathogen interactions

Galectins interact with β-galactoside-enriched glycoconjugates present in several pathogens [24–26]. While the nature of these interactions is not well-characterized, galectin oligomerization and/or lattice formation are likely to play a role.

It has been demonstrated that galectin-1 inhibits envelope-mediated cell-cell fusion of some paramyxoviruses by binding to specific *N*-glycans on viral glycoproteins and inducing its oligomerization [27]. However, galectin-1 can also promote human immunodeficiency virus infectivity by stabilizing viral attachment to host cells and cross-linking viral glycoproteins with the target cells [28].

Specific interactions have been described between galectin-3 and -9 and the intracellular protozoan *Leishmania*. Galectin-3 binds the lipophosphoglycan (LPG) of *L. major* [26]. This binding leads to the proteolytic removal of the galectin-3 N-terminal domain, preventing oligomerization. In this way, *L. major* destroys galectin-3 lattice formation, leading to a

decreased threshold for signal transduction [26]. Galectin-9 also recognizes *L. major* by binding to LPG and promotes *L. major*-macrophage interactions, which may be critical for the clinical outcome of leishmaniasis [25].

Recently, a novel galectin type with four CRDs was discovered in the eastern oyster, *Crassostrea virginica*. This molecule binds to endogenous ligands at the surface of the oyster's phagocytic cells and recognizes exogenous carbohydrate ligands on microbial pathogens and phytoplankton components [29]. Although this novel galectin binds β-galactosyl residues, it exhibits broader saccharide specificity than mammalian galectins, which may confer upon this oyster galectin biological functions involved in immune recognition, as well as in feeding and intracellular digestion [29]. Thus galectin-carbohydrate lattices may have evolved to ensure host-pathogen interactions during the initiation and resolution of microbial infections.

Galectin-glycoprotein lattices in innate immunity

Galectin-carbohydrate lattices may also modulate the biology of innate immune cells at inflammatory foci [6,15,16]. Galectin-3-mediated ligand clustering triggers neutrophils to phagocytose, produce reactive oxygen species, release proteases, and secrete interleukin (IL)-8 [15,16,30]. In addition, galectin-3 induces mast cell degranulation: recent studies have revealed a critical role for this protein in mast cell function since galectin-3-deficient mast cells show reduced histamine release and IL-4 secretion [14].

Also critical for innate immune responses, macrophages require GlcNAcT-V-dependent galectin-glycoprotein lattice formation to maintain sufficient cell-surface cytokine receptor density to drive motility and phagocytosis [11]. Moreover, by interacting with specific saccharide ligands, galectin-1 differentially regulates Fcγ receptor I-dependent phagocytosis and inhibits major histocompatibility complex (MHC)-II-dependent antigen presentation by monocytes/macrophages [31].

Recently, fluorescence resonance energy transfer (FRET) was employed to visualize physiological galectin-3 oligomerization on the surface of neutrophils and endothelial cells [15]. Removal of the *N*-terminal domain of galectin-3 by proteolytic cleavage prevented oligomerization. These studies further suggested that galectin-3 lattices are robust, stable, and rigid, with slow lateral movement on the surface of cells, and could thus easily restrict receptor clustering and modulate cell signaling [15].

Galectin-glycoprotein lattices in T cell functions

Cross-linkage of T-cell surface receptors by galectins can trigger different transmembrane signaling events through which diverse processes such as survival, activation, and cytokine secretion are modulated [6].

The T-cell receptor (TCR) α/β is decorated by GlcNAcT-V -modified *N*-glycans which restrict nonspecific TCR aggregation through binding to galectins. Multivalent galectin-3-TCR complex lattices limit TCR clustering at the immune synapse by restricting lateral TCR movement within the plane of the membrane, thus increasing agonist threshold for TCR signaling [32,33]. Conversely, deficiency in GlcNAcT-V lowers T-cell activation threshold by enabling TCR clustering and signaling characterized by increased TCR-dependent tyrosine phosphorylation and proliferation [32,33].

The authors recently extended their observations, showing that β1,6GlcNAc-branched *N*-glycans on T cells are regulated by the nutrient environment and metabolite supply of the hexosamine pathway. Thus the production of high affinity ligands for galectins is controlled in T cells by the availability of key metabolic intermediates [34]. Increasing β1,6GlcNAc-branched *N*-glycans in T cells by hexosamine supplementation suppresses TCR signaling,

CTLA-4 endocytosis, Th1 differentiation, and development of autoimmune inflammation [34] (Figure 2).

A critical process for dampening potential harmful T-cell responses is the fine-tuning of T-cell survival. Galectin-1, -2, -3 and -9 bind distinct cell surface glycoprotein receptors and trigger distinct intracellular signaling pathways to promote T-cell death [17,19,35–37]. Remarkably, a number of factors determine the responsiveness of cells to galectin-mediated signals which include the repertoire of glycosylated molecules expressed on the cell surface and the activities of specific glycosyltransferases, which are responsible for creating or masking galectin ligands [5,6,18]. These variables can dramatically change during thymic-development and peripheral activation and differentiation of T cells. [5,6,18,38–41].

Compelling evidence indicates that galectin-1 treatment suppresses chronic inflammation, modulates T-cell survival and skews the balance towards a Th2 cytokine profile *in vitro* [42] and *in vivo* in animal models, including experimental autoimmune uveitis [43] and autoimmune diabetes [44]. Furthermore, selective blockade of galectin-1 in tumor tissue results in increased Th1-mediated anti-tumor responses, suggesting potential involvement of this protein in tumor-immune escape [45]. Recent work provides a molecular explanation for galectin-1-mediated Th2 skewing, demonstrating that Th1 and Th17 effector cells express the repertoire of cell surface glycans that are essential for the formation galectin-glycoprotein lattices. In contrast Th2 cells are protected from galectin-1 binding through differential α2,6-sialylation of cell surface glycoproteins (Figure 2). Galectin-1-deficient mice consistently developed greater antigen-specific Th1 and Th17 responses compared to wild-type mice [40]. In addition, other galectin members may contribute to this immunoregulatory effect, including galectin-9 which acts as a specific binding partner of Tim-3, a Th1-specific receptor, and selectively eliminates Th1 cells *in vivo* [46]. Furthermore, it has been proposed that phosphatidylserine exposure induced by galectins may serve as an alternative homeostatic mechanism, to favor phagocytosis, modulate secretion of anti-inflammatory cytokines and influence the resolution of inflammatory responses [47]. In contrast to the inhibitory actions of galectin-1, galectin-4 contributes to exacerbated intestinal inflammation by promoting CD4^{+} T cell activation and favoring IL-6 secretion through a protein kinase Cθ-dependent mechanism [48].

In addition to activation-induced cell death, avoidance of collateral damage to the host is also achieved by active immunosuppression mediated by regulatory T cells. We found that treatment with recombinant galectin-1 in the efferent phase of autoimmune ocular inflammation results in increased IL-10 and TGF-β production and expansion of regulatory T cells *in vivo* [43]. Interestingly, recent studies demonstrated that galectin-1 and -10 are over-expressed in regulatory T cells, and are critical for the suppressive activity of these cells [49, 50]. Further studies are needed to establish a role of galectin-carbohydrate lattices at synapse formation between regulatory and effector T cells.

Galectin-glycoprotein lattices in B-cell functions

VpreB, a surrogate immunoglobulin light chain that functions in early stages of B-cell receptor (BCR) maturation in pre-B cells, interacts with galectin-1 to modulate essential B-cell maturation activities [51]. An immune developmental synapse is formed between pre-B and stromal cells in a galectin-1-dependent manner: pre-BCR binding to stromal cells depends upon galectin-1 binding to glycosylated $\alpha_4\beta_1$, $\alpha_5\beta_1$, and $\alpha_4\beta_7$ integrins [51,52]. Pre-B cell integrins and their stromal cell ligands, together with pre-BCR and galectin-1, form a homogeneous lattice at the contact area between pre-B and stromal cells [52]. The resulting synapse formation initiates intracellular tyrosine kinase activity and signal transduction from the pre-BCR [51, 52]. In mature B cells, the B cell-specific transcriptional coactivator OCA-B, important for B cell activation and germinal center formation, interacts with galectin-1. The authors showed that galectin-1 negatively regulates B-cell proliferation and tyrosine phosphorylation upon

BCR stimulation [53]. Finally, anergic B cells show up-regulated expression of galectin-1 and -3, suggesting a possible role for these lectins in the control of B-cell tolerance [54].

Conclusions

Research over the past few years has illuminated critical functions of galectin-glycoprotein lattices in receptor turnover and cell signaling, thus dictating the choice among cell proliferation, differentiation and survival, and serving as "on-an-off switch" that controls the decision between immune cell responsiveness and tolerance. Given the broad spectrum of immunoregulatory effects in autoimmune diseases and cancer, galectin-carbohydrate lattices are postulated as targets of novel anti-inflammatory and anti-cancer therapies. However, before galectin- or glycan-based therapeutic strategies can be fully realized, a more thorough understanding of the mechanisms by which galectin-carbohydrate lattices modulate cell function is required. To what extent is there functional redundancy and specificity of action within the galectin family? What is the precise explanation of the different functions exerted by the same galectin in different environmental contexts? What are the levels of galectins attained *in vivo* during an inflammatory reaction, infectious process or tumor dissemination? Increased understanding of the biochemistry and biology of galectin-glycoprotein lattices will provide insights into how the regulation of galectin expression and activity can be exploited for therapeutic purposes.

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Abbreviations

BCR

B cell receptor

CRD

Carbohydrate recognition domain

CTLA-4

Cytotoxic T-lymphocyte-associated protein-4

EGFR

Epidermal Growth Factor Receptor

FGFR

Fibroblast Growth Factor

FRET

Fluorescence resonance energy transfer

GLUT-2

Glucose transporter-2

IGFR

Insulin-like growth factor receptor

IL

Interleukin

LacNAc

N-acetyllactosamine

LPG

Lipophosphoglycan

GlcNAcT-IVa

N-acetylglucosaminyltransferase IVa

GlcNAcT-V

N-acetylglucosaminyltransferase V

MHC

Major histocompatibility complex

PDGFR

Platelet-derived growth factor receptor

TCR

T cell receptor

TGF-β

Transforming growth factor-β

TGFβR

Transforming growth factor-β receptor

Th

T helper

Tim-3

T cell immunoglobulin mucin-3
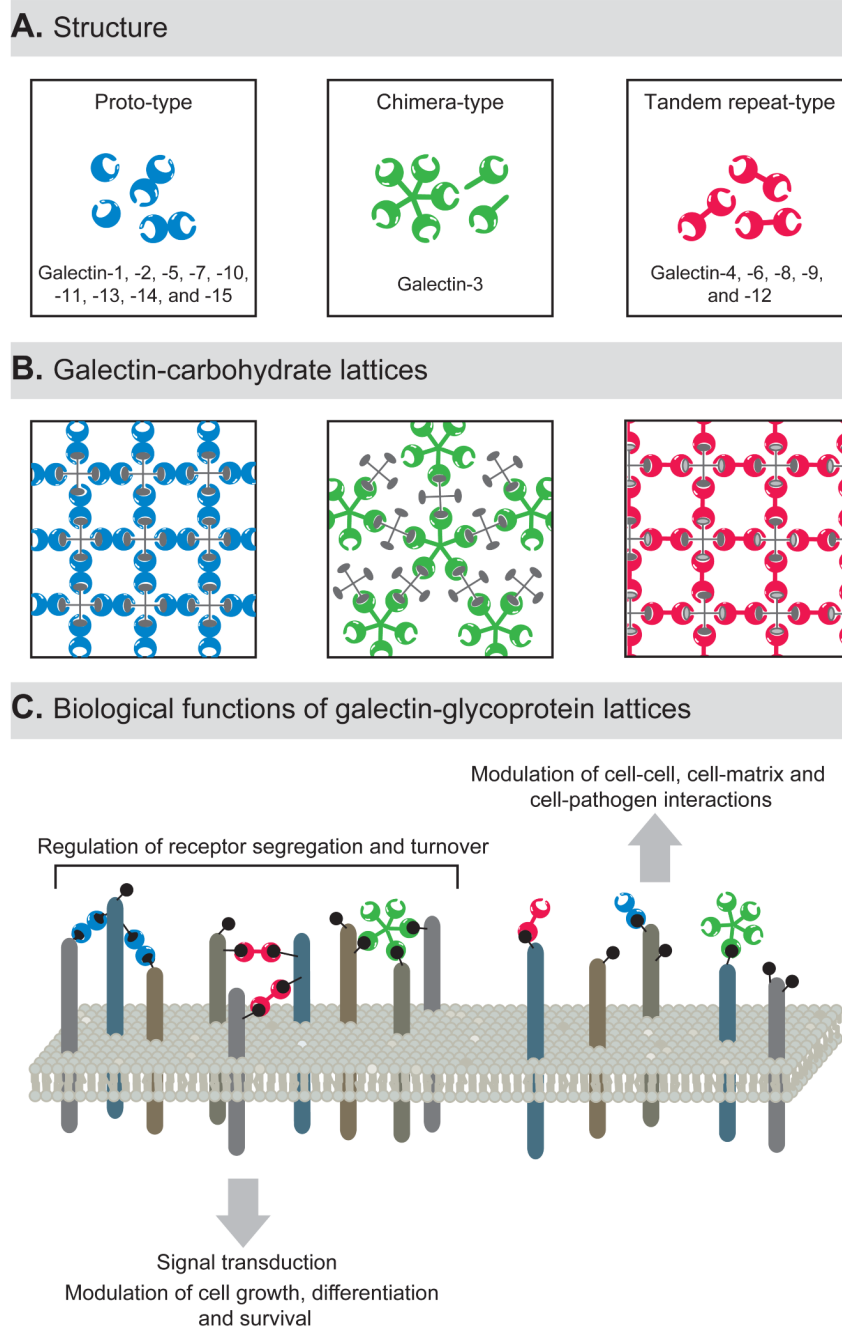
Figure 1. Biochemistry and functional relevance of galectin-glycoprotein lattices
(A) Schematic representation of the structure of different monomeric and oligomeric members of the galectin family. Proto-type galectins contain one CRD and exist in solution as homodimers. Chimera-type galectins are thought to undergo a conformational change following carbohydrate ligand binding which enables their oligomerization as pentamers. Tandem-repeat type galectins contain two distinct CRDs in tandem, connected by a linker of up to 70 amino acids, and are thus inherently dimeric. **(B)** Schematic representation of lattice formation between multivalent galectins and multivalent carbohydrate ligands. **(C)** Biological relevance of galectin-glycoprotein lattices.
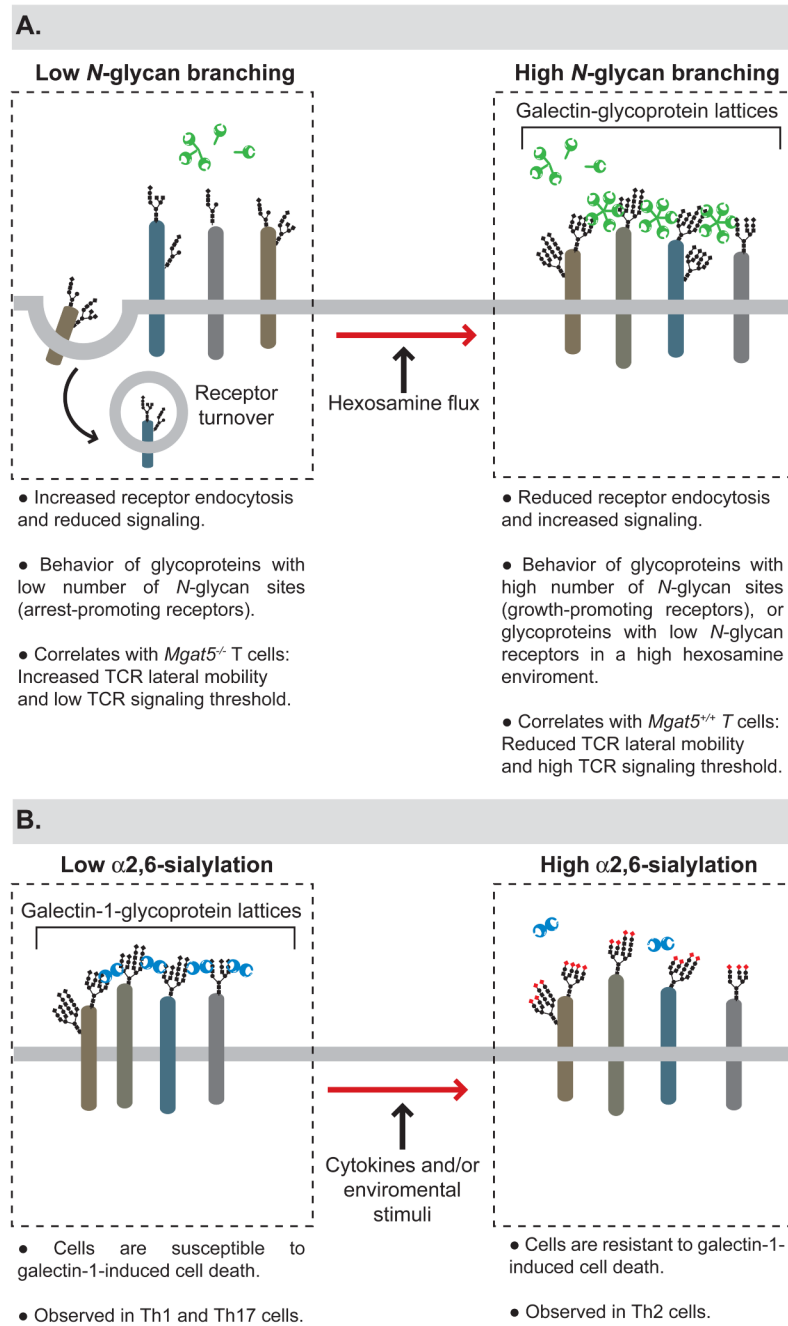
Figure 2. Galectin-glycoprotein lattices in the regulation of receptor turnover, cell signaling and survival

(A) The degree of *N*-glycan branching controls galectin-glycoprotein lattice formation, which in turn modulates receptor turnover and signaling. **(B)** Differential sialylation of cell surface glycoproteins selectively influences the formation of galectin-glycoprotein lattices in distinct T-helper cells, thus regulating their susceptibility to galectin-1-induced cell death.