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CELL SCIENCE AT A GLANCE

The galectin lattice at a glance

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ABSTRACT

Galectins are a family of widely expressed β-galactoside-binding lectins in metazoans. The 15 mammalian galectins have either one or two conserved carbohydrate recognition domains (CRDs), with galectin-3 being able to pentamerize; they form complexes that crosslink glycosylated ligands to form a dynamic lattice. The galectin lattice regulates the diffusion, compartmentalization and endocytosis of plasma membrane glycoproteins and glycolipids. The galectin lattice also regulates the selection, activation and arrest of T cells, receptor kinase signaling and the functionality of membrane receptors, including the glucagon receptor, glucose and amino acid transporters, cadherins and integrins. The affinity of transmembrane glycoproteins to the galectin lattice is proportional to the number and branching of their N-glycans; with branching being mediated by Golgi N-acetylglucosaminyltransferase-branching enzymes and the supply of UDP-GlcNAc through metabolite flux through the hexosamine biosynthesis pathway. The relative affinities of glycoproteins for the galectin lattice depend on the activities of the Golgi enzymes that generate the epitopes of their ligands and, thus, provide a means to analyze biological function of lectins and of the 'glycome' more broadly.

KEY WORDS: MGATs, Endocytosis, Galectin, Glycolipid, Glycosylation, Receptor

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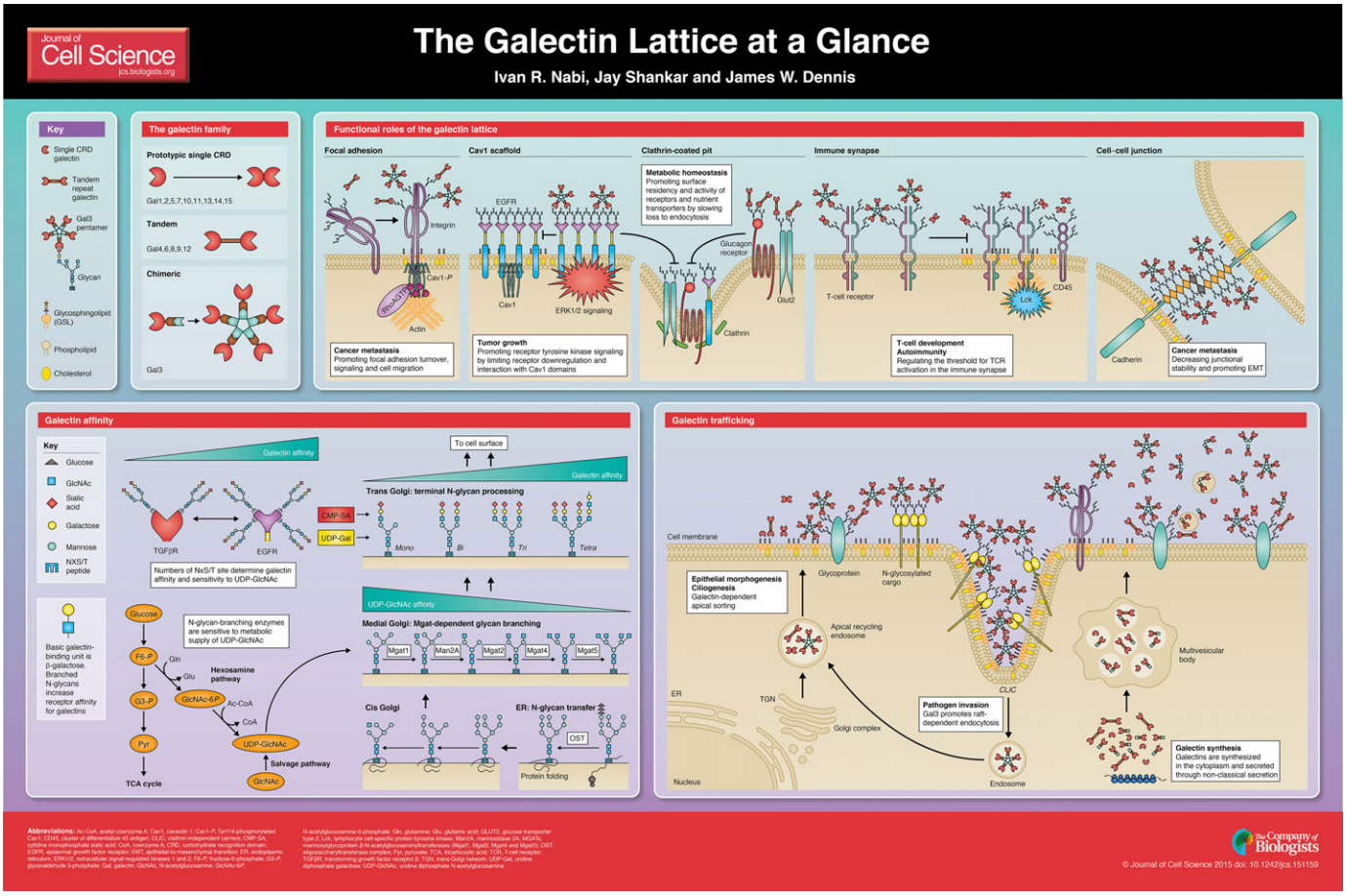
Introduction

Galectins were initially isolated as β-galactoside -binding proteins and, subsequently, characterized as a family of 15 genes in mammals with one or two conserved ∼130 amino-acid-long carbohydrate recognition domains (CRDs) (Cooper, 2002; Drickamer and Fadden, 2002; Seetharaman et al., 1998). Galectins are synthesized in the cytoplasm and interact with cell surface glycans following their secretion by a non-classical exocytic pathway (i.e. not via the ER/Golgi secretory route), that is likely to be an exosome-mediated secretory route (Hughes, 1999; Jones et al., 2010). The β-galactoside epitopes are di- to tetra-saccharide sequences that are widely found in



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oligosaccharide modifications (N- and O-glycans) to glycoproteins and glycolipids of vertebrate tissues. The galectins are a subfamily of glycan-binding proteins or 'lectins', a designation coined many decades ago for proteins in plants and microbial extracts that display hemagglutin activity (Sharon, 2008). The galectins are: (1) prototypic single-CRD galectins that can form non-covalent homodimers (such as Gal1, Gal2, Gal5, Gal7, Gal10, Gal11, Gal13, Gal14 and Gal15), (2) tandem-repeats of two CRD motifs (Gal4, Gal6, Gal8, Gal9 and Gal12) or, (3) the chimera-type galectin-3 (Gal3) with a single CRD and an intrinsically disordered sequence at the N-terminal domain that promotes oligomerization (see poster). Single-site-binding affinities are generally low (K_d in the micromolar range) (Hirabayashi et al., 2002), but interactions between galectins and glycoproteins are largely multivalent and, therefore, depend on critical glycan concentrations (Dam et al., 2005; Lee and Lee, 2000). In turn, the affinity of glycoproteins for galectins is determined by the number of glycosylation sites (sequence-encoded information) and the glycosyltransferase activities of the Golgi complex that generate the various β-galactoside-binding epitopes present in glycoproteins (Demetriou et al., 2001), Ser/Thr O-linked glycans (Nguyen et al., 2001) and glycolipids (Boscher et al., 2012). The Galβ1,4GlcNAcβ-epitope is widely present in complex, branched N-glycans of transmembrane glycoproteins, which is the main class of ligands for Gal1 and Gal3 (Patnaik et al., 2006). These glycoproteins are central to biological effects of the galectins, as they include cytokine receptors, nutrient transporters and adhesion receptors, which all are crucial sensors of the environment at the cell surface. In this Cell Science at a Glance article and accompanying poster, we review the biophysical nature of the galectin lattice at the cell surface, how N-glycans regulate the glycoprotein composition within the lattice, and provide examples of lattice function in immunity, metabolism and cancer biology.

The galectin lattice: a dynamic planar gel-like polymer

Gal1, Gal3 and Gal9 cluster and restrict mobility of cell surface transmembrane glycoproteins (Demetriou et al., 2001; Johswich et al., 2014; Lajoie et al., 2007; Pace et al., 1999). Fluorescence resonance energy transfer revealed the oligomerization of Gal3, induced by cell surface glycoproteins (Nieminen et al., 2007). The galectin oligomers form bridges between glycoproteins, thereby both promoting but also interfering with protein–protein interaction. Homotypic binding through the N-terminus of Gal3 enables the formation of oligomers up to pentamers that contribute to variable geometries of the crosslinked Gal3 lattice; there is an optimal molar ratio of Gal3 to multivalent ligands, and crosslinking can be inhibited by monovalent ligands (Ahmad et al., 2004). Indeed, proteolysis of the N-terminal extension of Gal3 by metalloproteinases results in the formation of Gal3 monomers that interfere with Gal3-dependent oligomerization (Gong et al., 1999; Ochieng et al., 1994). As might be expected, tandem-repeat galectins are often more potent than the prototype, single-CRD galectins in crosslinking glycoproteins and triggering cell responses (Earl et al., 2011). As such, specific galectins might form segregated lattices, or different galectins might cooperate or compete in lattice formation. However, these particular features of the galectins have not been well characterized and remain to be explored.

Gal3 oligomerization and crosslinking of its ligand glycoproteins represents a demixing or phase transition from soluble complexes into gel-like polymer lattices or liquid droplets (Ahmad et al., 2004; Li et al., 2012). Mixing experiments with Gal3 and a bivalent N-glycan *in vitro* have revealed rapid phase transitions at critical molar ratios between Gal3 and ligands (Ahmad et al., 2004); dynamics are similar to that of phase transitions by adaptor proteins in receptor signaling (Li et al., 2012) or stress-induced RNA-protein granules (Han et al., 2012). N-glycans generally extend away from the protein surface and are flexible due to rotational freedom of the glycosidic bonds (Guttman et al., 2013). Multivalent interactions and a flexible geometry contribute to the intrinsic disorder (i.e. the many microstates) of the lattice. The N-glycans on extracellular domains are often hundreds of Ångstroms above the plasma membrane, where the galectin lattice extends laterally (see poster). The plasma membrane is also compartmentalized by lipid microdomains (or rafts) and the submembranous cytoskeleton into nanoscale compartments (Kusumi et al., 2012). The galectin lattice represents an additional layer of membrane organization that controls diffusion, complex formation and domain interactions in the plasma membrane (Lajoie et al., 2009). Indeed, proteomic analysis of detergent-resistant membranes (DRMs, or rafts) revealed that the galectin lattice blocks the distribution of many glycoproteins, including solute transporters, into DRM fractions (Boscher et al., 2012). In other words, the galectin lattice is a dynamic microdomain assembled as a gel-like polymer that regulates the distribution of glycoproteins at the cell surface.

Galectin lattice composition

In vertebrates, ∼30% of the transcriptome is translated in the secretory pathway and most of the proteins are modified on the lumenal side of the membrane by the N-glycosylation pathway. Galectins bind to glycan motifs found on many glycoconjugates at the cell surface and, in this regard, might appear to lack specificity for individual effectors. Indeed, proteomic analyses of Gal3-interacting partners, Mgat5-dependent raft association and L-PHA-binding proteins (the higher affinity galectin ligands) indicate binding of many glycoproteins in parallel (Abbott et al., 2008; Boscher et al., 2012; Lakshminarayan et al., 2014). Nonetheless, a systems-based approach has revealed a selective association of transmembrane glycoproteins with the galectin lattice (Lau et al., 2007). Importantly, the relative affinity of transmembrane glycoproteins for the galectin lattice is proportional to their number of Asn-x-Ser/Thr (NxS/T) N-glycan sites (where x can be any amino acid except Pro) and modification through the Golgi N-glycan branching pathway (Lau et al., 2007) (see poster). In addition, these affinities can vary owing to modifications of the branched N-glycan that occur in the *trans* Golgi. For example the presence of polylactosamine (repeating units of Gal–GlcNAc) enhances the affinity of glycoproteins for Gal3 and other galectins, whereas α2-6-sialylation reduces the affinity for Gal1 (Amano et al., 2003). By considering the relationship between N-glycan number and branching modification we suggested a model for glycoprotein-galectin interactions that accounts for the multivalency and additive effects of the branched N-glycans on galectin binding (Dennis and Brewer, 2013; Dennis et al., 2009). Strong selective forces appear to have acted on NxS/T sites during vertebrate evolution in a manner that supports the lattice model (Lau et al., 2007; Williams et al., 2014).

More than 97% of N-glycans are attached at the NxS/T motif within mammalian glycoproteins (Zielinska et al., 2010); they are transferred from the glycolipid $Glc_3Man_9GlcNAc_2$-pyrophosphate-dolichol to Asn by the oligosaccharyltransferase complex (OST) (Varki et al., 2009). This N-glycan is a ligand for the ER protein chaperones calnexin and calreticulin, which enhance protein folding efficiency (Deprez et al., 2005). After folding in the ER, most glycoproteins transit the Golgi complex, where the glucose (Glc) and mannose (Man) residues are trimmed by glycosidases –

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including mannosidase 2A (Man2A) – and remodelled by branching N-acetylglucosaminyltransferases, which form an ordered enzyme cascade that comprises Mgat1, Mgat2, Mgat4a/b/c and Mgat5 (Schachter, 1986). Each Mgat enzyme transfers GlcNAc in a specific linkage to N-glycans, followed by the addition of β-linked galactose; therefore, each branch is a potential ligand for galectin (Galβ1,4GlcNAcβ). The affinity of the N-glycans for galectins increases with branching and poly-N-acetyllactosamine extensions (repeating Galβ1,4GlcNAcβ-units) (Hirabayashi et al., 2002; see poster). The branching enzymes reduce the binding affinity for their common substrate UDP-GlcNAc, (~300-fold), whereby the Michaelis constant (K_m) changes from 0.04 to 10 mM, with Mgat4 and Mgat5 operating near and below their K_m, making the synthesis of tri- and tetra-antennary glycans highly sensitive to the supply of this metabolite (Lau et al., 2007). Glucose, glutamine and acetyl-CoA supply the hexosamine pathway and biosynthesis of UDP-GlcNAc and N-glycan branching, thereby regulating glycoprotein retention in the galectin lattice (Abdel Rahman et al., 2013; Grigorian et al., 2007).

Regulation of receptor kinases by the galectin lattice

The N-glycan-branching pathway generates heterogeneity that is distributed over multiple NxS/T sites in glycoproteins; thus, the number of potential glycoforms (i.e. the glycome) can become very large. However, a computational model based on grouping receptor kinase glycoforms owing to their affinity for Gal3 revealed that the glycome has functional implications at the cellular level (Lau et al., 2007). For example, human receptor kinases involved in growth and proliferation (e.g. EGFR) have approximately five times more NxS/T N-glycosylation sites than receptors that mediate organogenesis, differentiation and cell cycle arrest [e.g. TGF-β receptor (TGFβR)] (see poster). Receptors with five or more glycosylated sites are largely associated with the galectin lattice and their cell surface levels increase only modestly when the supply of UDP-GlcNAc increases (Lau et al., 2007). However, receptors with only one or few glycosylation sites are below the threshold for lattice association; the fraction of receptors associated with the lattice increases in a switch-like manner when the synthesis of tri- and tetra-antennary N-glycans is stimulated by UDP-GlcNAc. For instance, ligand-driven activation of EGFR (with eight N-glycans) – which is involved in growth signaling – stimulates metabolism and UDP-GlcNAc biosynthesis. This leads to the recruitment of TGFβR (with two N-glycans) into the galectin lattice, thereby promoting autocrine TGF-β and/or Smad signaling, resulting in reduced cell proliferation and inducing epithelial-to-mesenchymal transition (EMT) (Lau et al., 2007).

Other factors that regulate surface residency of a receptor include the presence of AP1- or AP2-adaptor-binding sites that promote clathrin-mediated endocytosis through clathrin-coated pits. Receptors that have only few NxS/T sites, are expressed at low levels or show rapid turnover at the cell surface and tend to be more dependent on the galectin lattice for their retention at the surface. Furthermore, because a high degree of membrane remodeling occurs in proliferating cells, macrophage and tumor cells, there is greater demand on the lattice in these cells in order to support the appropriate surface retention of receptors and transporters. TGFβRII (Lau et al., 2007), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) (Mkhikian et al., 2011) and glucagon receptor (Johswich et al., 2014) are examples of receptors with few NxS/T sites that oppose growth signaling and display a marked dependence on UDP-GlcNAc and/or N-glycan branching for their localization to the cell surface (Dennis and Brewer, 2013; Dennis et al., 2009). Therefore, both genetic inputs, such as the number of NxS/T sites and trafficking motifs, as well as metabolic cues (the availability of UDP-GlcNAc) control the association of receptors with the galectin lattice (see poster).

T-cell receptor and the immune response

Gal1 has been ascribed a broad spectrum of functions in immune cells, as well as in cancer (Bacigalupo et al., 2015), and during angiogenesis (Croci et al., 2014) and other processes (Toscano et al., 2011) (see poster). Gal1 binds to the T-cell-surface glycoproteins CD45, CD43, CD71 and CD7, which induces cell death and determines T-cell fate (Nguyen et al., 2001; Stillman et al., 2006). The main ligand for Gal3 on B-cell lymphoma is the phosphatase CD45; whose binding to Gal3 reduces its activity and opposes apoptosis (Clark et al., 2012). In fact, endogenous Gal3-mediated crosslinking of activated T cells was the first description of a galectin lattice that restrained T-cell receptor (TCR) mobility and reduced their sensitivity to antigen. In this context, Mgat5-deficiency weakens the lattice on T cells, resulting in autoimmunity in mice and a predisposition to multiple sclerosis in humans (Demetriou et al., 2001; Mkhikian et al., 2011). Early during immune synapse signaling, the galectin lattice and actin cytoskeleton segregate TCRs to the outside of raft microdomains that contain CD45, which – in turn – regulates Lck activity and the threshold for activation (Chen et al., 2007). Another study has confirmed that the Gal3 lattice negatively regulates the stability of the immune synapse and of TCR sensitivity in mature cytotoxic T cells (Chen et al., 2009). These studies identify the lattice as a key regulator of TCR sensitivity that can be fine-tuned to defend against pathogens while avoiding autoimmunity.

In T cells, the proliferative response to antigen is terminated by recruitment of CTLA-4 to the cell surface. A polymorphism in CTLA-4 that reduces the number of its N-glycans from two to only one is associated with increased risk of autoimmune disease (Mkhikian et al., 2011). Human polymorphisms in MGAT1, MGAT5, and interleukins 2 and 7 (IL7 and IL2) have been shown to genetically interact with CTLA-4 polymorphism through their effect on N-glycosylation and result in a higher combined risk of multiple sclerosis (Mkhikian et al., 2011). Experimental autoimmune encephalomyelitis induced in Gal3-deficient mice revealed a complex role for Gal3 in immune function and disease progression (Jiang et al., 2009). Thymic development of T-cell diversity is also dependent on N-glycan branching and provides another example of opposing but cooperative adaptations (i.e. recognition of pathogens versus self antigens). N-glycan branching regulates the variety of TCR-antigen affinities and acts as both a promoter and destabilizer of receptor dynamics (Zhou et al., 2014). Similarly, dichotomy in the effects of lattice dynamics at the molecular level is also observed for cell adhesion and motility (Partridge et al., 2004; Thiemann and Baum, 2011).

Metabolism

The galectin lattice also regulates metabolic homeostasis through nutrient transporters (see poster). *Mgat4a*$^{-/-}$ mice display hypoinsulinemia, hyperglycaemia and excess weight gain on a high-fat diet, whereas *Mgat5*$^{-/-}$ mice are resistant to weight gain and display hypoglycemia (Cheung et al., 2007; Ohtsubo et al., 2005). The Mgat4a-generated branched N-glycan on glucose transporter 2 (Glut2; also known as Slc2a2) binds to Gal9, which slows its lateral mobility and endocytosis, thereby increasing the transport of glucose and the secretion of insulin into pancreatic β cells (Ohtsubo et al., 2005). Furthermore, the presence of Mgat5-

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induced branched N-glycans on the glucagon receptor (GCGR) promotes crosslinking by Gal9, which reduces its lateral mobility and increases responsiveness to glucagon in hepatocytes. Mgat5 expression and UDP-GlcNAc availability cooperate to form a positive-feedback loop that further increases the responsiveness of the receptor to glucagon (Johswich et al., 2014). In cultured HEK293 epithelial cells, inducible expression of Mgat5 has been shown to promote glutamine transport and to rescue cell growth under conditions of low nutrient availability (Abdel Rahman et al., 2015). Upregulation of Glut1 activity through increased N-glycan branching also promotes its surface residency in tumor cells (Kitagawa et al., 1995). Furthermore, the surface residency of Glut4 has also been shown to increase in cells simulated with insulin or UDP-GlcNAc (Haga et al., 2011; Lau et al., 2007). Taken together, UDP-GlcNAc levels appear to act as sensors of metabolism that function through the galectin lattice to adapt the levels and activity of cell surface receptors and solute transporters in response to nutrient conditions (Dennis et al., 2009).

Cancer

Galectins also have a role in cancer progression. For instance, Gal3 was identified as a cancer-cell-associated protein (Raz and Lotan, 1981) and shown to be upregulated in many cancers, where it promotes cell migration and metastasis (Bresalier et al., 1998; Le Marer and Hughes, 1996). Gal8 promotes growth signaling, adhesion and motility in cancer cells (Levy et al., 2003). Furthermore, increased levels of N-glycan branching is closely associated with tumor malignancy (Dennis et al., 1987, 2009). Accordingly, Mgat5 deficiency reduces tumor growth and metastasis in mouse mammary cancer models (Cheung and Dennis, 2007; Granovsky et al., 2000), and sensitivity to stimulation with growth factors (Partridge et al., 2004). Sensitivity to these growth factors could be rescued by either (1) re-expressing Mgat5, (2) inhibition of constitutive endocytosis or, (3) increasing the level of UDP-GlcNAc by supplementation with GlcNAc (Partridge et al., 2004). GlcNAc supplementation restores the galectin lattice by stimulating the catalytic activity of Mgat1, Mgat2 and Mgat4, thereby generating N-acetyllactosamine branches in Mgat5$^{-/-}$ cells that compensate for the lack of Mgat5-generated branched N-glycans. Recruitment of EGFR to the galectin lattice prevents the inhibition of its signaling by the non-glycosylated membrane lipid raft protein caveolin-1 (Cav1), and late-stage growth of Mgat5$^{-/-}$ mammary tumors is rescued by loss of Cav1 (Lajoie et al., 2007) as well as by an acquired dependency on reactive oxygen species (ROS) inhibition of protein phosphatases (Mendelsohn et al., 2007). Other interventions that enhance the expression of Mgat-branching enzymes (Buckhaults et al., 1997) and/or increase UDP-GlcNAc levels might be able to exert similar effects in rescuing the galectin lattice, pointing to a network of pathways that, together, supports the galectin lattice and its functions at the cell surface (Lau and Dennis, 2008) (see poster).

The galectin lattice and expression of Mgat5 are associated with EMT in cancer cells (Lajoie et al., 2007; Partridge et al., 2004), and Gal1 has been recently implicated as an inducer of EMT (Bacigalupo et al., 2015). Cell–cell adhesion is primarily mediated by members of the cadherin family – which are also galectin ligands; for instance, murine epithelial (E)-cadherin carries two N-glycans and neuronal (N)-cadherin has seven N-glycan sites. Mgat5-dependent N-glycan branching and expression of Gal3 destabilizes N-cadherin at cell–cell junctions and, so, promotes the motility of mammary epithelial tumor cells (Boscher et al., 2012; Guo et al., 2009, 2003). Gal3 also increased junctional mobility of N-cadherin and of the raft marker GM1 ganglioside, as measured by fluorescence recovery after photobleaching (FRAP) (Boscher et al., 2012). In addition, binding of Gal3 to the N-glycans of β1-integrin stimulates integrin activation and endocytosis, as well as integrin-dependent fibronectin fibrillogenesis and focal adhesion turnover (Furtak et al., 2001; Goetz et al., 2008; Lagana et al., 2006; Lakshminarayan et al., 2014) (see poster). Although the Gal3 lattice competes with Cav1-positive lipid rafts to promote EGFR signaling to ERK1/2 (MAPK3, MAPK1) (Lajoie et al., 2007), Gal3 stimulation of integrin-mediated RhoA signaling, focal adhesion turnover and cell migration is dependent on phosphorylated Cav1 (Cav1-*P*) (Goetz et al., 2008; Lagana et al., 2006; Shankar et al., 2012). EGF signaling to RhoA – but not to ERK1/2 – and, in turn, cell migration and fibronectin remodeling, requires Gal3–Cav1-*P*-dependent integrin activation linking Gal3-dependent receptor kinase and integrin signaling (Boscher and Nabi, 2013). The combined effects of the galectin lattice on receptor kinases, cadherins and integrins, therefore, enhance tumor cell motility.

Galectin trafficking

A number of galectins (Gal1, Gal3, Gal4, Gal8 and Gal9) exhibit low-affinity interactions with raft-associated glycosphingolipids (GSLs) (Boscher et al., 2011) (see poster). Direct interactions between Gal3 and GSLs induce membrane bending on protein-free giant unilamellar vesicles (GUVs) and raft-dependent endocytosis through clathrin-independent carriers (CLICs) in cells (Lakshminarayan et al., 2014). Intriguingly, binding of pentameric forms of Shiga toxin and SV40 virus targeting GSL also mediate CLIC-dependent endocytosis (Ewers et al., 2010; Römer et al., 2007). Similarly, the pentameric conformation of Gal3, which is induced by high-density glycolipid ligands might have geometric features that promote GSL clustering, membrane bending and raft-dependent endocytosis (Lakshminarayan et al., 2014). Gal3 promotes raft endocytosis of CD44 and integrins (Furtak et al., 2001; Lakshminarayan et al., 2014), as well as Mgat5-dependent parvovirus internalization and infection (Garcin et al., 2015). Indeed, host galectins recognize microbial glycans and might play a role in pathogen attachment and invasion by viruses, bacteria, fungi and parasites (Baum et al., 2014).

In polarized MDCK cells, Gal3 is endocytosed at the apical membrane following activation of a raft-dependent pathway to recycling endosomes (Schneider et al., 2010; Straube et al., 2013). Internalized Gal3 interaction with glycosylated cargo in apical recycling endosomes sorts glycoproteins to the apical membrane (Delacour et al., 2007; Schneider et al., 2010). Similarly, in the absence of the basolateral sorting adaptor AP-1B, Gal4 promotes transcytosis and apical sorting of the transferrin receptor through apical recycling endosomes (Perez Bay et al., 2014). Gal3, Gal4, Gal7 and Gal9 have all been shown to be determinants of apical sorting, and are required for epithelial morphogenesis and polarity, the development of the apical lumen and ciliogenesis (Delacour et al., 2006, 2008; Mishra et al., 2010; Mo et al., 2012; Rondanino et al., 2011; Stechly et al., 2009; Torkko et al., 2008). Gal3 trafficking, therefore, involves non-classical secretion from the cytoplasm and glycan-dependent raft endocytosis to recycling endosomes, where it and other galectins play roles in post-Golgi sorting (see poster).

Conclusions and perspectives

The galectin lattice is a dynamic, extracellular planar gel-like polymer with crosslinking avidities that depend on the glycan profiles of surface-resident glycoproteins and glycolipids. The

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relative affinity of glycoproteins for the galectin lattice varies with the number of binding sites and is conditionally regulated by N-glycan modification/branching due to Golgi enzyme activity and nucleotide–sugar metabolism. This model for galectin function has been validated in autoimmune disease, T-cell development and cancer cell biology. The role of the galectin lattice in autoimmunity illustrates the power of a biological framework or model to help make predictions that can be tested by using genetics and biochemistry experiments (Li et al., 2013; Mkhikian et al., 2011).

Although our understanding of galectin lattice organization and structure is taking shape, it is still quite limited. Future studies should develop an understanding of the interplay between multiple galectins at the systems-level, and of the tissue-specific expression patterns of Golgi enzymes as well as glycoconjugates. Gal1 and Gal3 also regulate Ras signaling on the inner leaflet of the plasma membrane as well as mRNA splicing in nuclear ribonucleoprotein complexes – activities that are independent of their CRD-mediated binding to glycans (Haudek et al., 2010; Shalom-Feuerstein et al., 2008). However, like the galectin lattice, these activities may also depend on the multivalent protein–protein adaptor-like functions of these two proteins. The relationship between the extracellular carbohydrate-binding functions of galectins and their intracellular roles is an interesting aspect that will require further studies.

Competing interests

The authors declare no competing or financial interests.

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