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Role of laminin carbohydrates on cellular interactions

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Role of laminin carbohydrates on cellular interactions. Laminins, a family of large multidomain glycoproteins of the basal lamina, have been implicated in the development and maintenance of cellular and tissue organization. Considerable interest has arisen concerning the ways in which laminin carries out its biological functions. Previously these biologic responses have been primarily attributed to the peptide sequences of laminin, however, newer studies suggest that laminin carbohydrates may also participate in such cellular activities. Recently, a subpopulation of laminin molecules purified from EHS sarcoma by lectin affinity chromatography has been shown to contain about 25 to 30% carbohydrate. Most of the carbohydrates present are complex-type asparagine-linked oligosaccharides encompassing many different structures, some of which are unique to laminin. To date, the biological function of the carbohydrates of laminin remains somewhat unclear. They do not appear to be needed for heparin binding or to enhance proteinase stability, however, current evidence suggests they are important in cellular spreading and neurite outgrowth. It is our hypothesis that the covalently-linked carbohydrate moieties of laminin will ultimately prove to be involved in information transfer to responsive cells. It is the purpose of this review to delineate current concepts of the structure and function of this unique glycoprotein's sugar chains.

The basement membrane is a specialized extracellular complex of macromolecules which forms a barrier to segregate epithelial and endothelial cells from the stromal tissues. This sheet-like structure appears early in development and has been thought to play roles in the promotion of cell differentiation and growth, selective permeability, and cell attachment [1]. This structure has also been shown to be of importance in the alterations which occur during carcinomatous cell invasion and metastasis [2]. The extracellular macromolecules of basement membranes include type IV collagen, noncollagenous glycoproteins, and proteoglycans [3].

The major noncollagenous glycoprotein of the extracellular matrix is laminin, which is localized in the lamina lucida of basement membranes [4]. Laminin was first isolated by Timpl et al in 1979 from neutral salt extracts of the mouse Engelbreth-Holm-Swarm (EHS) tumor [5]. Since that time, laminin has been found to promote cell adhesion, growth, migration, differentiation, and neurite outgrowth [1]. Previously these biologic responses have been primarily attributed to the peptide sequences of laminin, however, recent studies suggest that laminin carbohydrates may also participate in such cellular activities [6–8].

Laminin carbohydrates are recognized by several different kinds of integrin and non-integrin cell surface components [9]. Among the non-integrin components are: (1) a 67 kDa protein which has both a peptide recognition site and a lectin-like galactoside binding site [10], (2) two S-type lectins, the Mac-2 surface antigen of macrophages [11] and one obtained from cardiac tissue [12], and (3) a cell surface galactosyl transferase which has also been demonstrated to play an important role in the mediation of biological responses to laminin [13].

Considerable interest has arisen concerning the ways in which laminin carries out its biological functions. As a prerequisite, a clear understanding of the biological function of laminin requires a knowledge of its structure. Although the function of laminin carbohydrates has not yet been definitively determined, it will be the purpose of this review to delineate current concepts of the structure and function of this unique glycoprotein's sugar chains.

Molecular structure and organization of laminin

Laminin, a large basement membrane glycoprotein (M_r = 900,000), is made up of three distinct polypeptide chains (B1, B2, and A) linked by disulfide bonds [1, 14]. The three chains of laminin are arranged in a unique cruciform shape (Fig. 1) as viewed by electron microscopy, with three arms of similar length (about 37 nm) and one longer arm (about 77 nm) [15]. The A chain has one large terminal globule on its long arm and another at its carboxy-terminus. Two smaller globules evenly spaced along its shorter arm, are also present. The B chains each have a small globular region at their amino-termini with another globular region closer to the center of molecule. Intrachain disulfide bonds also contribute to the structure of this molecule and numerous biologically-active domains have been identified [16, 5]. The cruciform shape of laminin is remarkably conserved through evolution from sea urchin [17] and Drosophila [18, 19] to humans [20, 21]. Isoforms of laminin have been found that differ only in the ratio or size of the three component chains compared to the prototypical A-B1-B2 molecule of EHS tumor-derived laminin [22, 23].

The complete amino acid sequence of the B1 (M_r = 220,000) [24], B2 (M_r = 200,000) [16] and A (M_r = 400,000) [25] chains of mouse and human [20, 21, 26] laminin have been deduced by molecular cloning. The three globular regions of the short arm of the A chain are separated by intervening cysteine-rich, epidermal growth factor-like (EGF) rods. Similarly, the B chain globular domains are also separated by EGF-like repeats. The long arm α-helix of the A chain forms a super helical coiled-coil region with corresponding α-helices of the B1 and B2 chains,

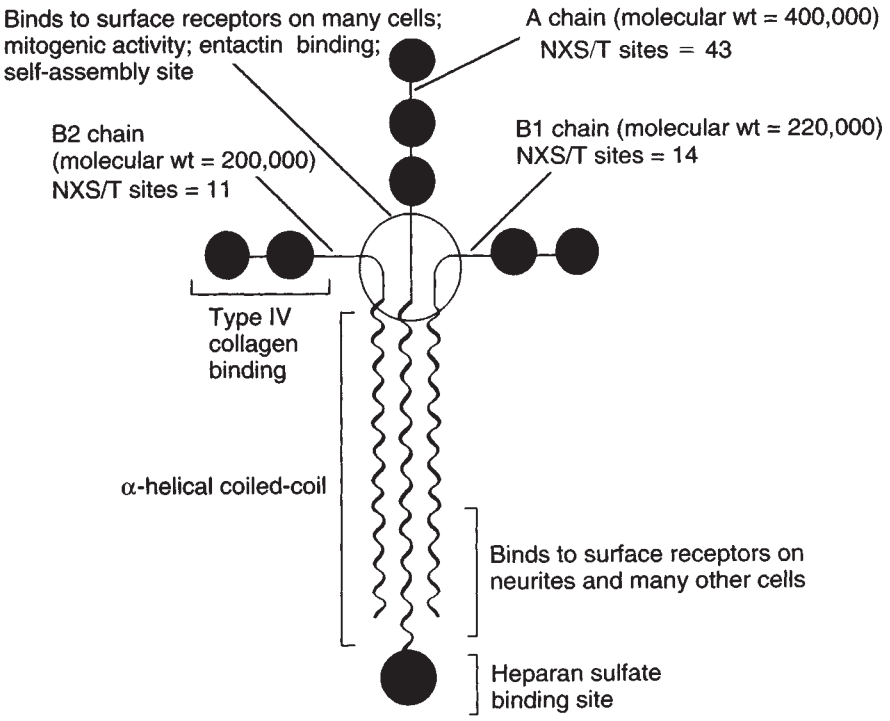


Fig. 1. *Diagram of laminin structure and binding domains adapted from reference 1.* NXS/T sites signify the number of consensus sequences for asparagine glycosylation.

ending in a carboxy-terminal globule composed of five homologous repeats [27]. The B chains are structurally similar to each other but lack the carboxy-terminal globule of the A chain. However, the amino terminus of the A chain is homologous to the corresponding regions of the B chains [28]. The B chains from mouse and human laminin are 90% homologous while the A chains exhibit 74% homology [26]. Thus, laminin structure is highly conserved, reflecting its cross-species prominence in basal laminae.

Glycosylation of laminin

Two studies show that laminin contains 12 to 15% carbohydrate [29, 30] although the most recent data suggest that the actual percentage might reach twice that amount. Recently, a subpopulation of laminin molecules purified from EHS sarcoma by lectin affinity chromatography (*Griffonia simplicifolia* agglutinin I) has been shown to contain about 25 to 30% carbohydrate [31]. Sixty-eight consensus sequences for potential asparagine glycosylation have been identified in mouse laminin, with the majority of sites concentrated in the long arm [5]. The A chain has 43 potential N-glycosylation sites [25], the B1 chain has 11 potential sites [24] and the B2 chain has 14 potential sites [16] (Fig. 1). Mouse EHS tumor laminin has about 40 different glycosyl substituents on these 68 potential sites [30]. Most of the carbohydrates present are complex-type asparagine-linked oligosaccharides encompassing many different structures [29–31], some of which are unique to laminin.

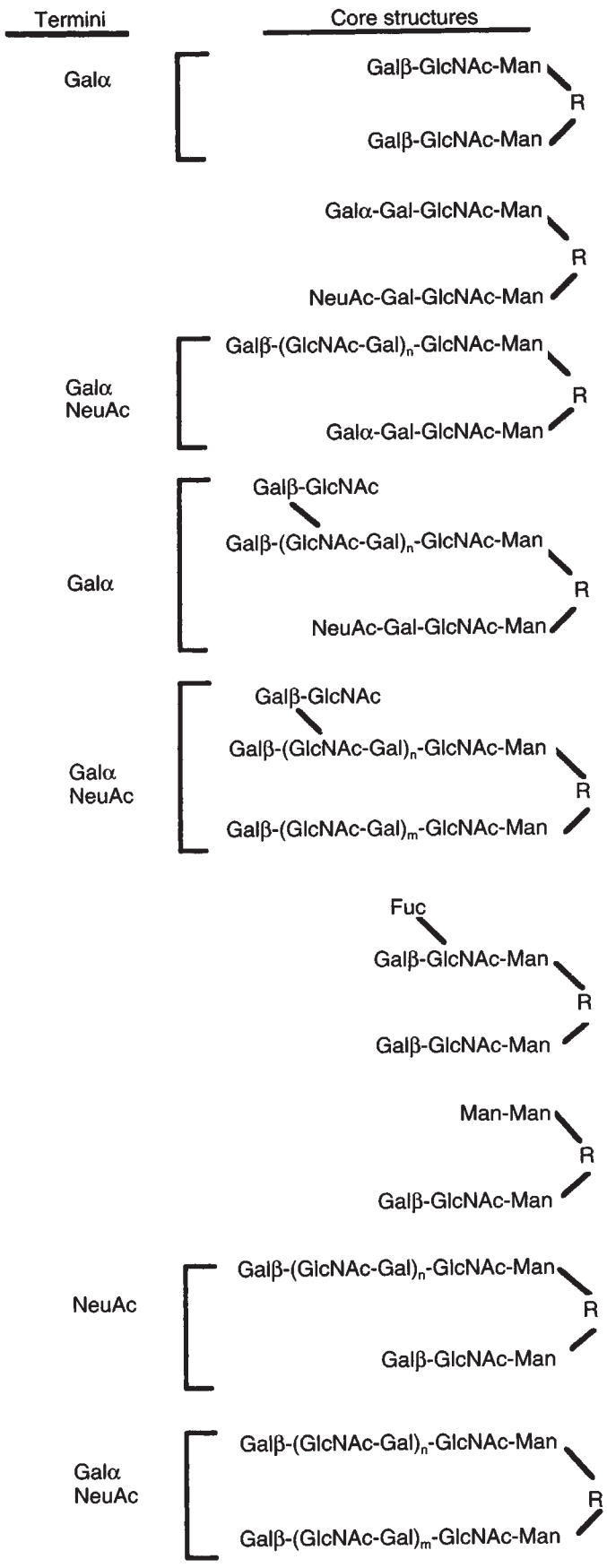
The structural studies of laminin carbohydrates carried out to date suggest that laminin contains exclusively N-linked oligosaccharides [29–31]. Unlike some glycoproteins, the presence of carbohydrates on laminin does not seem to confer protection from proteolytic degradation nor are they necessary for binding to heparin [32]. The biosynthetic assembly of laminin chains into a molecule also seems to be independent of N-glycosylation [33].

All the N-linked sugar chains share a common $Man_3GlcNAc_2$ core. Methylation analysis has revealed that the oligosaccharides of laminin contains bi- and triantennary chains and repeating sequences of 3Galβ1,4GlcNAcβ1 units [31]. Prominent features of the oligosaccharides are the presence of terminal α-galactose moieties, terminal N-acetyl-neuraminic acid residues, and poly-N-acetyllactosamine-containing chains. The latter have both linear blood group i and branched blood group I structures [31]. Figure 2 shows representative structures of the N-linked oligosaccharides of laminin, adapted from references [29–31]. Similar to other glycoproteins, laminin glycosyl substituents may vary with the species and tissue source of the molecule, the stage of tissue or organ development, and oncogenesis, among other factors [34].

Within the same set of oligosaccharides in any given group, extensive microheterogeneity appears to be present [29]. For example, the poly-N-acetyllactosamine-containing chains contain α-galactosyl, β-galactosyl and sialyl end groups in addition to the polylactosaminyl chain. The relative distribution of the lactosamine repeating units on the branched N-linked oligosaccharides is not yet known. Furthermore, it is not clear whether sialic acid and α-galactosyl residues occur at the non-reducing ends of the branches of the same oligosaccharide molecule [29, 35].

Interactions of laminin's sugar chains

N-linked oligosaccharides are often found as signals which incite lectin-mediated responses by animal cells [36–38]. The prototypical example has been hepatic cell receptors which recognize terminal galactose residues of oligosaccharides of circulating glycoproteins [39]. The recognition signal designates



removal of the glycoprotein from the circulation, followed by its intracellular degradation [40]. In this instance, and in other examples [41], it is the carbohydrate moiety which is critical for biological activity, rather than the polypeptide backbone of the molecule.

Correct and complete glycosylation has been shown to be essential for complete biological activity in some target cells; receptor binding to the α subunit of unglycosylated gonadotropic hormones is intact, but subsequent intracellular signalling via a second messenger pathway is defective when specific N-linked glycosyl groups are cleaved from the polypeptide structure of the hormone [42, 43]. This phenomenon may provide a clue to the ways in which cells progressively respond to a laminin surface, initially adhering, then spreading. Conceivably, the information encoded by both the protein and carbohydrate domains of laminin may be important for the sequence of cellular responses.

Several receptors that may mediate the biological effects of laminin carbohydrate moieties have recently been found. A newly discovered 67 kDa laminin receptor has been shown to be structurally and functionally similar to the 67 kDa elastin receptor [10]. This receptor interacts with a hydrophobic sequence in the B1 chain of laminin, similar to the cell recognition domain of elastin. Of special interest is the finding that this receptor contains a galactoside binding domain which shows immunological similarity to a rat lung lectin; apparently the lectin-like domain has a regulatory function in the 67 kDa receptor [44]. The receptor can be eluted from an elastin or laminin column by 1 mM lactose and is thought to function in vivo in the macromolecular assembly of elastic fibers [44].

Another example of a lectin's potential interaction with basement membrane components is the recent isolation of a lectin which is specific for poly-N-acetyllactosamine chains from calf heart [45]. When this lectin was used in an affinity column, laminin was bound with high affinity, leading to speculation about the possible role of this soluble animal lectin in extracellular matrix interactions [12].

A second category of molecules that have shown to interact with laminin's sugar chains, the cell surface glycosyltransferases, have been implicated in cell-cell adhesion and cell migration on extracellular matrix substrates. These molecules were first shown to be active in sperm/egg binding interactions [46]. It was reported in 1982 that the only glycosyltransferase present in significant quantity on the surface of embryonal carcinoma cells was galactosyltransferase and that it acted as a surface receptor for poly(N)-acetyllactosamine glycoconjugates [46, 47]. Subsequently, Runyan, Maxwell and Shur reported that neural crest cells expressed these cell surface galactosyltransferase molecules during migration and that laminin, with its many carbohydrate residues was the preferred substrate in vitro [48]. There is some evidence indicating that there are two separate populations of β-1,4-galactosyltransferase molecules that are similar yet distinct, one for Golgi processing of glycoproteins and the other for cell surface attachment [49, 50].

A further indication of the importance of laminin in the

Fig. 2. *Representative structures of the N-linked oligosaccharides of laminin adapted from references 29–31.* R = Man-GlcNAc-GlcNAc.

phenomena involving cell surface galactosyltransferase was the finding by Eckstein and Shur that laminin containing matrices induced the stable expression of this enzyme on the surface of migratory cells [51]. By using antibodies to the enzyme, they found that the increased numbers of galactosyltransferase molecules were primarily localized to the active lamellipodia of the cells, and could be co-localized with actin-containing microfilaments. Conversely, fibronectin was found to have no inductive effect on the expression of cell surface galactosyltransferase and cells tended to remain stationary on this substrate [51]. Also, cell surface galactosyltransferase participates in the initiation of neurite outgrowth from PC12 cells on laminin substrates [52]. These results highlight the potential role of the oligosaccharide chains present on laminin and present intriguing possibilities for cell surface galactosyltransferase in regulating cell motility and outgrowth of processes.

As noted earlier, Woo et al have also reported a major, non-integrin binding protein in macrophages that can bind to laminin with high affinity. This molecule has been determined to be an S-type lectin which appears to be identical to the carbohydrate binding protein Mac-2 [11]. Concurrently, Zhou and Cummings have reported that an S-type lectin from calf heart tissue selectively binds to the carbohydrate chains of laminin [12]. This receptor primarily recognizes poly-N-acetyllactosamine units.

Dennis, Waller and Schirrmacher reported in 1984 that cell attachment to laminin could be partially modulated by the expression of specific cell surface-associated oligosaccharide structures [53]. Recently, a cell surface receptor [54] responsible for concanavalin A induced inhibition of fibroblast spreading on laminin [55] has been isolated. The finding that this carbohydrate-containing receptor is operant in cell spreading on laminin but not on fibronectin highlights the diversity and specificity of these systems.

Chammas et al report that carbohydrates from both laminin and the $\alpha 6/\beta 1$ integrin play a role in their interaction [56]. They postulate alternative mechanisms of: (1) lectin-lectin interactions; and (2) carbohydrate-carbohydrate interactions. They also postulate that integrin glycosylation may modulate functional specificities of similar or identical integrins.

The importance of carbohydrates in development has been demonstrated by Bronner-Fraser who was able to disrupt avian neural crest cell migration in vitro on a laminin substrate by introducing an antibody (HNK-1) known to bind to a carbohydrate epitope on the cell surfaces [57]. A monoclonal antibody known to react with fucosyl residues in large poly-N-acetyllactosamine carbohydrates was found to inhibit cell substrate adhesion of F9 embryonal carcinoma cells [58]. This antibody also caused previously bound and spread cells to round up three hours after its addition to cultures. The studies of Trinkaus-Randall et al have shown that different lectins prevent cell binding and spreading on rabbit corneal basal laminae in vitro [59]. They reported that some lectins prevented cell binding while others allowed binding but inhibited cell spreading.

Deutzmann et al report that significant changes in cell adhesion, spreading and neurite outgrowth are produced by impairing the secondary and tertiary structure of the E8 fragment of laminin [60]. Using limited protease digestion these authors created a repertoire of degraded and denatured E8 fragment substrates; they found certain native conformations to be essential for full cell responsiveness. Yet circular dichroism studies [7] have indicated that unglycosylated laminin has a similar conformation to glycosylated laminin, but lacks full biologic activity. Therefore it can be concluded that the glycosylation state of the molecule is at least as important in determining cell responsiveness.

Work from our laboratory

Work in our laboratory has also focused upon how the carbohydrate moieties of laminin might influence its cellular interactions. In 1988 we reported that two lectins, namely, wheat germ agglutinin (WGA) and *Griffonia simplicifolia* agglutinin I (GSA I), when bound to laminin substrates were capable of preventing the adhesion of a mouse melanoma cell line (B16 F1) and a rat pheochromocytoma cell line (PC12) [6]. We postulated that the lectins became bound to N-linked carbohydrates adjacent to polypeptide recognition sequences and blocked cellular access to them. When the lectin concanavalin A was used, both cell types were able to bind, but no spreading or neurite outgrowth was subsequently found. This result suggested that the carbohydrates on laminin might play a role in cell spreading and neurite outgrowth. Bouzon et al also indicate the importance of laminin carbohydrates in the spreading of B16 F1 cells [8]. Their results differ from our findings in that they found reduced cell spreading on laminin substrates using wheat germ agglutinin whereas we found WGA inhibited cell attachment. However, there were methodological differences between the two studies and Bouzon et al did not mention testing other lectins.

We next produced unglycosylated laminin by using tunicamycin with a cell line that constitutively produces laminin. The resultant unglycosylated laminin was isolated from cell lysates by sequentially using an anti-laminin monoclonal antibody affinity column and a GSA I lectin affinity column [7]. When either the neuron-like PC12 cells or the B16 F1 melanoma cells were seeded onto unglycosylated laminin substrates, as many of them attached as did cells seeded onto glycosylated laminin. However, they failed to extend neurites or become spread, respectively [7]. We concluded that the carbohydrate moieties of laminin did indeed have a biological role, that is, to specifically signal certain types of cells to spread or others to extend neurite processes.

This conclusion has recently been reinforced by our ability to restore the biological responses to the attached, arrested cells either using: (1) appropriate mixtures of glycosylated and unglycosylated laminin as substrata; (2) laminin containing certain immature glycosyl groups; and (3) a Pronase digest of glycosylated laminin [61]. We find that a higher concentration of the Pronase digest, compared to intact laminin, is required to restore cell spreading, similar to results in other systems when the biological activity of released peptides or oligosaccharides was examined [62, 63].

Conclusions

In contrast to proteins and nucleic acids, carbohydrates are capable of encoding considerably more information per monomer unit, due to a repertoire of positional and anomeric linkages. This feature is both a strength and a weakness. Many unique structures can be generated, and indeed, are found. Such diversity of structure has sometimes been viewed as

implying lack of specificity. This point, coupled with the fact that the structures must be generated by posttranslational pathways has often tempered interest in the biological roles of glycoconjugates. However, a resurgence of interest has occurred, in part due to elucidation of important functional roles for animal lectins [35–38, 56]. It is our hypothesis that the covalently-linked carbohydrate moieties of laminin are intimately involved in information transfer to responsive cells.

Figure 3 schematically illustrates possible mechanisms by which laminin carbohydrates could signal cells. Cell surface lectins have been isolated by many investigators [11, 64, 65]; such lectins could potentially interact with the sugar chains of laminin. Carbohydrate-carbohydrate interactions may also occur as noted elsewhere [56, 66]. Cell surface galactosyltransferase have already been shown to help mediate neural crest cell migration [48] and thus represents still another possibility which must be considered. Finally, soluble lectins may mediate attachment between carbohydrates on cell surfaces and those on laminin [12] or perhaps there are dual receptors that contain both peptide-specific and lectin-like domains [10].

There is growing evidence that these "dual receptors" are responsible for binding to laminin in several biological systems. Recently an elastin receptor of this nature has been discovered that shows structural and functional similarities to the 67 kDa tumor cell laminin receptor and, in fact, may be the same protein. A conspicuous characteristic of this receptor is that its affinity for elastin or laminin is highly influenced by its carbohydrate-binding lectin domain [10]. This receptor model could also explain the mechanism by which both PC12 cells and B16 F1 melanoma cells bind equally to wells coated with either an unglycosylated or glycosylated laminin substrate but fail to exhibit neurite outgrowth or cell spreading unless laminin sugar chains are present [7]. In other words, these cells may have a dual receptor that differentially triggers binding and spreading depending on whether one or both recognition domains are occupied.

Finally, it is noteworthy that clinical interest has recently focused on the terminal α-galactosyl residues of glycoproteins including laminin. Human antibodies to the epitope, Galα1-3Galβ-4GlcNac, are normally present in the serum as 1% of circulating IgG [67]; the epitope itself is not ordinarily expressed on human cells and glycoproteins [68]. In at least two human disease states, namely metastatic cancer and Chagas' disease, it is thought that these antibodies may participate in defense processes. Human cancer cells often express the epitope. The antibody will inhibit adhesion of those cells to laminin [69]. The parasitic trypanosome which causes Chagas' disease carries the same epitope [70]. The markedly elevated levels of circulating antibody in infected individuals may be causative in the chronic phase of the disease, perhaps by means of an autoimmune response [71].

Clearly, further understanding of how cells interact with laminin will require additional study of its carbohydrate moieties and related receptors. Despite the recent attention paid to laminin sugar chains, both in the laboratory and clinic, there remain many more questions than answers. Additional information is required to allow assignment of function to the numerous different oligosaccharide chains found on laminins and to comprehend cell-to-laminin interactions which play a key role in so many physiological processes.

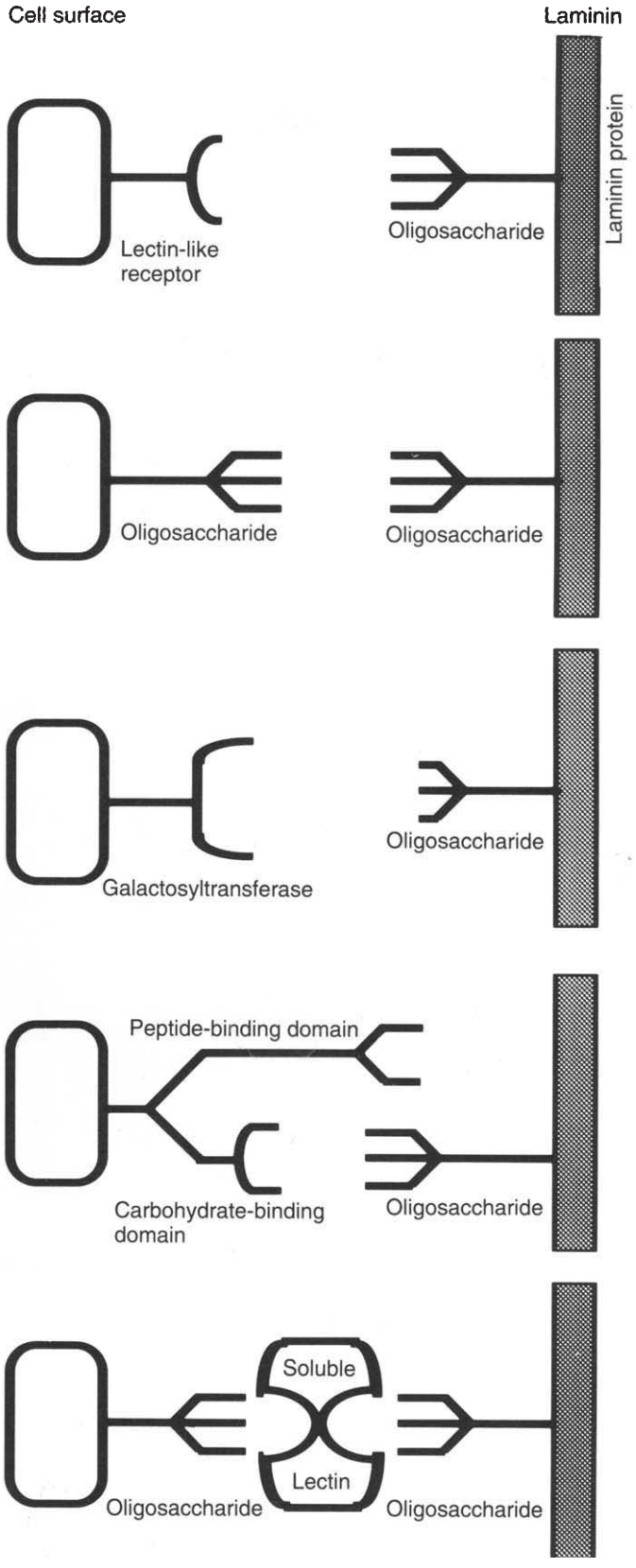


Fig. 3. *Potential modes of recognition of laminin oligosaccharides by the cell surface.*

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Note added in proof

Subsequent to the submission of this manuscript, high-mannose oligosaccharides and structurally similar compounds have been used in reconstitution experiments to determine which of these oligosaccharides could provide essential information to potentially metastatic melanoma cells. The results indicated that of all the various branched oligosaccharide structures tested, only mannose-based structures similar to the type of high mannose oligosaccharides found on laminin were capable of eliciting a cellular response. Furthermore, these mannose containing oligosaccharides were alone sufficient to promote spreading of laminin-adherent melanoma cells [72].

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