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PROSPECT

Polysialic Acid at the Cell Surface: Biophysics in Service of Cell Interactions and Tissue Plasticity

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Abstract Polysialic acid (PSA) is a long polymer of negatively-charged sialic acid associated with the neural cell adhesion molecule. PSA serves as a potent negative regulator of cell interactions via its unusual biophysical properties. During development the abundant and regulated expression of this carbohydrate is closely correlated with axon pathfinding and targeting, and with certain aspects of muscle formation. Its level can also be modulated by synaptic activity. PSA expression is more restricted in the neonatal and adult brain, being primarily associated with regions capable of morphological or physiological changes. Studies on the function of PSA studies suggest that its primary role is to promote developmentally-controlled and activity-dependent plasticity in cell interactions and thereby facilitate changes in the structure and function of the nervous system. The presence of PSA on a variety of metastatic tumor lines has also attracted the attention of oncologists, and its late appearance in evolution raises interesting questions about the phylogeny of complex tissue formation. J. Cell Biochem. 70:304–312, 1998.

Key words: polysialic acid; neural and muscle development; tissue plasticity

The study of cell interactions is presently dominated by membrane-bound receptors, with focus on their identification, expression patterns, binding specificities, relationship with intracellular components, and phenotype in targeted mutations. This impressive progress largely reflects the opportunities afforded by modern molecular and genetic techniques, and has provided for rapid expansion of the field. However, a downside to this rich harvest is that it has displaced or eclipsed other avenues of investigation that are essential to a fundamental understanding of receptor function at the cell membrane. One of these avenues is cell surface biophysics. Given the unusual and highly constrained environment of the cell surface, particularly when closely apposed to another cell, it is hardly surprising that biophysical factors should be important players in cell surface biology. Of particular note in this respect is the abundance of large cell surface carbohydrates, whose unusual biophysical properties are likely to be relevant, and whose functions are for the greater part poorly understood or ignored.

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This review features a conspicuous example of the importance of carbohydrates in cell surface biophysics: the polysialic acid (PSA) moiety associated with the neural cell adhesion molecule (NCAM), and its role in regulation of cell-cell interactions [Rutishauser, 1991]. PSA is a long, linear polymer composed entirely of negatively-charged sialic acid, and thus belongs to a class of anionic carbohydrate chains, such as hyaluronic acid, with distinct biophysical properties. It was discovered as a major component of vertebrate brains by Finne [1982], largely on the basis of its unusual composition and size. The cell biology of PSA on NCAM indicates that this carbohydrate functions as a negative regulator of cell interactions, and does so via its physical properties rather than a specific affinity for a receptor. The developmental biology of PSA reveals a highly regulated expression pattern in embryonic tissues, with the abundant and persistent expression of NCAM being punctuated by periods and sites with a high content of PSA. The presence of high-PSA NCAM is closely correlated with alterations in cell migration, axon pathfinding and targeting, and muscle development. Finally, the continued expression of PSA in certain brain regions suggests that it is associated with their ability to exhibit physiological plasticity.

Introduction to PSA

As a linear homopolymer (n = 8 to over 100) of alpha-2,8-linked sialic, PSA is a remarkably simple macromolecule (Fig. 1) [Finne et al., 1983]. The minimum chain length reflects characteristic formation of a helical conformation [Michon et al., 1987] whose unique structure provides for two essential tools in the study of PSA, specific monoclonal antibodies [Frosch et al., 1985], and a bacteriophage-derived endoneuraminidase (endo N) [Vimr et al., 1984]. The value of this fortuitous enzyme lies in the fact that it does not degrade any other known sialic acid-containing structure [Hallenbeck et al., 1987] and is suitable for both in vitro and in vivo studies [Rutishauser et al., 1985].

The phylogenetic distribution of PSA in biology is peculiar, being a component of the surface coat of Gram-negative bacteria (the natural target of the phage-derived endo N) [Troy et al., 1982], the zona pellucida of some vertebrate eggs [Kitajima et al., 1986], the sodium channel in the electric organ of electroplax [James and Agnew, 1987], and an abundant component of many developing vertebrate tissues. Surprisingly, this structure appears to be absent [Rutishauser, unpublished results] or at least much more restricted [Roth et al., 1992] in invertebrates. Unlike most carbohydrates found on the cell surface, PSA in the vertebrate embryo appears to be confined to the NCAM polypeptide [Acheson et al., 1991], in particular the fifth Ig domain (Fig. 1) [Crossin et al., 1984]. For comparison, the HNK-1 carbohydrate found on NCAM is also expressed on a wide variety of glycolipids and glycoproteins [Kruse et al., 1984]. An unfortunate consequence of the PSA antigen being expressed both by bacteria and in tissues of young children is that Group B meningicoccus infections may be immunologically sheltered, vaccines have potential problems with host reactivity, or autoimmune meningitis may occur [Finne, 1985].

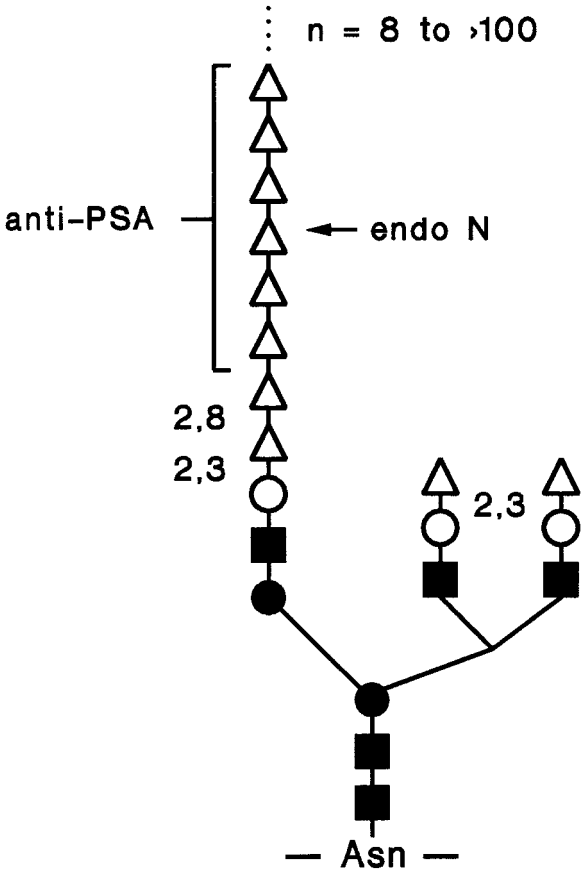

Fig. 1. Proposed structure of polysialic acid attached to NCAM via typical N-linked core glycosylation [Finne et al., 1983]. The unique structure of the α2,8-linked polymer allows for its specific recognition by monoclonal antibodies (anti-PSA) and by a phage-derived endoneuraminidase (endo N).

PSA as a Biophysical Regulator of Cell Interactions

The initial recognition of PSA function in vertebrate development was through its ability to decrease NCAM-mediated membrane-membrane adhesion in vitro [Sadoul et al., 1983]. Subsequently it was observed that a negative regulation of other cell interactions could also occur in the absence or independent of NCAM-binding function [Rutishauser et al.,1988]. For example, even the artificial agglutination of membrane vesicles by plant lectins can be reduced by this large polymer. Of greater biological relevance is the finding is that removal of PSA from cells can enhance the function of other CAMs as much or even more so than NCAM itself. This is particularly true for the L1/NgCAM class of adhesion molecules [Acheson et al., 1991], with important consequences for neural development as described below.

It has been proposed that PSA-mediated regulation of cell-cell interactions stems directly from its steric properties. A space-filling role for PSA is obvious in the surface coat of bacteria or the peri-vitelline space of vertebrate eggs. But can it also serve as a thin glycocalyx on embryonic cells? In fact, manipulations of ionic strength indicate that the steric properties of PSA directly underlie its effects on embryonic membrane adhesion [Yang et al., 1994]. In con-

sidering mechanisms by which steric effects could act on CAMs other than NCAM, two distinct modes are evident: those in which PSA impedes *trans* interactions between receptors on apposing cells, and those that involve a change in *cis* interactions between receptors on the same cell (Fig. 2).

In the *trans* mode, an interference with overall membrane-membrane apposition would affect other receptors as well as NCAM. This mechanism has two critical predictions: that enough space is influenced by PSA to affect overall membrane-membrane apposition, and that intercellular space is actually changed upon removal of PSA. Compatible evidence to this effect includes the high surface density of NCAM and the large steric impact of PSA (over 10 times that of an equivalent mass of protein).

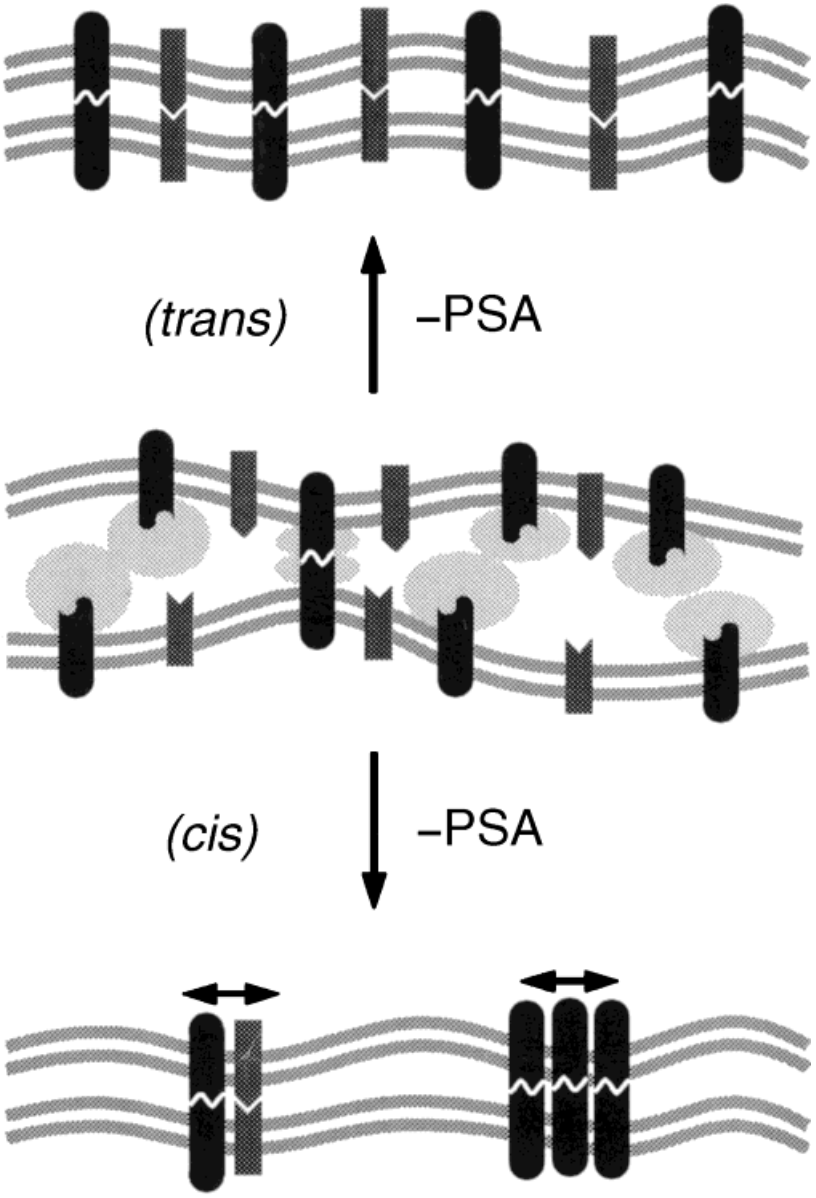

Fig. 2. Steric mechanisms by which PSA could affect cell-cell interactions. In the *trans* mechanism, the highly hydrated polymer (shaded ellipses) attached to NCAM serves as a impediment to membrane-membrane contact, and therefore decreases the efficiency of encounter between complimentary receptors on apposing cells. In *cis* mechanisms, the steric action of PSA is more local, in that its presence affects cell-cell interactions via interference with a clustering of NCAMs **(right)** or by association of NCAM with other receptors on the same cell **(left)**. Such *cis* effects could alter intracellular signaling as well as adhesion.

Furthermore, the distance between apposed cell membranes upon enzymatic removal of PSA decreases by 10–15 nm and thus would be expected to have a substantial effect on receptor-mediated interactions [Yang et al., 1992]. The *cis* mode is more complex, in that it could involve changes in an adhesion-promoting interaction of NCAM with other receptors on the same cell [Kadmin et al., 1990], or an indirect augmentation of interactions as a result of intracellular signaling [Doherty and Walsh, 1992] and/or clustering of NCAMs within the plane of the membrane [Doherty and Walsh, 1992; Singer, 1992]. At present it is not possible to conclude whether either or both of these mechanisms operate. What is now necessary to resolve this issue is a more direct biophysical approach that readily discriminates between interactions that occur across or within lipid bilayers. Fortunately such tests, employing measurements of receptor mobility, molecular proximity, and membrane-membrane forces are being developed [Isrealachvili, 1991].

Developmental Regulation of Cell Interactions by PSA In Vivo

Regardless of the precise cellular mechanism by which PSA operates, it is now clear that the resulting regulation of cell surface events is a fundamental parameter in several aspects of vertebrate development. Studies of PSA function in live embryos have been carried out by a combination of immunohistochemistry to detect sites of rapid changes in expression, and in vivo perturbation using injected endo N to eliminate cell surface PSA from those sites. The biological systems that express PSA are diverse in nature, but a common theme to the findings has been that the up-regulation of PSA creates a permissive condition for the rearrangement of cells and axonal processes.

Expression of PSA during development. In the developing vertebrate, PSA is expressed in stage and tissue-specific patterns that often correlate with the reorganization of cells or guided extension of cellular processes. Examples include its abundant and regulated expression on growing axons in many neural systems [Chuong and Edelman, 1984], and during development of a variety of non-neural tissues such as skeletal muscle [Fredette et al., 1993], kidney [Lackie et al., 1990], heart [Lackie et al., 1991], and feathers [Marsh and Gallen, 1992]. Although such indirect correlations do not by themselves establish a causal relationship between PSA and the reorganization of a tissue, the combination of these expression patterns and the anti-adhesive properties of PSA have provided a basis for more direct analyses, as in the examples described below.

PSA is associated with cell rearrangement and migration. The movement of cells involves the breaking of cell interactions, both in an initial detachment phase from their site of origin and in the adhesion-deadhesion process of locomotion along a substrate. An example of detachment occurs in the sorting out of skeletal muscle myotubes [Fredette et al., 1993]. Although muscle does not express PSA during most of its development, a transient expression of high-PSA NCAM does occur during the separation of myotubes to form individual muscles in the chick hindlimb. In addition to this correlation, it is known that the ability of myotubes to separate is dependent on synaptic activity, and the fact that the neurotoxin curare also inhibits this expression of PSA argues that the carbohydrate's expression is directly related to myotube rearrangement. Another suggestive aspect of the process is that the form of NCAM being synthesized at this time changes from a transmembrane to a lipid-linked form, and this PSA-rich form of the molecule appears to be selectively associated with regions of the myotube surface that had recently separated from neighboring cells. Together these findings argue that selective targeting of PSA to certain regions of a cell can be used to promote separation of that cell from its neighbors.

Evidence that PSA can affect long distance migration of cells has come from studies of precursor cells in the developing brain. Three types of migrating brain cells have been found to express PSA, LHRH cells as they move from the olfactory placode to the forebrain Murakami et al., 1991], the migration of oligodendrocyte precursors [Trotter et al., 1989], and subependymal cells that travel to the olfactory bulb [Miragall et al., 1988]. The enzymatic removal of PSA from neurohypophyseal explants in vitro inhibits the spread of oligodendrocyte precursors onto the culture substrate [Trotter et al., 1989], indirectly suggesting that PSA may affect migratory processes in the animal.

The most complete case for a causal link between PSA and cell migration in vivo comes from analysis and comparison of subependymal cell distributions in NCAM-mutant and endo

N-injected mice. As indicated above, NCAM is the sole carrier of PSA in the developing embryo, and homologous recombination has been used to generate mice deficient in their NCAM and PSA expression [Tomasiewicz et al., 1993]. The most conspicuous abnormality in these mutants is an impaired ability of precursor olfactory bulb cells, born at the lateral ventricle, to migrate through the subependymal zone to the olfactory bulb [Luskin, 1993]. To confirm the direct role of PSA in the mutant phenotype, it was demonstrated that this defect in cell migration can be duplicated in wild-type mice by injection of endo N [Ono et al., 1994]. In addition to the ectopic location of the precursors in the mutant or endo N-treated animals, the precursor cells are morphologically altered with respect to growth cone-like processes oriented along the migration route. In contrast to this tangential movement of neuronal precursors, radial migration and histogenesis of granule cells in the olfactory bulb or cerebellum does not appear to be affected by loss of PSA.

PSA serves as a permissive regulator in axon pathfinding and targeting. Much of the functional analysis of PSA has concerned the behavior of axons as they grow toward and innervate their specific targets. For example, the patterns of motorneuron innervation are distinct for fast and slow muscle regions. Although the relative levels and distribution of the relevant adhesion molecules (NCAM and L1/NgCAM) are not different for fast and slow regions, there is considerably more PSA on axons in the fast region than in the slow [Landmesser et al., 1988]. Moreover, when endo N was used to remove the PSA, all the axons grew toward the slow region. The interpretation of these results is that high levels of PSA serves to limit axon-axon interactions and thereby allow other environmental cues to attract fibers toward the fast region. Conversely, the lower levels of PSA on slow fibers produces thick fascicles that continue to grow along muscle fibers extending into the slow region.

Similarly, PSA appears to be a critical factor in the establishment of specific motorneuron pathways after they emerge from the neural tube [Tang et al., 1992]. Motorneurons upregulate their expression of PSA as their growth cones begin to enter the plexus region. Within this region they normally alter their course, crossing over each other, in order to segregate into motoneuron pool-specific groups. When PSA was removed by endo N during this period of axonal outgrowth, errors in pathfinding were common, resulting in the innervation of inappropriate muscles. As in the fast/slow decision described above, the endo N-induced defect involved an inability of the axons to break away from fascicles to respond to environmental cues. Moreover, when anti-L1/NgCAM (but notably not anti-NCAM) was co-administered with endo N, the normal behavior of the axons was restored, confirming that loss of PSA had increased fasciculation mediated by L1/NgCAM.

Despite these differences in biological context, the common theme is that PSA does not in of itself provide a specific signal for target interaction and pathfinding. Instead it expression appears to represent a regulated permissive signal that allows the axons to respond to external cues at the appropriate time and place.

Neural Input Alters PSA Expression and Promotes Tissue Remodeling

One of the fundamental features of the nervous system is the alteration of its structure according to changes in input from axons. Such alterations in architecture also require modification of cell-cell interactions, and thus provide another potential avenue for facilitation by PSA. In fact, three different modes of input-dependent change have already been found to involve expression of PSA: synaptic activity-dependent changes in motorneuron innervation [Landmesser et al., 1988], neurosecretory axon input-dependent remodeling of the neurohypophysial system that produces vasopressin and oxytocin [Theodosis et al., 1991; Kiss et al., 1993], and light- and activity-dependent reorganization of the visual cortex [Udin et al., 1996]. The variety of relationships between PSA, tissue plasticity and input exhibited by these examples serves to illustrate the versatility of PSA in promoting the remodeling of cell contacts.

In addition to its activity-dependent role in muscle development, PSA serves as a molecular link in the feedback loop from neuromuscular synaptic activity to changes in the pattern of motorneuron branching and sprouting [Landmesser et al., 1988]. As in the study of fast/slow muscle innervation, this analysis features L1/NgCAM, NCAM, and PSA, but with addition of synaptic activity blockade by the neurotoxin curare. Whereas the increased sprouting and branching produced by curare is not reflected by changes in NCAM or L1/NgCAM, levels of

PSA on the motoraxons is dramatically increased. A causal relationship between activity, PSA and innervation pattern is supported by the observation that the morphological effects of curare on innervation are reversed by co-administration of endo N. As in fast/slow muscle targeting, the inhibition of axon-axon interaction with antibodies against L1 (but again not against NCAM) both mimics the effects of curare and reverses the effects of the endo N.

The hypothalamo-neurohypophysial has become a model system for input-dependent morphological plasticity, with changes in axon-glial cell architecture affecting hormone release. Recent studies have revealed that the maintenance of PSA expression by the glial cells of the adult neurohypophysis [Theodosis et al., 1991] requires the presence of innervation by the neurosecretory axons of the hypothalamus [Kiss et al., 1993]. It has been proposed that the expression of PSA by the glial cells of the neurohypophysis plays a role in their ability to regulate release of hormone from nerve terminals, possibly by promoting plasticity in physical blockade of release by the surrounding glial processes.

In the visual system of the frog, the target region of the brain (tectum) transiently expresses high levels of PSA during the activity-dependent reorganization of ipsilateral axon inputs to match with contralateral input. Both PSA expression and this form of plasticity can be restored in the adult either by depriving the animals of visual input or via application of the glutamate-receptor agonist NMDA [Udin et al., 1996].

PSA in the Adult Brain and During Nerve Regeneration

Most tissues that express PSA during development tend to show a progressive loss of this carbohydrate as mature, adult structures are formed. For this reason NCAM with a high PSA content is sometimes referred to as "embryonic NCAM" and with a low content as "adult NCAM" [Edelman and Chuong, 1982]. Thus, the expression of "embryonic NCAM" in the adult is of particular interest in that it is suggestive of a maintenance of morphoplastic properties. This suggestion is bolstered by the fact that such patterns of expression are often associated with brain tissues that exhibit physiological plasticity [Aaron and Chesselet, 1989]. In addition to the neurohypophysis and olfactory bulb mentioned above, examples include newly-generated granule cells in the adult dentate gyrus that can add new neuronal circuits [Seki and Arai, 1993], the medial basal hypothalamus which is associated with the onset of sexual maturation [Perera et al., 1993], the suprachiasmatic nucleus whose plasticity is reflected in circadian rhythms and function is altered both in NCAM mutant and endo N-treated animals [Glass et al., 1994; Shen et al., 1997], and a variety of specific subregions of cortex, brain stem and spinal cord. A role for PSA on NCAM in higher brain function including behavior is also suggested indirectly by reports that polysialylation of NCAM by is transiently increased during passive avoidance responses [Doyle et al., 1992] and that NCAM-mutant mice have deficits in spatial learning that may be related to the demonstration that PSA is required for long-term potentiation in the hippocampus [Cremer et al., 1994].

Another correlation with PSA expression can occur when neural tissues react to chemical, disease-related or physical damage. Although such major insults result in complex changes involving many molecular and cellular components, the re-expression of PSA has been observed and proposed to be a factor in the response process. The types of damage and affected systems that exhibit enhanced PSA expression can be quite diverse, ranging from the effects of a neurotoxic agent (kainic acid) on reactive glia in the hippocampus [Le Gal La Salle et al., 1992], to lesion of axons in the optic system of fish and amphibians [McQuarrie and Rutishauser, 1986], cerebellum [Miller et al., 1994], and sciatic nerve [Daniloff et al., 1986] as well as in the pathology of human muscle diseases involving dystrophy or denervation [Figarella-Branger et al., 1990]. A critical test of the role of PSA in these systems, as in the activity-dependent phenomena, will be to establish a causal relationship between PSA and regeneration, for example through co-administration of endo N.

PSA as an Oncofetal Marker on Tumor Cells

The expression of fetal antigens on tumor cells is a common phenomenon, perhaps reflecting an immature state that features a high rate of proliferation. Among those antigens is PSA, with abundant expression in Wilms' tumor cells [Roth et al., 1988], small cell lung cancer [Kibbelaar et al., 1989], neuroendocrine and neuro-

ectodermal tumors [Heitz et al., 1990], neuroblastoma [Moolenaar et al., 1990], and teratomas [Metzman et al., 1991]. In the case of PSA, however, there is the additional question as to whether this expression directly contributes to the tumorigenicity of the cells. While no clear correlation has been made, it has been reported that growth of clonal sublines of human small cell lung carcinoma can be modulated by PSA [Scheidegger et al., 1994] and in view of the established influence of PSA on cell migration, there are reasons to suspect that tumor metastasis might be affected as well.

Phylogeny of PSA—Why Not in Invertebrates?

A curious aspect of PSA is that it appears to be an important regulatory element in cell migration, axon guidance and targeting, and neural plasticity, yet appears to be absent from invertebrates whose cells can exhibit similar phenomena. In fact, the attenuation of cell interactions does appear to be a feature of synaptic plasticity in Aplysia [Nadja et al., 1994], except that in contrast to vertebrates, the decrease is accomplished through down-regulation of the adhesion receptors themselves rather than the up-regulation of an anti-adhesive mechanism. Similarly, in Drosophila the over-expression of axon adhesion molecules produces aberrant axon pathfinding that resembles the effects produced by enzymatic removal of PSA in vertebrates [Lin and Goodman, 1994]. Thus it would appear that the evolution of PSA as a cell surface component on NCAM does not so much represent a fundamentally new process, but rather an improved mechanism for producing optimal levels of cell interaction. A possible driving force for this change in mechanism could well be the increase in the number of interaction receptors required for very large and complex tissues. With such a large repertoire, the specific regulation of so many different genes might have proven cumbersome, so that instead a different mechanism was introduced that was capable of global attenuation of cell interactions.

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