Cell adhesion, junctions and the cytoskeleton

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NCAM and its polysialic acid moiety: a mechanism for pull/push regulation of cell interactions during development?

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Summary

Many cell adhesion molecules have a distinct pattern of expression and well-defined role in cell-cell recognition. In contrast, NCAM is broadly expressed and perturbations of its function affect many diverse aspects of embryonic development. Evidence has been obtained suggesting that the molecule and its polysialic acid moiety serve not only to contribute to specific interactions, but also to regulate overall cell-cell apposition. In this latter mode, the molecule can have both a positive and a negative effect on a wide variety of contact-dependent cellular events.

Key words: neural cell adhesion molecule, regulation of cell interactions, adhesive preferences, axon outgrowth.

Introduction

Being the first cell adhesion molecule to be identified in vertebrate embryos, NCAM tended to be considered as a candidate receptor in nearly every cell-cell interaction that occurs during development. The molecule itself encouraged this practice by its expression on diverse cell types in many tissues, and observations that antibodies that block NCAM-associated adhesion interfere with a wide variety of cellular events. Subsequently, other adhesion molecules were discovered that were more specifically associated with particular events. Eventually it became clear that the role of NCAM in these same events needed re-evaluation, and a rationale provided for the extraordinarily broad influence of this molecule. This chapter reviews the biological role of NCAM from this perspective, focussing on its ability to act both as a mediator of specific interactive preferences, and as a general regulator of cell-cell contact.

NCAM expression in development

The broad distribution of NCAM in vertebrate embryos includes the primitive neural ectoderm of the neural plate, transient expression in morphogenetically active structures (such as notochord, somites, placodes, epidermis, mesenchyme, mesonephros), a uniform and persistent presence on neurons, and a spatially and temporally regulated expression by glial and muscle cells (Thiery et al., 1982; Jacobson and Rutishauser, 1986; Keane et al., 1988; Grumet et al., 1982; Silver and Rutishauser, 1984; Maier et al., 1986). Therefore, a substantial portion of embryonic tissues are composed of cells that produce, or whose precursors have produced, detectable amounts of NCAM. On this basis alone, it is clear that NCAM by itself is not well suited for identification of individual cells or specification of precise cell-cell interactions.

NCAM is a complex molecule with multiple functions

There is a tendency to label a molecule's function in the framework of its initial discovery, which for NCAM was as a homophilic cell adhesion molecule. It is now clear from its large size and diverse structural domains and variants (see Rutishauser, 1991, for review), that this simplistic view will require expansion. For example, the splicing of mRNA encoding both intracellular and extracellular regions of the polypeptide, an affinity for heparan sulfate, the remarkable postranslational addition of polysialic acid, and evidence that the molecule can influence second messenger systems, all need to be considered in a comprehensive view of NCAM function. As yet such an integration of NCAM functions has not been made at the molecular level, and its observed biological effects undoubtedly reflect a mixture of different mechanisms.

For these reasons, the ensuing paragraphs will use the relatively vague terms 'interaction' or 'recognition' to refer to the combined functions of NCAM, with the specific term 'adhesion' reserved for the physical association of membranes via receptors. Thus in schematic figures depicting cell-cell interactions, the illustration of membranes becoming more closely apposed can also be viewed in the larger context of interaction.

NCAM as a recognizer: combination and competition with other CAMs

In some biological situations, NCAM does in fact behave like a simple homophilic recognition molecule. That is, a phenomenon involving a specific interaction between two NCAM-positive cells appears to reflect directly the adhesion properties of this molecule. In most of these cases, the term 'specific' is operationally defined by the ability to inhibit the phenomenon with antibodies against NCAM. Leaving aside the more artificial cell aggregation assays, these situations have been best documented in the developing nervous system, including fasciculation of retinal ganglion cell neurites in the optic nerve (Thanos et al., 1984), migration of growth cones on cellular substrata both in vitro (Rutishauser et al., 1983; Bixby et al., 1988; Covault et al., 1987; Neugebauer et al., 1988) and in vivo (Silver and Rutishauser, 1984), innervation of muscles by motorneurons both in development (Landmesser et al., 1988) and regeneration (Rieger et al., 1988), and paralysis-induced sprouting (Booth et al., 1990).

While NCAM function in these cases appears relatively straightforward, it is important to emphasize that the molecule's influence is only appreciated in terms of the simultaneous action of other CAMs. For neuronal processes, these include the axon-associated CAMs (L1, F11, neurofascin, etc.) as well as the more broadly expressed cadherin and integrin families. For example, in studies of axon-axon bundling or growth cone migration in vitro, the action of individual CAMs appears to be additive or synergistic with others, relative contributions varying for different cell types and developmental ages (Bixby et al., 1987; Neugebauer et al., 1988; Rathjen et al., 1987; Tomaselli et al., 1988; Bixby et al., 1988; Drazba and Lemmon, 1990). When axon bundling and growth combine in the context of in vivo innervation, they tend to oppose each other as illustrated in Fig. 1. In the case of motor innervation of the chick hindlimb, the competition, which is most easily described in terms of adhesion but may be more complex, appears to be between L1's promotion of fasciculation and NCAM's support of nerve growth on muscle (Landmesser et al., 1988). While less information is available, it is reasonable to suspect that such principles also apply in the proposed role of NCAM in optic nerve bundling (Thanos et al., 1984) and guidance along epithelial endfeet (Silver and Rutishauser, 1984).

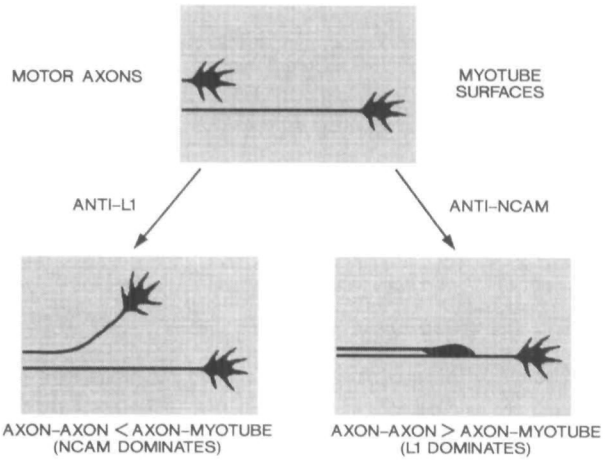

Fig. 1. Competition between CAMs in the outgrowth and bundling of axons. The top part of the drawing depicts one axon that has extended over the surface of a myotube, while a second has just begun to grow. As the second axon grows, a dominance of L1-mediated axon-axon interactions over NCAM-mediated axon-muscle interactions (lower right) tends to produce elongation along the first axon. Conversely, if axon-myotube interactions are stronger (lower left), the two axons will diverge. These different patterns can be manipulated experimentally by the addition of antibodies that specifically block L1 or NCAM adhesion.

Regulation of non-NCAM interactions by NCAM

Other studies suggest that NCAM's influence goes beyond a tug-of-war between CAMs. For example, antibodies against NCAM can inhibit cell-cell interactions whose intrinsic mechanism does not directly involve NCAM itself: gap junctional communication in the neural plate (Keane et al., 1988), contact-dependent regulation of neurotransmitter enzymes (Acheson and Rutishauser, 1988), and possibly cadherin function (Rutishauser et al., 1988; Knudsen et al., 1990). These findings are accompanied by an increasing awareness that adhesion can trigger other cellular events, even avoidance reactions between neurites (Kapfhammer and Raper, 1987; Kapfhammer et al., 1986) and de-adhesion (Dustin and Springer, 1989). The central theme is that adhesion can be a regulatory element that promotes recognition or interaction, but is not the whole process in of itself.

This permissive influence is most easily described in the case of junctional communication (Keane et al., 1988). NCAM is an early positive marker for neural induction (Jacobson and Rutishauser, 1986), and its expression appears to precede junctional communication. In addition, there is a precise correlation between the ability of two cells to exchange a fluorescent dye and the presence of NCAM on their surfaces. Finally, whereas the block of junctional communication by the *src* gene product does not prevent NCAM expression, the addition of anti-NCAM Fab to histotypic cultures of neuroepithelial cells delays the establishment of extensive communication among cells that express NCAM. Since there is no reason to believe that NCAM itself contributes to the structure of the channels, it is more likely that, in neuroepithelium, NCAM-mediated adhesion is required to allow sufficient interaction between cells for the assembly of stable junctions (Fig. 2).

If such a role represents the simple augmentation of cell-cell apposition, then NCAM's function should be readily replaced by other CAMs. In fact, transfected E-cadherin (L-CAM) (Musil et al., 1990) also has been shown to be capable of promoting formation of junctions. Moreover, gap junctions are capable of assembly in tissues that do not express NCAM.

Such a two-step mechanism for cell interaction has some useful attributes. It provides for additional specificity through independent regulation of the CAM and the information-generating entities. That is, a cell can decide not only what array of specific communications are desirable,

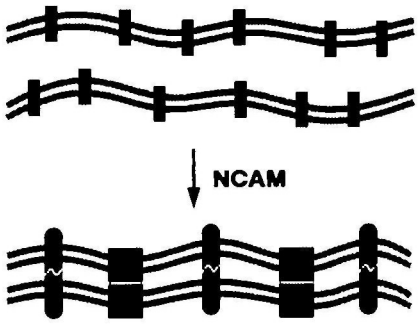

Fig. 2. Proposed mechanism for positive regulation of junctional communication between cells by NCAM-mediated adhesion. Two membranes containing junctional subunits (rectangles) are not sufficiently apposed (top) to allow the subunits to assemble into a stable junction. When NCAM is expressed, the resulting homophilic adhesion promotes junctional assembly (bottom).

but also when and where this ensemble is functionally engaged. There is also the potential for biosynthetic economy, since signals can often be generated with far fewer molecules than are required for macroscopic adhesion between cells.

NCAM polysialic acid: turning pull into push

Theoretical models and indirect evidence for multi-step recognition have been around for decades. The demonstration that NCAM can serve in such a capacity is therefore a welcome but not surprising finding. What is more novel and unanticipated is the fact that the molecule, through an unusual form of glycosylation, can provide for negative as well as positive regulation.

Perhaps the most remarkable structural variant of NCAM is its post-translational modification with polysialic acid (PSA) (see Rutishauser, 1989, for review). For nearly 10 years, it has been known that the presence of this very large linear homopolymer on NCAM decreases the molecule's ability to promote adhesion (Cunningham et al., 1983; Hoffman and Edelman, 1983; Rutishauser et al., 1985). In the context of the positive regulatory function for NCAM described above, PSA could therefore serve as a negative regulator of other cell interactions through direct effects on NCAM.

However, this negative regulation also can occur in the absence or independent of known NCAM-binding functions. For example, the presence of PSA on the surface of a cell can inhibit its attachment to a laminin substratum, a process that is blocked by antibodies against laminin but not by antibodies against NCAM (Acheson et al., 1991). Even agglutination of membrane vesicles by plant lectins is reduced by this large polymer (Rutishauser et al., 1988). Another surprising finding is that removal of PSA from cells can enhance the function of other CAMs even more so than NCAM itself. This is particularly true for the L1 adhesion molecule (Acheson et al., 1991), the consequences of which have important biological consequences as described below.

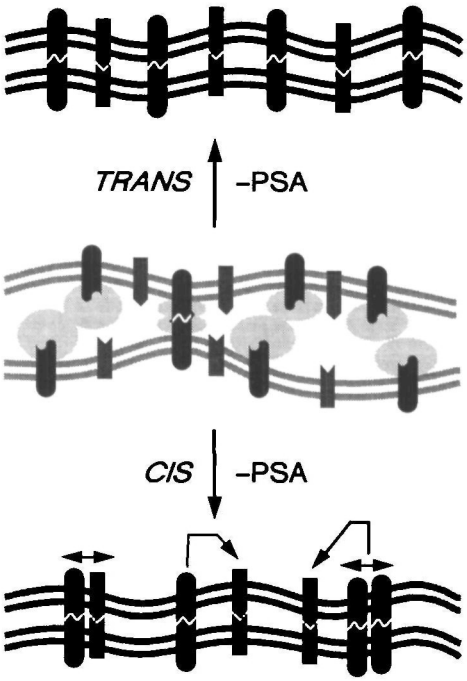

Fig. 3. Different modes by which removal of PSA (shaded elipses) could affect cell-cell interactions. Top: loss of PSA removes a physical impediment to membrane apposition and thereby enhances the *trans* encounter between receptors on apposing cells. Bottom: loss of PSA augments *cis* interactions among receptors which in turn promote cell-cell interaction. Such *cis* effects could include heterophilic or homophilic receptor clustering (double arrows) with NCAM, and could generate intracellular signals (single bent arrows) as well.

In considering mechanisms by which PSA may act in molecules other than NCAM itself, two distinct modes must be considered: those in which PSA would impede *trans* interactions between receptors on apposing cells, and those that would involve a change in *cis* interactions between receptors on the same cell (Fig. 3). The *trans* mode could include not only the specific inhibition of NCAM-NCAM homophilic interaction (Hoffman and Edelman, 1983), but also, if overall membrane apposition is affected, the interaction of other receptors as well (Rutishauser et al., 1988). The *cis* mode is more complex, in that it could reflect changes in an adhesion-promoting interaction of NCAM with other receptors on the same cell (Kadmon et al., 1990), an indirect augmentation of interactions as a result of intracellular signalling, or clustering of NCAMs within the plane of the membrane (Singer, 1992).

At present, there is too little information to choose definitively among these possibilities. The importance of *cis* interactions among membrane components has been established in a variety of systems, and the presence of the bulky and charged PSA moiety could easily disrupt binding of proteins to NCAM within the plane of the cell membrane. Some studies argue against mechanisms involving intracellular signalling or specific *cis* interactions between NCAM and other receptors. For example, PSA can alter the adhesion of purified membranes vesicles (Rutishauser et al., 1985), and of reconstituted bilayers containing only lipid

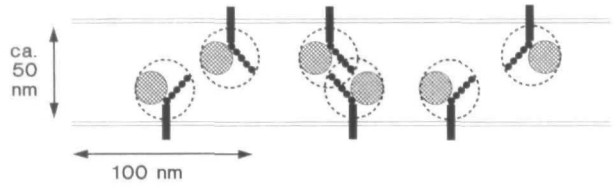

Fig. 4. The density and excluded volume of polysialylated NCAM are sufficient to affect overall membrane-membrane contact. This figure is a scale representation of the interaction of two cell membranes, each bearing polysialylated NCAM at the density measured on live cells (Yang et al., 1992). The size of the NCAM polypeptide was estimated from electron micrographs and the increase in radius of hydration of the polysialylated molecule. The dashed circle around each NCAM represents the ability of the molecule to rotate rapidly in the plane of the membrane.

and NCAM (Hoffman and Edelman, 1983). These findings, however, do not exclude the possibility that *cis* interactions among NCAMs might be a requisite for both NCAM-mediated adhesion between membrane vesicles, and a consequent signalling to other receptors in live cells (Fig. 3, bottom right). Evidence for cooperativity among NCAMs has in fact been observed with both membrane vesicles and cells transfected with NCAM cDNA (Hoffman and Edelman, 1983; Doherty et al., 1990b).

The *trans* mechanism, in which PSA impedes cell-cell contact, has two critical predictions: that enough space is occupied by PSA to affect overall membrane-membrane apposition, and that intercellular space is actually changed upon removal of PSA. Recently, evidence to this effect has been obtained (Yang et al., 1992). The abundance of NCAM in the membrane (about 1 molecule per 100 × 100 nm patch of bilayer) and the large hydrated volume of PSA (over ten times that of an equivalent mass of protein) is compatible with overall steric effects (Fig. 4). Furthermore, the distance between apposed cell membranes upon specific enzymatic removal of PSA decreases by about 25%. Such a decrease in space is equivalent to about two immunoglobulin domains and would be expected to have a substantial effect on interaction between such domains on apposing cells.

The potential value of regulation at the level of overall membrane-membrane contact is that changes in the closeness, extent, or duration of apposition can be a basis for selection between different interactions. Some receptors may be too small or too large to work effectively at a particular membrane-membrane distance; others may be both big and flexible enough to minimize the effects of this variable. Similarly, the surface area and stability of contacts can be factors depending on the number of molecular bonds required, or the speed of effective interaction. Although the model is easier to illustrate schematically as a complete disengagement in the presence of PSA (Fig. 3), much of its attractiveness lies in the ability to affect interactions partially as well as selectively. In this manner, a cell could choose advantageous interactions (or avoid deleterious ones), and the ability of PSA to promote axonal elongation on cellular substrata may well reflect this capacity (Landmesser et al., 1990; Doherty et al., 1990a).

PSA as an in vivo regulator

Whatever the exact mechanism underlying PSA's effect on cell interactions, there is substantial evidence that this type of regulation is an important developmental parameter in living embryos. PSA is highly regulated during development, with patterns quite distinct to those of NCAM itself (except of course that PSA expression requires NCAM expression). The original documentation of naturally occurring variation in NCAM sialylation focussed on the state of the molecule isolated from the brains of embryonic and adult tissues (Chuong and Edelman, 1984). This pattern of temporal regulation was found in other tissues as well, leading to the description of heavily sialylated NCAM as the embryonic form and the less sialylated molecule as the adult form. Additional studies, however, have revealed that low PSA NCAM also predominates very early in development (Sunshine et al., 1987), that the level of PSA is regulated independently in many tissues (Rougon et al., 1982; Chuong and Edelman, 1984; Boisseau et al., 1991) and can vary even within the same cell (Schlosshauer et al., 1984), and that high PSA NCAM persists in some adult tissues such as the olfactory system (Chuong and Edelman, 1984) and hypothalamus (Theodosis et al., 1991). It should also be mentioned that although PSA is most thoroughly studied in neural tissues, it is also abundant and regulated in many non-neural structures, including skeletal muscle, heart, kidney, and mesenchymal tissue.

To date, almost all functional characterization of PSA has been with neurons. As discussed above and illustrated in Fig. 1, L1 and NCAM appear to compete with each other to produce patterns of motorneuron innervation of muscle (Landmesser et al., 1988). Nevertheless, in the naturally occurring variations in pattern (age-related changes, fast versus slow fibers), neither of these molecules appears to be regulated in its expression. Instead, it is the PSA content of NCAM on axons that is developmentally correlated with in vivo patterns, and removal of this carbohydrate with a PSA-specific endoneuraminidase produces a predictable change in these patterns (Landmesser et al., 1990). Moreover, these predictions center on modulation of L1's function, not NCAM's, which as described above is consistent with an overall effect of PSA on membrane apposition.

This analysis of PSA regulation in vivo has also revealed an unexpected influence of the carbohydrate on synaptic activity-dependent sprouting and branching of motor neurons (Landmesser et al., 1990). Broadly stated, it appears that PSA can serve as a molecular link between synaptic activity and changes in the pattern of axonal innervation. The experimental system used was again innervation of the chick limb, and combined the analysis of L1, NCAM and PSA with blockade of synaptic activity by the neurotoxin curare. As in normal development, neither NCAM nor L1 expression is correlated with the increased sprouting and branching produced by curare, whereas PSA is dramatically increased on axonal surfaces of curare-treated animals. A causal relationship between activity, PSA and innervation pattern (Fig. 5) is supported by the observation in vivo that the morphological effects of curare on innervation are reversed by co-administration of an endoneuraminidase that specifically destroys PSA.

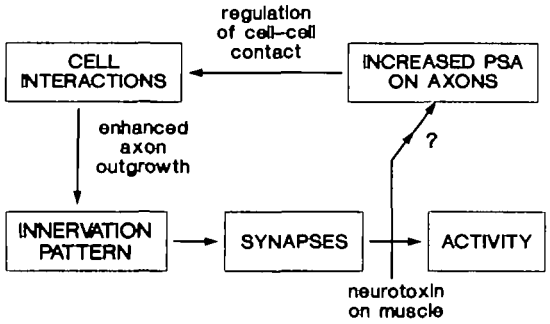

Fig. 5. Schematic representing the functional relationship between the molecular, cellular and physiological parameters that contribute to synaptic activity-dependent remodelling of muscle innervation. In vivo studies (Landmesser et al., 1990) have revealed that enhanced PSA expression after blockade of synapses leads directly to alteration of cell interactions involved in this remodelling.

Conclusion

It is now clear that NCAM did not evolve to serve a specific recognition function, such as axon guidance or synaptogenesis. Instead the molecule appears to part of the machinery that mediates and/or regulates a fundamental cellular property, the ability to form close contacts with other cells. As with other basic cell behaviors, such as mitosis, migration and differentiation, this parameter is incorporated into a myriad of physiological contexts both within and outside the nervous system. NCAM function must therefore be closely integrated into cellular biochemistry through which it is coordinated with other major cell processes. Such coordination can be achieved through a number of mechanisms involving second messengers, postranslational modification and cytoskeletal elements as well as gene transcription.

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