Taniguchi Symposia on Brain Sciences No 9

MOLECULAR GENETICS IN DEVELOPMENTAL NEUROBIOLOGY

Edited by
Yasuzo Tsukada

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PREFACE

Molecular genetics in neurobiology is a very young research field and is developing rapidly with the introduction of the new and productive methodologies of genetic engineering and cell manipulation. Particularly in the field of developmental neurobiology, molecular genetics is having a great impact in research on the molecular mechanism of development and differentiation in the nervous system. It is also anticipated that a molecular genomic understanding of inherited neurological diseases will contribute greatly to future human welfare.

To advance the frontier in this field, a symposium entitled "Molecular Genetics in Developmental Neurobiology" was held in Kyoto/Katata Japan, November 10–14, 1985 with 10 foreign and 10 Japanese scientists participating. It took place under the auspices of the Taniguchi Foundation which was established for the dual purpose of promoting research in the basic sciences and promoting mutual understanding and friendship among young scientists internationally.

This volume is composed of 20 articles presented in 4 parts: cell recognition, embryo and gene manipulation, gene analysis and manipulation, and neural regeneration. The authors have reviewed and interpreted their

most recent results. It is hoped that the reader will discover on these pages new concepts and ideas in the molecular approach to neurobiology.

Publication was made possible through the cooperation of the participants and the sponsoring organizations, the Taniguchi Foundation and the Ministry of Education, Science and Culture of Japan.

I wish to express my sincere appreciation to all members of the organizing and executive committees and to the participants, and also to express my deep gratitude to the sponsoring organizations for promoting and sponsoring both the symposium and this publication.

June 1986

Y. Tsukada

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STRUCTURE AND MOLECULAR BIOLOGY OF CELL ADHESION MOLECULES

JOHN J. HEMPERLY, GEOFFREY C. OWENS, AND BRUCE A. CUNNINGHAM

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Interactions among neurons and between neurons and glia are the fundamental bases for the formation and function of the nervous system. Two cell-adhesion molecules (CAMs) (*11*), the neural cell adhesion molecule N-CAM (*44*) and the neuron-glia cell adhesion molecule Ng-CAM (*24*), have been shown to play crucial roles in these interactions (*13*). In addition, a third cell adhesion molecule, L-CAM (*20*), which is not found in the mature nervous system, appears with N-CAM in early embryos and probably has a major influence on early neural morphogenesis.

N-CAM and L-CAM are designated primary CAMs because they appear on embryonic cells and on derivatives of all three germ layers (*5*). Throughout development they are expressed in dynamic patterns, particularly at sites of induction, suggesting that their expression is a crucial event in both morphogenesis and histogenesis (*12*). Ng-CAM, in contrast, appears relatively late in development and only on derivatives of the neural ectoderm (post-mitotic neurons and Schwann cells) (*45*). It is expressed most intensely on fiber tracts and is apparently important in neuronal migration.

All three CAMs were isolated by variations of the same general pro-

cedure. Antibodies against whole cells or membranes were used to inhibit short-term cell-cell adhesion *in vitro*, and the CAMs were detected during purification by their ability to neutralize the activity of the antibodies (*2*). Monoclonal antibodies, prepared against the partially purified material, were then used to affinity purify the CAM, which was then used for structural analyses and to raise polyclonal antibodies for biological studies.

Although our studies have focused on CAMs from embryonic chickens, all of the molecules have counterparts in other species. N-CAM has been detected in humans (*34*), rats (*4*, *33*), mice (*4*, *28*), and frogs (*17*), as well as in sharks, salamanders, newts, turtles, snakes, and lizards (*29*). Rodent Ng-CAM has recently been found (*19*) to be identical to the NILE protein (*35*, *43*) and is also closely similar to the L1 antigen (*38*). Ng-CAM expression, like NILE expression, is enhanced in PC12 rat pheochromocytoma cells by nerve growth factor (*19*). L-CAM shares nearly all of its features with a molecule associated with the compaction of mouse blastomeres and the aggregation of teratocarcinoma cells (*32*, *48*) and with an adhesion molecule from human carcinoma cells (*9*).

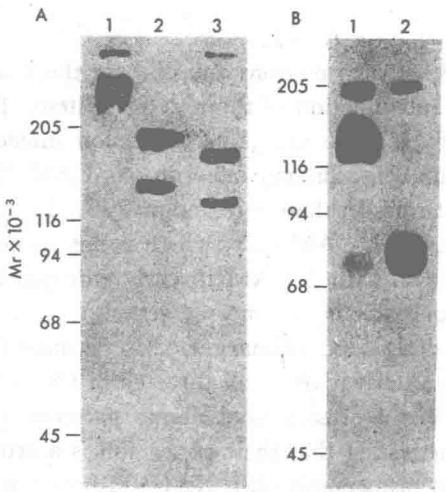

Fig. 1. Polypeptide components of N-CAM and Ng-CAM. A: electrophoresis of N-CAM (lane 1), neuraminidase-treated N-CAM (lane 2), and endoglycosidase F-treated N-CAM (lane 3) from embryonic chicken brain on polyacrylamide gels containing SDS. B: electrophoresis of immunoaffinity purified Ng-CAM detected by staining with Coomassie brilliant blue (lane 1) or of ^{32}P-labeled Ng-CAM detected by autoradiography (lane 2). The migration of protein molecular weight standards (in Kd) is shown on the left of each panel.

All three CAMs are large cell surface glycoproteins and, as such, share a number of structural features. They are distinct chemical species, however, in accord with their different activities, cellular distributions, and antigenic properties. To identify features that might regulate CAM expression or activity, we have identified chemical features of each molecule and have produced cDNA probes for determining their amino acid sequences and describing the regulation of their expression.

I. N-CAM

N-CAM from embryonic chicken brain migrates on SDS-polyacrylamide gel electrophoresis (SDS-PAGE) as a diffuse zone (M_r=150,000 to 250,000) (Fig. 1A) (*31*) due to large amounts of sialic acid present in an unusual polymeric form (*16, 31*). Treatment of N-CAM with neuraminidase produces two glycoproteins with M_r=140,000 and 170,000, while treatment with endoglycosidase F to remove all Asn-linked oligosaccharides gives two polypeptides of M_r=130,000 and 160,000. The two polypeptides are very similar to each other, having the same amino acid sequences for at least the first 14 residues at the amino terminus and giving similar peptide maps (*7*).

Our working model of N-CAM is shown in Fig. 2A (*7*). The model is based on the characterization of two large, independently derived fragments. One fragment (Fr 1, M_r=65,000) was spontaneously produced by protease activity that is either intrinsic to N-CAM or is a trace contaminant in N-CAM preparations (*31*). The amino terminal sequence of the fragment is identical with that of each of the intact polypeptides (*7*), and antibodies that recognize this fragment recognize both N-CAM polypeptides; it also appears to include the N-CAM binding region and one Asn-linked oligosaccharide, but lacks the bulk of the sialic acid. The second fragment (Fr 2, M_r=108,000) was generated by treating membranes with V8 protease. This fragment includes all of fragment Fr 1 plus the bulk of the sialic acid. It reacted with an additional monoclonal antibody that recognizes both N-CAM polypeptides and is directed against peptide determinants near the sites where polysialic acid is attached. These and other data suggest that the smaller component differs from the larger in the remaining carboxyl terminal third of the polypeptide, either lacking a terminal segment or having a deletion in this area.

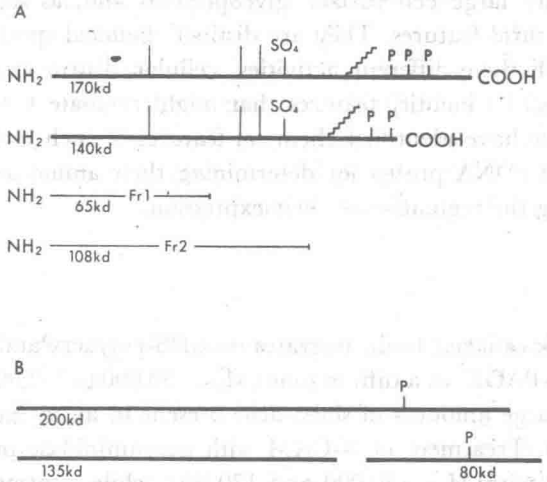

Fig. 2. Schematic representations of N-CAM and Ng-CAM. A: the top two horizontal lines represent the two glycopeptides (M_r=140,000 and 170,000) obtained after neuraminidase treatment of N-CAM. Underneath these lines are indicated the relative positions of fragments Fr 1 and Fr 2. Vertical lines denote Asn-linked oligosaccharides. At least one of the oligosaccharides in the central region contains polysialic acid. The stairstep symbol denotes covalently bound fatty acid, P indicates phosphate moieties on phosphothreonine and phosphoserine residues, and SO_4 indicates carbohydrate-linked sulfate. B: the upper horizontal line represents the larger 200 Kd polypeptide of Ng-CAM. The lower lines represent the 135 Kd and 80 Kd polypeptides presumably derived from the 200 Kd species by proteolytic cleavage. P indicates covalently attached phosphate groups.

A number of results suggest that N-CAM is an integral membrane protein. Because Fr 2 apparently does not contain the region of difference, the polypeptides probably differ near the sites where they penetrate the bilayer or in a cytoplasmic domain. This view is supported by analyses of the phosphorylation of the molecule (*22*, *42*). Both N-CAM polypeptides were phosphorylated on serine and threonine when brain tissue was cultured with $^{32}PO_4$, but the ratio of the phosphoamino acids differed significantly between the two. When membranes from these cells were treated with V8 protease (*42*), a number of components were released from the membranes including fragment Fr 2 but none were labeled with radioactive phosphate, indicating that the $^{32}PO_4$ was incorporated into the carboxyl terminal portion of the molecule. Peptide maps revealed ^{32}P-labeled peptides unique to each polypeptide indicating that different sites may be phosphorylated in each (B.C. Sorkin *et al.*, unpublished results).

Because transmembrane proteins are often phosphorylated on cytoplasmic domains, these results support the notion that the two N-CAM polypeptides span the membrane and may differ in the size of their cytoplasmic domains or in the number of times they span the lipid bilayer.

Preliminary results indicate that the membrane-associated region of N-CAM can also be acylated (B.C. Sorkin *et al.*, unpublished results). Cultures of embryonic brain tissue incorporated ^{3}H-palmitate into both polypeptides of N-CAM, and the label was not released by endoglycosidase F. Treatment of membranes with V8 protease released no palmitate, indicating that the fatty acid is in the same general area as the phosphoamino acids. Current data suggests that such fatty acid acylation helps anchor proteins in the lipid bilayer (*41*).

The most distinctive feature of N-CAM is its high content of sialic acid (30 g/100 g protein) (*31*). Most of this negatively charged sugar is present in α 2–8 linked polymers at least five residues long (*16*). The sialic acid does not appear to be within the N-CAM binding region (*7*), but its removal enhances the rate of N-CAM binding 4-fold (*30*). Whether this effect is steric, ionic, or conformational is as yet unknown.

All of the sialic acid can be released by endoglycosidase F (*6*) and no sialic acid is added to N-CAM synthesized in the presence of tunicamycin (*7*), indicating that it is attached to Asn-linked oligosaccharides. Essentially all of it is contained in the central region of the polypeptide (*6*, *7*), which can be isolated from N-CAM as a CNBr fragment with three Asn-linked oligosaccharides and a polypeptide portion of M_r=35,000. When isolated from embryonic tissue, this component migrates on SDS-PAGE as a broad zone of M_r=42,000 to 60,000 that stained blue with Stains-all, reflecting the fact that it contains the bulk of sialic acid.

In addition to the negatively charged sialic acid residues, N-CAM can be sulfated when brain tissue is incubated with $^{35}SO_4$ (*42*). The ^{35}S-label is removed by endoglycosidase F, but not by neuraminidase, indicating that it is on Asn-linked oligosaccharides but not on sialic acid.

Of the many posttranslational modifications of N-CAM, the sialic acid appears to have the most direct effect on activity. N-CAM from adult tissue appears as two discrete components that resemble, on SDS-PAGE, embryonic N-CAM that has been treated with neuraminidase (Fig. 1A, lane 2). The conversion of N-CAM from embryonic (E) to adult (A) forms proceeds throughout development and at different rates and to dif-