Rapid Axonal Transport of the Neural Cell Adhesion Molecule

Judy A. Garner, Michiko Watanabe, and Urs Rutishauser

Department of Developmental Genetics and Anatomy, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106

The neural cell adhesion molecule (NCAM) is a cell-surface glycoprotein that mediates cell-cell interactions in the nervous system during development. In the present study, we demonstrate that NCAM is axonally transported in 3-d-old chick retinal ganglion cells and that it travels within the fast component of axonal transport (FC). Proteins were radiolabeled in retinal ganglion cell bodies after intraocular injection of ^{35}S -methionine. The presence of radiolabeled NCAM in the optic nerves and contralateral tectum was detected by specific immunoadsorption to a monoclonal antibody. Major radioactive polypeptide bands at relative mobilities of approximately 200,000, 150,000, and 120,000 Mr (after SDS-PAGE) were recognized by the anti-NCAM antibody. These bands comigrated in 1-dimensional gels with components of purified NCAM from chick brain. The 2 largest NCAM polypeptides (at 200,000 and 150,000 Mr) were found to be transported in this system, while the 120,000 Mr form was apparently not transported. The ratio and electrophoretic profiles of the 2 transported forms of NCAM remained similar in the retina, optic nerve, chiasm, tract, and tectum, suggesting that there is no interconversion of the 2 major polypeptides. The fraction of NCAM in the ^{35}S -labeled FC proteins appears to be at least an order of magnitude less than in the plasma membrane, suggesting that the turnover rate of NCAM at this age is slower than for other membrane proteins of the CNS.

The neural cell adhesion molecule (NCAM), a cell-surface glycoprotein, mediates cell-cell adhesion during development of nerve tissue (for review, see Rutishauser, 1983). NCAM plays an important role during neurite fasciculation (Rutishauser et al., 1978a), in axon-muscle interaction (Rutishauser et al., 1983), and in the association of nerve growth cones with glial precursors (Silver and Rutishauser, 1984).

In the present study, we have examined the axonal transport characteristics of the neural cell adhesion molecule. Since there is no protein synthetic machinery within the axon, the molecule must be supplied to the neurite and its terminal by transport within 1 of the 3 major axonal transport rate-components. Each of the 3 major rate-components is thought to convey a cytoplasmic structure within the axon: the fast component (FC) (moving at approximately 250 mm/d) provides membranes and membrane proteins, while the 2 slow components (moving at 0.2 and 2 mm/d) convey cytoplasmic matrix and cytoskeleton, respectively (Lasek, 1980; Lasek et al., 1984).

NCAM is an integral membrane protein (Cunningham et al., 1983; Gennarini et al., 1984a, b) with a largely uniform distribution on the growth cone, axon shaft, and cell soma (Rutishauser et al., 1978b). It was therefore predicted that NCAM would be transported within the FC of axonal transport.

Although the FC of axonal transport has been the subject of study for a number of years, only a few rapidly transported proteins have been well characterized: notably the $\text{Na}^+\text{-K}^+$ ATPase (Baittinger and Willard, 1981; Specht and Sweadner, 1984), the growth-associated proteins (Skene and Willard, 1981), and several transmitter-related enzymes (for review, see Grafstein and Forman, 1980). FC proteins are usually studied by radiolabelling them during synthesis in the cell body, then allowing them to be transported down the axons. Specific proteins have thus far been difficult to isolate or study using this method alone because the FC group contains a large number of membrane-associated glycoproteins of unknown function, each of which represents but a small fraction of the total FC (Garner and Mahler, 1982; Lorenz and Willard, 1978). In the present work, we have combined the classical radiolabeling approach with monoclonal antibody techniques to identify and characterize NCAM in the FC and to compare neuronal, axonally transported NCAM with that synthesized by more heterogeneous populations of nervous system cells (e.g., retina, whole brain).

Materials and Methods

Injection of radiolabeled precursors

^{35}S -Methionine (New England Nuclear) was lyophilized to dryness and resuspended in glass-distilled water to a concentration of 50 mCi/ μl . Three days after hatching, white Leghorn chicks (*Gallus gallus*) were anesthetized with ether, and radiolabeled concentrate (10 μl) was injected into the right eye of each animal using a 26 gauge needle attached to a Hamilton repeating dispenser (Brady and Lasek, 1982).

Harvest and dissection of chicks

Chicks were anesthetized with ether at specified times after injection and sacrificed by decapitation. Optic tectum contralateral and ipsilateral to the injected eye, and/or optic nerves, chiasm, and tracts were rapidly excised. For experiments in which the delivery of radioactive FC to the terminal regions was monitored, control (ipsilateral) and experimental (contralateral) optic tecta were excised at injection-sacrifice intervals of 2, 6, 12, 24, 48, 72, and 96 hr and homogenized in isotonic saline. Either 7 or 8 chicks were used at each time point. Equal aliquots were either directly counted in an LKB 1211 Rackbeta liquid scintillation counter or were placed on Whatman filters for trichloroacetic acid precipitation of protein at 4°C for 1 hr. The filters were washed with ethanol and ether, dried, and counted as above. The quantity of FC radioactivity in the terminals due to axonal transport was considered to be the difference between the total quantity of radioactivity in the contralateral (experimental) and the ipsilateral (control) tecta (or $E - C$). Since several different batches of isotope were used, data were normalized essentially as described in McEwen and Grafstein (1968). The data are presented as experimental minus control values for total radioactivity per tectum, divided by the control value [($E - C$)/C].

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Correspondence should be addressed to Dr. Judy A. Garner, Department of Anatomy and Cell Biology, USC School of Medicine, 1333 San Pablo St., Los Angeles, CA 90033.

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Figure 1. Axonally transported polypeptides of the contralateral (experimental) tectum were immunoadsorbed by a monoclonal antibody to NCAM. Lane 1 is the Coomassie Brilliant Blue-stained electrophoretic profile of affinity-purified standard chick NCAM from optic tecta (CBB). (There is relatively little of the 120,000 Mr NCAM polypeptide in NCAM immunoadsorbed from optic tecta compared to other brain areas, so the 120,000 Mr band does not reproduce well photographically.) Lanes 2 and 6 are radiolabeled species immunoadsorbed from the control tecta ipsilateral to the injected eye at 2 and 16 hr after injection, respectively. Lanes 4 and 8 are the radiolabeled species immunoadsorbed in the experimental tecta contralateral to the injected eye after 2 and 16 hr, respectively. The remaining lanes are controls for non-specific binding of radiolabeled polypeptides to ethanolamine-derivatized beads; lanes 3 and 7 are controls for the ipsilateral tecta, and lanes 5 and 9 are controls for the contralateral tecta. Large arrows indicate relative mobilities of major immunoadsorbed polypeptides in thousands of daltons; small arrows indicate less abundant polypeptides immunoadsorbed by anti-NCAM (7.5% polyacrylamide gel; for molecular-weight standards, refer to Fig. 3).

Immunoadsorption of NCAM

In initial experiments testing whether NCAM is axonally transported, optic tecta of 3 d hatched chicks were excised at 2 or 16 hr after intraocular injection of radiolabeled precursor and subjected to immunoadsorption immediately after excision. To determine whether radiolabeled, axonally transported NCAM was present in the axon and terminal regions of retinal ganglion cells, optic nerve, chiasm, and tectum were subjected to the same immunoadsorption procedure either immediately after excision or after storage at -80°C . In experiments in which axonally transported NCAM was compared with NCAM synthesized by whole retina, retinæ from experimental and control eyes were removed and prepared in the same way as other visual system tissues.

The monoclonal antibody chosen for this study specifically recognizes the polypeptide portion of the NCAM molecule (Thanos et al., 1984). Monoclonal antibodies were covalently attached to activated agarose beads (Affigel 10, Biorad) according to manufacturer's instructions (2 mg anti-NCAM/ml beads in PBS, pH 7.4). Fresh or thawed tissues were either sonicated or homogenized (12 strokes, glass-Teflon homogenizer) in 0.5–2.0 ml ice-cold NP-40 buffer (0.5% Nonidet P-40, 0.8% NaCl, 0.14% Na_2HPO_4 , 200 U/ml Aprotinin, pH 7.4), and undissolved material removed by centrifugation at $100,000 \times g$ for 10 min. Sample supernatants were incubated with the antibody-derivatized beads at 4°C for 2–12 hr with end-over-end agitation. The beads were washed 5 times with NP-40 buffer, and the radiolabeled antigen released from the derivatized beads for subsequent SDS-PAGE and fluorography as described below.

Two types of controls were used. First, competition experiments were performed in which derivatized beads were preincubated with purified NCAM (50 $\mu\text{g}/50 \mu\text{l}$ beads at 37°C for 5 min prior to addition of radiolabeled tectal homogenate. As a control for nonspecific binding of

radiolabeled polypeptides to the agarose itself, beads that had not been derivatized with antibody and whose active sites had been blocked by treatment with 1 M ethanolamine hydrochloride were incubated with radiolabeled samples. In a separate set of control experiments, beads derivatized with monoclonal IgG directed against *Drosophila* yolk protein were found to be incapable of binding any of the NCAM polypeptides (not shown).

SDS-PAGE and fluorography

Radiolabeled antigen bound to antibody-derivatized beads was released by addition of BUST (2% SDS, 5% 2-mercaptoethanol, 8.0 M urea, 100 mM Tris, pH 6.8) at room temperature for at least 30 min. FC samples (containing the entire spectrum of FC proteins for comparison purposes) were prepared by homogenizing tissues containing radioactive FC in isotonic saline followed by a 1:1 dilution with BUST. Proteins from the samples were separated by electrophoresis on 7.5 or 10% SDS-polyacrylamide gels using a modification of the method of Laemmli (1970) in which the ratio of acrylamide to bis-acrylamide was 100:1 and the running buffer was 0.1% SDS, 50 mM Tris, and 0.38 M glycine, pH 8.3. Radioactivity in the gels was visualized by fluorography (Bonner and Laskey, 1974; Laskey and Mills, 1975). X-ray film (X-AR5 Kodak) was exposed by the fluor-impregnated gels for 1–3 weeks at -80°C .

Two-dimensional gel electrophoresis was performed essentially as described by O'Farrell (1975) and modified by Tytell et al. (1981). Isoelectric focusing gels (IEF) were prepared using a 4:1 mixture of pH 5–7 and pH 3–10 ampholines (LKB). IEF gels were run in the second dimension according to the modified Laemmli procedure described above, using 4–17.5% gradient polyacrylamide gels.

Quantification of radioactivity in polypeptide bands immunoadsorbed by the anti-NCAM antibody was performed as described by

Figure 2. *A*, Total acid precipitable radioactivity entering the control and experimental tecta was normalized as described in the Materials and Methods (experimental – control/control) at varying times after intraocular injection. SDS-PAGE revealed that the first peak (between 6 and 48 hr) contained proteins characteristic of the FC, while new proteins entering in the second wave appear to be slow component proteins (not shown). Eight animals were averaged for each time point, and vertical lines represent 1 SD from the mean. *B*, Amount of radioactivity immunoadsorbed from the 2 major NCAM polypeptides in control (ipsilateral, open circles) and experimental (contralateral, closed circles) tecta at varying times after intraocular injection. Data from 4 experiments at each time point were compared. Vertical lines represent 1 SD from the mean. The 96 hr time point (in parentheses) was estimated from 2 experiments.

Garner and Lasek (1981). Bands were localized in dried gels by overlaying with exposed fluorographs and marking the extent of the band with pins. Bands were excised, dissolved in H_2O_2 at 60°C overnight, and counted in a liquid scintillation counter.

Results

Axonal transport of NCAM

The first series of experiments tested whether chick NCAM is axonally transported from retinal ganglion cell bodies to neuron terminal regions in the optic tecta. The great majority of retinal ganglion cell axons in chicks of this age cross at the optic chiasm to terminate in the contralateral optic tectum (O'Leary et al., 1983). Therefore, the ipsilateral optic tectum serves as a control for uptake of blood-borne radiolabeled precursors and thus local synthesis of radioactive protein.

In these experiments, ^{35}S -methionine was injected in the right eyes of 3 d hatched chicks, and the chicks sacrificed after either 2 or 16 hr. Anti-NCAM-derivatized beads were used to immunoadsorb radiolabeled NCAM from both ipsilateral (control) and contralateral (experimental) optic tecta. The specifically bound radiolabeled species were subsequently visualized by fluorography of SDS gels (Fig. 1). The anti-NCAM antibody

recognized 3 major polypeptide species with relative mobilities in SDS gels corresponding to apparent molecular weights of 200,000, 150,000, and 120,000 Mr at both times (Fig. 1, lanes 4 and 8). The electrophoretic mobilities of these radiolabeled bands were identical to those of the 3 major polypeptide bands of purified chick brain NCAM (Fig. 1, lane 1), and correspond to the 180,000, 140,000, and 120,000 Mr polypeptides described in previous reports (mouse: Hirn et al., 1983; chicken: Chuong and Edelman, 1984). The identity of the 3 major polypeptides as NCAM was confirmed by the inhibition of their adsorption to anti-NCAM-derivatized beads by preincubation of the beads with purified chick brain NCAM (not shown).

There was significantly more radiolabeled material immunoadsorbed by the anti-NCAM antibody from the contralateral tecta (Fig. 1, lane 4) than in the ipsilateral control (Fig. 1, lane 2), as early as 2 hr after intraocular injection of precursor. By 16 hr, the amount of radiolabeled polypeptide recognized by the anti-NCAM antibody had increased substantially (Fig. 1, lane 8). Quantitation of the radioactivity in the individual polypeptide bands revealed that the ratio of radiolabel in the 200,000 and 150,000 Mr polypeptides was approximately 2:1 at both times. Between 2 and 16 hr, there was only a slight increase in the amount of radiolabeled material immunoadsorbed from the control, ipsilateral tecta (Fig. 1, lane 6). The conclusion drawn from these experiments was that at least the 2 major NCAM polypeptides are, in fact, axonally transported in this system.

The radiolabeled 120,000 Mr polypeptide behaved unlike the other 2 major immunoadsorbed polypeptides. The amount of radioactivity in the 120,000 Mr band was approximately the same in both the experimental (Fig. 1, lanes 4 and 8) and control (Fig. 1, lanes 2 and 6) tecta. This observation suggested that the amount of radiolabeled 120,000 Mr polypeptide found in each tectal homogenate resulted from utilization of blood-borne radiolabel during local protein synthesis in the tectum rather than due to axonal transport.

In addition to the major polypeptides recognized by the anti-NCAM antibody, there were several minor radiolabeled bands visible in the SDS-PAGE profile of immunoadsorbed material (most easily seen in Fig. 1, lane 8, small arrows). Whether these species are breakdown products of the major species or are distinct members of the NCAM family of proteins was not tested.

Nonspecific binding of radioactive proteins to ethanolamine-blocked beads was minimal, as seen in lanes 3, 5, 7, and 9 of Figure 1.

Time course of arrival of NCAM in terminal regions

The first experiments confirmed that NCAM was axonally transported and suggested that the transport occurred within the FC. In order to delineate more clearly the time course of arrival of the NCAM polypeptides in the terminal regions, we immunoadsorbed NCAM from experimental and control tecta at 2, 6, 12, 24, 48, 72, and 96 hr after intraocular injection of precursor and compared its arrival with that of the bulk of FC proteins.

Figure 2*A* presents the normalized acid precipitable FC radioactivity in control and experimental tecta over the same period of time. The FC radioactivity peaked early (at 6 hr) and remained high for at least 24 hr. By 48 hr, a new and larger surge of radioactivity started to enter the terminal regions, and it continued to increase over the length of time examined. SDS-PAGE analysis of the second peak of radioactive material entering the terminal regions revealed that the new peak represented the onset of entry of slow component b proteins (J. A. Garner, unpublished observations). It should be emphasized, however, that throughout the entire period of time examined, all the major FC proteins persisted in the terminal regions.

Figure 2*B* compares the sum of radioactivity in the 2 major

Figure 3. NCAM comigrates with relatively minor amounts of FC radioactivity in SDS-PAGE fluorographic profiles. *A*, One-dimensional radiolabeled polypeptide profiles of FC proteins in experimental optic tecta (2 and 16 hr after intraocular injection) were compared with the radiolabeled proteins immunoadsorbed from the optic tecta by anti-NCAM at 16 hr (NCAM). The arrows indicate the major NCAM polypeptides separated on a 7.5% polyacrylamide gel. Molecular-weight standards are indicated to the left of the 2 hr profile: 210,000, 96,000, 68,000, 43,000, 31,000, and 21,000 Mr. *B*, Radiolabeled FC proteins from optic tecta were separated on 2-dimensional gels and subjected to fluorography. The location of stained NCAM standard is indicated with brackets. Little radioactivity was localized to the NCAM region. Acidic and basic ends of the gel are as indicated (4–17.5% gradient SDS-PAGE).

immunoadsorbed NCAM bands from control and experimental tecta at each time. The time course of arrival and persistence of the major NCAM polypeptides was very similar to that of the FC: It appeared to peak early and was present over the entire 96 hr time period. The half-life of the NCAM polypeptides in the terminal regions was not determined in this study because the quantity of NCAM had not decreased sufficiently by the end of 96 hr to allow extrapolation for a half-life estimate.

Amount of NCAM in the FC

Once it was established that the major NCAM polypeptides were transported in the FC of axonal transport, the proportion of the ^{35}S -FC radioactivity that could be attributed to NCAM was determined. Comparison of 1-dimensional gel electrophoretic patterns of radiolabeled FC polypeptides with the radiolabeled NCAM polypeptides (Fig. 3*A*) revealed that the NCAM polypeptides comigrated with FC polypeptide bands containing only a small amount of radioactivity. Separation of FC proteins on 2-dimensional gels also demonstrated that only a relatively small amount of radioactivity comigrated with purified chicken brain NCAM (indicated in brackets). It was determined, after

quantification of the radioactivity from both 1- and 2-dimensional gels, that less than 0.05% of the total FC radioactivity was recovered during our immunoadsorption of the NCAM polypeptides from the tectum.

Forms of transported NCAM

Since there were several polypeptides immunoadsorbed by the anti-NCAM antibody in the terminal regions, we asked whether the NCAM species were transported in multiple forms or whether the different forms were generated once NCAM reached the terminal regions. Figure 4 shows the radiolabeled polypeptides immunoadsorbed from sequential 1 mm segments of the optic nerve, chiasm, and tract of the injected eye, as well as those from the retina and tectum at 5 hr after intraocular injection. The 2 higher-molecular-weight forms of radiolabeled, axonally transported NCAM appeared to be present throughout the optic nerve and tract. This suggested that the 2 major forms of NCAM were not generated in the terminal regions through some molecular interconversion, consistent with the hypothesis that NCAM is axonally transported as more than one species. The presence of the 2 higher-molecular-weight NCAM polypeptides

Figure 4. Radiolabeled polypeptides immunoadsorbed by anti-NCAM(pp) from retina (*Ret.*), tectum (*Tect.*), and sequential 1 mm segments of the optic nerve (*ON*), chiasm (*OC*), and tract (*OT*) at 5 hr after intraocular injection are compared. At 5 hr after injection, the peak of FC radioactivity was still within the visual system axons. The arrows at left indicate the major radiolabeled NCAM species (120,000, 150,000, and 200,000 Mr) detected in the retina. The arrows at right indicate the 2 higher-molecular-weight NCAM polypeptide bands that appear to be transported in the rest of the optic system. Extreme overexposure of the fluorographs reveals a small quantity of the 120,000 Mr species in the 1 mm segments of optic axons, most likely the result of local blood-borne labeling (not shown).

throughout the optic nerve and tract at the same time as the other FC proteins could also be considered further evidence that NCAM is axonally transported in the FC. Although the ratio of radioactivity between the 200,000 and 150,000 Mr forms of NCAM appears different in proximal nerve segments in Figure 4 from that seen in tectal samples in Figure 1, quantitation revealed no significant difference in ratio.

Comparison of axonally transported NCAM with retinal NCAM

Since axonally transported NCAM is purely neuronal in source, it was of interest to determine whether transported retinal ganglion cell NCAM exhibited any obvious biochemical differences from the NCAM synthesized by a more diverse population of retinal cells. Figure 5A displays the NCAM polypeptides immunoadsorbed from the radiolabeled retina at 2 and 16 hr after intraocular injection of precursor. The 3 major NCAM polypeptides were the most prominent radiolabeled bands immunoadsorbed to the beads from retinal homogenates. Most low-molecular-weight minor bands appeared to be a result of nonspecific binding (see controls as described in the figure legend).

Figure 5B presents a comparison between the NCAM immunoadsorbed from the injected retina and contralateral tectum (both 16 hr after intraocular injection). While the relative proportion of 200,000 to 150,000 Mr NCAM species was approximately the same in retina, optic axons, and tectum, the proportion of radioactivity in the 120,000 Mr species was much higher in the retinal samples than in either the optic axons (Fig. 4) or the experimental tectum (Figs. 1 and 5B).

There were several other, relatively minor, differences exhibited by the immunoadsorbed NCAMs from the 2 sources. The amount of very high molecular weight material recognized by the antibody was significantly greater in the retinal sample (brackets) than in the tectal sample. In addition, the distribution of radioactivity in the minor polypeptide bands recognized by the antibody varied in the samples from the 2 sources. In the tectal sample, there were 2 polypeptide bands (at 140,000 and 130,000) that did not appear in the retinal sample, and the relative proportion of the minor 180,000 band (when compared to amount of 200,000 or 150,000) was consistently different in the 2 samples.

Discussion

NCAM, a major integral membrane protein of neurons, has been identified in the FC of axonal transport in 3 d hatched chick retinal ganglion cells. Radiolabeled, immunoadsorbed NCAM that is axonally transported is synthesized not only by a single cell type, neurons, but is synthesized by 1 particular class of neurons, the retinal ganglion cells. Since the NCAM synthesized in whole tissue or cell culture is made by both neuronal and non-neuronal cells, this finding has made a comparison between neuronal and mixed-tissue NCAM possible.

The NCAM synthesized in whole tissue or cell culture is resolved as 3 major polypeptides at relative mobilities of 180,000, 140,000, and 120,000 Mr in SDS gels (Chuong and Edelman, 1984; Hirn et al., 1983), corresponding to the 200,000, 150,000, and 120,000 Mr polypeptides, respectively, in our study. Examination of the NCAM transported to the tectum reveals that though retinal ganglion cells transport the 200,000 and 150,000 Mr NCAM polypeptides, they do not appear to transport the 120,000 Mr form. One explanation for the selectivity might be that retinal ganglion cells do not synthesize the 120,000 Mr form at all; the 120,000 Mr form that is present in the preparation would be synthesized by other populations of neural cells. Characterization of NCAM synthesized by rat brain cultures (Noble et al., 1985) suggests that the 120,000 Mr form is synthesized by glial cells but not by neurons. It is also possible that retinal ganglion cells do synthesize all forms, but the 120,000 Mr form is either unstable or not rapidly transported. Alterations in the cytoplasmic domain of newly synthesized viral membrane glycoproteins delay or stop their intracellular transport to the cell surface (Doyle et al., 1985). It may be impossible for the 120,000 Mr NCAM polypeptide to be axonally transported for a similar reason: The 120,000 Mr form of NCAM is missing most, if not all, of the cytoplasmic domain present in the 150,000 and 200,000 Mr NCAM forms (Gennarini et al., 1984a, b).

There has been some question as to whether these major molecular weight forms are synthesized within the same cell or are uniquely produced by different cell types (Williams et al., 1985). The 2 major higher-molecular-weight forms share antigenic determinants and have very similar proteolytic digest patterns (Cunningham et al., 1983; Hoffman et al., 1982). There is evidence that all 3 forms may originate from 1 gene that produces different mRNAs (Goridis et al., 1985; Hansen et al., 1985; Murray et al., 1984). Although the retinal ganglion cell population is much more homogeneous than whole tissue, there are some morphological and physiological differences within the group: Synthesis of different forms of NCAM by different cell types has not been ruled out.

Although posttranslational cleavage of protein (Brown et al., 1982; Gainer and Sarne, 1977; Nixon et al., 1982) and glycosylation (Ambron and Treisman, 1977) occur during the axonal transport of other proteins, our studies provided no evidence for interconversion or modification of the 2 major NCAM polypeptides detected along the optic nerve, chiasm, or tract. Furthermore, the ratio of radioactivity in the 2 higher-molecular-weight NCAM forms in the tectum did not differ between samples harvested 2 or 16 hr after injection. Together, the evidence suggests that, like Na⁺-K⁺ ATPase (Specht and Sweadner, 1984), the NCAM polypeptides are relatively stable by the time they exit the cell body and travel towards the tectum. This is in agreement with the evidence from pulse-labeled neuronal cultures that 2 high-molecular-weight NCAM polypeptides forms do not interconvert (Lyles et al., 1984).

Although there is no apparent interconversion of the 2 major forms of axonally transported NCAM, the terminal regions of retinal ganglion cells do contain small quantities of minor radiolabeled polypeptide bands recognized by the anti-NCAM

Figure 5. *A*, Radiolabeled polypeptides immunoadsorbed by the anti-NCAM antibody from the whole chick retina at 2 and 16 hr after intraocular injection of radiolabeled precursors. Lanes 1 and 5 show radiolabeled polypeptides immunoadsorbed from the injected retina by anti-NCAM. Little radiolabeled material was specifically immunoadsorbed by anti-NCAM from the opposite noninjected, control retina (lanes 2 and 6). Lanes 3 and 7 show radiolabeled proteins from the injected retina that are non-specifically bound to ethanamine-derivatized agarose beads, while lanes 4 and 8 demonstrate radiolabeled, non-specifically bound proteins from the control retina. *B*, Profiles of immunoprecipitated NCAM species from the contralateral tectum (LT) and the injected retina (RR) are directly compared. High-molecular-weight species recognized from retinal samples but not tectal samples are indicated with brackets, while minor polypeptide species recognized by the antibody are indicated with arrows (7.5% polyacrylamide gel).

antibody that are not detectable in retina or axons. NCAM may be processed somewhat differently in the terminal regions than it is in the cell bodies or axons. It is not known whether this processing is simply protein degradation or a further specialization of the molecule for function in the terminal region.

It is theoretically possible that radiolabeled precursors are transported to the terminal regions where they (along with radiolabeled precursors released by FC protein degradation) are released from the terminal and reutilized in local cell synthesis of NCAM (Heacock and Agranoff, 1977). Two findings suggest that this is not the case. First, it would be expected that newly synthesized NCAM in the contralateral (experimental) tectum, resulting from delivery of transported precursors, would be incorporated into the individual NCAM polypeptides in proportions similar to those seen in the ipsilateral (control) tectum, resulting from local synthesis using blood-borne precursors. However, the amount of radioactivity in the 2 high-molecular-weight NCAM species relative to that in the 120,000 Mr form in the 2 tectal samples is significantly different. Second, there are significant levels of the 2 high-molecular-weight forms of NCAM in the 1 mm segments of optic nerve, chiasm, and tract. Synthesis of radiolabeled proteins by non-neuronal cells surrounding radiolabeled axons is considered to be negligible (Droz, 1969), and transported acid-soluble material is apparently not converted to protein while undergoing transport (Ochs et al., 1970), providing further support that the radiolabeled NCAM present in the samples is furnished by axonal transport.

Proportion of FC radioactivity in NCAM polypeptides

NCAM is an abundant membrane protein, representing about 1% by weight of embryonic brain membrane proteins (Hoffman et al., 1982). However, the relative proportion of radiolabeled NCAM in the entire labeled membrane fraction in FC of 3 d

hatched chicken retinal ganglion cells is very low (0.05%) compared to these values.

It is unlikely that this discrepancy arises from the differences in composition between the labeled FC membrane proteins (neuronal) and the total membrane fraction of brain (neuronal plus non-neuronal). Immunostaining studies indicate that, if anything, NCAM is enriched in neuronal membranes compared to non-neuronal membranes (Rutishauser, 1983). Another explanation might be that not all of the NCAM was recovered by immunoadsorption. To reduce the chance of this possibility, the incubations were done in antibody excess, and the procedure followed was much the same as that used to determine the percentage of NCAM in chick brain (Hoffman et al., 1982).

A third intriguing possibility is that the maintenance of high NCAM levels in the surface membrane in 3 d hatched chick may result from a lower turnover rate of the molecule relative to other proteins of the plasma membrane. Others have performed biochemical studies that suggest that the more "mature" forms of NCAM (such as those seen in 3 d hatched chick) turn over much more slowly than the forms seen earlier in development (Linneman et al., 1985). A low rate of degradation for NCAM might reflect unusually high stability of the molecule when it has been incorporated into cell-cell bonds. A direct comparison of NCAM turnover with that of other cell-surface proteins will be necessary to test this hypothesis.

Axoplasmic localization of the transported NCAM

Previous work, including electron-microscopic autoradiography (Hendrickson, 1972; Tessier et al., 1980) and physiological studies (Ellisman and Lindsey, 1983; Tsukita and Ishikawa, 1980), strongly suggests that the great majority of FC proteins are associated with membranes, membrane proteins, and membranous organelles located in the axoplasm rather than in associ-

ation with the plasma membrane. However, biochemical (DiGiamberardino et al., 1973; Lorenz and Willard, 1978; Specht and Swadner, 1984) and autoradiographic (Bennett et al., 1973; Schonbach et al., 1971) studies both link renewal of axonal plasma membranes and membrane proteins to the fast component. In addition, the supply of axonal membrane proteins from internal membrane stores has previously been shown to occur in developing neurites (Griffin et al., 1981; Pfenninger and Maylie-Pfenninger, 1981). As a result of such studies, newly synthesized plasma membrane proteins are thought to be transported internally in the axoplasm and inserted in the plasma membrane at the point at which they are needed.

The NCAM polypeptides have been described primarily as integral plasma membrane proteins, and details concerning regulation of their insertion into the plasma membrane have not been fully elucidated. In view of the present and previous studies, it is reasonable to suggest that the transported NCAM, like other membrane proteins, is inserted in intracellular membranes destined for transport, transported internally in the axon, and then intercalated into the plasma membrane at different regions of the distal axon. Since NCAM is believed to be involved in lateral associations among axon shafts (Rutishauser, 1983), as well as in interactions of the growth cones with the environment (Silver and Rutishauser, 1984), the mechanics and spatiotemporal regulation of its insertion in the axolemma are likely to be an important parameter in neurite outgrowth, guidance, and fasciculation.

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