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Isolation of cDNA clones for the chicken neural cell adhesion molecule (N-CAM)

(*in vitro* translation/recombinant DNA/brain development/molecular embryology)

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ABSTRACT **Enriched mRNA coding for the neural cell adhesion molecule (N-CAM) was prepared from 9-day embryonic chicken brains by immunoprecipitation of polysomes with antibodies to N-CAM. This mRNA programmed the translation *in vitro* of N-CAM polypeptide chains in a rabbit reticulocyte lysate system. Two independent N-CAM cDNA clones (designated pEC001 and pEC020) were derived from the enriched RNA. The specificity of pEC001 for N-CAM mRNA was verified by hybrid selection experiments. Both plasmids hybridized to two discrete 6- to 7-kilobase-long RNA species in poly(A)$^+$ RNA from embryonic chicken brain and to lesser amounts of polydisperse material of smaller sizes (probably degradation products of the large RNAs). No hybridization was detected to poly(A)$^+$ RNA from embryonic liver. Southern blotting experiments with pEC001 detected only one hybridizing fragment in chicken genomic DNA digested with several different restriction enzymes, suggesting that sequences corresponding to those within this region of N-CAM mRNA are present at most only a few times, and possibly only once, in the chicken genome.**

Specific cell–cell adhesion is likely to be of critical significance in embryonic development and in the maintenance of histological integrity (1, 2), and its abrogation may alter the behavior of neoplastic cells (3). Previous studies from this and other laboratories have identified several cell-surface molecules that appear to mediate cell–cell adhesion in vertebrates (1–20). One of the most extensively studied of these is the neural cell adhesion molecule (N-CAM). At least two structurally related N-CAM polypeptide chains with molecular weights of 130,000 and 160,000 have been identified (6).

N-CAM exhibits a dynamic pattern of spatial and temporal expression in neural and nonneural tissues during early embryogenesis and becomes widely distributed in the nervous systems of embryonic and adult vertebrates (7, 11–13). Native embryonic N-CAM is a highly glycosylated molecule containing a large amount of sialic acid in an unusual polysialic acid linkage (4, 21). The level of sialylation varies at a given time from one region of the brain to another and, in general, declines greatly during development (12–14, 16); changes in levels of sialylation are inversely correlated with changes in the rate of N-CAM to N-CAM binding and, hence, presumably with changes in cell–cell adhesion (9, 17). Furthermore, certain monoclonal antibodies distinguish between N-CAM molecules that carry comparable levels of sialic acid but are present in different regions of the brain (13). It is not known to what extent these modulations of N-CAM expression and structure reflect changes in the expression of N-CAM genes.

Recombinant DNA technology provides an approach to analyzing the structure and expression of N-CAM gene(s) and mRNA(s) in different tissues and at different times during development. To perform such studies, it is necessary to obtain nucleic acid probes complementary to regions of the N-CAM gene(s) or mRNA(s). In this paper, we report the isolation and preliminary characterization of two cDNA clones derived from chicken N-CAM messenger RNA.

MATERIALS AND METHODS

Materials. The preparation of rabbit polyclonal and mouse monoclonal antibodies specific for N-CAM has been described (4, 5, 7). For some experiments, polyclonal anti-N-CAM antibodies were affinity-purified by chromatography on immobilized N-CAM (5). Materials from commercial sources included: protein A-Sepharose (Pharmacia); oligo(dT)-cellulose, type T3 (Collaborative Research, Waltham, MA); calf liver tRNA (Boehringer Mannheim); rabbit reticulocyte lysate *in vitro* protein synthesis reagents, placental ribonuclease inhibitor, agarose, and oligo(dG)-tailed pBR322 plasmid (Bethesda Research Laboratories); [^{35}S]methionine (New England Nuclear or Amersham); EN^{3}HANCE autoradiography enhancer (New England Nuclear); and reverse transcriptase (Life Sciences, St. Petersburg, FL). Restriction enzymes and other enzymes were obtained from Bethesda Research Laboratories, P-L Biochemicals, or New England Biolabs and were used as recommended by the manufacturers.

Preparation of Polysomes and Isolation of RNA. Precautions were taken throughout to avoid contamination with ribonuclease (22). Polysomes were prepared from 100–200 fresh 9-day embryonic chicken brains (10–20 g wet weight) by Mg^{2+} precipitation (22). The polysomes were passed over a column of protein A-Sepharose to remove nonspecifically bound material (23) and were incubated overnight at 4°C with 0.5 mg (unfractionated IgG) or 0.05 mg (N-CAM affinity-purified IgG) of rabbit antibodies against N-CAM per original 1 g wet weight of brains. Polysomes that bound to anti-N-CAM IgG were collected by protein A-Sepharose chromatography, RNA was released by treatment with buffered EDTA, and poly(A)$^+$ RNA was collected by chromatography (one cycle) on a column (0.2 ml) of oligo(dT)-cellulose (24). Calf liver tRNA (62.5 μg) was added as a carrier, and the RNA was ethanol-precipitated overnight—once from 0.3 M sodium acetate (pH 5) and twice from 0.3 M potassium acetate (pH 5). The final pellet was dissolved in 10 μl of H_2O, lyophilized, redissolved in 10 μl of H_2O, and stored frozen at −70°C.

Poly(A)$^+$ RNA (not enriched by immunoprecipitation) was isolated by two cycles of oligo(dT)-cellulose chromatography from polysomes (25) or from guanidine thiocyanate extracts (26) of 9-day embryonic chicken brains or 9-day or 14-day embryonic chicken livers.

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Abbreviations: N-CAM, neural cell adhesion molecule; poly(A)$^+$ RNA, polyadenylylated RNA isolated by chromatography on oligo(dT)-cellulose.

***In Vitro* Translation and Immunoprecipitation.** RNA (1 μl) enriched by immunoprecipitation was translated *in vitro* in a rabbit reticulocyte lysate system. Translations were incubated for 90 min at 30°C with 50 μCi of [^{35}S]methionine in a 30-μl reaction mixture (1 Ci = 37 GBq). Each reaction mixture was supplemented with 5 μg of calf liver tRNA and 15 units of placental ribonuclease inhibitor. Labeled translation products were precipitated from sodium dodecyl sulfate- and nonionic detergent-treated translation mixtures by using protein A-Sepharose (27). [^{35}S]Methionine-labeled unglycosylated N-CAM was prepared from overnight cultures of tunicamycin-treated 9-day embryonic chicken brains by affinity chromatography on insolubilized monoclonal antibody anti-N-CAM no. 1 (6). The polypeptides were separated on a 7.5% sodium dodecyl sulfate/polyacrylamide gel (28), which subsequently was treated with EN^{3}HANCE autoradiography enhancer, dried, and exposed to Kodak XAR-5 x-ray film at −70°C with a Dupont Cronex Lightning-Plus intensifying screen.

cDNA Synthesis and Cloning. Procedures for oligo(dT)-primed reverse transcription of poly(A)$^+$ RNA, S1 nuclease digestion, homopolymer extension, and hybridization with oligo(dG)-tailed pBR322 were as described (29). Alternatively, cDNA synthesis was primed with random oligodeoxynucleotides from calf thymus, *Eco*RI and *Sal* I synthetic linkers were added, and cDNA was cloned in plasmid pUC8 (29, 30). Plasmids were introduced (31) into *Escherichia coli* strains MM294 (32) or DH1 (31), respectively. Transformants were replicated and stored on nitrocellulose filters (33).

Plasmid Isolation and Restriction Mapping. Plasmids were isolated from chloramphenicol-amplified cultures by an alkaline lysis method and were purified once by centrifugation to equilibrium in CsCl (29) in a Beckman VTi 50 vertical rotor at 20°C for 16 hr at 50,000 rpm. The restriction map of the 640-base-pair insert in pEC001 was deduced from the pattern of DNA fragments detected after single and multiple restriction enzyme digestions of the purified plasmid DNA (29).

Hybridization Probes. Chloramphenicol-treated filters carrying transformants were lysed and probed with ^{32}P-labeled cDNA or nick-translated plasmid DNA (29). ^{32}P-labeled cDNA probes were prepared from RNA by reverse transcription; plasmid DNA fragments were purified by agarose gel electrophoresis and were labeled by nick translation to a specific activity of 1–2 × 10^8 cpm/μg (29).

Hybrid Selection. Plasmid DNA (20 μg) was bound to 1-mm^2 nitrocellulose filters (34). Poly(A)$^+$ RNA that had been enriched by immunoprecipitation of polysomes from 10 g of 9-day embryonic chicken brains was heated for 10 min at 65°C, cooled on ice, and hybridized to the filters at 40°C for 5–7 hr in 100 μl of 50% formamide/0.4 M NaCl/10 mM Pipes, pH 6.4/2.7 mM EDTA containing 0.1 mg of *E. coli* tRNA per ml, 1 mM dithiothreitol, and 850 units of placental ribonuclease inhibitor. The filters were washed nine times with 150 mM NaCl/10 mM Tris·HCl, pH 8.0/1 mM EDTA/0.1% sodium dodecyl sulfate and twice with the same buffer without detergent—all at 65°C. The hybridized RNA was eluted by boiling individual filters for 2 min in 300 μl of water containing 6 μg of *E. coli* tRNA as a carrier. The samples were concentrated by precipitation with ethanol for translation *in vitro*.

Gel Blot Analysis. For RNA analysis, poly(A)$^+$ RNA (5 μg per lane) was electrophoresed in a 0.8% agarose gel containing formaldehyde (29). For DNA analysis, DNA was prepared (35) from a single adult chicken liver, digested to completion with restriction enzymes, and electrophoresed (21 μg per lane) in a 0.7% agarose gel in 0.089 M Tris borate/0.002 M EDTA, pH 8.0. In either case, the nucleic acids were transferred to nitrocellulose (29) and hybridized with nick-translated probes in the presence of 50% formamide and 10% dextran sulfate (36) at 42°C for 16–36 hr (29). The nitrocellulose filters were washed several times in 2× NaCl/Cit/0.1% sodium dodecyl sulfate at room temperature, followed by 1–2 hr in 2× NaCl/Cit (RNA blots) or 0.2× NaCl/Cit (DNA blots), both with 0.1% sodium dodecyl sulfate at 68°C (1× NaCl/Cit = 0.15 M NaCl/0.015 M Na citrate, pH 7.0). Bound probe was detected by autoradiography at −70°C with an intensifying screen. *Hin*dIII fragments of bacteriophage λ DNA were labeled with ^{32}P using the Klenow fragment of *E. coli* DNA polymerase or T4 polynucleotide kinase (29), for use as molecular weight markers.

RESULTS

***In Vitro* Translation of N-CAM mRNA.** In preliminary experiments, N-CAM polypeptides were not detected in *in vitro* translation assays with poly(A)$^+$ RNA isolated from 9-day embryonic chicken brains. Therefore, specific immunoprecipitation of polysomes was used to enrich the N-CAM mRNA. The enriched poly(A)$^+$ RNA programmed the synthesis of at least nine polypeptides (with molecular weights ranging from 40,000 to 160,000) that were precipitated by highly specific rabbit anti-N-CAM antibodies but not by antibodies from unimmunized animals (nonimmune IgG) or by antibodies directed against a chicken liver cell adhesion molecule (8) (Fig. 1, lanes a–c). The relative intensities of the different immunoprecipitable bands varied among different preparations of RNA (compare Figs. 1 and 3), but the qualitative pattern of bands observed was consistently reproducible from experiment to experiment. The precipita-

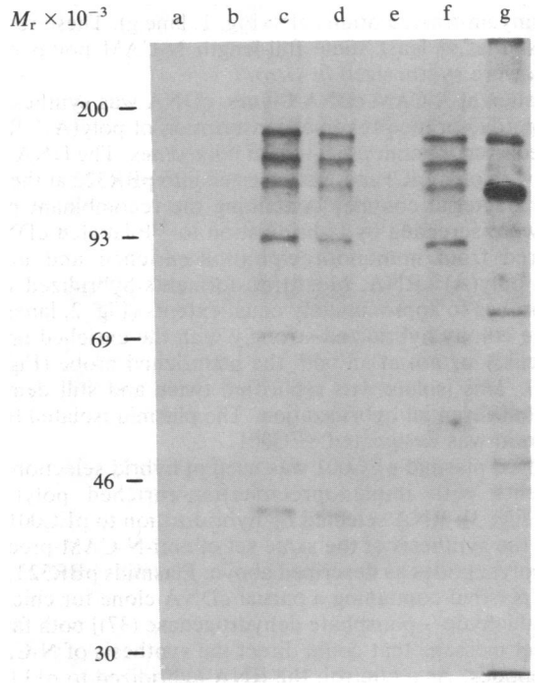

FIG. 1. *In vitro* translation and immunoprecipitation of N-CAM polypeptides. Apparent molecular weights determined by the migration of markers run in parallel are shown. Poly(A)$^+$ RNA enriched by immunoprecipitation (1 μl) was translated *in vitro*, and the translation products were immunoprecipitated with the following rabbit polyclonal antibodies: 2 μg of nonimmune IgG (lane a), 2 μg of anti-chicken liver cell adhesion molecule (L-CAM) IgG (8) (lane b), 2 μg of anti-N-CAM IgG (lane c), 2 μg of anti-N-CAM IgG plus 2 μg of purified N-CAM (lane d), 2 μg of anti-N-CAM IgG plus 20 μg of purified N-CAM (lane e), 2 μg of anti-N-CAM IgG plus 20 μg of bovine serum albumin (lane f). [^{35}S]Methionine-labeled unglycosylated N-CAM isolated from tunicamycin-treated 9-day embryonic chicken brain cells was electrophoresed in lane g. The immunoprecipitated polypeptides were separated on a 7.5% polyacrylamide gel and autoradiographed.

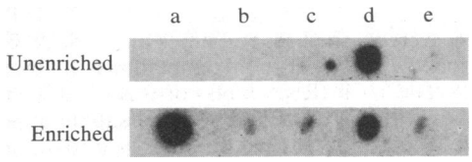

FIG. 2. Differential hybridization of N-CAM clones with cDNA. Bacterial colonies (strain MM294) immobilized on nitrocellulose filters were hybridized with cDNA synthesized from poly(A)$^+$ RNA prepared from unenriched (top row) or anti-N-CAM immunoprecipitate-enriched (bottom row) brain polysomes from 9-day chicken embryos. The bacteria contained the following plasmids: pEC001 (lane a), pBR322 (lane b), and other recombinant plasmids constructed in the experiment yielding pEC001 (lanes c–e).

tion of all nine polypeptides was prevented when an excess of nonradioactive N-CAM, but not of bovine serum albumin, was added to the translation mixture before immunoprecipitation (Fig. 1, lanes d–f). These experiments provide evidence that N-CAM mRNA was enriched by the polysome immunoprecipitation procedure and that this mRNA could direct the *in vitro* synthesis of N-CAM polypeptide chains.

Direct comparison of the *in vitro* translation products with authentic N-CAM was complicated by the fact that N-CAM synthesized *in vivo* is extensively glycosylated (4). Therefore, tunicamycin was used to block asparagine-linked glycosylation of N-CAM synthesized in overnight cultures of embryonic chicken brain cells (6). Two of the high-molecular-weight polypeptides synthesized *in vitro* comigrated with the major [^{35}S]labeled N-CAM polypeptides synthesized by tunicamycin-treated brain cells (Fig. 1, lane g). These results suggest that at least some full-length N-CAM polypeptide chains were synthesized *in vitro*.

Isolation of N-CAM cDNA Clones. cDNA was synthesized by oligo(dT)-primed reverse transcription of poly(A)$^+$ RNA isolated from immunoprecipitated polysomes. The DNA was tailed with oligo(dC) and was inserted into pBR322 at the *Pst* I site. Bacterial colonies containing the recombinant plasmids were screened by hybridization to ^{32}P-labeled cDNAs prepared from immunoprecipitation-enriched and unenriched poly(A)$^+$ RNA. Most transformants hybridized with both probes to approximately equal extents (Fig. 2, lanes c–e). One colony hybridized strongly with the enriched probe but weakly or not at all with the unenriched probe (Fig. 2, lane a). This isolate was repurified twice and still demonstrated differential hybridization. The plasmid isolated from this strain was designated pEC001.

Purified plasmid pEC001 was used in hybrid selection experiments with immunoprecipitation-enriched poly(A)$^+$ RNA (Fig. 3). RNA selected by hybridization to pEC001 directed the synthesis of the same set of anti-N-CAM-precipitable polypeptides as described above. Plasmids pBR322 and p13 [a plasmid containing a partial cDNA clone for chicken glyceraldehyde-3-phosphate dehydrogenase (37)] both failed to select message that could direct the synthesis of N-CAM polypeptides. As a control, the RNA hybridized to p13 (but not the RNA hybridized to pBR322 or pEC001) was shown to be greatly enriched in a message that directed the synthesis of an abundant translation product with a molecular weight appropriate for glyceraldehyde-3-phosphate dehydrogenase (unpublished data). These results suggest that pEC001 contains DNA sequences complementary to N-CAM mRNA.

The inserted DNA in pEC001 could be reexcised as a 640-base-pair fragment by digestion with *Pst* I. A partial restriction map of this fragment is presented in Fig. 4.

The *Pst* I fragment was isolated by agarose gel electrophoresis, nick-translated, and used to screen further cDNA clones that had been inserted into plasmid pUC8 after the addition of synthetic *Eco*RI and *Sal* I linkers (30). These

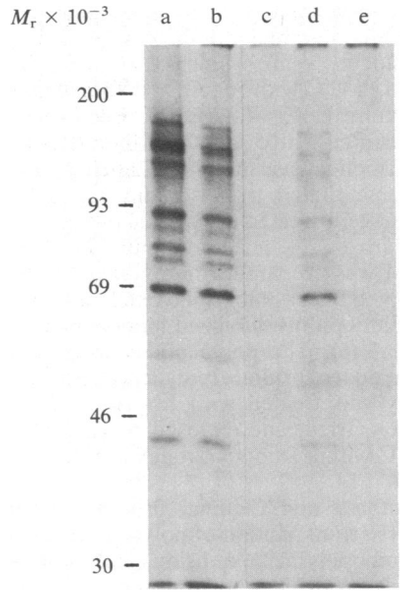

FIG. 3. Hybrid selection of N-CAM mRNA using pEC001. Apparent molecular weights of marker proteins are indicated. Poly(A)$^+$ RNA enriched by immunoprecipitation was hybridized to filters containing bound plasmid DNA. Aliquots of the hybridization mixture were removed for *in vitro* translation before (lane a) or after (lane b) hybridization to the filters. Hybridized RNA was eluted for *in vitro* translation from filters carrying plasmids pBR322 (lane c), pEC001 (lane d), or p13 (lane e). The translation products were immunoprecipitated with rabbit polyclonal anti-N-CAM antibodies, separated on a 7.5% polyacrylamide gel, and autoradiographed for three days (lanes a and b) or 2 weeks (lanes c–e).

cDNAs had been reverse-transcribed, with random oligodeoxynucleotides from calf thymus DNA as primers, in an attempt to obtain sequences away from the 3′ ends of the mRNAs. One strain reproducibly showed hybridization with the insert from pEC001; the plasmid in this strain was designated pEC020. Agarose gel electrophoresis of this plasmid after digestion with *Eco*RI and *Sal* I indicated that the inserted DNA in pEC020 is 150 to 200 base pairs long.

Expression of N-CAM mRNAs. Electrophoretically separated poly(A)$^+$ RNA from embryonic chicken brain and liver was transferred to nitrocellulose filters and analyzed by hybridization with the nick-translated inserts purified from pEC001 or pEC020 (Fig. 5, lanes a–d). Both probes hybridized to two large discrete RNA species between 6 and 7 kilobases long in embryonic brain poly(A)$^+$ RNA and to variable amounts of polydisperse, faster-migrating material (probably degradation products derived from the large RNAs). With either probe, no specific hybridization was detected to embryonic liver poly(A)$^+$ RNA isolated at day 9 (unpublished data) or day 14 (Fig. 5) of development.

Analysis of Genomic Sequences. To examine the occurrence of sequences complementary to pEC001 in the chicken genome, adult chicken liver DNA was digested with four restriction enzymes that did not cut within the 640-base-pair *Pst* I fragment (Fig. 4). The DNA was electrophoresed in a 0.7% agarose gel and transferred to nitrocellulose for hybrid-

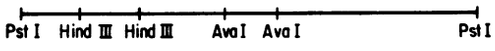

FIG. 4. Partial restriction map of the 640-base-pair *Pst* I fragment from pEC001. The *Pst* I sites were generated by the cloning process. The following restriction enzymes failed to cut within this fragment: *Bst*EII, *Cla* I, *Eco*RI, *Eco*RV, *Hin*fI, *Pvu* I, *Sst* I, and *Xho* I. The *Eco*RI site of pBR322 lies closer to the right side of the map in the orientation shown.

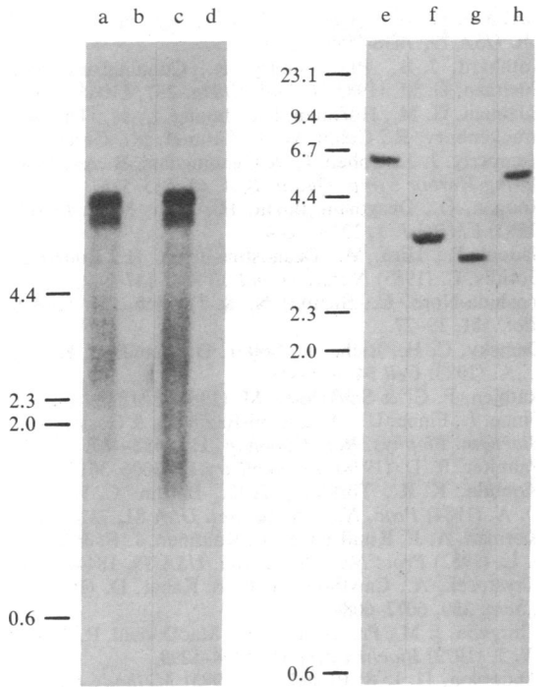

FIG. 5. Hybridization analyses of RNA and DNA sequences. Hybridized ^{32}P-labeled probes were detected by autoradiography. ^{32}P-labeled *Hin*dIII fragments from bacteriophage λ of the indicated sizes (in kilobase pairs) are shown to the left of each group of lanes. (*Left*) Hybridization analysis ("Northern blot") of chicken embryonic brain and liver RNA. Five micrograms of 9-day embryonic chicken brain poly(A)$^+$ RNA (lanes a and c) or 14-day embryonic chicken liver poly(A)$^+$ RNA (lanes b and d) were electrophoresed in a 0.8% agarose-formaldehyde gel. The RNA was transferred to nitrocellulose and hybridized with the nick-translated 640-base-pair *Pst* I fragment from pEC001 (lanes a and b) or the nick-translated *Eco*RI–*Sal* I fragment from pEC020 (lanes c and d). (*Right*) hybridization analysis ("Southern blot") of adult chicken liver DNA. Adult chicken liver DNA (21 μg) was digested to completion with *Eco*RI (lane e), *Pst* I (lane f), *Sst* I (lane g), or *Eco*RV (lane h). The digested DNA was electrophoresed in a 0.7% agarose gel, transferred to nitrocellulose, and probed with the nick-translated 640-base-pair *Pst* I fragment from pEC001.

ization analysis with the 640-base-pair *Pst* I fragment from pEC001 as a probe. In each digest, only one hybridizing fragment was detected (Fig. 5, lanes e–h). The *Eco*RI and *Pst* I digests were also probed with the nick-translated *Eco*RI–*Sal* I fragment from pEC020. In each case only one fragment was detected; this fragment migrated to the same position as the fragment detected using pEC001 as the probe (unpublished data).

DISCUSSION

We have isolated and partially characterized two putative N-CAM cDNA clones made from poly(A)$^+$ RNA purified from 9-day embryonic chicken brain. The conclusion that these clones contain sequences complementary to N-CAM mRNA relies on the identification of N-CAM polypeptides that were translated *in vitro*. Several lines of evidence suggest that *in vitro* translation of N-CAM polypeptides was achieved. Immunoprecipitation of brain polysomes with highly specific antibodies to N-CAM yielded poly(A)$^+$ RNA populations with a greatly enhanced capability to program the *in vitro* synthesis of polypeptide chains that were precipitated by anti-N-CAM antibodies. Two of the largest of these *in vitro* translation products comigrated on gel electrophoresis with authentic N-CAM polypeptides. Finally, the precipitation of the polypeptides by anti-N-CAM antibodies was specifically prevented by the addition of excess authentic N-CAM that had been affinity-purified using a highly specific monoclonal antibody.

It should be noted that the mRNA coding for N-CAM did not appear to be abundant, inasmuch as no *in vitro* translation of N-CAM polypeptides was detected unless the N-CAM mRNAs had been enriched by precipitation of polysomes with anti-N-CAM antibodies. This result may reflect the fact that N-CAM is not a highly abundant protein species *in vivo*, comprising only about 1% of the total membrane protein in embryonic chicken brain (4).

The appearance of multiple protein species recognized by anti-N-CAM antibodies in the *in vitro* translation experiments, although not unprecedented (ref. 23 and references cited therein), was unexpected. Because immunoprecipitation of all of the *in vitro* translation products was prevented in the presence of excess nonradioactive N-CAM, we assume that the components that did not comigrate with authentic N-CAM polypeptides were partial translation products of N-CAM mRNA. Whether these products resulted from premature termination of translation, initiation at internal codons, or some other process is as yet unknown.

Several lines of evidence suggest that plasmids pEC001 and pEC020 contain sequences complementary to N-CAM mRNA. Plasmid pEC001 hybridized much more strongly to labeled cDNA synthesized from immunoprecipitation-enriched brain poly(A)$^+$ RNA, which was shown to be enriched for N-CAM mRNA sequences, than to cDNA synthesized from unenriched brain poly(A)$^+$ RNA. Other brain clones isolated in the same experiment did not exhibit this strong differential hybridization. In addition, the RNA species recognized by pEC001 and pEC020, like N-CAM protein (7), were expressed in embryonic brain but not in embryonic liver. Moreover, pEC001 but not other plasmids [including p13, a plasmid recognizing an abundant mRNA species in brain and other organs (37)] hybridized to mRNA that could be translated *in vitro* to yield N-CAM polypeptides.

The observation that the RNA species hybridizing to pEC001 and pEC020 were expressed in embryonic brain but not in liver implies that regulation of message abundance is one mechanism by which N-CAM expression is controlled. Both pEC001 and pEC020 hybridized to two discrete RNAs from brain, both of which were large enough (between 6 and 7 kilobases) to code for either the 130-kilodalton or the 160-kilodalton N-CAM polypeptide chain. Although we have not directly demonstrated that these large RNA species are functional N-CAM mRNAs, it is an attractive hypothesis that they code, respectively, for the two major N-CAM polypeptides. Further experiments will be required to resolve this question in a conclusive fashion.

As suggested by the DNA hybridization experiments, the sequences in pEC001 and pEC020 appear to be represented at most only a few times, and perhaps only once, in the chicken genome. If the latter possibility is verified, then both of the large RNAs contain regions complementary to a single genomic sequence. Similar results have been reported for fibronectin (38). Several different fibronectin polypeptides appear to be generated from mRNAs formed by differential splicing of transcripts of a single gene (38–41), although these fibronectin mRNAs have not been resolved by electrophoresis. The two large RNA species detected here in brain RNA may similarly arise from differential splicing of common precursors. Alternatively, the difference in size might reflect differences in the sites of transcriptional initiation or poly(A) addition for the two RNAs. Finally, it is a remote possibility that DNA rearrangements, as in the case of the immunoglobulin genes (42, 43), might generate genes with differing 5′ ends but common 3′ ends, from which different mRNAs could be transcribed. The cDNA probes used here, which presumably contain sequences near the 3′ ends of the

mRNA, probably would not detect such rearrangements, and in any case such rearrangements might not occur in the liver DNA used in these experiments. Because we do not know whether our clones contain protein coding information, we cannot exclude the possible existence of other N-CAM genes having divergent 3′ untranslated sequences that could not be detected by our probes.

It should now be possible to extend previous studies of the temporal and spatial modulations of N-CAM abundance and structure (6, 12–14) to the nucleic acid level and to determine whether these modulations are reflected in changes in the sizes, structures, or relative abundances of the N-CAM mRNAs. For example, rat neuroblastoma cell lines transformed by temperature-sensitive variants of Rous sarcoma virus do not express N-CAM at the permissive (transformed) temperature but do express the molecule after being shifted to the nonpermissive (not transformed) temperature (3). Studies of this phenomenon can now be extended to the level of mRNA synthesis and decay. Such studies should give new insights into the relationships between the structure, the biochemical activities, and the *in vivo* functions of N-CAM.

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