The Mouse Neuronal Cell Surface Protein F3: A Phosphatidylinositol-anchored Member of the Immunoglobulin Superfamily Related to Chicken Contactin

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Abstract. Several members of the Ig superfamily are expressed on neural cells where they participate in surface interactions between cell bodies and processes. Their Ig domains are more closely related to each other than to Ig variable and constant domains and have been grouped into the C2 set. Here, we report the cloning and characterization of another member of this group, the mouse neuronal cell surface antigen F3. The F3 cDNA sequence contains an open reading frame that could encode a 1,020-amino acid protein consisting of a signal sequence, six Ig-like domains of the C2 type, a long premembrane region containing two segments that exhibit sequence similarity to fibronectin type III repeats and a moderately hydrophobic COOH-terminal sequence. The protein does not contain a typical transmembrane segment but appears to be attached to the membrane by a phosphatidylinositol anchor. Antibodies against the F3 protein recognize a prominent 135-kD protein in mouse brain. In fetal brain cultures, they stain the neuronal cell surface and, in cultures maintained in chemically defined medium, most prominently neurites and neurite bundles. The mouse *f3* gene maps to band F of chromosome 15. The gene transcripts detected in the brain by F3 cDNA probes are developmentally regulated, the highest amounts being expressed between 1 and 2 wk after birth.

The F3 nucleotide and deduced amino acid sequence show striking similarity to the recently published sequence of the chicken neuronal cell surface protein contactin. However, there are important differences between the two molecules. In contrast to F3, contactin has a transmembrane and a cytoplasmic domain. Whereas contactin is insoluble in nonionic detergent and is tightly associated with the cytoskeleton, about equal amounts of F3 distribute between buffer-soluble, nonionic detergent-soluble, and detergent-insoluble fractions. Among other neural cell surface proteins, F3 most resembles the neuronal cell adhesion protein L1, with 25% amino acid identity between their extracellular domains. Based on its structural similarity with known cell adhesion proteins of nervous tissue and with L1 in particular, we propose that F3 mediates cell surface interactions during nervous system development.

THE Ig superfamily consists of a variety of proteins that serve diverse functions both within and outside the immune system (for review see Williams and Barclay, 1988). The criterion for inclusion in the superfamily is the presence of one or several structural motifs called the Ig homology units or domains (Edelman, 1970; Amzel and Poljak, 1979; Hood et al., 1985). According to structural similarity, Ig domains have been divided in V-, C1-, and C2-type domains (Williams, 1987), which may occur alone or in combination in individual members of the superfamily. The function of these proteins ranges from antigen recognition to cell–cell contact formation and a role as cell surface receptors for growth factors and other ligands. In spite of this apparent diversity of function, most members of the Ig superfamily seem to have in common a role in mediating cellular interactions that control cell adhesion, migration, or differentiation.

A growing number of proteins with Ig domains are found to be expressed predominantly on cells of the nervous system. These include neural cell adhesion molecule (N-CAM)[1] (Barthels et al., 1987; Cunningham et al., 1987), myelin-associated glycoprotein (MAG) (Lai et al., 1987; Salzer et

1. *Abbreviations used in this paper*: Endo F, endoglycosidase F; MAG, myelin-associated glycoprotein; N-CAM, neural cell adhesion molecule; PI, phosphatidylinositol.

al., 1987), L1 (Moos et al., 1988), P_o (Lemke et al., 1988), contactin (Ranscht, 1988)—which are expressed in vertebrates—and two recently identified insect proteins, fasciclin II (Harrelson and Goodman, 1988) and amalgam (Seeger et al., 1988). With the exception of P_o, they contain C2-type Ig domains, and their sequence similarity extends in some cases beyond the Ig-like region (Harrelson and Goodman, 1988).

The neural surface proteins with Ig domains of the C2 type seem to serve similar functions since four of them—N-CAM (Edelman et al., 1983; Goridis et al., 1983; Rutishauser, 1983), MAG (Poltorak et al., 1987), L1 (Keilhauer et al., 1985; Fischer et al., 1986), and fasciclin II (Harrelson and Goodman, 1988)—have been shown to be involved in surface interactions between neural cells and their processes. It has been proposed (Lai et al., 1987; Harrelson and Goodman, 1988) that a common, phylogenetically ancient role of C2-type domains may be to mediate cell adhesion or recognition phenomena during nervous system development. Another common trait shared by N-CAM, L1, and MAG is the presence of the L2/HNK-1 carbohydrate determinant (Kruse et al., 1984). This sugar moiety is also found on cell adhesion molecules from nervous tissue not belonging to the Ig superfamily and may directly participate in cell-cell or cell-matrix adhesion (Kruse et al., 1985; Keilhauer et al., 1985; Riopelle et al., 1986; Hoffman and Edelman, 1987; Künemund et al., 1988).

We have recently reported the identification and cloning of the F3 antigen, a mouse neuronal cell surface glycoprotein, which also expressed the L2/HNK-1 determinant. In the developing cerebellum, this antigen was found on postmitotic neurons and most prominently on axons and neuropil in the molecular layer (Gennarini et al., 1989*a*,*b*). In this paper, we describe the sequence and structure of the F3 protein as deduced from overlapping cDNAs. Conceptual translation of the single long open reading frame yielded a protein of 1,020 amino acids containing six domains that met the criteria for inclusion in the C2 set of Ig domains. When compared with the other members of the Ig superfamily, F3 was found to be strikingly similar to contactin, the recently characterized chicken neuronal surface protein (Ranscht, 1988). In contrast to contactin, however, the F3 sequence did not encode a typical transmembrane segment. In accord with this finding, we present evidence showing that part or all of F3 at the cell surface is associated with the membrane via phosphatidylinositol (PI). The common structural features F3 shares with known cell adhesion molecules, L1 in particular (Moos et al., 1988), together with its conspicuous localization in neuropil and fiber tracts, suggest that it may also be involved in specifying cell contact formation.

Materials and Methods

Isolation of F3 Homologous cDNA Clones and Sequence Analysis

The isolation of the 280-bp cDNA clone D1.1 by antibody screening of a postnatal day-2 mouse brain cDNA library (Barthels et al., 1987; a kind gift of D. Barthels and W. Wille, Institut für Genetik der Universität zu Köln, Cologne, FRG) prepared in lambda gt 11 has been described (Gennarini et al., 1989*a*). A rescreen of the same library with clone D1.1 yielded clones 25.1 and 25.2 (Fig. 1). Clone 25.1 was 1.5 kb in size, contained an internal Eco RI site, and encoded a beta-galactosidase fusion protein reacting with F3 antibodies affinity purified on the 135-kD F3 protein from mouse brain (results not shown). Clone 25.2 was 1 kb in size with a 3′ end corresponding to the internal Eco RI site of clone 25.1. Clone D1.1 extended 150 bp further 5′ than both clones. A 130-bp Eco RI-Taq I fragment corresponding to the 5′ half of D1.1 was then used to screen a lambda gt 10 cDNA library prepared from postnatal day-8 mouse forebrain (Moos et al., 1988; a kind gift of K. Früh, Department of Neurobiology, Universität of Heidelberg, Heidelberg, FRG). Twelve clones were isolated, ranging in size from 1.3 to 3.4 kb and containing an internal Eco RI site. The longest clone, called 7.1.1, was 3.4 kb in size and contained most of the 25.1 sequence. Screening of the lambda gt 10 library with a 300-bp Eco RI-Ava I fragment derived from the 5′ end of this clone allowed isolation of the 900-bp clone 19.1, which was found to contain the 5′ end of the open reading frame.

Lambda DNA was prepared from plate lysates purified on DEAE cellulose columns according to the method of Helms et al. (1985). DNA in amounts sufficient for both insert size determination and subcloning was obtained by collecting phage lysates from three 8.5-cm agar plates. The isolated inserts were subcloned into the Eco RI site of either pBR328 or pGEM 7Zf (+) (Promega Biotec, Madison, WI). The insert of clone 25.1, which contained an internal Eco RI site, was cut out by Pvu II and subcloned into the Pvu II site of pBR328.

Suitable restriction fragments were subcloned in opposite orientation into M13 mp18 and mp19 vectors, and their sequence was determined on both strands by the dideoxy chain termination method (Sanger et al., 1977) using a sequencing kit (Sequenase; United States Biochemical Corp., Cleveland, OH). When fragments >400 bp were generated, synthetic oligonucleotides were also used as sequencing primers. The oligonucleotides were synthesized by the phosphoramidite method on a synthesizer (System I; Beckman Instruments, Inc., Fullerton, CA) and used without further purification.

RNA Analysis

Total RNA was prepared from adult and developing mouse forebrains by the method of Chomczynsky and Sacchi (1987), and poly $(A)^+$ RNA was enriched by oligodT cellulose chromatography. The RNA preparations were fractionated by electrophoresis on 1% agarose gels containing formaldehyde and blotted to nitrocellulose (Thomas, 1980). Prehybridization and hybridization conditions were as described (Gennarini et al., 1986). Probes were labeled by random priming (Feinberg and Vogelstein, 1984) and used at 2×10^6 cpm/ml.

Comparison of Protein and cDNA Sequences

The ALIGN program of Dayhoff et al. (1983) was used to define internal homologies within the deduced F3 protein sequence or to compare the six Ig-like F3 domains with those of other members of the Ig superfamily. Ig domains were arbitrarily defined by selecting sequences extending for 15 residues in both directions from the cysteines assumed to be involved in disulfide bonds. The program was run with a gap penalty of six, and 100 random permutations were performed. Scores >3.1 (corresponding to a chance probability of 10^{-3}; Williams and Barclay, 1988) were considered significant. Protein and nucleotide sequences were also compared by using the ALIGN or dot matrix programs contained in the Microgenie sequence analysis package (Beckman Instruments, Inc.). Searches of the Genpro (a protein version of GenBank) and National Biochemical Research Foundation data bases were done using the algorithm of Goad and Kanehisa (1982) adapted to the comparison of protein sequences.

Preparation and Analysis of Brain Extracts and Subcellular Fractions

Mouse forebrains were homogenized in 5 vol of Hepes-buffered (5 mM, pH 7.8) 10% sucrose containing 5 mM iodoacetamide, 0.5 mM PMSF, 10 U/ml aprotinin, 5 μM pepstatin, 40 μM leupeptin, and 5 μg/ml alpha-2 macroglobulin as protease inhibitors. After centrifugation (30 min at 1,000 *g*) to pellet cell debris and nuclei, the supernatant was centrifuged at 140,000 *g* for 90 min to separate soluble and membrane fractions. The soluble material was concentrated fivefold by filtration on Centricon 30 (Amicon Corp., Danvers, MA). Membranes were lysed in 50 mM Tris-HCl (pH 8), 1% NP-40, and protease inhibitors (lysis buffer). Detergent-soluble and -insoluble fractions were separated by centrifugation at 140,000 *g* for 90 min. The insoluble material was extracted once more with lysis buffer. The pellet was dissolved in electrophoresis sample buffer; all other fractions were mixed 1:1 with 2× electrophoresis sample buffer.

Proteins were separated on 6.67% SDS-polyacrylamide gels and blotted

onto nitrocellulose as previously described (Gennarini et al., 1986). Molecular weight markers, with their apparent relative molecular masses given in parentheses, were myosin (220,000), beta-galactosidase (120,000), phosphorylase b (94,000), BSA (68,000), ovalbumin (43,000), and carbonic anhydrase (30,000). The blots were developed using either F3 antiserum at a 10^{-3} dilution or F3 antibodies immunoaffinity purified on the 25.1 fusion protein and ^{125}I-protein A.

Phospholipase C Digestion of Brain Membranes

Crude membranes prepared as described above were suspended at a protein concentration of 10 mg/ml in 50 mM Tris-HCl buffer (pH 7.5) containing the aforementioned protease inhibitors (phospholipase buffer), preincubated for 2 h at 37°C, and then repelleted (30 min at 25 psi in an airfuge [Beckman Instruments, Inc.]) to remove material spontaneously released from the membrane vesicles. After an additional wash in phospholipase buffer, the membranes were resuspended in the same buffer, and 150-μl aliquots were incubated in the presence or absence of 3 U PI-specific phospholipase C from *Bacillus thuringiensis*. The enzyme was prepared by one of us (Rougon, G.; Jean et al., 1988) from *B. thuringiensis* culture filtrates as described (Taguchi et al., 1980). After incubation for 40 min at 37°C, a freeze–thaw cycle was carried out to open the sealed membrane vesicles, and the incubation was continued for 40 more min after addition of another 3 U of enzyme. After another freeze–thaw cycle and a 40-min incubation, the reaction was stopped by adding 1 mM EDTA and soluble and particulate fractions were separated again by centrifugation for 30 min at 25 psi in an airfuge (Beckman Instruments, Inc.). The pellet was resuspended in 150 μl of phospholipase buffer and both fractions were boiled for 2 min after adding an equal volume of 2× electrophoresis sample buffer. Control samples received phospholipase buffer only; alternatively, digestion was performed in the presence of 5 mM $ZnCl_2$.

Endoglycosidase F (Endo F) Treatment of Immunoprecipitates

Treatment with Endo F was performed on the F3 immunoprecipitates prepared as described (Gennarini et al., 1989*a*). To the washed protein A-Sepharose beads (12 μl packed gel), 10 μl of 0.1 M phosphate buffer (pH 6.5) containing 0.1% SDS, 1% mercaptoethanol, and 50 mM EDTA were added. After boiling for 2 min, the sample was put on ice and 30 μl of 0.1 M phosphate buffer, pH 6.5, containing 1% NP-40, 50 mM EDTA, and protease inhibitors were added together with 3 U (=2 μl) of Endo F (Boehringer Mannheim Biochemicals, Indianapolis, IN). Control samples received buffer only. After 2 h of incubation at 37°C, the digestion was stopped by boiling the samples for 2 min with an equal volume of 2× electrophoresis sample buffer. The supernatants were analyzed by immune blotting using F3 antiserum.

Cell Culture and Immunofluorescence Labeling

Cultures were prepared from day-15 embryonic mouse forebrains according to Rougon et al., (1983). They were maintained either in DME supplemented with 10% FCS and 50 mM KCl or in the defined medium described by Bottenstein (1985) for neural cells. The cultures were used 5 d after plating.

Live cultures were stained either with rabbit anti-F3 serum diluted 1:500 or with an IgG fraction of anti-D1.1 fusion protein serum (prepared by $(NH_4)_2\,SO_4$ precipitation and diluted 10^{-3} with respect to the original serum). Bound antibodies were detected by FITC-conjugated $(Fab)_2$ fragments of goat anti-rabbit IgG (Immunotech, Marseille, France) used at a 1:50 dilution. The coverslips were mounted on slides in PBS/glycerol (1:1 [vol/vol]) and examined under an epifluorescence microscope (Carl Zeiss, Ober-Rochen, FRG).

Antibodies

The preparation of the two rabbit antisera directed against the F3 antigen has been described (Gennarini et al., 1989*a*): one, designated anti-F3 serum, was raised against gel-purified F3 glycoprotein; the other one, designated anti-D1.1 serum, was prepared by immunizing with the beta-galactosidase fusion protein encoded by clone D1.1. In some cases, the anti-F3 serum was microaffinity purified on the beta-galactosidase fusion protein encoded by clone 25.1. The protein was expressed in *Escherichia coli*, blotted to nitrocellulose, and F3 antibodies were immunoaffinity purified on the fusion protein band as reported previously (Gennarini et al., 1989*a*).

In Situ Hybridization on Metaphase Spreads

In situ hybridization experiments were carried out using metaphase spreads from a WMP male mouse in which all the autosomes, except 19, were in the form of metacentric Robertsonian translocations. Con A-stimulated lymphocytes were cultured at 37°C for 72 h with 5-bromodeoxyuridine added for the final 6 h of culture (60 μg/ml of medium) to ensure a chromosomal R banding of good quality.

Clone 7.1.1 subcloned in pGEM1 was tritium labeled by nick translation to a specific activity of 10^8 dpm/μg. The radiolabeled probe was hybridized to metaphase spreads at a final concentration of 12 ng/ml of hybridization solution as previously described (Mattei et al., 1985). After coating with nuclear track emulsion (NTB_2; Eastman Kodak Co., Rochester, NY) the slides were exposed for 14 d at 4°C and then developed. To avoid any slipping of silver grains during the banding procedure, chromosome spreads were first stained with buffered Giemsa solution and metaphases photographed. R banding was then performed by the fluorochrome–Giemsa method, and metaphases were rephotographed before analysis.

Results

Isolation and Sequence Analysis of cDNA Clones

One F3-related clone, designated D1.1, has been isolated in the initial screening of a neonatal mouse brain expression library probed with anti-F3 antibodies (Gennarini et al., 1989*a*). A rescreen of the same library with the D1.1 insert yielded clones 25.1 and 25.2, which were found to extend the cloned region further 3′. Two additional clones, designated 7.1.1 and 19.1, were then isolated by screening a lambda gt 10 cDNA library from young postnatal mouse brain with restriction fragments of D1.1 and 7.1.1, respectively. The longest clone, 7.1.1, contained almost the complete coding sequence for a mouse F3 protein; clone 19.1 contained the missing 5′ part of it plus 249 bp of presumed untranslated sequence. Restriction analysis and hybridization experiments indicated that the five clones were identical in their regions of overlap (Fig. 1). The insert of clone 25.1 had been cloned in the correct orientation and reading frame since the anti-F3 serum reacted with the beta-galactosidase fusion protein expressed from this clone (not shown).

Clones 19.1, 25.2, almost all of clone 7.1.1, and the 3′ half of 25.1 were sequenced on both strands (Fig. 1). Except for the first 43 bp of 7.1.1 and the very 5′ and 3′ ends of D1.1 (Gennarini et al., 1989*a*), the regions of overlap of the different clones were identical in sequence. Most likely, the divergent end structures of these two clones were artifacts that have arisen as a consequence of the S1 nuclease digestion carried out during library construction. A composite sequence of 3,843 bp is shown in Fig. 2.

The longest coding sequence started with the methionine codon at position 250, which was preceded by an in-frame stop codon 63 nucleotides further upstream. There was a second methionine codon starting at nucleotide 256. The surrounding nucleotides did not match well the consensus sequence for eukaryotic initiation of translation; however, both potential initiation sites met the stringent requirement for a purine in position -3 (Kozak, 1987). A single long open reading frame of 3,060 nucleotides extended from the first ATG to a stop codon at position 3,310 followed by 533 bp of presumed 3′ untranslated region. A potential polyadenylation signal was found at position 3,510, which was, however, not succeeded by a poly (A) tail. Hence, the 3′ end sequences of the mRNA were not present in the cDNA clones we have isolated.

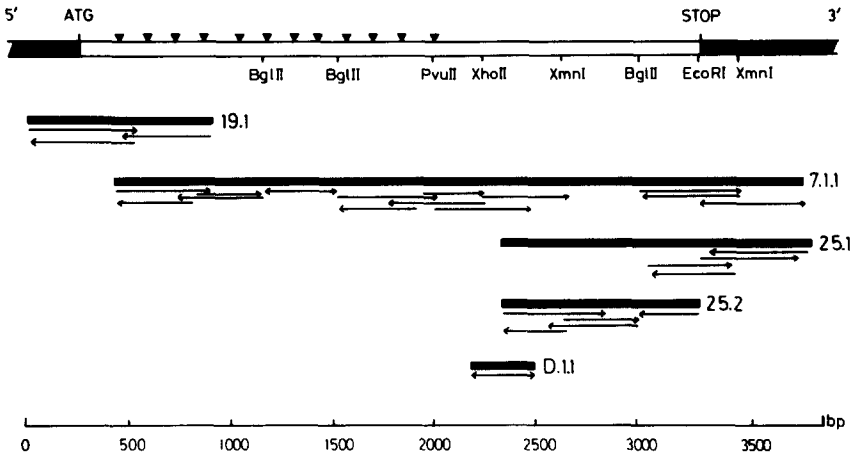

Figure 1. Organization of the F3 cDNA and alignment of the clones. The upper bar gives the overall structure deduced from the five overlapping cDNA clones that are shown below. The white portion of the bar represents the putative protein coding region with the positions of the initiation ATG and of the stop codon as indicated. Solid triangles mark the position of the 12 regularly spaced cysteine codons defining the six Ig domains. The hatched regions correspond to the 5' and 3' untranslated regions. The restriction endonuclease sites used for M13 subcloning are given. The cDNA clones are symbolized by solid black bars; the arrows underneath them indicate extent and direction of sequencing.

Analyses of the Deduced Protein Sequence

The open reading frame, which started at the first methionine, predicted a polypeptide of 1,020 amino acids. The sequence was largely hydrophilic but had hydrophobic segments at both ends (Fig. 2). The hydrophobic NH_2-terminal sequence had the characteristics of a signal peptide. Several residues at its 3' end matched the consensus sequence preceding a signal peptide cleavage site (Von Heijne, 1983). Cleavage at this site would leave the aspartic acid at position 21 as the NH_2-terminal amino acid of the mature protein. The following 999 residues would make up a polypeptide with a calculated 110,800 M_r. This value was in good agreement with the apparent 110,000 M_r estimated by SDS-PAGE for the Endo F–digested 135-kD F3 protein (Fig. 4 *A*). There were nine potential sites for asparagine-linked glycosylation (Figs. 2 and 3). Attachment of sugar side chains at some or all of these sites would explain the large relative molecular mass shift of the F3 protein from 135 to 110 kD after deglycosylation.

The moderately hydrophobic COOH-terminal sequence consisted of 27 uncharged amino acids, with the exception of one glutamic acid. Its proximity to the COOH terminus and the absence of a stop–transfer sequence suggests that it does not span the membrane.

The hydrophilic portion of the F3 protein sequence contained 14 cysteines (Fig. 2). Nonreduced F3 protein migrated in SDS-PAGE faster than after reduction (results not shown). This is typical for proteins with multiple intrachain disulfide bridges and excludes the possibility that some of the cysteines were engaged in interchain bonds. Twelve of the cysteines were evenly spaced in the NH_2-terminal half of the sequence (Fig. 1). This part of the protein sequence consisted of six repeating units with two cysteines each. The ALIGN program of Dayhoff et al. (1983) was used to assess the significance of their sequence similarity. As shown in Table I, significant scores (>3.1; i.e., 3.1 standard deviations from random with a probability of occurring by chance of 10^{-3}; Williams, 1987; Williams and Barclay, 1988) were obtained for all comparisons except for the alignments of repeat II with repeat III and IV.

We can thus subdivide the deduced protein sequence (after cleavage of the putative signal sequence) into eight domains: the six NH_2-terminal repeats each containing two cysteines, the premembrane region of around 400 amino acids, and the COOH-terminal hydrophobic segment which, however, may not be present in the mature protein.

Homology with Chicken Contactin

A search of the National Biochemical Research Foundation and Genpro data bases for potentially homologous proteins did not yield significant polypeptide sequence similarities other than those expected for a protein containing C2-type Ig domains (see below). However, a comparison with the recently published sequence of contactin, the chicken neuronal surface glycoprotein (Ranscht, 1988), revealed a striking degree of positional identity at both the nucleotide and the protein level. In an optimized alignment of the entire F3 cDNA sequences with the 3,843 5' nucleotides of the contactin cDNA clone 183, 71% of the nucleotides were in identical positions. Starting at nucleotide 238, the similarity extended over the entire length of the F3 cDNA sequence as the dot matrix shown in Fig. 3 *A* demonstrates.

Amino acid identities ranged from 64.2 to 86.4% for the extracellular domains of contactin and domains I–VII of F3 (Fig. 3 *B*). However, the two protein sequences diverged at both ends. At the NH_2 terminus, the similarity started at residue 33 of the F3 sequence, 13 residues behind the presumptive NH_2 terminal aspartic acid. Towards the COOH terminus, the sequence similarity broke down near the beginning of the putative membrane-spanning domain in the contactin (Ranscht, 1988) and the COOH-terminal hydrophobic segment in the F3 sequence. The predicted protein sequence of contactin then continued with a cytoplasmic domain of 88 residues, whereas the open reading frame of the F3 cDNA ended just behind the stretch of uncharged residues.

At the nucleotide level, the sequence similarity between F3 and contactin was interrupted at the point where the two protein sequences diverged (Fig. 3 *A*, *arrow*) but it resumed again further downstream and continued past the stop codons in the two cDNAs. To exclude the possibility that a reverse transcriptase error or a mutation during propagation of our clones had artificially introduced a shift in the reading frame, we sequenced the corresponding region in three independent clones (25.1, 25.2, and 7.1.1) isolated from two different libraries (Fig. 1). The sequences of all three clones were found to be identical. Evolutionary conservation of noncoding sequences is rather unusual and the possibility should be

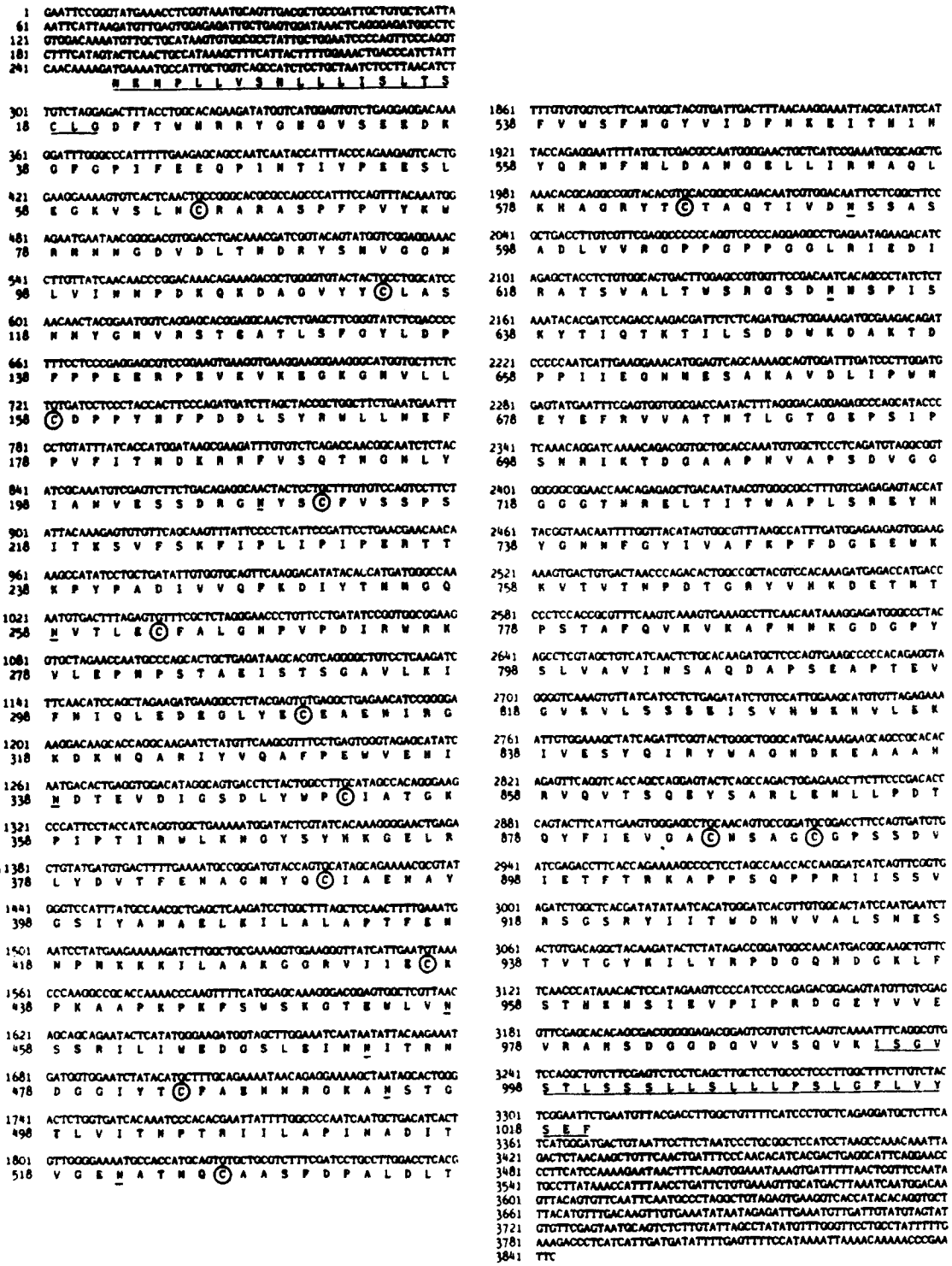

Figure 2. Composite nucleotide and deduced amino acid sequences of the cDNA clones. The 3,843-nucleotide sequence shown includes an open reading frame of 3,119 nucleotides beginning at position 190. The protein sequence is given starting with the nearest methionine codon. The 14 cysteine residues (12 in the Ig domains and 2 in the premembrane domain) are circled. The putative signal peptide, the COOH-terminal extension of 27 uncharged amino acids, and nine potential Asn-linked glycosylation sites are underlined. The sequence data contained in this paper will appear in the EMBL/GenBank/DDBJ nucleotide sequence databases under the accession number X14943F3. After acceptance of this manuscript, Brümmendorf et al. (1989) have published the sequence of the F11 protein, which appears to be the chicken homologue of F3.

Table I. Alignment Scores for Comparisons of the Six Ig Domains of F3 Between Themselves and Selected V-, C1-, or C2-type Domains

	F3.1	F3.2	F3.3	F3.4	F3.5	F3.6	IgλV	IgκC	N-CAM5	L1.6	Cont. 3	Cont. 6
F3.1		**4.2**	**4.6**	**3.6**	**8.3**	**4.3**	**4.8**	**3.2**	**3.2**	**4.1**	**3.6**	**4.4**
F3.2	**4.2**		1.5	1.9	**5.2**	**7.4**	2.2	2.2	1.5	2.4	0.6	**7.0**
F3.3	**4.6**	1.5		**7.5**	**7.6**	**4.1**	2.4	0.9	**5.0**	2.7	**29.0**	**5.1**
F3.4	**3.6**	1.9	**7.5**		**3.3**	**4.0**	−0.4	2.0	**3.2**	2.0	**7.0**	**4.8**
F3.5	**8.3**	**5.2**	**7.6**	**3.3**		**3.2**	2.0	1.0	2.6	**4.6**	**5.9**	**4.8**
F3.6	**4.3**	**7.4**	**4.1**	**4.0**	**3.2**		1.2	0.5	1.4	**7.2**	2.9	**29.0**
N-CAM3	**5.7**	**4.2**	**7.3**	**5.5**	**9.2**	**6.0**	**4.0**	**3.1**	**4.9**	**7.0**	**7.1**	**6.3**
L1.3	**4.9**	**3.8**	**8.8**	**7.9**	**7.8**	1.6	2.4	2.6	**6.0**	2.3	**6.3**	2.9
MAG3	**7.7**	**4.1**	**5.2**	**8.8**	**9.3**	**3.2**	2.5	2.6	**7.3**	**4.2**	**5.6**	**3.1**
THY-1	**3.4**	0.8	**3.2**	0.9	1.6	0.4	**5.6**	1.3	2.1	0.7	2.2	2.6

Domains were defined by choosing the sequences that encompass the cysteines assumed to be involved in intrachain bonds and extend for 15 residues in both directions. The ALIGN program (Dayhoff et al., 1983) was run using a gap penalty of 6 and 100 random permutations. Scores >3.1 were considered significant; they are shown in boldface. Limits (*F3.1*, domain I from the NH_2 terminus, etc.; *cont.*, contactin) were (*F3.1*) 133–212; (*F3.2*) 226–309; (*F3.3*) 334–408; (*F3.4*) 419–489; (*F3.5*) 504–582; (*F3.6*) 594–683; (*Cont. 3*) 251–328; (*Cont. 6*) 513–601 (Ranscht, 1988); (*IgλV*) 26–124 (L1MS4E); (*IgκC*) 11–101 (K3HU); (*N-CAM3*) 201–285; (*N-CAM5*) 482–563 (Barthels et al., 1987); (*L1.3*) 207–285; (*L1.6*) 391–470 (Moos et al., 1988); (*MAG 3*) 246–320 (Lai et al., 1987); (*Thy-1*) 23–119 (TDR). The sequences are referenced either by their National Biochemical Research Foundation library accession code or by a bibliographic reference.

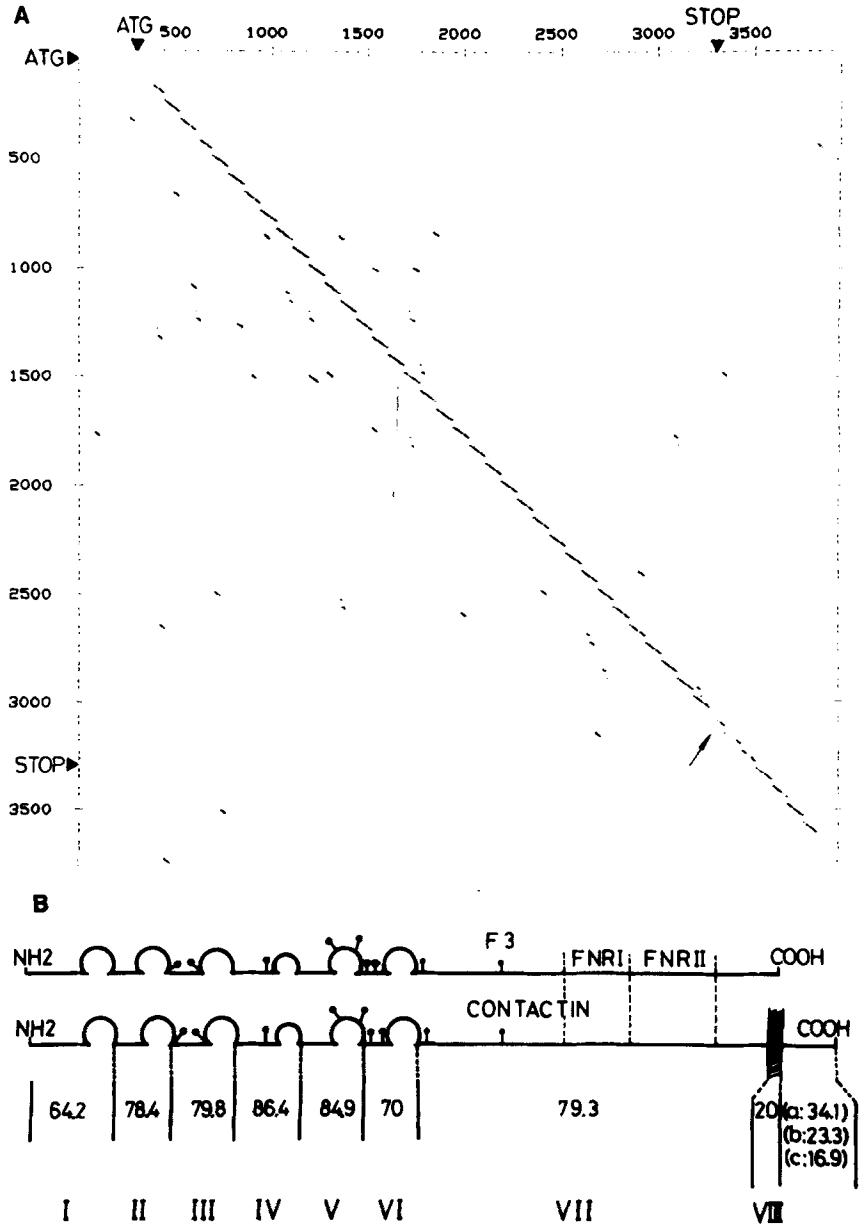

Figure 3. Comparison of F3 with contactin cDNA and protein sequences. (*A*) Dot matrix comparison of contactin (ordinate) and F3 (abscissa) nucleotide sequences using the Microgenie program (Beckman Instruments, Inc.). The positions of the initiation ATG and the stop codons are indicated. The program was run with parameters set to select sequences having 70% similarity over a stretch of 19 nucleotides. The arrow points to an interruption in the nucleotide sequence similarity (nucleotide 3,238–3,258). (*B*) Overall structure and percent amino acid identity between F3 and contactin. The percent positional identity of amino acids between F3 and contactin is given for the individual six Ig domains (numbered I–VI), the premembrane region (domain VII), and the hydrophobic COOH terminus and the transmembrane segment of F3 and contactin, respectively (domain VIII). The values in brackets are the percent matches between the cytoplasmic domain of contactin and the protein sequences deduced from the three possible reading frames (designated *a*, *b*, and *c*) of the corresponding part of the F3 cDNA. (↑) Potential sites for Asn-linked glycosylation.

Table II. Alignment Scores for Comparison of the F3 with L1 Ig-like Domains

	F3.1	F3.2	F3.3	F3.4	F3.5	F3.6
L1.1	**6.6**	2.4	4.9	5.5	5.2	4.1
L1.2	4.3	**7.7**	3.9	2.9	4.3	4.2
L1.3	4.9	3.8	**8.7**	7.9	7.9	1.6
L1.4	4.3	1.4	4.2	**3.8**	4.1	2.8
L1.5	5.5	3.6	5.3	6.3	**7.4**	3.9
L1.6	4.1	2.4	2.8	2.0	4.6	**7.2**

Sequences were aligned as described in Table I. The scores obtained for comparisons between the same domains (i.e., domain I with domain I; domain II with domain II) are shown in boldface.

considered that the stop codon in the F3 sequence is due to insertion of an alternatively used exon. Conceptual translation of the three reading frames of the presumed 3′ noncoding region of F3 revealed amino acid identities of 34, 23, and 17%, respectively, with the COOH-terminal region of contactin. This might be taken to indicate that mRNAs exist in which the first reading frame codes for protein. However, there were several in-frame stop codons in all reading frames and we conclude that the segment represents untranslated region. This conclusion is also supported by the observation that the nucleotide differences between the F3 and contactin sequences in this part of the cDNAs did not fall preferentially in the third position of the contactin codons as would be expected if there was selective pressure to preserve the amino acid coding capability.

F3 Contains Ig Domains of the C2-type and Two Segments Resembling Fibronectin Type III Repeats

The size and structural organization of the six repeats which made up the NH_2-terminal half of the protein bore striking similarity to the homology units of Ig-like proteins (Hood et al., 1985; Williams, 1987; Williams and Barclay, 1988). Most of the canonical features of Ig-like domains were indeed present in these repeating units, such as alternating hydrophobic and hydrophilic residues preceding the presumptive first and the DXGXYXCXA/V consensus sequences around the second cysteine as well as the conserved tryptophan in the middle of each domain. All six F3 domains showed significant (>3.1) ALIGN (Dayhoff et al., 1983) scores with other Ig-related sequences. Typical examples of a more extensive analysis are given in Table I.

Ig domains have been divided into three classes: V domains with nine β strands; C1 domains with seven β strands; and C2 domains which, although folded like C domains, show greater sequence similarity to V domains (Williams, 1987). In our analysis, the highest and most consistently significant scores were obtained in the comparisons with C2-type sequences as exemplified in Table I for domains III and V of N-CAM, domains III and VI of L1, and domain 3 of MAG. The six F3 domains did not seem to be more closely related to each other than to the N-CAM, L1, and MAG domains; however, much higher scores were obtained when corresponding domains of F3 and contactin were compared.

Ranscht (1988) has already shown for contactin that the premembrane region contains two segments that share sequence similarity with type III repeats of fibronectin. Most of the residues shared between contactin and fibronectin were also conserved in F3 and alignment of the first (position 768–846) and the second (position 846–946) segment of the deduced F3 protein sequence matched a type III repeat fibronectin sequence (Peterson et al., 1983) at 24 and 23% of the positions, respectively (results not shown).

Among the other mammalian neural cell surface proteins belonging to the C2 set of Ig-like proteins, F3 most resembled L1 (Moos et al., 1988) in overall structure, but the comparison of their individual Ig domains did not reveal a particularly close relationship between F3 and L1 (Table I). Upon closer inspection of the data, however, it appeared that for five of the six domains the highest scores were obtained when corresponding domains were compared; i.e., domain I matched best with domain I, domain II with domain II, and so on (Table II). This was not the case when F3 was compared with other molecules of this type (results not shown). Furthermore, the ALIGN program did not produce reasonable alignments between the F3, N-CAM, and MAG sequences beyond the region of homology with Igs. By contrast, an optimized alignment between the entire F3 protein sequence and the extracellular part of L1 matched at 25% of the positions, a value which increased to 37% when conservative exchanges were taken into account (results not shown). It seems reasonable, therefore, to consider L1, contactin, and F3 as prototypes of a subgroup of neural surface proteins.

Evidence for PI Linkage and Solubility Characteristics of the F3 Protein

A COOH-terminal segment of 18–30 moderately hydrophobic amino acids can be considered diagnostic for glypiation and membrane attachment via PI (Cross, 1987; Low, 1987; Ferguson and Williams, 1988). To test this point more directly, we investigated whether PI-specific phospholipase C would release soluble F3 from the membranes. A microsomal fraction was prepared from a homogenate of adult mouse brain, and the F3 proteins were revealed by immune blotting using anti-F3 antibodies microaffinity purified on the 25.1 fusion protein. The membrane vesicles were first incubated at 37°C in the absence of enzyme to remove any material that may be spontaneously released from the membranes; only small amounts (<10%) were recovered in the corresponding supernatants (not shown). The membranes were then incubated in the presence or absence of PI-specific phospholipase C from *B. thuringiensis* (Taguchi et al., 1980; Jean et al., 1988); in additional controls, 5 mM $ZnCl_2$, a specific inhibitor of the enzyme (Taguchi et al., 1980), was added together with the enzyme. Two freeze-thaw cycles were carried out during the incubation to allow access of enzyme to outside-in vesicles and to release the material that may be trapped inside closed vesicles. As seen in Fig. 4 *B*, substantial amounts of F3 protein were recovered in a high-speed supernatant after treatment with the *B. thuringiensis* phospholipase C, and this release was completely inhibited by addition of $ZnCl_2$. To provide a more quantitative estimate of the release, the corresponding regions of the nitrocellulose filters were cut out and counted. The results showed that 9% of the 135-kD band present in the starting material was recovered in the supernatant after incubation in the absence of enzyme, a value which increased to 40% in its presence. Although we cannot formally exclude that part or all

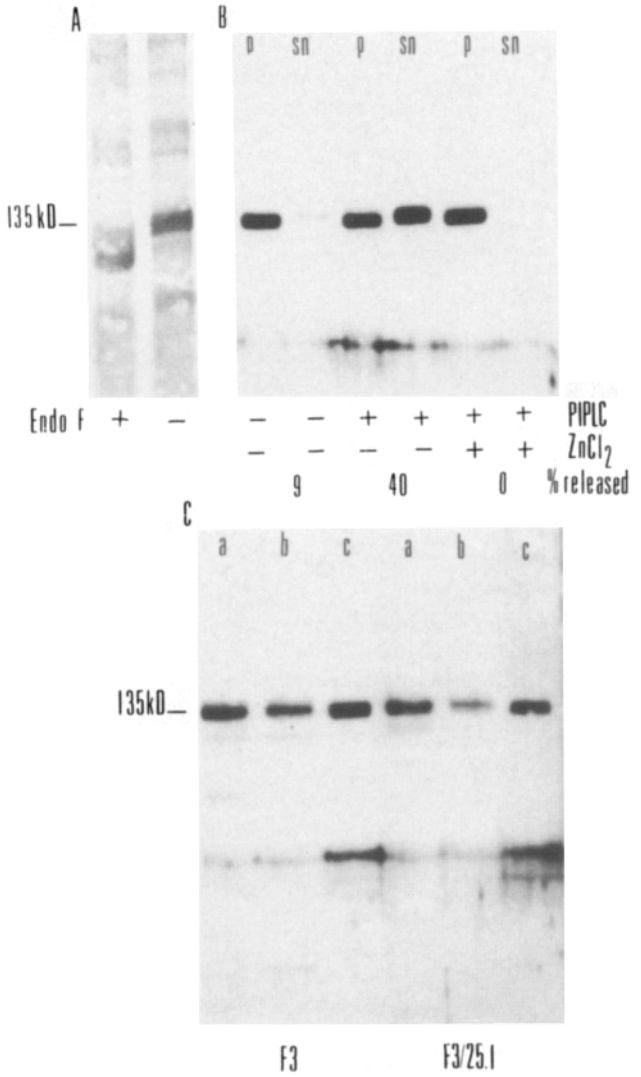

Figure 4. F3 protein in mouse brain is sensitive to Endo F and phospholipase C digestion and distributes among buffer-soluble detergent-soluble, and detergent-insoluble fractions. (*A*) Endo F digestion of immunoisolated F3 antigens. Material isolated by immunoprecipitation with F3 antiserum from an NP-40 extract of mouse brain was digested with Endo F and analyzed by immune blotting using the same antibodies. (*Left lane*) The digested material migrates with a 110-kD M_r; (*right lane*) control incubated without enzyme. (*B*) Release of F3 antigen from brain membranes by treatment with PI-specific phospholipase C. Mouse brain microsomal membranes were first incubated for 2 h at 37°C in the absence of enzyme to remove the spontaneously released material (not shown). The membranes were then incubated in the presence or absence of PI-specific phospholipase C from *B. thuringiensis* as indicated (*PIPLC*). Controls were also performed by incubating the membranes with enzyme in the presence of 5 mM $ZnCl_2$. Membrane (lanes *p*) and soluble (lanes *sn*) fractions were then separated by centrifugation. All fractions were electrophoresed on a 6.7% polyacrylamide gel, blotted to nitrocellulose, and probed with F3 antibodies affinity purified on the beta-galactosidase fusion protein encoded by the clone 25.1. Bound antibody was revealed with ^{125}I–protein A. The fuzzy material in the lower part of the blot reacts nonspecifically since it is also revealed by preimmune serum (not shown). After autoradiography, the 135-kD region was cut out of the nitrocellulose. The quantities of F3 antigen released, expressed in percent of the radioactivity present in the 135-kD band of the starting material, are given below. (*C*) Distribution of F3 antigen in isotonic and detergent extracts of adult mouse brain. Buffer-soluble (lanes *a*), NP-40–soluble (lanes *b*), and NP-40–insoluble (lanes *c*) material (250 μg of protein) were prepared from adult mouse brains, fractionated on a 6.67% acrylamide gel, and transferred to nitrocellulose. The blots were then reacted either with the F3 antiserum (*F3*) or with F3 antibodies immunopurified on the beta-galactosidase fusion protein encoded by clone 25.1 (*F3/25.1*). Bound antibody was revealed with ^{125}I–protein A. The quantities recovered in each fraction were determined by scanning suitable exposures of the autoradiographs and correcting for volume changes.

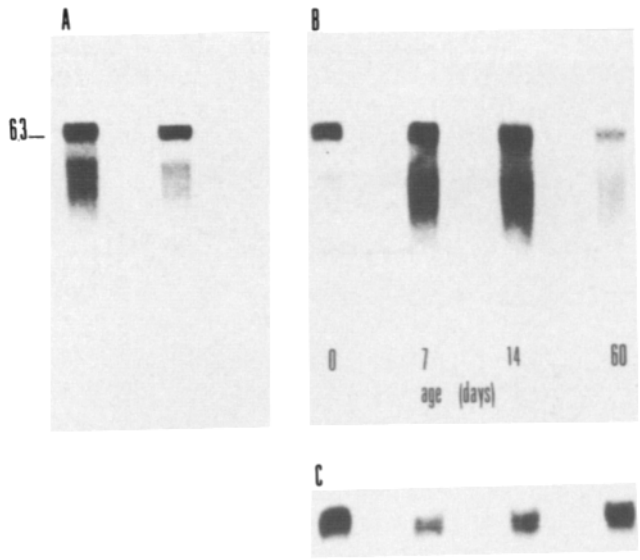

Figure 5. Northern hybridization of adult and developing mouse brain poly $(A)^+$ RNA to F3 cDNA. (*A*) Adult mouse brain poly $(A)^+$ RNA was hybridized to the 3-kb 5′ Eco RI fragment (*left*) or to the 0.5-kb 3′ Eco RI fragment (*right*) of clone 7.1.1. (*B*) Poly $(A)^+$ RNA was prepared from mouse forebrains of different developmental stages and probed with the 3-kb Eco RI fragment of clone 7.1.1. A discrete mRNA species was detected at 6.3 kb together with material at positions corresponding to 3.3–3.9 kb. (*C*) The same blot was reprobed with a glyceraldehyde phosphate dehydrogenase cDNA (Hanauer and Mandel, 1984) to show that intact RNA was present in each lane and to serve as internal control.

of the release was due to a contaminating protease activity rather than to phospholipase action, this seems unlikely for the following reasons: (*a*) in a previous study (Jean et al., 1988) the same enzyme preparation has been found to be free of detectable protease activity; (*b*) the incubation was performed in the presence of a mixture of protease inhibitors; (*c*) no degradation products were detected after incubation in the presence of enzyme (Fig. 4 *B*); and (*d*) it is improbable that a contaminating protease would be completely inhibited by $ZnCl_2$. It is worth noting that the Zn ions also prevented the spontaneous release, and the possibility should be considered that $ZnCl_2$ also inhibits an endogenous phospholipase.

A striking difference between F3 and contactin is that the latter has been identified by virtue of its insolubility in non-

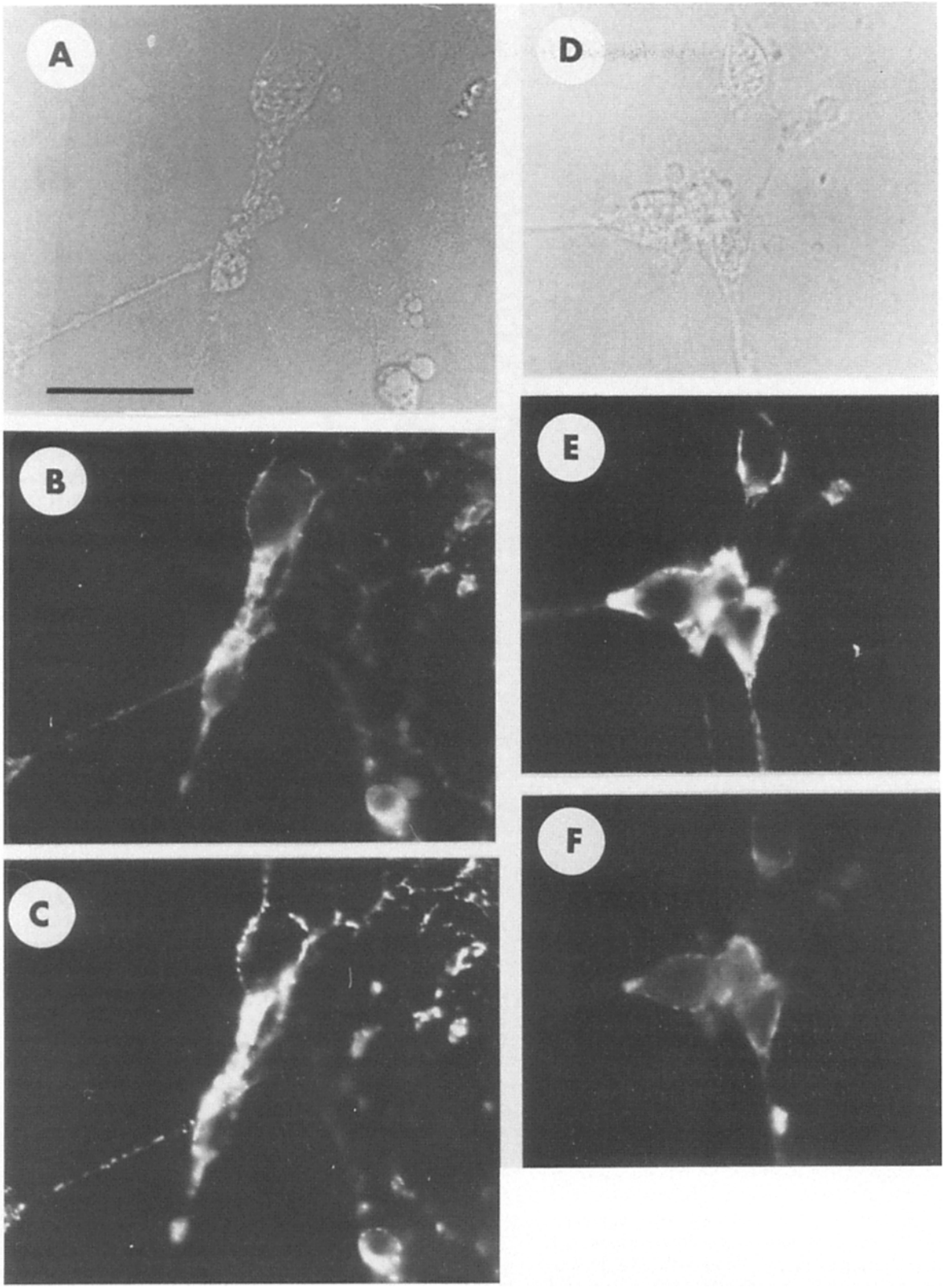

Figure 6. Indirect immunofluorescence on fetal brain cultures using rabbit anti-F3 sera, anti-D1.1 sera, and anti-N-CAM mAb. Cultures prepared from embryonic day-15 mouse forebrains were maintained for 5 d in DME supplemented with 10% FCS. Live cultures were double stained either with anti-F3 serum (*C*) plus anti-N-CAM mAb (*B*) or with anti-D1.1 fusion protein antibodies (*F*) plus anti-N-CAM mAb (*E*). The rabbit antibodies were revealed with TRITC-coupled $(Fab)_2$ fragments of anti-(rabbit IgG) antibodies, the mAb with FITC-coupled $(Fab)_2$ fragments of anti-(rat IgG) antibodies. *A* and *D* show the corresponding phase-contrast pictures. All three antibodies label cells of neuronal morphology and their processes. Bar, 50 μm.

ionic detergent (Moss, 1983, 1986; Ranscht et al., 1984), whereas the original immunogen used for preparing anti-F3 antiserum consisted of a soluble protein shed or secreted from brain slices (Gennarini et al., 1989*a*). We thus fractionated a mouse brain homogenate into a buffer-soluble and a membrane fraction which was again separated into an NP-40-soluble and an NP-40-insoluble fraction. As shown in Fig. 4 *C*, the 135-kD F3 protein was found in all three fractions. The relative amounts were estimated by densitometry of suitable exposures of the immune blots. Of the total amount present in the homogenate, 42% was recovered in the soluble fraction, 26% in the NP-40 extract, and 32% in the remaining pellet. The results were the same whether crude F3 antiserum or antibodies purified on the 25.1 fusion protein were used. Obvious differences in molecular weight among the F3 proteins in the three fractions were not observed. Conceivably, soluble and insoluble F3 proteins could represent distinct protein isoforms. However, it is also possible that soluble F3 is produced by the action of an endogenous phospholipase. In this case, the NP-40-insoluble molecules may consist of extracellularly released proteins that either form aggregates refractory to detergent extraction or associate with extracellular matrix components.

The Expression of F3-related RNA Is Developmentally Regulated

Northern blot analysis of adult mouse brain poly $(A)^+$ RNA using the originally isolated F3 cDNA D1.1 showed a prominent hybridizing RNA species of 6.3 kb plus several fainter bands migrating in the 3–4-kb region (Gennarini et al., 1989*a*). We obtained similar results with either the 5′ located 3-kb or the 3′ 0.5-kb Eco RI fragment of the 7.1.1 insert (Fig. 5 *A*). However, with these longer probes the smaller RNAs were relatively more intense. Striking changes in the level of expression of F3-related RNAs were found when poly $(A)^+$ RNA from mouse brains of different ages was analyzed (Fig. 5 *B*). The highest levels of RNA hybridizing with the 5′ Eco RI fragment of clone 7.1.1 were expressed between 1 and 2 wk after birth. The blots were reprobed with a cDNA coding for the glycolytic enzyme glyceraldehyde phosphate dehydrogenase (Hanauer and Mandel, 1984) to serve as internal standard. The blots were scanned by densitometry and the values obtained normalized for differences in the amounts applied to each lane by dividing through the value obtained with the latter probe. The results showed that the 6.3-kb transcript decreased approximately eightfold between 14 and 60 postnatal d. The amount of the 3.3–3.9-kb material decreased to a very similar extent.

The disperse character of the lower relative molecular mass bands may indicate that this material was produced by degradation of the 6.3-kb band. However, the discreteness of both the 6.3-kb band and the signal obtained with the glyceraldehyde phosphate dehydrogenase probe argue against this possibility. Upon shorter exposure, a band of ~3.3 kb and a doublet of 3.9 kb could be discerned in the material of lower relative molecular mass. Since these RNA species hybridized to probes from both the 5′ and 3′ parts of the F3 cDNA they may differ from the 6.3-kb transcript by 5′ or 3′ untranslated regions of different lengths. The possibility remains, however, that these mRNA arise through differential splicing or are the products of different but closely related genes.

Immunofluorescence Labeling of Fetal Mouse Brain Cultures

We have previously shown that neurons in fetal brain cultures express the F3 antigen (Gennarini et al., 1989*a*,*b*). As shown in Fig. 6, both anti-F3 serum and an antiserum raised against the D1.1 fusion protein stained live cells of neuronal morphology in cultures prepared from embryonic day-15 mouse forebrain. In cultures maintained for 5 d in serum-containing media, cell bodies and their processes were labeled (Fig. 6, *C* and *F*), and the staining pattern given by both anti-F3 and anti-D1.1 sera was virtually indistinguishable from the one observed with anti-N-CAM mAb. By contrast, in the same type of cultures maintained in chemically defined medium, F3 antigen appeared to be expressed more prominently on neurites than on neuronal cell bodies (Fig. 7). Anti-D1.1 serum stained neurites and neurite bundles intensely in a characteristic punctuate pattern, whereas the cell bodies were only weakly labeled. Identical results were obtained when anti-F3 serum was used (not shown). Apparently, serum-free culture conditions induced a redistribution of F3 antigen expression, perhaps related to a more rapid differentiation of the neurons in the absence of serum.

Mouse Gene Mapping by In Situ Hybridization

The Southern blot pattern on genomic DNA given by the D1.1 insert (Gennarini et al., 1989*a*) was consistent with there being only one copy of the *f3* gene per mouse haploid genome. In accord with these data, only one main location was found for the *f3* gene, on band F of chromosome 15 (Fig. 8).

Discussion

We have used a rabbit antiserum raised against a 135-kD glycoprotein shed or secreted from slices of newborn mouse brain to identify and clone the F3 antigen, a novel cell surface glycoprotein of mouse nervous tissue (Gennarini et al., 1989*a*). In this study, we define the primary structure of what appears to be the 135-kD F3 protein. Key features of the structure of this molecule are (*a*) the presence of Ig domains of the C2 type and of a sequence resembling type III fibronectin repeats; (*b*) its membrane attachment via PI; and (*c*) a striking similarity with the recently described chicken neuronal surface glycoprotein, contactin (Ranscht, 1988). To acknowledge the differences in structure and biochemical properties between contactin and the mouse protein we have cloned, we will continue for the time being calling it the F3 protein.

The NH_2-terminal segment of the F3 protein sequence has the characteristics of signal sequences common to secreted and membrane-bound proteins. The remainder of the F3 protein can be divided into eight domains. The sequences that place F3 in the Ig superfamily are found in the six NH_2-terminal domains which share the structural features characteristic for the C2 subgroup of Ig domains as defined by Williams (1987).

The Ig domains are followed by a segment of ~400 amino acids which contains two regions of ~85 residues each which exhibit similarity to fibronectin type III repeats involved in cell and heparin binding (Peterson et al., 1983; Kornblihtt et al., 1984; Odermatt et al., 1985; Obara et al., 1988). A conspicuous Pro-Gly repeat and a stretch of five

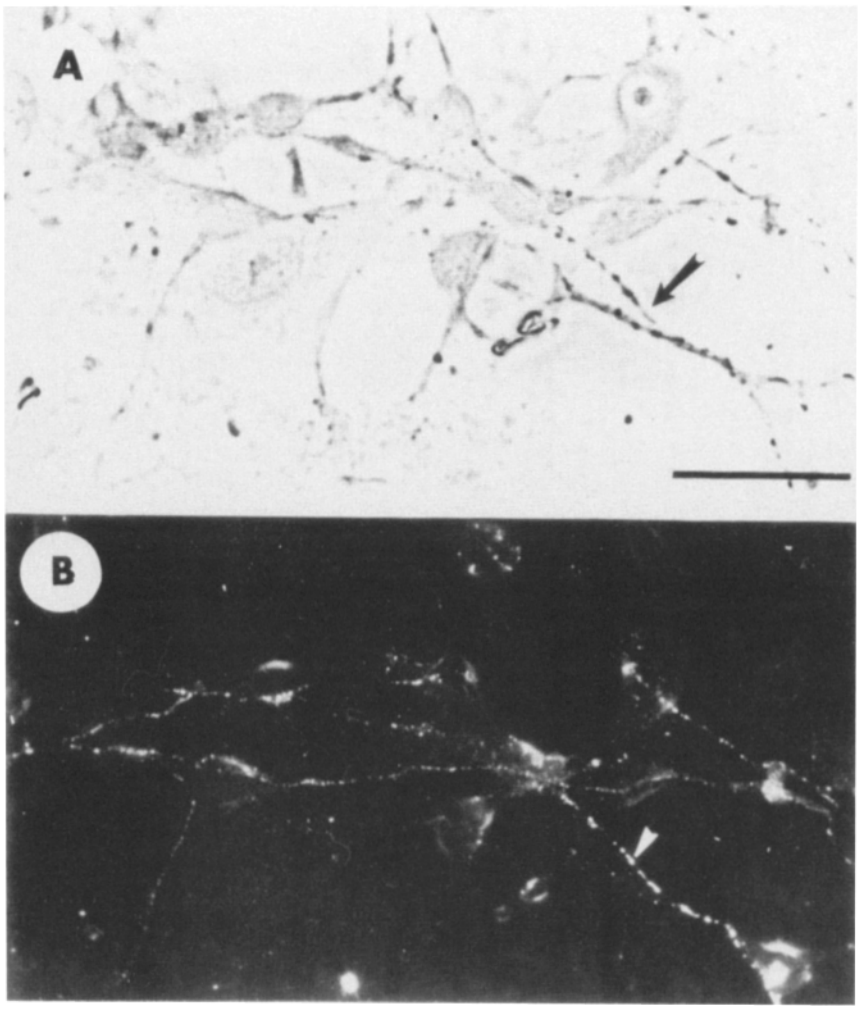

Figure 7. F3 antigenic sites become concentrated on neurites in cultures maintained in chemically defined medium. Cultures prepared from embryonic day-15 mouse forebrains were grown for 5 d in the medium formulated by Bottenstein (1985). Live cultures were stained with anti-D1.1 antibodies using FITC-conjugated anti-(rabbit IgG) $(Fab)_2$ (*B*). *A* shows the corresponding phase-contrast picture. Neurites and neurite bundles (*arrow*) are much more intensely labeled in a punctuate pattern than the corresponding cell bodies. Care was taken that cell bodies and processes were in the same plane of focus. Bar, 50 μm.

glycines just behind domain VI may constitute a hinge region as often found in Ig-like proteins (Amzel and Poljak, 1979; Hall and Rutishauser, 1987).

The eighth and last domain consists of a moderately hydrophobic segment as found in proteins that are attached to the membrane via a covalently bound glycolipid containing PI as lipid component (for reviews see Cross, 1987; Low, 1987; Ferguson and Williams, 1988). These proteins can be released by phospholipase C and this holds also true for F3. However, with our present data we cannot formally exclude the possibility that F3 binds strongly to another protein that is anchored in the membrane by a phospholipid. As discussed below, we cannot exclude either that an isoform of F3 exists that spans the membrane.

By in situ hybridization, we could assign the F3 gene to band F of mouse chromosome 15. No other Ig superfamily gene has as yet been found in this region, underlining the fact that the present day genes coding for these proteins are widely dispersed in the mammalian genome.

In immune blots of mouse brain homogenates the anti-F3 serum recognizes a prominent 135-kD species (Gennarini et al., 1989*a*; see also Fig. 4 *C*). Antibodies raised against the D1.1 fusion protein recognized an additional protein species of 90 kD (Gennarini et al., 1989*a*). As shown in the present study, F3 antibodies affinity purified on the 25.1 fusion protein, which comprises most of domains VII and VIII of the F3 sequence and starts in the middle of clone D1.1, react only with the 135-kD protein. Size considerations further support our conclusion that the protein we have cloned is the 135-kD species since Endo F treatment reduces the apparent relative molecular mass of the 135-kD band to 110 kD, which is very close to the value of 111 kD encoded by the sequenced cDNA.

Comparison of the F3 sequence with the recently published sequence of chicken contactin (Ranscht, 1988) shows a high degree of similarity in the region beyond the Ig domains. Most strikingly, the nucleotide sequences are very similar too, with 71% of the residues in identical positions. Hence, F3 may be considered the mouse homologue of chicken contactin.

There are, however, important differences between the two proteins. In contrast to F3, contactin is a transmembrane protein and has a cytoplasmic domain. The differences in biochemical properties we detect may be the consequence of

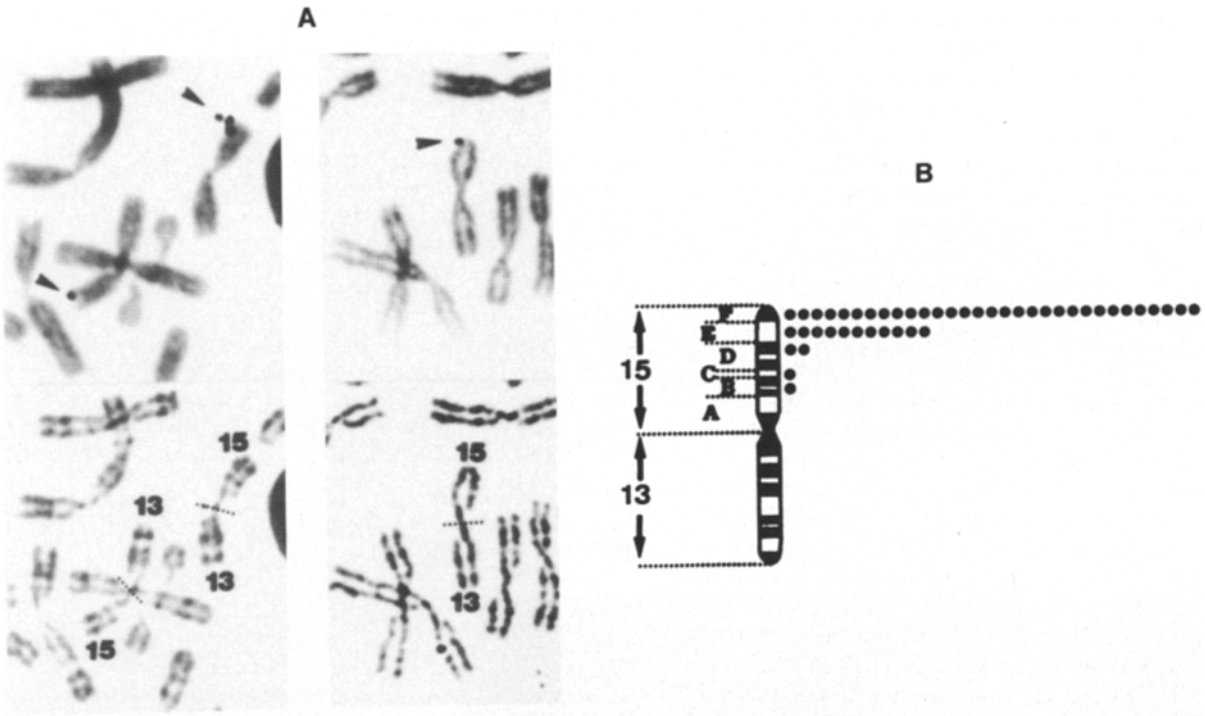

Figure 8. Localization of the gene encoding F3 cDNA to mouse chromosome 15 by in situ hybridization. (*A*) Two partial WMP mouse metaphases, showing the specific site of hybridization to chromosome 15. (*Top*) Arrowheads indicate silver grains on Giemsa-stained chromosomes after autoradiography. (*Bottom*) Chromosomes with silver grains were subsequently identified by R banding. (*B*) Diagram of WMP mouse Rb (*13*; *15*) chromosome, indicating the distribution of labeled sites on chromosome 15.

these different modes of membrane association. Whereas contactin has been identified by virtue of its insolubility in nonionic detergent (Moss, 1983; 1986), sizable fractions of F3 are buffer and NP-40 soluble. We should thus consider the possibility that F3 exists both as a transmembrane and a lipid-linked protein, perhaps also as a genuinely secreted protein, as recently shown for N-CAM (Gower et al., 1988). Such isoforms may be generated by alternative splicing as in the case of several proteins including two members of the Ig superfamily, N-CAM (Cunningham et al., 1987; Barbas et al., 1988; Gower et al., 1988) and LFA-3 (Dustin et al., 1987). The other possibility is that both, the soluble and the NP-40–insoluble forms are derived from PI-linked F3 molecules. In this case, soluble F3 may be generated by cleavage of the phospholipid anchor. The insoluble form on the other hand, may consist of released protein that becomes associated with extracellular matrix, a mechanism which may also generate the insoluble fraction of N-CAM-120 (He et al., 1987). The fact that phospholipase C solubilized only part of the F3 proteins cannot be taken as evidence for the existence of a transmembrane form since phospholipase C–resistant fractions of PI-linked proteins have been documented in other systems (for review see Low, 1987). Finally, contactin has been reported to attain its highest levels in the adult brain (Ranscht et al., 1984), whereas F3 mRNA expression in mouse brain peaks during the second postnatal week followed by an eightfold drop into adulthood. The F3 protein has also been found to be present in lower amounts in adult than in perinatal brain (Gennarini et al., 1989*b*).

Among the other proteins with C2-type Ig domains F3 seems most closely related to L1 (Moos et al., 1988). Both molecules have six Ig domains and a long premembrane region containing fibronectin type III repeats, and optimized alignment between F3 and the extracellular sequences shows that 25% of the residues are in identical positions. Some resemblance with fibronectin type III repeats is also seen in the membrane-proximal region of N-CAM (Harrelson and Goodman, 1988), but the sequence similarity is less obvious than it is in F3 and L1 (our unpublished observation). Finally, F3 seems also related to L1 in terms of its expression. In fact, the staining patterns of young postnatal mouse cerebellum obtained with F3 (Gennarini et al., 1989*a*,*b*) and L1 (Faissner et al., 1984) antibodies are virtually indistinguishable.

Apart from the L1 family, to which NgCAM (Daniloff et al., 1986), NILE (Bock et al., 1985), and G4 (Rathjen et al., 1987*a*) appear to belong as well, other proteins that may be related to F3 are (*a*) F11 (Rathjen et al., 1987*a*) and neurofascin (Rathjen et al., 1987*b*), which are both adhesion molecules comprising a prominent 135-kD component involved in neurite–neurite interactions; and (*b*) TAG-1, a 135-kD glycoprotein selectively expressed on subsets of axons (Dodd et al., 1988). However, sequence data for these molecules are not yet available.

The propensity of Ig-like molecules to bind to each other is best documented by the various interactions between components of the immune system: the T cell receptor, CD8, and CD4 bind to MHC class I and/or class II molecules; LFA3 binds to CD2; and the fibronectin receptor for IgG binds to IgG constant domains (for reviews see Kronenberg et al., 1986; Littman, 1987; Springer et al., 1987; Unkeless et al., 1988). The nervous system may be another tissue where the

mutual binding between proteins belonging to the Ig superfamily plays key roles in mediating cell interactions. N-CAM is involved in contact formation between neural cells mainly by binding to other N-CAM molecules (Edelman, 1986, 1987). The principal function of L1/NgCAM/NILE may be the fasciculation of axons, at least in part also involving a homophilic mechanism (Stallcup and Beasley, 1985; Fischer et al., 1986; Grumet and Edelman, 1988), and MAG appears to be a ligand mediating neurone–oligodendrocyte and oligodendrocyte–oligodendrocyte adhesion (Poltorak et al., 1987). The structural resemblance of F3 to these cell adhesion molecules from nervous tissue and in particular to L1, supports the hypothesis that F3 may also be involved in cell surface interactions among neural cells and their processes. In addition to its similarity in polypeptide structure, F3 shares with L1, N-CAM, MAG, and other cell adhesion molecules from nervous tissue the L2/HNK-1 carbohydrate epitope (Gennarini et al., 1989*a*; Kruse et al., 1984; Keilhauer et al., 1985; Hoffman and Edelman, 1987) which may directly participate in cell–cell and cell–substrate adhesion (Künemund et al., 1988).

Over the last several years, it has become clear that most neural cells express more than one type of cell adhesion molecule on their surface, and it has been proposed that their adhesive properties result from a combination of, or competition between, different binding specificities (for review see Rutishauser and Jessell, 1988). This leads us to the question of how many different molecules are required to specify the multiple interactions among neural cells and their processes during neuroontogeny. The identification of contactin and F3 adds new candidates to the list, but many more, possibly related, molecules are likely to be discovered. All adhesion molecules expressed by the same neuron need not carry out identical functions, since they are often distributed differently between cell bodies and processes. From the limited data available (Ranscht, 1988; Gennarini et al., 1989*a*,*b*), contactin and F3 seem to be most concentrated in fiber-rich areas of the brain, and we show in this paper that F3 is more abundant on the processes than on the cell bodies of cultured neurons maintained in chemically defined medium. These molecules may thus mainly be involved in fiber–fiber interactions. Functional differences may also result from different modes of membrane attachment and from different associations with submembrane structures, a situation exemplified by the 180- and 120-kD isoforms of N-CAM (He et al., 1987; Pollerberg et al., 1987). Contactin and F3 may represent another example of this type since the latter, which lacks a cytoplasmic tail does not seem to, and probably cannot, undergo the tight association with the cytoskeleton which has led to the discovery of the former (Moss, 1983; Ranscht et al., 1984). In fact, the phospholipid-mediated membrane attachment of F3 may provide for a distinctive role of this molecule. However, the functional significance of this type of membrane anchorage, which is displayed by very different proteins (for review see Cross, 1987; Low, 1987; Ferguson and Williams, 1988), is unknown at present and may not be the same for all molecules. One possibility is that such proteins serve dual roles both as cell surface molecules and as extracellular proteins after cleavage of the phospholipid anchor. The prospective role of F3 in neural cell surface interactions can now be studied by the use of antibodies prepared against bacterial fusion proteins containing different parts of the molecule and by expressing full-length cDNA. The probes described here will also help to identify the full complement of F3/contactin-like molecules in mammalian and avian species.

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