Nectinepsin: A New Extracellular Matrix Protein of the Pexin Family

CHARACTERIZATION OF A NOVEL cDNA ENCODING A PROTEIN WITH AN RGD CELL BINDING MOTIF*†‡§¶

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Christine Blancher‡, Boubaker Omri, Laure Bidou§, Bernard Pessac, and Patricia Crisanti¶

CNRS 9035 Développement et Immunité du Système Nerveux Central, Université Paris VI, Faculté de Médecine Broussais Hôtel Dieu, 15 rue de l’Ecole de Médecine, 75270 Paris cedex 06

We report the isolation and characterization of a novel cDNA from quail neuroretina encoding a putative protein named nectinepsin. The nectinepsin cDNA identifies a major 2.2-kilobase mRNA that is detected from ED 5 in neuroretina and is increasingly abundant during embryonic development. A nectinepsin mRNA is also found in quail liver, brain, and intestine and in mouse retina. The deduced nectinepsin amino acid sequence contains the RGD cell binding motif of integrin ligands. Furthermore, nectinepsin shares substantial homologies with vitronectin and structural protein similarities with most of the matricial metalloproteases. However, the presence of a specific sequence and the lack of heparin and collagen binding domains of the vitronectin indicate that nectinepsin is a new extracellular matrix protein. Furthermore, genomic Southern blot studies suggest that nectinepsin and vitronectin are encoded by different genes. Western blot analysis with an anti-human vitronectin antiserum revealed, in addition to the 65- and 70-kDa vitronectin bands, an immunoreactive protein of about 54 kDa in all tissues containing nectinepsin mRNA. It seems likely that the form of vitronectin found in chick egg yolk plasma by Nagano et al. (1992) J. Biol. Chem. 267, 24863–24870 is the protein that corresponds to the nectinepsin cDNA. This new protein could be an important molecule involved in the early steps of the development.

The differentiation of the central nervous system is a highly complex process that involves transient cell-cell interactions, including neuronal migration, neuritogenesis, synaptic junction formation, and axonal growth and guidance. These mechanisms depend largely on adhesive interactions between cells and substrates, which can be either extracellular matrix (ECM)* material or the surface of other cells (2). To understand the functions of morphoregulatory molecules in the early development of the central nervous system, avian neuroretina has been frequently used as model. Indeed, it is a functional nervous unit with a highly stratified organization. Furthermore, avian retina is easily accessible in early (days 5–7) embryos and is free of vascularization and of contaminating connective tissue.

Numerous cell adhesion molecules have been identified in the nervous system and implicated in cell aggregation, attachment, and migration, and in neurite outgrowth and differentiation (3). Secreted glycoprotein constituents of basal lamina and ECM are one class of “neurile-promoting molecules”; they include laminin, fibronectin, vitronectin, thrombospondin, tenascin, collagens, and a variety of glycosaminoglycans and proteoglycans, which are localized in extracellular spaces in developing neural tissues (4–8).

Vitronectin is present very early in avian development from ED 2.5 in quail embryos (9) and from ED 5 in chick retina, optic stalk, and nerve (10). Vitronectin is a multifunctional adhesive glycoprotein originally defined as S protein (11), produced primarily in the liver and identified in various tissues, including blood. There is clear experimental evidence that vitronectin is a regulatory link between cell adhesion, humoral defense mechanisms, and cell invasion (12, 13, 14). Vitronectin promotes cellular attachment, spreading and migration of a wide variety of cell types. A vitronectin-like molecule has been identified in chick egg yolk (1). However, although yolk vitronectin has a cell-spreading activity similar to blood vitronectin, the size of the molecules, heparin and collagen binding activities, and bound carbohydrates are different.

Integrins are primarily responsible for the attachment of cells to the ECM (15). Several reports show that the binding of integrins to the adhesion proteins is at least partially mediated by the interaction of integrin with the short hydrophilic amino acid sequence RGD, which is present in many ECM components, including fibronectin, vitronectin, laminin, collagens, thrombospondin, and other adhesive proteins (16). This cell-binding domain is also involved in determining cell morphology, in differentiation and division, and in facilitating cell locomotion (17).

We report here a new cDNA that encodes a protein named nectinepsin. The nectinepsin cDNA identifies a major 2.2-kb mRNA that is increasingly expressed in neuroretina during embryonic development. A nectinepsin mRNA is also found in mouse retina and in quail liver, brain, and intestine. The deduced amino acid sequence presents the integrin-binding sequence RGD, the somatomedin B domain and the hemopexin repeat homology unit of the pexin family (18), which includes replication-competent avian vector; PCR, polymerase chain reaction.

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† C. Blancher was supported by Ministère de la Recherche et de la Technologie Grant 91776 and by Association de Recherche sur le Cancer fellowships.
‡ Present address: Institut de Génétique et Microbiologie, Université Paris-Sud, CNRS Unité de Recherche Associée 1354, BATiment 400, 91405 Orsay cedex, France.
¶ To whom correspondence and reprints request should be addressed. Tel: 33-1-44-41-61-71; Fax: 33-1-44-41-61-72.
§ Present address: Institut de Génétique et Microbiologie, Université Paris-Sud, CNRS Unité de Recherche Associée 1354, BATiment 400, 91405 Orsay cedex, France.
¶ To whom correspondence and reprints request should be addressed. Tel: 33-1-44-41-61-71; Fax: 33-1-44-41-61-72.
1 The abbreviations used are: ECM, extracellular matrix; ED, embryonic day; PA-I, plasminogen activator-inhibitor 1; kb, kilobase; RCAS,
hemopexin (19, 20), vitronectin (21, 22), and most metalloproteinases.

EXPERIMENTAL PROCEDURES

Cell Culture Conditions—Primary cultures of quail neuroretina cells were obtained after dissection and dissociation of 7-day quail embryos (Coturnix coturnix) as described previously (23). Cells were plated in Eagle’s basal medium containing 10% fetal bovine serum (complete medium) and incubated for one week at 36°C.

RNA Isolation, Blotting, and Hybridization—Total RNA was purified by a guanidine-isothiocyanate/cesium chloride method (24), denatured at 60°C in a formamide/formaldehyde mixture and fractionated by electrophoresis on 1% agarose gels. As a check on the quality and quantity of RNA loaded, agarose gels were stained with ethidium bromide and the RNA was visualized under UV light before Northern blotting. RNAs were transferred to a nylon membrane (Hybond-N, Amersham Corp.) in 20× standard saline citrate and hybridized overnight at 42°C under standard conditions (25), with cDNA probes labeled by random primer synthesis (Multiprime cDNA labeling kit, Amersham Corp.).

DNA Isolation, Blotting, and Hybridization—Genomic DNA was extracted from whole bodies of 3 day post-hatching quail, following standard procedures (25). Ten micrograms of DNA digested independently with the restriction enzymes EcoRI, BamHI, and HindIII were separated on a 1% agarose gel, treated with 0.4N NaOH for 10 min and alkali blotted onto a nylon membrane (Hybond-N, Amersham Corp.), using 0.4 N NaOH. After washing three times in 6× standard saline citrate, the nylon filter was prehybridized and hybridized in 5× standard saline citrate, 5× Denhardt’s solution, 0.5% (w/v) SDS, and 100 μg/ml herring sperm DNA, according to the manufacturer’s instructions. The complete nectinepsin cDNA and the nectinepsin-specific cDNA fragment (described in Fig. 1) were labeled by random primer synthesis and used as probes (Multiprime cDNA labeling kit, Amersham Corp.). The final washes were done in 1× standard saline citrate/0.1% SDS.

cDNA Library Construction—Total RNA was isolated from 2 day post-hatching quail neuroretina. Poly(A)+ RNAs were then isolated by oligo(dT) cellulose chromatography (26). The library was constructed according to the method described by Gubler and Hoffman (27) and primered with oligo(dT) and random hexanucleotides. Double-stranded cDNAs were size-fractionated on a 5%–20% (w/v) sucrose gradient. After ligation to EcoRI linkers, cDNA fractions were ligated into the EcoRI site of the vector λgt11 according to the manufacturer’s instructions (Amersham Corp.).

cDNA Sequence Analysis—cDNA sequences were determined by the double-stranded DNA sequencing method (dideoxy-chain termination procedure) with the T7 sequencing kit as described by the manufacturer (Pharmacia). Sequences were initiated with T3, T7, and internal primers.

In Situ Hybridization—In situ hybridizations were performed on eyes and cerebellum sections as described previously (28). We used as probes (a) a digoxigenin-labeled RNA probe (RNA-labeling kit, Boehringer Mannheim), using entire nectinepsin cDNA; and (b) a 30-mer oligonucleotide nectinepsin-specific probe 3’-CCGTCGTTGTGTCTGAGGG-GACTCCTGTGTGGTTGTCC-5′ tail were digoxigenin-labeled nucleotide (dUTP) as described by the manufacturer (oligonucleotide tailing kit, Boehringer Mannheim).

Translation of Synthetic mRNAs in Vitro—To synthesize mRNAs in sufficient quantities, template cDNA was transcribed in vitro using the SP6/T7 Translation kit as recommended by the supplier (Boehringer Mannheim). To synthesize capped mRNAs, the protocol was modified by adding an analog of the cap structure (m7G(5′)ppp(5′)G) (Boehringer Mannheim) to the transcription reaction. In addition, after a 90-min incubation at 37°C, 10 units of T3 or T7 RNA polymerase were added to each reaction mixture, which was then incubated for 1 h more. Then, 20 units of RNase-free DNase I was added and mixtures were incubated for 15 min at 37°C. Capped mRNAs were then purified by phenol/chloroform extraction and LiCl/ethanol precipitation. These mRNAs were translated using the Translation kit, Reticulocyte type II as recommended by the suppliers (Boehringer Mannheim).

Incorporation of Retina Extracts—Primary cultures of 7-day quail embryo neuroretinas and whole retinas from 3 day post-hatching quail were metabolically labeled for 16 h in the presence of [35S]methionine and [35S]cysteine (Amersham Corp.) at a concentration of 200 μCi/ml. The cultured cells and retinas were then washed twice with phosphate-buffered saline and were lysed as described under “Western Blot Analysis.” The lysates (200 μg of protein per sample) were precleared with Staphylococcus aureus protein A (Pansorbin, Calbiochem) and immunoprecipitated overnight at 4°C with anti-human vitronectin antisera (Life Technologies, Inc.) or with normal rabbit serum, diluted 1:200. Immune complexes were collected on Pansorbin, washed three times with lysis buffer, resuspended in Laemmli sample buffer, and analyzed by 10% SDS-PAGE.

Western Blot Analysis—Tissues were homogenized in 20 volumes of the following buffer: 50 mM Tris-HCl pH 7.4, 250 mM sucrose, 10 mM EGTA, 1% Triton X-100, 1 mM leupeptin, 1 mM phenylmethylsulfonyl fluoride, 1% Triton X-100. After 30 min at 4°C, homogenates were cleared by centrifugation at 10,000 × g for 15 min and supernatants were immediately treated for electrophoretic separation.

For immunoblot analysis, after separation by SDS-PAGE (10%), proteins (50 μg per sample) were transferred onto a nitrocellulose filter by electroblotting. After transfer, nonspecific binding sites were blocked by incubating the membrane in phosphate-buffered saline buffer containing 5% nonfat dry milk. The membrane was then incubated with the rabbit anti-human vitronectin polyclonal antisera (Life Technologies, Inc.) at 1/2000 dilution, in phosphate-buffered saline buffer with 5% nonfat dry milk, 0.1% Tween 20. Negative controls were performed with normal rabbit serum at the same dilution. Immunoreactivity was visualized with a goat anti-rabbit immunoglobulin (dilution 1/4000) conjugated to peroxidase (Caltag Laboratories) and enhanced by chemiluminescence reagents, according to the manufacturer’s protocol (Amersham Corp.).

PCR—The polymerase chain reaction (PCR) was performed by using 250 μM dNTP, 1.5 mM MgCl2, PCR buffer 1× (Life Technologies, Inc.), 0.5 μM oligonucleotide primers, 2.5 units Taq polymerase (Life Technologies, Inc.), in a final volume of 50 μl nectinepsin cDNA (150 ng), and standard PCR mixture were initially denatured at 94°C for 3 min. The samples were then subjected to 30 rounds of amplification (94°C for 45 s, 60°C for 30 s, and 72°C for 90 s).

Cloning of Nectinepsin into the RCAS Vector—A two-step cloning strategy was used to clone the specific portion (372–585 bp) of nectinepsin cDNA into the replication-competent avian vector RCAS (29). Synthetic oligonucleotide primers containing 5’ and 3’ ClaI sites were introduced by PCR. The 5’ RCAS was 5’-CCCCCCATCGGCA-TGCTCCGCGTACACCCGAC3’; the 3’ RCAS was 5’-CCCCCATCGGTCATCTCCCGAGGC3’. The second step involved cloning of the digested PCR product into the RCAS vector and isolation of recombinant plasmids. The sequences of RCAS sense and antisense plasmids were confirmed by DNA sequence analysis.

The recombinant RCAS vectors were transfected into PA12 White Leghorn chicken embryo fibroblasts by CaPO4 precipitation (30). Protein extracts from transfected fibroblast cultures were analyzed as described under “Western Blot Analysis.”

RESULTS

Cloning and Characterization of Nectinepsin cDNA—The nectinepsin cDNA clone was unexpectedly isolated from a λgt 11 total cDNA library prepared from post-hatching quail neuroretinas. The cDNA fragment was 1.2 kb long and hybridized with a major mRNA of 2.2 kb and a minor mRNA of 4.2 kb in neuroretinal RNA preparations. Its sequence was determined; it contains an uninterrupted open reading frame of 1077 nucleotides. The deduced amino acid sequence (359 residues) has an expected molecular mass of about 40 kDa and a pi of 5.16 (Fig. 1). The deduced nectinepsin sequence shows an overall similarity of up to 60% with vitronectin from other species (21, 22). It is most similar to human vitronectin. The functional and structural domains of human vitronectin (31) and nectinepsin can be aligned and show very similar organizations (Fig. 2, D and E).

The first 18 amino acids of both proteins (50% of similarity) appear to be a leader peptide, characteristic of membrane-bound and membrane-secreted proteins, since they are very hydrophobic and end with an alanine residue, which is a suitable cleavage site for signal peptidase (32). Between this signal peptide and amino acid 62, the sequence of both proteins is 100% identical. This domain encodes for a somatomedin B peptide (33). Amino acids 63–65 are the RGD cell binding motif; they are followed by a stretch of 13 highly acidic amino acids (60% of identity), in which Tyr-74 and probably Tyr-77 are posttrans-
lationally sulfated in vitronectin (34). Between amino acids 79 and 149, there is a hinge domain weakly hydrophobic with a large number of prolines but otherwise dissimilar in the two proteins (10% of homology). In nectinepsin, this domain is dissimilar to all databases sequences. This region is followed by the “hemopexin repeat” domains, conserved in the pexin family (Fig. 2, A–C): hemopexin (19, 20), vitronectin, and most of the metalloproteases, including interstitial collagenase (35) and stromelysin (36). The sequence of these hemopexin repeat domains can be aligned with the Hydrophobic Cluster Analysis method (HCA plot software, Doriane S.A.) and shows a great conservation in all these molecules (data not shown). Thus, hydrophobic amino acids involved in the secondary structures (α helix and β sheets) clearly show repeated clusters common to these proteins (31). Almost all the mature protein sequence of hemopexin is constituted in two border units (0 in Fig. 2) and eight repeated units (1–4 in Fig. 2) organized into two domains (hemopexin repeat) joined by a short hinge region. The amino-terminal half of metalloprotease members consist of an enzymatic domain proper to each molecule, and the carboxyl-terminal half is the hemopexin repeat domain.

Vitronectin and nectinepsin have a hemopexin repeat domain without the fourth repeat and the carboxyl-terminal part of the second repeat. The second hemopexin repeat of vitronectin contains an additional heparin binding domain that replaces the carboxyl-terminal part of the second repeat and the amino-terminal part of the third repeat. Nectinepsin contains a consensus motif of eukaryotic aspartyl protease active site; a potential N-glycosylation site at Asn-167; potential phosphorylation sites for protein kinase C at Ser-155, Ser-185, Ser-262, Ser-328, and Ser-358 and Thr-85, for casein kinase II at Thr-62, Thr-113, Thr-118, Thr-136, and Thr-287 and Ser-145, Ser-146, and Ser-310, and for tyrosine kinase at Tyr-264; a potential N-myristoylation site at Gly-168 and Gly-247 and potential microbodies carboxyl-terminal targeting signal at Cys 57, Ala 59, and Gly 349 (Prosite data file; Ref. 37). Thus nectinepsin mRNA encodes a protein with interesting features that suggest that it could be a new ECM protein. Indeed, the structure of nectinepsin is different from that of vitronectin due to the presence of a specific domain, its small size, and the lack of heparin and collagen binding site. Nectinepsin appears to have a similar, if not identical, structure to the biochemically characterized yolk vitronectin (1). Indeed, yolk vitronectin has been described as a distinct form of vitronectin, preferentially found in chick egg yolk plasma, with a molecular mass smaller than blood vitronectin (45 and 54 kDa for the yolk to 65 and 70 kDa for the vitronectin). Yolk vitronectin contains the RGD cell binding sequence but not the heparin or collagen binding domains of vitronectin. Moreover, the amino-terminal microsequence of the 45-kDa yolk vitronectin protein, derived from the 54-kDa protein by cleavage of the somatomedin B peptide, is very similar to the beginning of the specific domain of nectinepsin and not to the vitronectins of corresponding sequences of human (21, 22), rabbit (38), and mouse (39) (Fig. 3).

Northern Blot Analysis—We investigated the production of nectinepsin transcripts in neuroretina during development (Fig. 4) using a nectinepsin-specific probe. The number of bands increased in parallel until 3 days post-hatching. In some experi-
ments, a very faint 6.5-kb transcript was observed (data not shown). To determine whether nectinepsin is produced outside of the retina, we screened various tissues for nectinepsin mRNA 3 days post hatching. The 2.2-kb mRNA was abundant in brain and liver and at a much lower level in intestine (Fig. 5). No signal was detected in lung and heart.

In Situ Hybridization—We used in situ hybridization in neuroretina to follow nectinepsin mRNA during proliferation, migration, and differentiation. These experiments were performed on retina sections at various stages, using a digoxigenin-labeled riboprobe of entire cDNA and a digoxigenin-tailed oligonucleotide nectinepsin-specific probe (Fig. 1). The pattern of labeling was similar with both probes.

Nectinepsin mRNA was detected from ED 5 in neuroretina, the first stage that we have investigated (data not shown). On embryonic days 8 and 9, when retina is close to the end of proliferation but lamination and differentiation processes continue, staining was mainly localized in ganglion cells, amacrine cells, and soma and processes of numerous migrating neuronal cells (Fig. 6A). At this stage, only the ganglion and amacrine cells have stopped their proliferation and are organized in distinct cell layers.

On embryonic day 14, when the cells are quiescent and retina is organized, labeling was observed mainly in ganglion and amacrine cells (data not shown).

At 3 days (Fig. 6A) and 1 month (data not shown) post-hatching, the labeling pattern was similar to that of ED 14 but more intense.

In addition, nectinepsin mRNA was detected by in situ hybridization in the same cell layers in adult mouse retina (Fig. 6B). To analyze nectinepsin mRNA expression in other regions of the CNS, we also performed in situ hybridizations on cerebellum sections of 2 day post-hatching quails. A strong hybridization signal was detected in granule cells of the cerebellum and a few labeled cells were also observed in the molecular layer. These cells might be Lugaro, stellate, or basket cells (data not shown).

Southern Blot Analysis—The nectinepsin-specific probe hybridized with only a single fragment in Southern blot of EcoRI, BamHI, or HindIII digested DNA (Fig. 7A). Thus nectinepsin 2.2- and 4.2-kb mRNAs are encoded by a single gene and might
be the result of differential splicing. In addition, Southern blot analysis using the entire cDNA probe, which contains sequence similarities with vitronectin, identified two additional fragments (Fig. 7B). These results suggest that nectinepsin and vitronectin are encoded by two different genes.

Characterization of Nectinepsin Protein—In vitro translation of mRNAs transcribed from the nectinepsin cDNA resulted in three bands corresponding to polypeptides of about 18, 32, and 46 kDa. The apparent molecular mass of the latter is consistent with the expected molecular size of nectinepsin (data not shown). We then investigated the presence of nectinepsin protein in different tissues. The immunoprecipitations and Western blot analysis were probed with an anti-human vitronectin antiserum. This antibody was chosen because of the substantial similarity between human vitronectin and quail nectinepsin, such that the antibody may bind both vitronectin and nectinepsin.

Embryonic day 7 retina cultured cells (data not shown) and 3 day post-hatching quail neuroretina (Fig. 8) were metabolically labeled with [35S]methionine and [35S]cysteine. In both experiments, a 54-kDa protein was specifically immunoprecipitated, in addition to the 70-kDa vitronectin band (Fig. 8A). The molecular mass of the 54-kDa protein is similar to that reported for yolk vitronectin (1).

In addition, protein extracts from 3 day post-hatching quail retina and lung (Fig. 8B) were analyzed by immunoblotting; the 54-kDa immunoreactive protein was only present in quail retina and not detected in lung, which is in agreement with the nonexpression of the nectinepsin mRNA in this tissue. The 54-kDa immunoreactivity was also observed in quail liver, brain, and cerebellum, mouse brain, and rabbit retina extracts (data not shown).

Inhibition of Protein Production with a RCAS Vector Containing the Antisense Nectinepsin Sequence—To confirm that the 54-kDa protein seen in Western blot with an anti-human vitronectin antiserum is indeed nectinepsin, we have carried out experiments to specifically inhibit nectinepsin production. Preliminary analysis on chicken embryo fibroblasts has shown the presence of nectinepsin transcripts (data not shown). Therefore, chicken embryo fibroblasts were transfected with a RCAS (29) containing only the nectinepsin-specific sequence, in sense or antisense orientations, as described under “Experimental Procedures.”

Two weeks after infection, protein extracts of control and transfected fibroblasts were analyzed by Western blot with the anti-human vitronectin antiserum used above (Fig. 9). The 54-kDa band was only detected in control (data not shown) and RCAS sense-transfected fibroblasts, but not in RCAS antisense-transfected fibroblasts. In addition, the two protein extracts showed a 70-kDa band corresponding to vitronectin as well as a nonspecific 60-kDa band. These results confirm that the 54-kDa band corresponds to the nectinepsin cDNA deduced protein.

**DISCUSSION**

We report a cDNA encoding a protein that we have named nectinepsin. A major corresponding transcript is present in large amounts in the quail neuroretina, brain, and liver and in lower amounts in the intestine. Furthermore, a similar mRNA is present in mouse neuroretina.

The mRNA encoding nectinepsin in neuroretina is present on embryonic day 5, the first stage that we investigated. Its level increases during cell migration and lamination of the neuroretina. *In situ* hybridization experiments show that somas and probably processes of numerous migrating neuronal cells contain this mRNA. As soon as lamination ends (day 11 of embryogenesis), the nectinepsin mRNA staining is located in ganglion cells and in a part of the inner nuclear layer, corresponding to the amacrine cells. This labeling intensifies until the postnatal stage and is strong in adults.

The deduced amino acid sequence of nectinepsin is 60% identical to human vitronectin. Thus, nectinepsin shares the RGD motif involved in cellular adhesion mediated by several integrins (15), somatomedin B peptide, and hemopexin repeat domains of vitronectin. These similarities and the fact that synthetic peptides containing the RGD tripeptide inhibit the attachment of cells to vitronectin (40) make it virtually certain that the RGD sequence represents the cell attachment site of vitronectin.
nectinepsin. However, the presence of a specific domain downstream from the RGD motif and the lack of the heparin and collagen binding domains of the vitronectin indicate that nectinepsin is a new extracellular matrix protein. This new protein is a member of the pexin family, which includes hemopexin, vitronectin, and most matricial metalloproteases, which all contain a similar hemopexin repeat domain (18). It appears that nectinepsin is the molecule most homologous to vitronectin; however, Southern blot suggests that they are encoded by different genes. Similarity between vitronectin and nectinepsin allowed the use of an anti-human vitronectin antisera for immunoprecipitation and Western blot analysis of nectinepsin. An immunoreactive protein of about 54 kDa was detected in all tissues containing nectinepsin mRNA, in addition to the 70-kDa specific vitronectin band. The deduced nectinepsin amino acid sequence has an expected molecular mass of about 40 kDa, which could be shifted to 54 kDa by posttranslational modifications. Indeed, the fact that the 54-kDa band corresponds to the nectinepsin cDNA is demonstrated by the specific inhibition of this band in protein extracts of fibroblasts transfected with a RCAS (29), containing nectinepsin-specific cDNA sequence in antisense orientation. Therefore, it is likely that the lower protein(s) seen in many previous reports using antivitronectin antibodies correspond(s) to nectinepsin.

One of the original reports (41) using the monoclonal antibody 8E6, which has become the reference anti-human vitronectin antibody, shows in human plasma a protein of about 50 kDa that does not bind to heparin-Sepharose and is compatible to nectinepsin.

Vitronectin was reported in the chick embryonic neural retina by Neugebauer et al. (10). At ED 6, vitronectin was detected with an anti-chick vitronectin antibody in the extracellular spaces of the neural retina, and the strongest staining was observed on the basement membrane adjacent to the optic fiber layer. At ED 12, vitronectin staining was most intense in the optic fiber layer, in the inner and outer plexiform layers, and surrounding cell bodies of the ganglion cell layer. In addition, Western blot analysis with this anti-chick vitronectin antibody gave a weak 70-kDa protein band in the ED 7 and ED 11 retina, whereas in the ED 7 vitreous humor, large amounts of a 70-kDa protein and four minor proteins of about 28, 41, 45, and 54 kDa were found. The last two proteins are likely nectinepsin proteins. Furthermore, the localization of vitronectin immunoreactivity in neuroretina is in agreement with the distribution of nectinepsin mRNA. It would be interesting to know whether the same cells produce both vitronectin and nectinepsin during the development of the neuroretina.

Delannet et al. (9) also reported, in addition to the vitronectin 78- and 68-kDa proteins, a 43-kDa protein recognized by a monoclonal antibody raised against human vitronectin and present in quail embryos at the time of the neural crest development. Furthermore, using polyclonal antibodies to chicken vitronectin, they investigated by immunoprecipitation quail neural crest cells proteins metabolically labeled with [35S]methionine. They detected proteins of about 78 and 68 kDa corresponding to vitronectin and other bands of lower molecular masses, including two abundant proteins of about 54 and 45 kDa. It is likely that the proteins of 54 and 45 kDa reported by these authors are related to the nectinepsin described in this study.

Nectinepsin also contains the somatomedin B domain of vitronectin, which regulates plasminogen activator-plasmin system, which appears to play a central role in the pericellular cascade of proteolytic activity (43, 44). In this context, it is interesting to note that in a previous report, Seiffert et al. (45) have identified a 57-kDa ECM protein that is recognized by an anti-bovine vitronectin antibody and bound to PA-I 1. This experiment is in agreement with the binding of PA-I 1 to nectinepsin.

In nectinepsin, as in vitronectin, the cell-attachment sequence RGD is directly adjacent to the carboxy-terminal boundary of the somatomedin B domain (46). In spite of this, the binding of PA-I 1 to vitronectin does not appear to affect the cell attachment properties of this adhesive glycoprotein (47). Nevertheless, the RGD sequence at the carboxy-terminal boundary of the somatomedin B domain (46) may be destroyed by proteolysis between Thr-44 and Arg-45 or Arg-45 and Gly-46, thus releasing the somatomedin B peptide (48). These results pose interesting questions regarding the possible role(s) of nectinepsin in the extracellular compartment. The ECM binds many growth factors, proteases, and protease inhibitors (49). These interactions not only position these molecules in the pericellular environment but also modulate their biological activity. Thus, nectinepsin might be involved in releasing growth factors stored in the ECM or in the activation of latent growth factors. Furthermore, the RGD motif may allow nectinepsin to link to integrins and thus to localize in the vicinity of the ECM proteins. Therefore, nectinepsin might be involved in regulatory mechanisms between cellular adhesion and ECM degradation. This hypothesis is particularly interesting with regard to the persistent presence of nectinepsin mRNA in neuroretina after the end of development, in contrast to vitronectin.

The vitronectin form found in chick egg yolk plasma and in small amounts in blood plasma by Nagano et al. (1) could correspond to our cDNA-deduced product, nectinepsin. Indeed, the amino-terminal microsequence of the 45-kDa yolk vitronectin protein shows a strong identity with the beginning of the nectinepsin-specific domain, which is absent from vitronectin.

The presence of the 54- and 45-kDa proteins described by Nagano et al. (1) in the chick egg yolk indicates that nectinepsin is probably expressed very early, even before the development. It is noteworthy that Nagano et al. did not detect vitronectin and fibronectin in the chick egg yolk, and thus nectinepsin appears to be expressed first. In egg-laying species, the developing embryo depends completely on the egg components for its physiological and nutritional requirements. Cell adhesion and migration are important during early development, and the abundance of nectinepsin but not vitronectin and fibronectin suggests that this new adhesion protein may serve as a main component in organization of distinctive territories of the developing embryo. Furthermore, the very recent experiment in which vitronectin homozygous null mice demonstrate normal development, fertility, and survival (50) indicates that nectinepsin may have an important role.

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