Nerve Terminal Anchorage Protein 1 (TAP-1) Is a Chondroitin Sulfate Proteoglycan: Biochemical and Electron Microscopic Characterization

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Abstract. The plasma membranes of the nerve terminal and the postsynaptic cell of electric organ are separated by a basal lamina. We have purified, biochemically characterized, and visualized in the electron microscope a macromolecule which appears to anchor the nerve terminal to this basal lamina. This molecule, terminal anchorage protein 1 (TAP-1) is associated with the nerve terminal membrane of electric organ, has the properties of an integral membrane protein, and is tightly bound to the extracellular matrix (Carlson, S. S., P. Caroni, and R. B. Kelly. 1986. J. Cell Biol. 103:509–520). TAP-1 can be solubilized from an electric organ extracellular matrix preparation with guanidine-HCl/3-[(3-cholamidopropyl)-dimethylamminio]-l-propane sulfonate and purified by a combination of permeation chromatography on Sephacryl S-1000, sedimentation velocity, and ion exchange chromatography on DEAE Sephacel. The total purification from electric organ is 91-fold and results in at least 86% purity. Digestion of the molecule with chondroitin ABC or AC lyase produces a large but similar shift in the molecular weight of the molecule on SDS-PAGE. The presence of chondroitin-4- or 6-sulfate is confirmed by identification of the isolated glycosaminoglycans with cellulose acetate electrophoresis. Gel filtration of the isolated chains indicates an average molecular weight of 42,000. Digestion of TAP-1 with other glycosaminoglycan lyases such as heparitinase indicates that only chondroitin sulfate is present. These results demonstrate that TAP-1 is a proteoglycan. Visualization of TAP-1 in the electron microscope reveals a “bottlebrush” structure expected for a proteoglycan. The molecule has an average total length of 345 ± 17 nm with 20 ± 2 side projections of 113 ± 5 nm in length. These side projections are presumably the glycosaminoglycan side chains. From this structure, we predict that the TAP-1 glycosaminoglycan side chains should have a molecular weight of ~50,000, which is in close agreement with the biochemical studies. Both biochemical and morphologic data indicate that TAP-1 has a relative molecular weight of ≈1.2 × 10^6. The large size of TAP-1 suggests that this molecule could span the synaptic cleft and make a significant contribution to the structure of the nerve terminal basal lamina of electric organ.

The synaptic extracellular matrix (ECM) is capable of directing the precise rebuilding of the nerve terminal during nerve regeneration at the neuromuscular junction (Sanes et al., 1978; Sanes and Chiu, 1983; Sanes and Covault, 1985). The presence of a muscle fiber is not required during rebuilding. In muscle damaged so that it contains empty basal lamina sheaths without muscle fibers, the regenerating axon can locate the original synaptic site and reform a mature nerve terminal. A high percentage of these nerve terminals formed on empty synaptic basal lamina show precise rebuilding. The exocytotic zones of the presynaptic plasma membrane are reconstructed at their original position on the basal lamina (Sanes et al., 1978; Glickman and Sanes, 1983). Thus, components of the original synaptic basal lamina provide both identification cues as well as a synaptic foundation on which to build a new nerve terminal. These synaptic ECM components are presumably recognized by membrane proteins of the returning axon during the rebuilding process. Integral membrane proteins that link the mature nerve terminal to the synaptic basal lamina, nerve terminal anchorage proteins (Kelly et al., 1987), are likely candidates in this recognition. However, identification and purification of anchorage proteins from the neuromuscular junction is a formidable task, given the minute quantities of material available.

The electric organ of marine rays contains an abundance of synapses embryologically related to the neuromuscular junction. This tissue has served as a rich source of synaptic macromolecules, such as an ECM factor involved in the clustering of acetylcholine receptor and acetylcholinesterase.
(Fallon et al., 1985; Wallace et al., 1985). Recently, a molecule with the properties of a nerve terminal anchorage protein has been identified in electric organ (Carlson et al., 1986). This molecule is found on the nerve terminal surface, behaves as an integral membrane protein, and is tightly bound to an electric organ ECM fraction. However, the exact chemical nature of this molecule is not known. We now report the complete purification and characterization of this anchorage protein, called TAP-1 (terminal anchorage protein 1). TAP-1 under denaturing conditions is a large proteoglycan (relative molecular weight of ~1.2 million) containing chondroitin-4- or 6-sulfate side chains with a relative molecular weight ~42,000. When visualized in the electron microscope, TAP-1 has the typical proteoglycan "bottle brush" morphology with dimensions of ~3,500 x 2,100 Å. A synaptic molecule of this size could easily span the ~600-Å distance of the synaptic cleft and substantially contribute to the structure of the synaptic basal lamina.

**Materials and Methods**

**Materials**

Na<sup>125</sup>I was obtained from Amersham Corp. (Arlington Heights, IL); urea from Schwarz/Mann Div., Becton, Dickinson & Co. (Spring Valley, NY); di-[3-cholamidopropyl]-dimethylammonio)-propyl sulfate (CHAPS), Heps, SDS, diisopropylfluorophosphatase, iodoacetamide, pepstatin, leupeptin, chymostatin, guanidine-HCl, heparin, chondroitin sulfates A, B, and C, from Sigma Chemical Co. (St. Louis, MO); keratan sulfate and heparan sulfate from Miles Scientific (Naperville, IL). The electric ray, Discopyge ommata, was obtained from Marinus Inc. (Long Beach, CA).

**Purification of TAP-1**

TAP-1 was detected with a monoclonal antibody (mAb) directed against the synaptic vesicle antigen 4 (SV4) antigenic site in a nitrocellulose dot blot assay as described previously (Carlson et al., 1986). This assay was used throughout the purification to determine the TAP-1 concentration in the various fractions.

The progress of the purification was followed by monitoring the specific activity (i.e., TAP-1 antigenicity per microgram of protein) of the fractions generated by the isolation scheme. However, when measuring TAP-1, most protein assays were found to be too insensitive. Thus, a modified version of the filter-binding assay of Schaffer and Weissmann (1973) was used. The modifications were as follows. All sample dilutions were done in 8 M urea, 0.2% CHAPS, 50 mM Heps, pH 7.0. After the sample was applied to the nitrocellulose (0.45 μm) using a Bio-Dot microfiltration apparatus (Bio-Rad Laboratories, Richmond, CA), the nitrocellulose was stained overnight in 1 μl/ml India ink, 0.4 M NaCl, 10 mM Heps, 0.1% Tween 20, pH 7.0. As indicated by Hancock and Tsang (1983), the India ink must be Pelikan fount India drawing ink for fountain pens (Pellikan AG, Hanover, Federal Republic of Germany). The nitrocellulose was dried on a Slab Gel dryer ( Hoeffer Scientific Instruments, San Francisco, CA), and the stained samples on the nitrocellulose were quantitated with a Zeineh soft laser scanning densitometer, model SL-504-6L (Biomed Instruments, Inc., Fullerton, CA). A sample containing ECM proteins of known concentration was used as a standard in the assay. The assay was linear from 10 to 100 ng of protein.

The starting material for the TAP-1 preparation was an ECM preparation from electric organ (Carlson et al., 1986). The ECM pellet was solubilized by mixing each gram with 2 ml of 6 M guanidine-HCl, 2% CHAPS, 50 mM Heps, 10 mM EDTA, 0.02% sodium azide, pH 7.0. All solutions used in the purification were Millipore filtered (0.45 μm, Millipore Corp., Bedford, MA) before use. For each gram of pellet the following protease inhibitors were also added: 12 mg of iodoacetamide, 2.5 μl of diisopropylfluorophosphatase, and 25 μl of dimethylsulfoxide containing pepstatin, leupeptin, and chymostatin at 5 mg/ml. A homogenate was then generated with a Polytron (Brinkmann Instruments Co., Westbury, NY), using three 10-s bursts 10 min apart at 4°C. The homogenate was then mixed by rotation for 1 h; 6 μl of β-mercaptoethanol/g ECM pellet was added, then given 30 min more rotation mixing, and finally spun at 12,096 g for 30 min at 4°C.

All of the remaining purification steps were performed at room temperature. The 40-50 ml of ECM 12,096 g supernatant (obtained from ~300 g of electric organ) were applied to a 2.4-liter Sephacryl S-1000 column (5 x 122 cm) equilibrated with 4 M guanidine-HCl, 50 mM Heps, 0.2% CHAPS, 50 mM EDTA, 0.02% sodium azide, pH 7.0, 100 mg/liter of iodoacetamide, 8 μl/liter of diisopropylfluorophosphatase, and 50 μl/liter of dimethylsulfoxide containing 5 mg/ml each of pepstatin, leupeptin, and chymostatin (Sigma Chemical Co.). The flow rate was 50 ml/h and 17-ml fractions were collected. The peak fractions containing the highest specific activity (antigenicity per microgram protein) of TAP-1 were pooled and concentrated to a volume of ~15 ml with a YM 30 membrane in an Amicon Stirred Cell (Amicon Corp., Danvers, MA). Before concentration, protease inhibitors were added: for each milliliter of the pooled fractions, 0.5 mg of iodoacetamide, 0.04 μl of diisopropylfluorophosphatase, 0.25 μl of dimethylsulfoxide containing 5 mg/ml each of pepstatin, leupeptin, and chymostatin. The concentrated pool of Sephacryl S-1000 fractions containing TAP-1 was spun at 12,500 g for 15 min, and the resulting supernatant was subjected to sedimentation velocity centrifugation at 35.5 ml of 5-20% sucrose gradients containing all the constituents of the Sephacryl S-1000 column buffer. Three ml of the concentrated pool was applied to each gradient. Typically about five gradients were run for 300 g of starting electric organ tissue. The gradients were spun at 20°C for 18 h at 25,000 rpm in an SW-28 rotor (Beckman Instruments, Inc., Fullerton, CA), and 1.5-ml fractions were collected for each gradient. The peak fractions containing the highest specific activity (antigenicity per microgram of protein) from all the gradients were pooled and concentrated to 3-4 ml as described above.

For the last step in the purification, the concentrated TAP-1 solution obtained from sedimentation velocity centrifugation was chromatographed on DEAE Sephacel. The concentrated sample was spun at 12,500 g and the resulting supernatant diluted 1:40 with a solution containing 8 M urea, 0.11 M NaCl, 50 mM sodium acetate, 0.2% CHAPS, 2 mM EDTA, 0.02% sodium azide, 0.05 μg/ml iodoacetic acid, and 0.04 μl/ml diisopropylfluorophosphatase, pH 4.9. The diluted sample was applied to an 8 ml (0.5 x 10 cm) DEAE Sephacel column equilibrated with the same 8 M urea buffer used for the dilution. The column was then washed with 50 ml of this equilibration buffer and eluted in the forward direction with a 150-ml linear NaCl gradient. The gradient was formed with 75 ml of the equilibration buffer and 75 ml of equilibration buffer containing 1.4 M NaCl (instead of 0.11 M). 2-ml fractions were collected at a flow rate of ~30 ml/h.

**SDS-PAGE of Glicosaminoglycan Lyase-digested TAP-1**

TAP-1 was subjected to SDS-PAGE to evaluate purity of the TAP-1 preparation and determine the effects of glycosaminoglycan lyase digestion. TAP-1 was iodinated with <sup>125</sup>I as previously described (Carlson et al., 1986) either in a solution of 8 M urea, 0.2% CHAPS, 50 mM Heps, pH 7.0, or the same solution with 4 M guanidine-HCl rather than urea. Both completely purified TAP-1 and partially purified TAP-1 by DEAE Sephacel step (and partially purified material (purified through the sedimentation velocity step) were iodinated. In some cases partially purified <sup>125</sup>I-TAP-1 was completely purified by the DEAE Sephacel step of the purification or by immunoprecipitation with the anti-SV4 mAb.

To digest <sup>125</sup>I-TAP-1 with chondroitin ABC lyase, procedures similar to those of Oike et al. (1980) were used. The reaction mixture (20-30 μl, total volume) contained 0.1-0.01 μg of <sup>125</sup>I-TAP-1, 5-10 μl of chondroitin ABC lyase (Miles Scientific), 94 mM Tris/HCl, pH 8.0, 9 mM EDTA, 0.19% CHAPS, 0.05 mg/ml BSA, 0.9 mg/ml iodoacetamide, 0.4 mg/ml phenylmethanesulphonyl fluoride, and 0.1 mg/ml each of pepstatin, leupeptin, and chymostatin. (BSA and these protease inhibitors were added to prevent possible digestion of TAP-1 by contaminating proteases. On SDS-PAGE, the BSA showed no evidence of protease activity during any of the digestions.) The <sup>125</sup>I-TAP-1 was in 4 M guanidine-HCl, 8 M urea or 0.5% SDS before a 1:16 dilution into this reaction mixture. The digestion was carried out at room temperature for 3 h, then terminated by boiling after adding 20 μl of 4.8% SDS, 0.11 M Tris/HCl, 18% glycerol, 9.2% β-mercaptoethanol, 0.002% bromophenol blue, and 0.68. Digestions of <sup>125</sup>I-TAP-1 with chondroitin AC lyase (Miles Scientific) and keratanase (Miles Scientific) were done under the same conditions but at pH 7.4 and at concentrations of 9 U/ml and 2-5 U/ml respectively. Heparinase (Miles Scientific) and heparitinase (Miles Scientific) digestions of <sup>125</sup>I-TAP-1 were performed at pH 7.0, in 47 mM Heps, 2 mM calcium acetate, 19% CHAPS at enzyme concentrations of 13-25 U/ml; all other conditions were the same as used for chondroitin ABC lyase digestions. When chondroitin ABC lyase was used in combination with other glycosaminoglycan lyases, the digestion conditions of the other enzyme were used. In order to be sure a glycosami-
noglycan lyase was active under the digestion conditions used for TAP-1, a separate digestion was done with the enzyme and its substrate at a concentration of 2 μg of glycosaminoglycan (GAG)/μl. The products of these digests were analysed by cellulose acetate electrophoresis (Carlson, 1982) and stained with Alcian blue (Kanwar and Farquhar, 1979).

When chondroitin ABC lyase was used in combination with N-glycanase (Genzyme Corp., Boston, MA) to digest TAP-1, the chondroitin ABC lyase reaction buffer was used with the exception that 40 mM sodium phosphate, pH 8.6, was substituted for 94 mM Tris/HCl. N-glycanase was used at a concentration of 27 U/nil and the reaction was carried out at room temperature for 24 h. When TAP-1 was digested with both chondroitin ABC lyase and neuraminidase-Vibrio cholerae (Calbiochem-Behring Corp., San Diego, CA), the digests were performed sequentially. First the chondroitin ABC lyase digestion was carried out as described before, except that 38 mM Tris/HCl, pH 8.0, was used instead of 94 mM, and no EDTA was present. After a 3-h incubation at room temperature 270 μl of 75 mM sodium acetate, 2 mM calcium acetate, 0.2 mM EDTA, pH 5.0, and 10 μl of 1 U/ml of neuraminidase were added to the 30-μl reaction mixture. The reaction was incubated for 24 h at room temperature and then concentrated to 100 μl with a Centricon-30 (Amicon Corp.). 500 μl of 0.05% SDS, 0.1 M NH4HCO3, was added to the Centricon-30 and the resulting 600 μl was concentrated to 100 μl. The sample was then dried in preparation for SDS-PAGE.

Chondroitin ABC lyase digestions were also performed on TAP-1 which had been immunoprecipitated with the anti-SV4 mAb. To immunoprecipitate TAP-1, the 221I-labeled material was diluted sevenfold with 0.2 M NaC1, 1% SDS, pH 7.0, and incubated 30 min at room temperature. The sample was diluted another 22-fold with 0.4 M NaC1, 10 mM Hepes, 1.5% Triton X-100, 0.1% BSA, pH 7.0, and then immunoprecipitated with Immunobeads (Bio-Rad Laboratories) to which the anti-SV4 mAb had been previously bound. These procedures were as previously described (Carlson et al., 1986). The antigen was then freed from the Immunobeads by boiling the resuspended beads in 0.2 M NaC1, 10 mM Hepes, 0.5% SDS, pH 7.0, for 2 min. After removing the beads with a 12,000-g centrifugation for 10 min, an aliquot from the resulting supernatant was diluted about 1:16 with the chondroitinase reaction mixture containing 0.47% Triton X-100 instead of 0.19% CHAPS. The chondroitin ABC lyase digestion was then carried out as described above.

SDS polyacrylamide slab gel electrophoresis was performed with the buffer system described by Laemmli (1970). The gels were overloaded with 4 mg guanidine, 0.2% CHAPS with a Centricon-30. The concentrated TAP-1 sample was mixed with 110 μl of 0.5 M NaOH and 800 μl of 1.38 M sodium borohydride and heated to 45°C for 48 h. The reaction was terminated by adding 60 μl of concentrated acetic acid. The excess hydrogen gas was removed from the sample by evaporation for 10 min on a Speed-Vac (Savant Instruments, Inc., Hicksville, NY). The reaction mixture was then either dialyzed overnight (Spectra/Per 7, molecular weight cutoff 3,500, Spectrum Scientific, Inc., Houston, TX) against 2 liters 50 mM NH4HCO3 (two changes outer solution), or it was applied to a Sephacrose CL-6B column. 30 μl of a 5 mg/ml Phenol Red was added to the alkaline borohydride-digested TAP-1 as a chromatographic marker. The gel sample was applied to a Sephacrose CL-6B column (133 × 0.7 cm), and the chromatography was carried out according to Wasteson (1971). The column buffer was prefilled through DEAE nitrocellulose to reduce the background in the nitrocellulose-binding assay used to detect GAGs (see below). The Kay values for the half-height of the leading and trailing edges, as well as the central peak of the TAP-1 GAG chains were determined from the elution profile. The molecular weights corresponding to these elution volumes were then determined by using the calibration curves of Wasteson (1971) for chondroitin sulfate chains.

A nitrocellulose-binding assay was used to determine the GAG content of various column fractions which resulted during the chromatographic analysis of the isolated TAP-1 GAG side chains as well as during the TAP-1 purification. This assay for GAGs involves binding the samples to DEAE nitrocellulose and staining the paper with Alcian blue. For samples in guanidine-HCl or urea solutions, the DEAE nitrocellulose sheet was wetted with 6 M urea, 0.1 M NaCl, 0.2% CHAPS, 20 mM Hepes, 0.02% sodium azide, pH 7.3; the sheet was placed in the dot-blot manifold (see protein assay) above a sheet of regular nitrocellulose (for structural support); and the samples (diluted to 200–1,000 μl with the same buffer) were spotted. Samples in nondenaturing solutions were diluted in 0.1 M NaCl, 25 mM Hepes, pH 7.0, with three to four changes of solution. The DEAE nitrocellulose was dried and the stained samples quantitated as described for the protein assay. The assay was linear from 100–1,000 ng of heparin. The assay showed a specificity for GAGs over protein of at least 20 to 1. That is, chondroitin sulfate (type c) is stained 23 times more intensely per microgram than several purified proteins (BSA, ovalbumin, tryptsin, and lysozyme).

In order to identify the GAGs of TAP-1, the isolated chains were subjected to cellulose acetate electrophoresis. The peak fractions from the Sepharose CL 6B chromatography were pooled, dialyzed against 50 mM NH4HCO3, and lyophilized. Cellulose acetate electrophoresis, the staining of these electrophoretograms with 3H2O/Ruthenium Red, and quantitation of the resulting autoradiograms were performed as previously described (Carlson, 1982).

**Electron Microscopy**

Samples of purified TAP-1 (100 μg/ml) in 8 M urea, 50 mM sodium acetate, 0.65 M NaCl, 0.2% CHAPS, 2 mM EDTA, pH 4.9, were diluted 1:20 with 1 M ammonium acetate buffer, pH 5.0, to give a final concentration of 5 μg/mg protein. The sample was then mixed 1:1 with 0.01 M Tris, 0.01 M EDTA, pH 8.5, and enough of the following solution containing 25 mg/ml cytochrome c (Horse heart type III, Sigma Chemical Co.), 2 M Tris/HCl, 0.05 M EDTA, pH 8.5, was added to give a final concentration of 62.5 μg/ml cytochrome c. A 75-μl aliquot of sample was run continuously down a wet glass slide onto a deionized water surface. After spreading for 15 s, the monolayer film was touched by carbon stabilized Parlodion-coated grids and stained immediately with 0.0001% phosphotungstic acid in 90% ethanol for 5 s and 5 × 10-3 M uranyl acetate in 90% ethanol. After an alcohol rinse, the grids were air dried and rotary shadowed with platinum/palladium at an angle of 10° and a distance of 8 cm. Grids were examined with a JEOL 100 B electron microscope and photographed at a magnification of 25,000. Magnifications were calibrated using a Pelco magnification-calibration grid (Pelco, Redding, CA).

**Results**

**Purification of TAP-1**

Previously it has been shown that an antigenic determinant (SV4) identified by a monoclonal antibody is present on a synaptic vesicle proteoglycan and on another much larger protein in the ECM of electric organ (Carlson et al., 1986). This ECM protein also has the charge characteristics of a proteoglycan. It requires high salt (=0.7 M NaCl) to elute it from DEAE Sephacel. This molecule, called TAP-1, is found in an electric organ ECM fraction which it can be solubilized with 4 M guanidine-HCl and CHAPS (Carlson et al., 1986). Only low amounts of the synaptic vesicle proteoglycan are found in this fraction (Iwata, M., and S. S. Carlson, unpublished observations). Thus, this ECM fraction serves as a good starting material for the purification of TAP-1.

The scheme determined for the purification of TAP-1 from the ECM fraction involves three separation methods: (a) permeation chromatography on Sephacryl S-1000, (b) sedimentation velocity on sucrose density gradients, and (c) ion exchange chromatography on DEAE Sephacel. The purification is performed under denaturing conditions; 4 M guanidine-HCl with 0.2% CHAPS for steps a and b, and 8 M urea.
with 0.2% CHAPS for the last step. Throughout the purification, the TAP-1 content of the resulting fractions was determined by using the monoclonal antibody which binds the SV4 epitope in a dot-blot assay. Table I outlines the purification, the yield, and specific activity (antigenicity per microgram of protein) at each step.

The ECM fraction is an insoluble pellet made from electric organ by a series of high salt, low salt, and detergent extractions of electric organ tissue (Carlson et al., 1986). The components of this ECM pellet, including TAP-1, were solubilized by dissolving the fraction in 6 M guanidine/CHAPS extract of electric organ. The antigenic site (SV4) used to detect TAP-1 is also present on a synaptic vesicle proteoglycan (Carlson et al., 1986). Further, the 6 M guanidine/CHAPS extract contains both TAP-1 and the synaptic vesicle proteoglycan. To determine the total amount of TAP-1 in the extract, using SV4 antigenicity, the total amount of synaptic vesicle proteoglycan in the extract also had to be determined. This was done with a monoclonal antibody directed against an antigenic determinant (SV1) present only on the synaptic vesicle proteoglycan (Caroni et al., 1985). The total TAP-1 antigenicity was then calculated by correcting the total SV4 antigenicity in the extract for that contributed by the synaptic vesicle proteoglycan. In the ECM fraction the synaptic vesicle proteoglycan is only present in low amounts (Caroni et al., 1985; Carlson et al., 1986; Iwata, M., and S. S. Carlson, unpublished observations); therefore, this correction is not important for the subsequent steps of the purification.

### Table I. Purification of TAP-1*

| Purification step | Antigenicity per gram electric organ | Recovery | Specific activity | Fold purification |
|-------------------|-------------------------------------|----------|------------------|------------------|
| Electric organ§   | 221 x 10⁹ | 100 | 1,318 | 1 |
| ECM extract       | 185 x 10⁹ | 84 | 6,598 ± 472§ | 5 |
| Sepharose S-1000 column | 1.9% | 8.2 | 16,350 ± 2,044§ | 12.4 |
| Peak fractions    | 18.1 x 10⁹ | 8.2 | ND | ND |
| Side fractions    | 18.3 x 10⁹ | 8.3 | ND | ND |
| Velocity sedimentation | 4.3 x 10⁹ | 1.9 | 52,603 ± 8,483§ | 40 |
| Peak fractions    | 4.8 x 10⁹ | 2.2 | ND | ND |
| Side fractions    | 0.91 x 10⁹ | 0.41 | 120,000 ± 29,200§ | 91 |

ND, not determined.

* The data presented in this table represent three to four separate TAP-1 preparations.

§ 6 M guanidine/CHAPS extract of electric organ.

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Figure 1. Preparative sedimentation velocity centrifugation of TAP-1. (●) TAP-1 antigenicity and (▲) GAG concentration. The direction of the sedimentation is from right to left. 3 ml of a concentrated TAP-1 solution (isolated by chromatography on Sephacryl S-1000) was applied to a 35.5 ml of 5–20% sucrose gradient containing 4 M guanidine-HCl, 0.2% CHAPS, 50 mM Hepes, 10 mM EDTA, 0.02% sodium azide, pH 7.0, and protease inhibitors. The gradients were spun at 83,000 g, 20°C for 18 h, and 1.5-ml fractions were collected. Each point on the figure represents two determinations of TAP-1 antigenicity and three for the GAG concentration. For more experimental details see Materials and Methods.
Figure 2. TAP-1 elution profile for preparative ion exchange chromatography on DEAE Sephacel. (●) TAP-1 antigenicity, (△) GAG concentration, (●) protein concentration, and (◆) NaCl concentration. 2.8 ml of a concentrated TAP-1 solution (isolated by preparative sedimentation velocity centrifugation) was diluted 40-fold with 8 M urea, 0.11 M NaCl, 50 mM sodium acetate, 0.2% CHAPS, 2 mM EDTA, 0.02% sodium azide, 0.05 µg/ml iodoacetamide, 0.04 µl/ml diisopropylfluorophosphate, pH 4.9, and applied to an 8-ml DEAE Sephacel column equilibrated with the same 8 M urea buffer. After a 150-ml wash with this equilibration buffer, the column is eluted with a 150-ml linear gradient of NaCl. The gradient is formed with 75 ml of equilibration buffer and 75 ml of the same buffer containing 1.4 M NaCl instead of 0.11 M. 2.0-ml fractions were collected and the flow rate was 28 ml/h. Each point on the figure represents two determinations of TAP-1 antigenicity and three determinations of GAG and protein concentration. For more experimental details see Materials and Methods.

Fractions (115–134, Fig. 2) was 5.7 ± 0.5 (SEM). The specific activity of this peak increased 91-fold compared with the starting material. The total recovery was 0.41% (Table I).

The TAP-1 isolated from DEAE Sephacel appears to be at least 86% pure by SDS-PAGE. An electrophoretogram of this TAP-1 preparation labeled with ¹²⁵I is shown in lane E of Fig. 3. (Iodinations were done in the presence of 4 M guanidine or 8 M urea in CHAPS to ensure maximum accessibility of tyrosine residues to label.) As seen in the figure, TAP-1 enters only the very top of the 2.4–8% polyacrylamide gel, most likely due to its very high molecular weight (over 1 million as discussed below). It is the only major component in the preparation. The radioactivity at the front of the gel corresponds to molecular weights <22,000 and does not resolve into discrete components on lower percentage gels (data not shown). Densitometry of lane E shows that the minor components on the gel make up only 14% of the total radioactivity. In some preparations a smaller molecular weight (~500,000) antigenic component was also present (as indicated by the immunoprecipitation of this smaller molecule). From preparation to preparation this minor component varied from 4% to 30% of TAP-1. One possibility is that it is a breakdown product of TAP-1.

Fig. 3 demonstrates that purified TAP-1 is the same material identified by the monoclonal antibody directed against SV4. Partially purified TAP-1, labeled with ¹²⁵I (lane A), was immunoprecipitated with the anti-SV4 mAb (lane B). The SDS-PAGE behavior of the immunoprecipitated TAP-1 and the TAP-1 purified by permeation chromatography, sedimentation velocity, and DEAE chromatography is identical (compare lanes B and E). Further, the immunoprecipitated TAP-1 and purified TAP-1 also migrate identically after digestion with chondroitin ABC lyase. This is apparent from a comparison of lanes D and F.

As Table I shows, the difficulty in the purification of TAP-1 was due to the large losses that occurred during the fractionation of the solubilized ECM components. The recovery was 10% from chromatography on Sephacryl S-3000, 23% from velocity sedimentation, and 22% from ion exchange chromatography on DEAE Sephacel. These large losses are probably due to the tendency of TAP-1 to nonspecifically adsorb to various surfaces. Because the purification involves two chromatographic steps as well as three concentrations by ultrafiltration, the overall yield from this purification scheme is particularly susceptible to this problem.

TAP-1 Is a Chondroitin Sulfate Proteoglycan

Previous characterization of TAP-1 in partially purified extracts suggested that it is a proteoglycan. Under denaturing
conditions it was found to behave as a very large, highly negatively charged molecule (Carlson et al., 1986).

When TAP-1 was exposed to chondroitin ABC lyase, its mobility on SDS-PAGE changed substantially. Intact TAP-1 just enters a 2.4–8% gradient gel (Fig. 3, lanes B and E); enzymatic digestion of the GAG side chains causes TAP-1 to move with a significantly greater mobility (Fig. 3, lanes D and F).

To determine whether any other GAG chains were present in TAP-1, the purified molecule was digested with a variety of glycosaminoglycan lyases and subjected to SDS-PAGE. We considered this a real possibility, since the chondroitin ABC lyase digested TAP-1 continued to migrate as a very broad band, suggesting that the molecule was still heavily glycosylated. Both chondroitin ABC lyase and chondroitin AC lyase digestion changed the mobility of TAP-1 to about the same extent (Fig. 4, lanes b and d). This indicates that TAP-1 contains chondroitin-4- or 6-sulfate and not dermatan sulfate. The AC lyase will digest only 4- or 6-chondroitin sulfate chains, while the ABC lyase will digest dermatan sulfate as well as the chondroitin sulfates (Saito et al., 1968).

We found that heparitinase also changed the mobility of TAP-1 (Fig. 4, lanes h and k). This enzyme is specific for heparan sulfate chains. Most likely, however, this result was simply due to a chondroitin sulfate lyase contaminating the heparitinase. The mobility shift produced by heparitinase was smaller than the shift produced by chondroitinase digestion (compare lanes h and i in Fig. 4). When TAP-1 was digested with both heparitinase and chondroitin ABC lyase, the mobility change was no greater than that caused by chondroitinase alone (Fig. 4, lane g). Such a result could be produced from a partial digestion of chondroitin sulfate chains by low amounts of a contaminating chondroitinase in the heparitinase preparation. This explanation was confirmed when we found that the presence of chondroitin sulfate (1.9 µg/ml) in the reaction mixture inhibited the heparitinase-induced mobility change in TAP-1, whereas heparan sulfate at the same concentration had no inhibitory effect (compare lanes m and k, Fig. 4). Presumably, the high concentration of unlabeled chondroitin sulfate protected the very small amounts of 125I-TAP-1 from digestion by saturating the contaminating enzyme with substrate. This contaminating activity must be at relatively low concentration. Parallel reactions with the same GAG and heparitinase concentrations showed complete digestion of heparan sulfate, but little change in chondroitin sulfate concentration when analyzed by cellulose acetate electrophoresis. The overall conclusion is that TAP-1 does not contain heparan sulfate chains.

Both heparinase (Fig. 4, lane f), specific for heparin and heparin-like sequences in heparan sulfate, as well as keratanase (Fig. 4, lane c), specific for keratan sulfate, had no effect on TAP-1. (Under these same conditions, heparinase and keratanase showed complete digestion of their GAG substrates.) Thus, TAP-1 does not appear to contain heparin or keratan sulfate.

The chondroitin ABC lyase digested material most likely contains carbohydrate side chains in addition to chondroitin sulfate, in that even after digestion it migrates as a very broad band on SDS gels. A combination of chondroitin ABC lyase and N-glycanase or neuraminidase failed to affect the mobility of TAP-1 compared with chondroitin ABCase alone (data not shown). Because TAP-1 does not migrate as a discrete protein on SDS-PAGE even after these digestions, the molecule is probably still heavily glycosylated. Presumably it contains other O-linked sugars, as has been demonstrated for a variety of other proteoglycans (Hascall and Hascall, 1981).

**Size of TAP-1 and Its Chondroitin Sulfate Chains**

To determine an approximate size for the entire TAP-1 molecule by column chromatography, we subjected TAP-1 to gel filtration on Sephacryl S-500 in 4 M guanidine-HCl/0.2% CHAPS. On the same column a smooth muscle cell chondroitin sulfate proteoglycan was chromatographed which had a molecular weight estimated to be 1.26 × 10^6 (Iozzo et al., 1982). These chromatographic separations are shown on Fig. 5. TAP-1 eluted with a K_v of 0.27 and the smooth muscle cell proteoglycan with a K_v of 0.21. Therefore, we would expect TAP-1 to have a molecular weight similar to the smooth muscle proteoglycan.

The size of the GAG side chains was estimated by gel filtrat-
Figure 4. SDS-PAGE of TAP-1 after digestion with glycosaminoglycan lyases. Purified $^{125}$I-TAP-1 is shown before and after digestion with several glycosaminoglycan lyases on 2.4–8% linear gradient polyacrylamide gels; the resulting autoradiograms are shown. TAP-1 was digested with the following enzymes: (a) no enzyme, (b) chondroitin AC lyase, (c) keratanase, (d) chondroitin ABC lyase, (e) no enzyme, (f) heparinase, (g) chondroitin ABC lyase and heparitinase, (h) heparitinase, (i) chondroitin ABC lyase, (j) no enzyme, (k) heparitinase and 1.9 µg/ml heparan sulfate, (l) heparitinase, 1.9 µg/ml heparan sulfate, and 1.9 µg/ml chondroitin sulfate-type C, (m) heparitinase and 1.9 µg/ml chondroitin sulfate-type C, and (n) no enzyme. Only chondroitin ABC lyase (d, g, i), chondroitin AC lyase (b), and heparitinase (g, h, and k) cause a change in the mobility of TAP-1. However, the change in mobility due to heparitinase must be due to a contaminating chondroitin sulfate lyase, because no shift was observed when the reaction mixture contained 1.9 µg/ml chondroitin sulfate (l and m). The same concentration of heparan sulfate did not interfere with the heparitinase digestion of TAP-1 (k). The other glycosaminoglycan lyases, which showed no effect on TAP-I, gave complete digestion of their normal GAG substrates at a concentration of 1.9 µg/ml under the same reaction conditions used for TAP-1. BSA, which was included in the digestion buffer at 50 µg/ml (1.5 µg per lane), showed no evidence of protease activity for any of the digestions on the Coomassie-stained SDS gel. All digestions contained protease inhibitors. The standards used were myosin (200 kD), β-galactosidase (116 kD), phosphorylase B (92 kD), and BSA (66 kD). S and R indicate the beginnings of the stacking gel and running gel, respectively. The $^{125}$I-TAP used for these digestions was iodinated with $^{125}$I after the velocity sedimentation step (Table I) and then purified by chromatography on DEAE sephacel. 30–50 µl of sample was applied to each gel lane. For further experimental details see Materials and Methods.

Figure 5. Elution profile of TAP-1 from Sepharose CL-6B. The GAG chains were first cleaved from TAP-1 by alkaline borohydride treatment. The elution profile for one such chromatography is shown in Fig. 6. The results of three cleavages and chromatographic separations give a $K_v$ for the major peak of 0.36 ± 0.02 (SEM); the $K_v$'s for leading and trailing half heights were 0.26 ± 0.01 (SEM) and 0.53 ± 0.01 (SEM), respectively. Using the chondroitin sulfate calibration curves of Wasteson (1971) for molecular weight vs. $K_v$, we calculated the corresponding molecular weights to be 42,000 (±4,000) with a range of 70,000–17,000 for the leading and trailing edges of the peak.

In order to directly identify the GAGs of TAP-1, the GAGs isolated by chromatography on Sepharose CL-6B were subjected to cellulose acetate electrophoresis. The results of this electrophoresis are shown in the inset of Fig. 6. One band is seen that comigrates with chondroitin-4- and 6-sulfate, but not with dermatan sulfate. This agrees with the glycosaminoglycanase digestion experiments discussed earlier, which demonstrate that TAP-1 is completely sensitive to chondroitin AC lyase.

A minor GAG, migrating more slowly, is also present on cellulose acetate electrophoretograms of TAP-1 GAGs. This component is present at 10% of the level of chondroitin sulfate. However, it is not readily apparent in Fig. 5 and is presumably just a contaminant of the TAP-1 preparation.

The model of TAP-1 which emerges from its biochemical characterization is that of a large proteoglycan of about a million molecular weight, possessing chondroitin sulfate side chains with an average molecular weight of 42,000.

Electron Microscopic Visualization of TAP-1

Individual proteoglycan molecules have distinctive morphologic features which can be seen in the electron microscope.
Figure 5. Chromatography of TAP-1 and a 1.26 x 10^6-mol wt smooth muscle chondroitin sulfate proteoglycan on Sephacryl S-500. Two separate chromatographic separations performed on the same column are shown: one with a guanidine-HCl ECM extract containing TAP-1 (o), and one with a 35SO4-labeled smooth muscle cell layer extract (A) which contained a 1.26 x 10^6-mol wt chondroitin sulfate proteoglycan (Chang et al., 1983; Iozzo et al., 1982). The smooth muscle cell proteoglycan elutes at a Kav of 0.21 (arrow A) and TAP-1 elutes at a Kav of 0.25 (arrow B). A smaller dermatan sulfate proteoglycan is also present in the smooth muscle cell extract and elutes at 0.7 Kav. The 0.9 g of electric organ ECM fraction was solubilized with 6 M guanidine-HCl (as described in Materials and Methods) and applied to a 350-ml (2.6 x 66 cm) Sephacryl S-500 column in 9 ml. The column was equilibrated with 4 M guanidine-HCI, 50 mM Hepes, 0.2% CHAPS, 200 mg/liter iodoacetamide, 15 µl/liter diisopropylfluorophosphate, 400 µg/liter each of pepstatin, leupeptin, and chymostatin, pH 7.0. The smooth muscle cell extract consisted of 1 ml of carrier proteoglycan (D1 fraction of rat chondrosarcoma extract (Chang et al., 1983)), 4 ml of 35SO4-labeled smooth muscle layer, and 6 ml of 6 M guanidine-HCI extraction buffer (see Materials and Methods). 10 µl of this extract were applied to the column. The chromatography was performed at a flow rate of 30 ml/h and 3.0-ml fractions were collected. The Vo and the Vt for the column were determined by the elution of blue dextran and NaUS1, respectively. The Kav for each fraction was calculated by using these values. Two additional alkaline borohydride cleavages and resulting chromatographic separations gave essentially the same results as shown here. Here 12 µg of protein of purified TAP-1 were subjected to β elimination and applied to the column. (Inset) Cellulose acetate electrophoresis of TAP-1 GAGs isolated by chromatography on this Sephacryl CL-6B column. The entire chromatographic peak was pooled and concentrated for the analysis. Autoradiograms of 35Ruthenium Red–stained electrophoretograms are shown. (Lane 1) Dermatan sulfate (CS-B); (lanes 2 and 4) TAP-1 GAGs; (lane 3) chondroitin-4- and 6-sulfate (CS-A,C). Or identifies the origin; the direction of travel is toward the anode.

Figure 6. Chromatography of TAP-1 GAG side chains on Sepharose CL-6B released by alkaline borohydride cleavage. GAG concentration (●) is plotted as determined by a quantitative Alcian blue assay on DEAE nitrocellulose. The Sepharose CL-6B column (133 x 0.7 cm) was equilibrated with 0.2 M NaCl, 50 mM Hepes, 0.02% sodium azide, pH 7.0; the flow rate was 3.8 ml/h, and 0.75-ml fractions were collected. The Vo and Vt for the column were determined by the elution of blue dextran and NaUS1, respectively. The Kav for each fraction was calculated by using these values. Two additional alkaline borohydride cleavages and resulting chromatographic separations gave essentially the same results as shown here. Here 12 µg of protein of purified TAP-1 were subjected to β elimination and applied to the column. (Inset) Cellulose acetate electrophoresis of TAP-1 GAGs isolated by chromatography on this Sephacryl CL-6B column. The entire chromatographic peak was pooled and concentrated for the analysis. Autoradiograms of 35Ruthenium Red–stained electrophoretograms are shown. (Lane 1) Dermatan sulfate (CS-B); (lanes 2 and 4) TAP-1 GAGs; (lane 3) chondroitin-4- and 6-sulfate (CS-A,C). Or identifies the origin; the direction of travel is toward the anode.

were present as clusters and associated with one another through their tail-like projections (Fig. 7 e).

Discussion

During nerve regeneration at the neuromuscular junction, the nerve terminal reforms the exocytotic zones of its plasma membrane opposite a specialized region of the postsynaptic membrane. It has been shown that the positional cues which the nerve terminal detects are actually present in the synaptic basal lamina (Sanes et al., 1978; Glickman and Sanes, 1983). A useful hypothesis is that the recognition of these ECM cues occurs via presynaptic integral membrane proteins which bind these ECM components. That is, this recognition probably occurs through a subset of nerve terminal anchorage proteins. Little is known about the number and kind of molecules which serve as anchorage proteins for the nerve terminal.

Previously it was shown that TAP-1 has the properties of a nerve terminal anchorage protein (Carlson et al., 1986). This protein was identified not from the neuromuscular junction, but from the related synapse of electric organ. Several properties suggest that TAP-1 is an anchorage protein. (a) It is exclusively associated with the nerve terminal plasma mem-
brane throughout the synaptic region, as determined by immunocytochemistry at the electron microscopic level (Carlson et al., 1986; Kelly et al., 1987). (b) The molecule binds lipids and can be incorporated into liposomes, indicating that it behaves as an integral membrane protein. (c) The molecule is tightly associated with an electric organ ECM fraction. Such characteristics suggest that this molecule is an integral membrane protein of the nerve terminal and acts as a link to the ECM. However, as yet we have no direct functional evidence that it fulfills this role.

We have isolated TAP-1 to at least 86% purity. The methods utilized to purify TAP-1 were based on the analytical procedures previously used to characterize this antigen in a less pure preparation (Carlson et al., 1986). Not surprisingly then, the molecule has the same physical properties (chromatographic size, sedimentation rate, and charge density) as the impure antigen. For example, at the last step of the purification TAP-1 elutes at about 0.7 M NaCl at pH 4.9. These are the same conditions required for the elution of the impure antigen (Carlson et al., 1986). Previously, 65% of TAP-1 in an impure preparation was found to behave as an integral membrane protein by liposome reconstitution (Carlson et al., 1986). About 80% of the purified TAP-1 is found to behave as an integral membrane protein (Iwata, M., and S. S. Carlson, unpublished observations). This was determined by the buoyant density of TAP-1 on CsCl density gradients containing Triton X-100 (Kjellen et al., 1981).

TAP-1 is a chondroitin sulfate proteoglycan of large hydrodynamic size. In the electron microscope TAP-1 has the typical "bottlebrush" morphology of a proteoglycan. In overall shape and size, the bottlebrush structure resembles similar structures of isolated and purified chondroitin sulfate containing proteoglycans from cartilage (Hascall and Hascall, 1981), blood vessels (Kapoor et al., 1986), skeletal muscle (Pechak et al., 1985), and arterial smooth muscle cell cultures (Wight and Hascall, 1983). The presence of a globular region at one end of the molecule is similar to preparations of the large chondroitin sulfate proteoglycans from cartilage. This domain is thought to represent a GAG-free area of the core protein involved in binding hyaluronic acid in the formation of high molecular weight aggregates (see review, Hassel et al., 1986). Whether TAP-1 is capable of interacting with hyaluronic acid is not known. It is interesting to note that a small fraction of the TAP-1 molecules spread for electron microscopic observation appear to interact with one another through these extended "taillike" regions. Because TAP-1 has the properties of an integral membrane protein (Carlson et al., 1986), TAP-1 might be associating nonspecifically through its hydrophobic domain. This is a consideration because TAP-1 was spread under low detergent condi-

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**Figure 7.** Electron microscopic visualization of TAP-1. Electron micrographs of purified TAP-1 spread on carbon-coated grids and rotary shadowed at low angle (10°). (a–d) Typical profiles of individual molecules. Each molecule possessed a globular region (arrow) at one end. (a) ×98,000; (b) ×101,000; (c) ×103,000; and (d) ×99,000. Occasionally, individual molecules were associated with one another through their "taillike" projections: (e) ×105,000. Bar, 0.25 μm.
Table II. Comparison of the Molecular Properties for TAP-1 Estimated from Biochemical and Electron Microscopic Data

| Property                  | Biochemical estimate | Electron microscopic estimate |
|---------------------------|----------------------|-------------------------------|
| Relative mol wt of GAG side chains | 42,000 (17,000-70,000) | 50,000                        |
| Relative mol wt of intact molecule | $10^6$ | $1.2 \times 10^6$ |
| GAG/protein (µg/µg)       | 5.7 ± 0.5            | 5.7                           |

Figure 8. (Upper diagram) Comparison of the size of TAP-1 and the synaptic cleft. The two-dimensional image of a TAP-1 molecule derived from electron microscopy is shown next to a cross section of the electric organ synapse; both the nerve terminal and TAP-1 are drawn to scale. TAP-1 is large enough to span the synaptic cleft. Thus, it could make a major structural contribution to the nerve terminal basal lamina. SyBL, Synaptic basal lamina; NT, nerve terminal; PSC, postsynaptic cell. (Lower diagram) Hypothetical placement of TAP-1 in the synaptic cleft. A small three-dimensional cutaway section of the synaptic cleft containing TAP-1 is shown. TAP-1 is represented as a transmembrane protein in the figure. However, it has only been shown to have the properties of an integral membrane protein (Carlson et al., 1986); it has not yet been determined whether it spans the nerve terminal membrane bilayer.

The model of TAP-1, which emerges from the biochemical and electron microscopic data, is that of an extremely large molecule. Visualized in two dimensions, as it is in the electron micrographs of the spread molecule, TAP-1 is 345 nm long and 214 nm wide. This is quite large compared with the electric organ synaptic cleft and the synaptic basal lamina of which TAP-1 is presumably a part. The synaptic cleft measures about 60 nm; thus, TAP-1 could easily touch both pre- and postsynaptic cells as diagrammed in Fig. 8. Unfortunately, none of the precise details of the extracellular location of TAP-1 in the synaptic basal lamina have been immunocytochemically determined. The placement of TAP-1 in the opaque central zone of the basal lamina in the lower section of Fig. 8 is simply a hypothesis. However, the comparison shown in Fig. 8 suggests that TAP-1 could be making a significant structural contribution to the basal lamina that runs through the cleft. The actual space that the molecule could occupy might be reduced considerably if the protein were complexed with other proteins. On the other hand, the large hydrodynamic volumes characteristic of the proteoglycans might be important for filling the synaptic cleft and holding the pre- and postsynaptic cells a fixed distance apart. The ability of the large cartilage proteoglycans to act as cushions and reversibly resist compressive force is well known (Hascall and Hascall, 1981). Proteoglycans might do this and at the same time provide an aqueous environment for the relatively unobstructed diffusion of neurotransmitters.

Several questions remain. The monoclonal antibody used to identify TAP-1 was originally raised against synaptic vesicles from the electric organ (Caroni et al., 1985). The characteristics of this antigenic site (SV4) are (a) it is present on TAP-1 and the synaptic vesicle proteoglycan in electric organ, (b) it is carbohydrate in nature, (c) it is a product of the nerve as indicated by axonal transport studies, and (d) it is enriched in the neurons innervating the electric organ (Caroni et al., 1985; Carlson et al., 1986). The exact nature of this antigenic site (SV4) remains to be elucidated. Further, the relationship between the synaptic vesicle proteoglycan and TAP-1 is not completely understood. It has been suggested that the much smaller synaptic vesicle molecule represents a breakdown product of TAP-1, picked up by the synaptic vesicle (Kelly et al., 1987; Carlson et al., 1986). However,
the results presented here make this hypothesis less likely. TAP-1 is a chondroitin sulfate proteoglycan, whereas the synaptic vesicle proteoglycan is thought to be in the heparin/heparan sulfate family (Carlson and Kelly, 1983; Stadler and Dowe, 1982). Maybe TAP-1 and the synaptic vesicle proteoglycan are unrelated polypeptides which share a unique carbohydrate for some other function, such as intracellular sorting (Kelly et al., 1987; Carlson et al., 1986).

Because TAP-1 appears to be an integral membrane protein, it makes sense that it is a product of the presynaptic cell in whose plasma membrane bilayer it resides. However, an ambiguity exists in the interpretation of previous axonal transport studies of the SV4 antigen (Caroni et al., 1985). These were done before it was realized that two molecules bearing the SV4 antigen exist in electric organ: the synaptic vesicle proteoglycan and TAP-1. Because the axonally transported SV4 antigens were identified only by their antigenicity, we cannot be certain that both antigens (the synaptic vesicle proteoglycan and TAP-1) were being axonally transported. It is possible that only the synaptic vesicle proteoglycan is being transported and TAP-1 is made by the postsynaptic cell. Entertaining this hypothesis, however, also involves the somewhat unlikely idea that one cell is contributing an integral membrane protein to another.

TAP-1 is one of the first purified nerve terminal integral membrane proteins which is thought to link the nerve terminal to the ECM. Integral membrane cell surface proteoglycans which are thought to act as anchorage proteins are known in a number of nonneuronal tissues, however (Hassel et al., 1986; Hook et al., 1984; Woods et al., 1985; Rapraeger et al., 1987; Garrigies et al., 1986). Presumably TAP-1 binds postsynaptic anchorage proteins directly or indirectly through other ECM proteins completing a pre- to postsynaptic bridge. Another element of a nerve terminal–postsynaptic ECM bridge has also been isolated from electric organ. This protein, known as agrin (Magill et al., 1986; Reist et al., 1986), will cluster acetylcholine receptor and acetylcholinesterase on the surface of myotubes. Its presence in the neuronal cell bodies of electric organ suggests that it, like TAP-1, is a product of the presynaptic cell (Magill et al., 1986). Unlike TAP-1, it is presumably not a membrane protein because it does not require detergent for its solubilization (Godfrey et al., 1986). Monoclonal antibodies that immunoprecipitate the agrin acetylcholine receptor–clustering activity identify two polypeptides with relative molecular weights of 70,000 and 95,000 (Magill et al., 1986). Both TAP-1 and agrin are purified from the same electric organ ECM preparation. It is not known whether they interact with one another.

An intriguing question for TAP-1 is: does it act as a general synaptic glue or a specific adhesive? In the former case, one might expect TAP-1 to be bound by the ECM only via proteins which are not synthetically localized, such as laminin (Sanes and Chiu, 1983). In the latter case, one might expect binding by synapse-specific proteins as well.

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Note added in proof: After this manuscript was submitted, Kiene and Stadler reported the association of the synaptic vesicle proteoglycan with an electric organ ECM fraction (Kiene, M. L., and H. Stadler. 1987. EMBO J. 6:2217-2221). We find that the majority of the synaptic vesicle antigen (SV4) in the ECM fraction is actually due to the presence of TAP-1 (Carlson et al., 1986). However, we also find low amounts of the smaller synaptic vesicle proteoglycan is also present (Iwata, M, and S. S. Carlson, unpublished observations).

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