# Immunocytochemical Localization of the Lens Main Intrinsic Polypeptide (MIP26) in Communicating Junctions

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ABSTRACT Plasma membranes of vertebrate lens fiber cells contain a major intrinsic polypeptide with an apparent molecular weight of 26,000 (MIP26). These plasma membranes are extremely rich in communicating junctions, and it has been suggested that MIP26 is a component of them. MIP26 was purified from cow lenses using preparative SDS gel electrophoresis followed by hydroxylapatite column chromatography. From gel electrophoresis patterns and aggregational properties it was concluded that the MIP26 preparation was homogeneous. The purified MIP26 was used to produce monospecific antibodies in rabbits as assessed by double immunodiffusion and crossed immunoelectrophoresis of purified MIP26 and solubilized lens plasma membranes against the antiserum. Indirect immunocytochemical studies were performed on open and closed lens plasma membrane vesicles by incubation in anti-MIP antiserum followed by ferritin-conjugated goat antirabbit IgG. The conjugate bound unequivocally to lens communicating junctions, indicating that MIP26 is a component of these structures.

The lens of the vertebrate eye is derived from ectoderm and is composed primarily of concentric layers of long, hexagonally-shaped units called lens fiber cells. The fiber cells differentiate from an epithelial monolayer that covers the anterior surface of the lens and terminates at the equator. During the process of differentiation, which occurs throughout life, the equatorial epithelial cells elongate and follow a curved course that extends from the anterior pole of the lens to the posterior pole. New lens fibers are deposited over the older ones, the latter being compressed towards the interior of the region known as the nucleus. As a result of this constant process of differentiation and in the absence of cell sloughing, the lens gradually increases in size with age. The fiber cells occupying the superficial layers, collectively termed the cortex, begin to lose their mitochondria, nuclei, and cytoplasmic membranes shortly after elongation commences, but retain their polyribosomes for a while (4). During this time, they synthesize a diverse class of proteins called crystallins along with their membrane proteins. The oldest fiber cells of the lens, compacted into the lens center (nucleus) and having lost all of their organelles, are incapable of protein synthesis (48).

Because the lens fiber cells are, on the average, very sparsely populated with cytoplasmic membranes, purification of their plasma membranes is simplified. During the past decade these plasma membranes have aroused interest because, like the erythrocyte membrane, they provide us with an easily accessible model for the study of membrane structure (4). Furthermore, they are extraordinarily rich in a unique type of communicating junction[1] (4, 6, 27, 31, 37) that is the subject of this investigation.

It is now generally agreed that vertebrate lens fiber cell plasma membranes contain a major intrinsic polypeptide species of ~26 kdaltons (2, 5, 7, 9), variously referred to as MIP (9) or MP26 (5). It should be stated however that this situation prevails only in the youngest of lens fiber cells. As the lens ages, or as the fiber cells are sampled in successively deeper and therefore older layers of the lens, an intrinsic polypeptide of 22 kdaltons is also found (25, 42). The lens of a 24-yr-old human has about equal amounts of the MIP26 and 22-kdalton intrinsic polypeptide (25). Horwitz and Wong (26) have shown that the 22-kdalton protein is derived from MIP26 by the posttranslational cleavage of a 4-kdalton segment. The 26-kdalton protein has been reported for isolated lens fiber cell

[1] The term communicating junction (43) rather than gap junction is used throughout this report for the lens because there is disagreement concerning the existence of an intrajunctional "gap" as originally described by Revel and Karnovsky (41). Reference 39 reviews the evidence on this subject and summarizes data relating to the intercellular coupling that is mediated by lens communicating junctions.

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junctions from various species (17). Because these junctions are so numerous (50–60% of the cell surface in some cases [27]), several authors have suggested on the basis of this evidence that MIP26 and the 26-kdalton junctional protein may be the same (1, 4, 8, 17). This report employs immunocytochemical methods in the investigation of this question.

## MATERIALS AND METHODS

### *Purification of MIP26*

After decapsulation, bovine lenses were partially crushed with a mortar and pestle in 20 mM Tris-HCl, 0.1 M NaCl buffer, pH 7.7. Lens nuclei were removed by straining the crushed lenses through sterile gauze. After this step, the cortical suspension was homogenized in the same buffer using a Ten Broeck glass homogenizer. The homogenate was centrifuged at 18,000 rpm for 25 min in a Sorvall SS-34 rotor (DuPont Co., Wilmington, Del.). The pellet was washed six times by resuspending with homogenization in a Ten Broeck glass homogenizer and repelleting the insoluble protein fraction by centrifugation. The final buffer-insoluble fraction was treated similarly six times with 7 M urea added to the buffer. Before each centrifugation step, the homogenate was diluted 1:4 with Tris-HCl buffer without urea. Urea was removed from the final insoluble fraction by two washes with the same buffer.

The resulting material was solubilized in 20 mM Tris-HCl buffer, pH 7.7, containing 1% SDS, and then centrifuged at 20,000 rpm for 15 min. 6 ml of the supernatant (total protein, ~15 mg) were applied to a 4 × 120 × 160 mm toothless slab gel, and electrophoresed at 20 mA for 18 h. Stacking and running gels were composed of 4.5% and 15% acrylamide, respectively. To prevent heat-induced aggregation of the lens main intrinsic polypeptide, the sample was not heated before electrophoresis (47). After electrophoresis, a 1-cm vertical strip, cut from the center of the gel, was scanned at 280 nm using an ACTA M VI spectrophotometer (Beckman Instruments, Inc., Palo Alto, Calif.) equipped with a model 2 Gel Scanner. This scan allowed the localization of MIP26 in the preparative slab gel (Fig. 1). A horizontal slice containing MIP26 was cut from the gel and crushed through a 6-ml syringe. The crushed gel was suspended in an equal volume of 0.1 M sodium phosphate buffer, pH 6.8, containing 1% SDS. The suspension of crushed gel and buffer was stirred gently at room temperature for 24 h to allow the protein to diffuse from the acrylamide. The supernatant was then applied to a 1 cm × 3 cm column of hydroxylapatite equilibrated with 0.1 M sodium phosphate buffer, pH 6.8, containing 1% SDS. After flushing the column with 5 bed volumes of the starting buffer, a two-step discontinuous gradient consisting of ~25 ml of 0.3 M sodium phosphate, pH 6.8, containing 1% SDS, followed by 0.5 M sodium phosphate, pH 6.8, with 1% SDS, was used to elute the proteins from the column. The material eluted with the 0.5 M sodium phosphate/1% SDS buffer was collected and pooled. Because the hydroxylapatite column was at times overloaded with protein, the fraction eluted with 0.3 M sodium phosphate contained a significant amount of MIP26. To recover the protein, this fraction was diluted again to a final concentration of 0.1 M sodium phosphate buffer, pH 6.8, with 1% SDS and rechromatographed as described above.

### *Removal of SDS and Concentration of MIP26 by KCl Precipitation*

Solid KCl was added to 5-ml aliquots of the 0.5 M sodium phosphate/1% SDS elution peak from the hydroxylapatite column to a final concentration of 0.5 M. The KCl precipitated the MIP26 as well as the bulk of the dodecyl sulfate ion. We centrifuged the suspension at 18,000 rpm for 10 min and discarded the supernatant. The pellet was washed by resuspension in 0.5 ml or 1 ml of 20 mM Tris-HCl buffer, pH 7.7, followed by centrifugation at 18,000 rpm for 10 min.

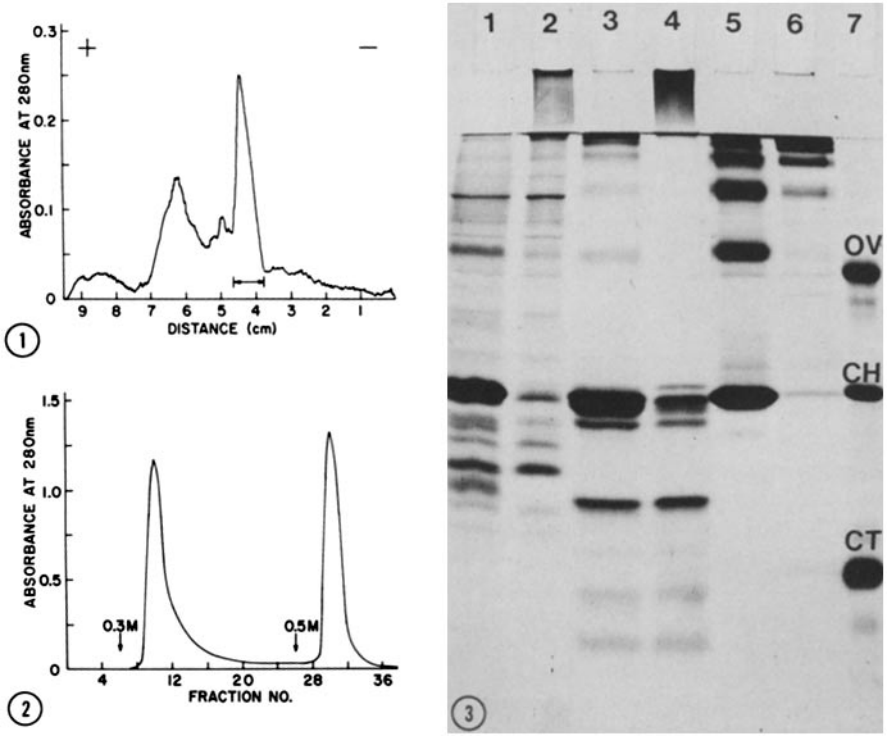



FIGURE 1 A representative absorption scan at 280 nm of a preparative polyacrylamide SDS gel. The urea-washed pellet containing lens plasma membranes was dissolved in buffer with 1% SDS and electrophoresed. The protein peak appearing at a distance of 3.8–4.7 cm and indicated by arrows, contains the 26-kdalton main intrinsic membrane polypeptide (MIP26).

FIGURE 2 Elution profile of MIP26, which was recovered from the region of the gel indicated by the arrows in Fig. 1 and then applied to a hydroxylapatite column. Membrane proteins were eluted with 0.3 M and 0.5 phosphate buffer containing 1% SDS. For more details see Materials and Methods section.

FIGURE 3 SDS PAGE of various fractions containing MIP26. Column *1*: plasma membrane fraction after urea-wash; column *2*: same as column *1*, but heated at 100°C for 4 min before electrophoresis; column *3*: MIP26 eluted from the hydroxylapatite column with 0.3 M sodium phosphate; column *4*: same as column *3*, but heated at 100°C for 4 min before electrophoresis; column *5*: purified MIP26 eluted with 0.5 M sodium phosphate from the hydroxylapatite column; column *6*: same as column *5*, but heated at 100°C for 4 min before electrophoresis; column *7*: standards (*OV*, ovalbumin; *CH*, α-chymotrypsinogen; *CT*, cytochrome *c*). Approximately 30 μg of protein was applied in each of columns *1–6*.

More than 90% of the polypeptide generally was recovered in the supernatant of the second and third washes, as determined by absorbance measurements at 280 nm.

The concentration of residual SDS present in the recovered protein was determined by introducing $^{35}S$-labeled SDS into the stock buffer-SDS solution. Results from several experiments indicated that the residual SDS in the protein sample was ≤0.05% of the SDS originally added. In addition to being invaluable insofar as bulk SDS removal was concerned, this technique also served to concentrate the samples 5- to 10-fold. From a total of 15 mg of membrane proteins that was applied to the preparative gel, 2 mg of highly purified MIP26 were obtained.

## Preparation and Characterization of Antisera

Female New Zealand rabbits were injected intradermally with 0.3 mg of antigen in Freund's adjuvant per immunization. At two successive 10-d intervals, the injections were repeated, after which the animals were bled for the first time. Thereafter, rabbits were bled monthly for ~6 mo with an injection of antigen administered 1 wk before each bleeding. Immunodiffusion assays were performed in 1% agar containing 0.2% SDS to prevent nonspecific precipitation as described previously (38). In addition, crossed immunoelectrophoresis was performed on pure antigen and solubilized membranes by the method of Converse and Papermaster (13) with the modification suggested by Chua and Blomberg (12).

## Preparation of Membranes for Immunocytochemical Studies

Bovine lens plasma membranes were prepared by discontinuous density centrifugation according to the procedure of Bloemendal et al. (6). A fraction rich in plasma membranes was collected from the 1.14/1.16 and 1.16/1.18 interfaces. After removal of the sucrose by two water washes, the membranes were washed twice with 7 M urea in 20 mM Tris-HCl, 0.1 M NaCl, pH 7.7. The remaining pellet was washed twice with phosphate-buffered saline (PBS), then aliquoted into several fractions, each containing 3–4 mg of the total protein. In some of the experiments the urea wash was omitted in order to test its effect on antibody binding.

## Indirect Immunocytochemistry

Each of the above membrane fractions was suspended in PBS containing 5% bovine serum albumin for 15 min. Immune serum was added to the membrane suspension at a final dilution of 1:3. Low dilutions of the antiserum were used due to the extraordinarily high concentration of antigen in each membrane fraction (3–4 mg of protein, 80% of which was MIP26), although dilutions of up to 1:100 gave positive results. The mixture was incubated for 1 h at 21°C. During that period, the suspension was gently homogenized once with a single stroke of the pestle. The suspension was then washed with PBS. Control fractions were prepared in an identical manner, except that the membrane suspensions were incubated in preimmune serum. After the PBS washes, the membrane fractions were again suspended in PBS containing 5% bovine serum albumin, and affinity-purified ferritin-conjugated goat antirabbit IgG (GAR-Fn, 1.0 mg/ml; Miles-Yeda, Rehovot, Israel). After incubation for 1 h at room temperature, the fractions were washed three times with PBS. At this point, the membrane fractions were suspended again in PBS and stirred gently overnight at 4°C to ensure thorough removal of loosely bound GAR-Fn. Each fraction was sedimented in a Sorvall SS-34 rotor at 20,000 rpm for 15 min. The pellets obtained were fixed in a solution of 2% glutaraldehyde, 0.1 M phosphate, pH 7.3, for two h, and further fixed in 1% osmium tetroxide for 1 h. All membrane pellets were dehydrated in a graded ethanol series and embedded in Araldite 502. Silver sections were prepared and viewed on Formvar-carbon-coated grids in a Siemens IA or JEOL 100C electron microscope.

# RESULTS

## Purification of MIP26 from Lens Fiber Cell Plasma Membranes

Fig. 1 shows a densitometric scan of a typical urea-washed membrane preparation recorded from a strip of preparative SDS polyacrylamide gel. In this figure, the MIP26 is contained in the peak appearing at a distance of 3.8–4.7 cm on the preparative gel. After removal of the protein from the gel, the

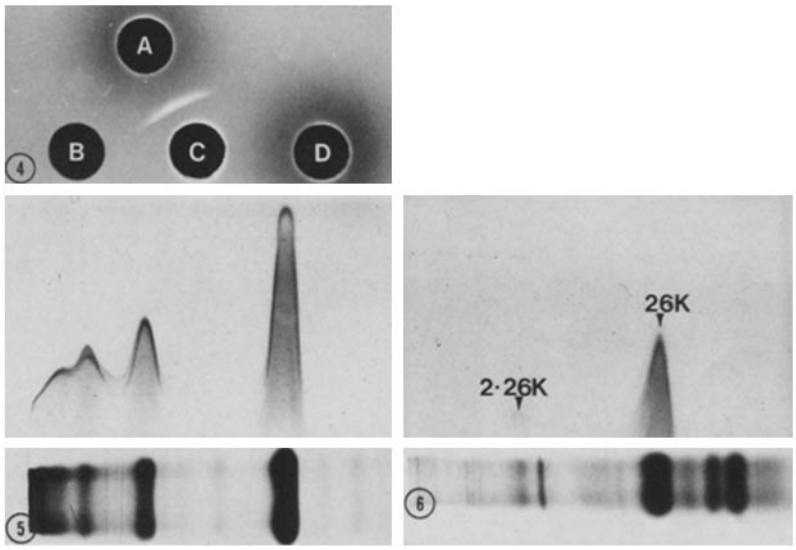



FIGURE 4 Double immunodiffusion of cow lens MIP26 against lens soluble crystallins, rabbit anti-MIP26 antiserum, and rabbit preimmune serum. Well *A*, 35 μl of immune serum. Well *B*, 20 μg of total lens soluble proteins solubilized in 0.1% SDS. Well *C*, 20 μg of KCl-extracted MIP26. Well *D*, 35 μl of preimmune serum. Dark-field photograph.

FIGURE 5 Crossed immunoelectrophoresis of MIP26 and its multimeric aggregates against rabbit antiserum raised against MIP26. 20 μg of purified MIP26 were first electrophoresed in a conventional SDS PAGE system, as shown in Fig. 3, column *5*. The lane containing the sample (2 × 6 × 10 mm) was cut out of the gel. A 2 × 2 × 80 mm strip containing the pertinent bands was then electrophoresed in the second dimension into the agarose antibody-containing gel, as described by Chua and Blomberg (12). The agarose gel contained 7% immune serum, and the intermediate gel contained 3% Lubrol PX. All other conditions were as described previously. Coomassie Blue stain.

FIGURE 6 Crossed immunoelectrophoresis of crude cow lens plasma membrane preparation against MIP26 antiserum. 20 μg of urea-washed membranes were first electrophoresed in SDS as shown in Fig. 3, column *1*. A 2-mm strip containing the pertinent bands was then electrophoresed in the second dimension into the agarose antibody-containing gel. All conditions were the same as Fig. 5. MIP26 (*26K*) and its dimer (*2·26K*) were the only bands to elicit rockets in the agar gel. Coomassie Blue stain.

protein solution was applied to a 1 × 3 cm column of hydroxylapatite. The first protein peak (Fig. 2), eluted with 1% SDS containing 0.3 M sodium phosphate buffer, comprises mainly the MIP26 component; however, appreciable amounts of smaller peptides can be detected in the electropherogram of this fraction (Fig. 3, lanes *3* and *4*). The protein fraction eluted with 0.5 M sodium phosphate buffer was found to be free of any low molecular weight contaminants. An SDS PAGE profile of a concentrated sample obtained from the 0.5 M sodium phosphate elution peak of the hydroxylapatite column is shown in Fig. 3, lanes *5* and *6*. In agreement with an earlier report, MIP26 formed multimeric aggregates with increased purification and concentration (26). Furthermore, MIP26 aggregated when the sample was heated before electrophoresis. Since MIP26 is the only protein associated with lens membranes that is heat sensitive, this behavior served as an additional criterion for the purity of the preparation (25).

### Characterization of Antisera against MIP26

The specificity of the anti-MIP26 serum was demonstrated in an earlier report by double immunodiffusion (49), and the data therefore is only partially repeated here. That study showed no cross-reactivity with any of the soluble bovine lens crystallins. Furthermore, no nonspecific precipitin lines were observed when 1% SDS was diffused against immune serum. Fig. 4 reemphasizes the lack of reactivity with soluble lens proteins and demonstrates in addition that no reaction was observed with preimmune serum.

To achieve a more rigorous demonstration of specificity of the anti-MIP26 antibody, we used the crossed immunoelectrophoresis system of Converse and Papermaster (13), which was recently improved by Chua and Blomberg (12). A highly purified preparation of MIP26 was first separated by SDS PAGE and subsequently electrophoresed at right angles to an agarose gel containing anti-MIP26 antiserum (Fig. 5). It is noteworthy that, in addition to MIP26, every multimer of this protein reacted specifically with the antiserum to produce a rocket which was proportional to the concentration of the corresponding MIP26 multimer. The result of crossed immunoelectrophoresis with a urea-washed lens membrane preparation is shown in Fig. 6. In the first dimension (SDS PAGE), many protein bands are observed in addition to MIP26. The three bands of molecular weight <26 kdaltons are known to be crystallins (26). Upon electrophoresis in the second dimension, MIP26 reacted with the immune serum to produce a rocket. We also observed consistently a second weak rocket at the position corresponding to 52 kdaltons, which represents the dimer of MIP26. This agrees with a previous observation that dimers of 26 kdaltons can be formed in solubilized membrane preparations (26). Thus combined, the immunodiffusion and crossed immunoelectrophoresis experiments demonstrate the specificity of the antiserum against MIP26.

### Indirect Immunocytochemistry Using GAR-Fn

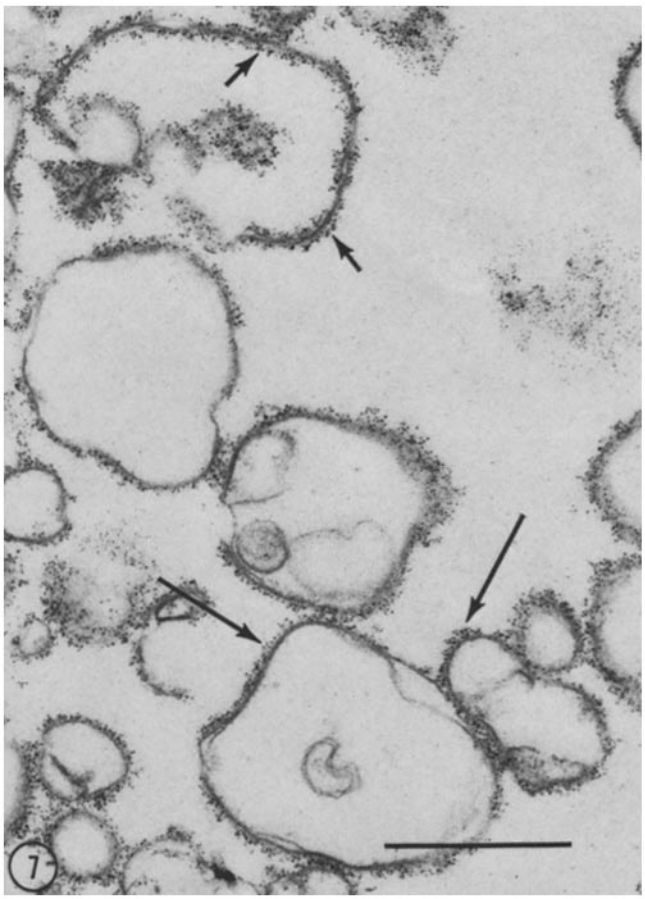
Purification of lens fiber cell plasma membranes by sucrose density centrifugation and washing in 7 M urea to remove adherent crystallins produced open and closed vesicles with a wide range of sizes (Figs. 7 and 8). The binding of anti-MIP26 antibody to the membranes was qualitatively the same whether or not the urea wash was included in the protocol. All results reported below are for urea-washed material. Fig. 7 illustrates a membrane preparation that was treated with immune serum followed by GAR-Fn. Each vesicle is labeled on its external surface and some are labeled on their internal surface as well, indicating that the latter were open during incubation in the immune reagents. Control vesicles that were incubated in preimmune serum followed by GAR-Fn showed no binding of the label (Fig. 8), although occasional nonspecific adsorption of ferritin particles or clumps was observed. Fig. 9 shows a higher magnification of a vesicle that was open during its treatment with immune serum and GAR-Fn. The vesicle is composed entirely of communicating junction as evidenced by its pentalaminar profile (16). Ferritin-labeled antibody is evenly distributed along both the external and internal surface. These two surfaces represent the cytoplasmic sides of lens fiber membranes that were connected *in situ*. Wherever the junction profile is favorably oriented, it can be observed that the ferritin molecules are separated from the membrane surface by a translucent zone. This is to be expected when first and second stage IgG molecules intervene between antigenic determinants on the membrane surface and the ferritin that is conjugated to the second stage antibody.

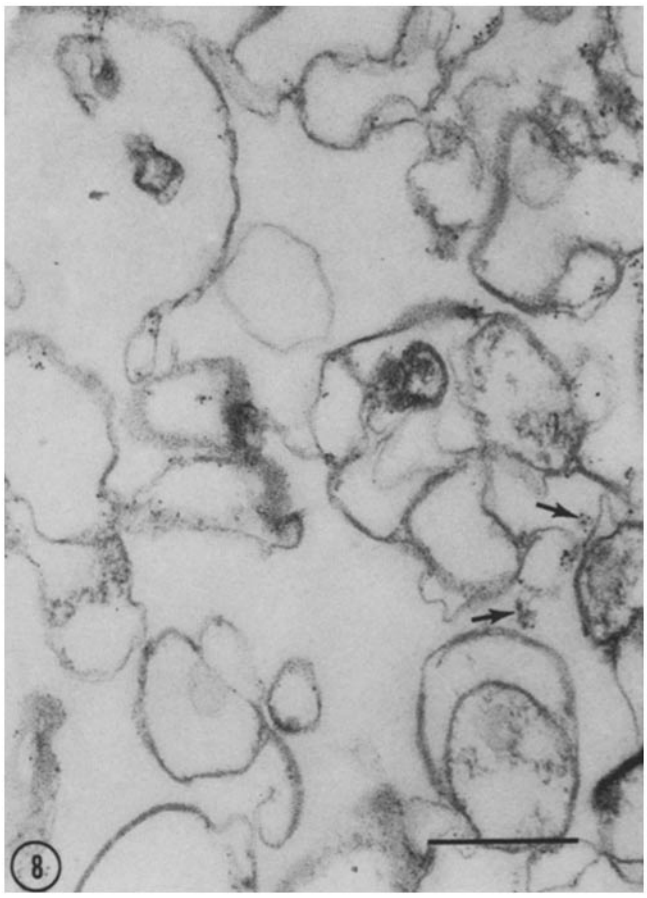
An even distribution of ferritin was not always observed over vesicles that were formed entirely of junctional membrane. Fig. 10 includes two vesicles that were closed during incubation in the immune reagents. The distribution of ferritin over the example on the left is interrupted in several places, whereas the vesicle on the right is saturated with label. It is possible that vacancies in the otherwise uniform labeling pattern result from imperfect delivery of either the primary or secondary antibody to the membrane surface during incubation. Alternatively, the vacancies in the staining may well be due to clumping of MIP26 in the plane of the membrane, with resultant denuded patches. This phenomenon has been observed in freeze-fracture replicas of lenticular junctions (17).

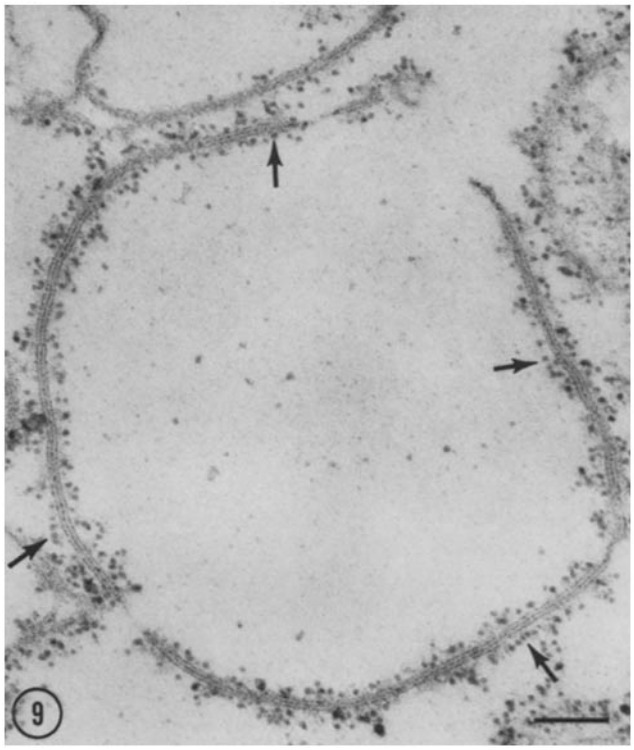
It was not possible to make a firm judgment concerning the binding of anti-MIP26 antibody to nonjunctional areas of the lens fiber cell plasma membrane because, in sectioned vesicles, one could never be certain of the past status of apparent nonjunctional regions. Without question, our vesicle preparations contained some nonjunctional membrane because they were formed in the absence of detergents which are known to selectively solubilize lens nonjunctional plasma membrane (17). Nonetheless, it is conceivable that communicating junctions were capable of dissociation under the conditions employed. Junctional splitting is known to occur in liver under hypertonic conditions (19). One might conclude that the vesicles illustrated in Figs. 11 and 12 demonstrate examples of labeled junctional and nonjunctional membrane. Alternatively, it could be argued that those segments that appear to be nonjunctional are in fact regions where the communicating junctions had split apart. We have evidence from indirect ferritin immunocytochemical staining of lens ultrathin frozen sections, however, that MIP26 is distributed at high density both junctionally and extrajunctionally (Fitzgerald, P. J., D. Bok, and J. Horwitz, manuscript in preparation).

## DISCUSSION

Earlier studies, based on biochemical and fluorescence immunocytochemical methods, showed that MIP26 is a component of the lens fiber plasma membrane (2, 5, 7, 9, 10, 14, 46). However, because lens communicating junctions had not been purified and because of the relatively low resolution of fluorescence microscopy, it could not be determined whether





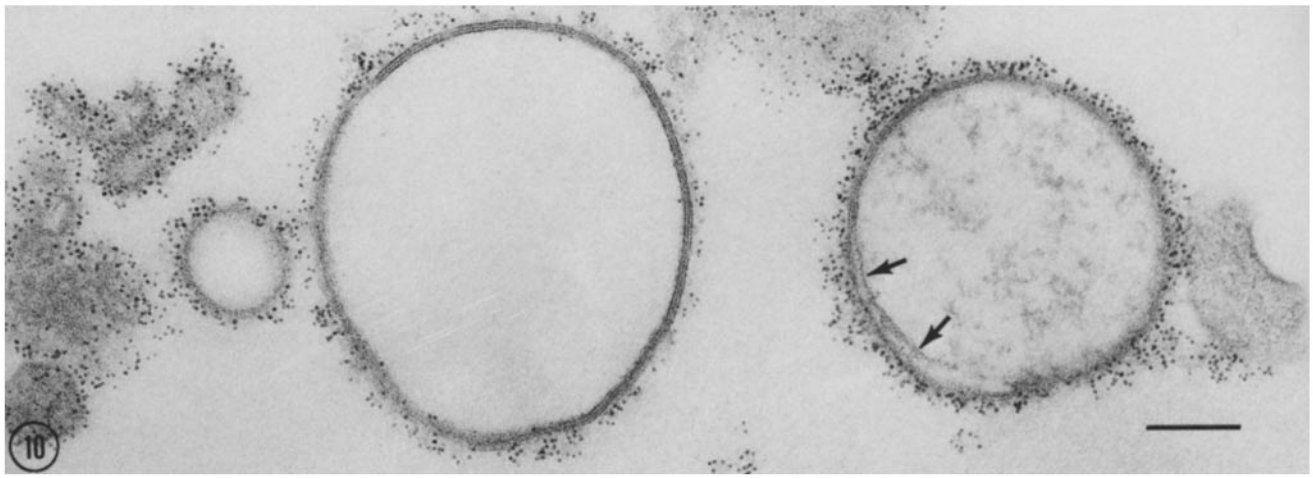


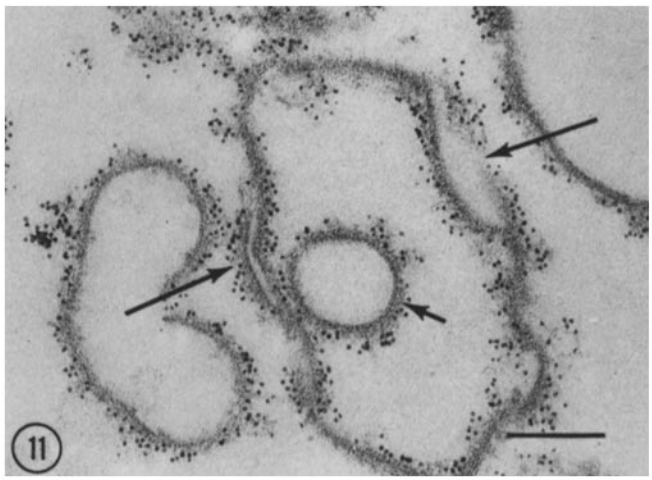
FIGURES 7–9 Lens fiber cell plasma membranes purified by sucrose density centrifugation, washed in 7 M urea and incubated in either preimmune or immune rabbit serum followed by ferritin-labeled goat antirabbit IgG (GAR-Fn).

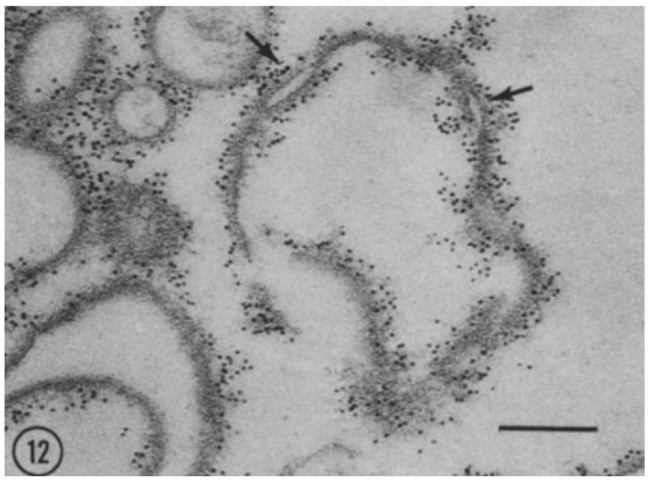
FIGURE 7 Membrane vesicles incubated in immune serum and GAR-Fn were always labeled. In some instances, ferritin was bound only to the external surface of a vesicle (long arrows) whereas other vesicles were labeled on both their external and internal surfaces (short arrows) indicating that the latter were patent during incubation. Bar, 0.5 μm. × 50,000.

FIGURE 8 Vesicles incubated in preimmune serum and GAR-Fn were not labeled. Occasional clumps of ferritin (arrows) are observed in the field. Bar, 0.5 μm. × 40,000.

FIGURE 9 Some vesicles were composed entirely of communicating junction. In this case, the external and internal surface of the vesicle represent the two cytoplasmic surfaces of the junction. Ferritin is uniformly distributed on both surfaces (arrows) of this vesicle which was incubated in immune serum and GAR-Fn. Bar, 0.1 μm. × 92,400.







FIGURES 10–12 Lens fiber cell plasma membranes purified by sucrose density centrifugation, washed in 7 M urea and incubated in immune rabbit serum and GAR-Fn.

FIGURE 10 Vesicles composed of communicating junctions were not always uniformly labeled. The closed vesicle on the right appears to be saturated with label whereas that on the left has an uneven distribution of ferritin on its surface. The latter may be due to the uneven delivery of immune reagents or to clumping of MIP26 in the plane of the membrane. The vesicle on the right has retained some lens crystallins in its interior (flocculent material). The junction forming it appears to be separating (arrows). Alternatively this region might represent nonjunctional membrane. Bar, 0.2 μm. × 62,000.

FIGURE 11 Vesicle profiles more complicated than those illustrated in Fig. 9 and 10 were also observed. A labeled single-membrane vesicle (short arrow) is observed within a larger labeled vesicle in this figure. It is deduced that the larger, obliquely sectioned vesicle consists, in part, of communicating junction, because portions of it are interspersed by separated membranes (long arrows). These labeled areas might represent dissociating junctional regions or they could be intervening nonjunctional membrane. Bar, 0.2 μm. × 65,000.

FIGURE 12 An example similar to that shown in Fig. 11 indicates labeled separated areas (arrows) that could be interpreted either as dissociated junctional or as nonjunctional membrane. Bar 0.2 μm. × 65,000.

MIP26 is a junctional or a nonjunctional component. Lens fiber communicating junctions have recently been purified to morphological homogeneity with a concomitant enrichment of MIP26 (7). The immunocytochemical data presented in this study support this biochemical evidence for MIP26 in communicating junctions. Because our identification of nonjunctional membrane had to be made with equivocation, we were not able to say with certainty from this study whether MIP26 is distributed throughout lens fiber plasma membranes or whether it is confined to communicating junctions. However, work in progress, utilizing frozen sections of lightly fixed lens cortex shows a general distribution of MIP26 in both junctional and nonjunctional regions. The functional consequences of this remain to be determined (Fitzgerald et al., manuscript in preparation).

It is obvious from our data that antigenic determinants sufficiently protrude from the cytoplasmic surface of the lens plasma membrane communicating junctions to allow binding of the first stage antibodies. This is not a surprising feature for a protein that is engaged in the formation of transmembrane channels. It is also consistent with the observation that proteolytic digestion of intact lens membrane cleaves the 26-kdalton component to a 20-kdalton "core" that is resistant to further proteolysis. These results suggest that the core 20-kdalton polypeptide is protected by the lipid bilayer, whereas a 6-kdalton component is accessible to the enzymes (44). Similar

results were obtained by others with proteolysis of liver communicating junctions (22). The extracellular surfaces of tightly apposed communicating junctions should not be accessible to enzymes or to immune reagents whose molecular weights exceed that of substances known to penetrate the extracellular gap between conventional communicating junctions. Because of this inaccessibility, and because no method exists for the controlled dissociation of lens communicating junctions before incubation in the primary antiserum and labeled second stage antibody, it could not be determined whether the rabbit anti-MIP26 antiserum included IgG species directed against antigenic determinants on the extracellular surface of this junctional protein.

During the past 10 yr, considerable effort has been expended in attempts at isolation and characterization of the subunits that make up the connexons (15) of communicating junctions. The question has been raised whether these subunits would be conservative in their structural and functional properties from one tissue to the next in a given individual and even among classes of vertebrates. However, in recent years, it has become apparent that this may not be the case, because communicating junctions between lens fibers have properties that are not shared by communicating or gap junctions in other tissues. One major difference may involve the matter of connexon crystallization in the plane of the membrane. Under conditions of increased proton or calcium ion concentration, the communicating junctions of nonlenticular tissues are uncoupled with respect to ion permeability (28, 45). It is felt by some that the morphological correlate of this uncoupling, when viewed by freeze-fracture electron microscopy, is that of a crystalline array of intramembranous particles or connexons with a center to center spacing of 8.5 nm, whereas the electrically coupled version displays a more fluid configuration (10.3–10.5 nm spacing) with a somewhat random distribution of connexons within the junction domain (32, 34). Others agree that connexon crystallization occurs under conditions of high calcium, low pH, and anoxia but feel that the relationship between crystallization and function is by no means clear (40). The crystalline junctional configuration is reported to be rare in lens fibers by some investigators (17, 20), although there is disagreement from one laboratory that has published freeze-fracture evidence for crystallization of isolated lens communicating junctions (33, 35, 36). In spite of a lack of consensus at the morphological level regarding comparative features of lenticular and other communicating junctions, more definitive statements can be made about differences in the chemistry of lenticular and other communicating junction subunits now that we are certain that lens MIP26 is a junctional protein. In this respect, it is best to compare the junctional subunits of liver and lens, since they are the best characterized of the lot. The molecular weight of liver junctional protein that has not been exposed to enzymes during purification (22, 24) and that of the lens are both ~26 kdaltons, but they are quite dissimilar in several other respects. Peptide maps prepared from liver and lens 26-kdalton proteins are different (23, 30), as are their $NH_2$-terminal amino acid sequences (30), whereas peptide maps of MIP26 from mammals, birds, reptiles, and amphibians are similar (44). Antibodies against MIP26 from lens do not cross-react with the 26-kdalton protein from liver gap junctions (23, 49), whereas they do cross-react against MIP26 from human, chicken, toad, gekko, and shark (Horwitz, J., and D. Bok, manuscript in preparation). Earlier reports claiming that avian MIP26 did not cross-react (3, 49) resulted from a lack of sensitivity in the immunoprecipitation methods used at the time. Evidence has recently been presented that connexon symmetry also varies between lens and liver communicating junctions. X-ray crystallography of centrifuged, unfixed pellets and image reconstruction methods applied to negatively stained junctions from liver have suggested a sixfold symmetry for connexons from this tissue (11, 22, 29). On the other hand, two other laboratories have recently suggested that lens connexons are tetramers rather than hexamers (36; and G. Zampighi, personal communication). Thus, not only is there ample evidence for significant differences with respect to amino acid sequence and immunochemistry, but the manner in which the connexon monomers interact with one another may also be strikingly different for lens and liver communicating junctions.

Two laboratories have reported that the epithelial layer from which the lens fiber cells differentiate is not stained by immunofluorescence with anti-MIP26 antibodies (10, 46), whereas, as was stated earlier, the lens fiber membranes stain intensely. Since the lens epithelium is known to contain communicating junctions (18), this could be taken as evidence that the protein in communicating junctions of lens epithelium and the MIP26 from the junctions in lens fibers are immunologically distinct. This interpretation could be disputed from the standpoint that the junction density in the epithelium might be too low to be detected by immunofluorescence, but it is strengthened by the observation that isolated, solubilized plasma membranes from lens epithelium do not cross-react with MIP26 antibodies (10).

The evidence is building that lens communicating junctions are constructed of an intrinsic protein that is very different from its counterpart in the liver. Much remains to be learned about the similarities and differences between communication junction proteins from other tissues such as heart, kidney, and the various epithelia within the eye itself. With respect to comparisons with the liver, however, lens junctional protein appears to be a unique gene product that appears upon elongation of lens epithelium into fiber cells. In spite of these surprising differences, electrophysiological evidence (39) and experiments involving the transcellular diffusion of fluorescent dyes injected intracellularly (21, 39) underscore the role of lens communicating junctions in the extensive coupling of lens fiber cells. Metabolic cooperation is an essential feature in an avascular structure such as the lens (18), which must rely upon a supply of nutrients from the ocular aqueous humor for its survival. Now that we are certain of the protein species that subserves these important functions, we are better equipped to explore mechanisms whereby lens communicating junctions exert their control over the transcellular transport of metabolites.

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