Immunocytochemical Localization of the Main Intrinsic Polypeptide (MIP) in Ultrathin Frozen Sections of Rat Lens

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ABSTRACT The in situ distribution of the 26-kdalton Main Intrinsic Polypeptide (MIP or MP 26), a putative gap junction protein in ocular lens fibers, was defined at the electron microscope level using indirect immunoferritin labeling of ultrathin frozen sections of rat lens. MIP was found distributed throughout the plasma membrane of the lens fiber cell, with no apparent distinction between junctional and nonjunctional membrane. MIP was not detectable in the basal or lateral plasma membrane of the lens epithelial cell, including the interepithelial cell gap junctions; nor was MIP detectable in the plasma membrane or gap junctions of the hepatocyte. Previous reports have indicated that the protein composition of the lens fiber cell junction differs from that of the hepatocyte gap junction. The evidence presented here suggests that the composition of the fiber cell junction and plasma membrane is also immunocytochemically distinct from that of its progenitor, the lens epithelial cell.

The gap junction has been described in a wide variety of tissues and cell types, both in vivo and in vitro. The gap junction is so common, in fact, that its absence is the exception to the rule. As increasingly detailed investigations into a wide variety of tissues occur it has become apparent that the term "gap junction," as defined morphologically, has come to represent a class of intercellular junctions exhibiting both morphological and biochemical heterogeneity (9). Differences in junctional thickness (20, 33), freeze-fracture particle packing (1, 21, 33), physiologic behavior (9, 27), biochemical composition (19), and immunologic cross-reactivity (14) have all been reported. Although no direct evidence exists, a broadly based body of circumstantial evidence supports the belief that the gap junction is the morphological entity by which electrical coupling and the intercellular transfer of small metabolites and injected probes is effected (see reviews 9 and 17).

The anatomy and developmental history of the ocular lens has made it a particularly attractive subject for the study of plasma membranes and what have been reported to be gap junctions (10). The lens is a pure cell population possessing no neural elements, connective tissue, or vasculature. It consists predominantly of greatly elongated, terminally differentiated fiber cells, with a monolayer of undifferentiated progenitor cells, the lens epithelium, on its anterior surface. In the course of maturing from an epithelial cell into a fiber cell there occurs a progressive loss of membranous organelles (see Fig. 4), and a tremendous synthesis of both soluble cytoplasmic proteins (the crystallins) and putative gap junctions (23). Although gap junctions occur only infrequently between epithelial cells, and between epithelial cell and fiber cell, they have been reported to be present in extraordinary numbers between fiber cells. Some estimates have claimed that upwards of 60% of the surface of the fiber cell is involved in gap junctions (16). Thus, membranes isolated from whole lenses are overwhelmingly plasma membranes of a single cell type, rich in intercellular junctions (2).

Physiologic studies of the lens support the belief that interfiber cell junctions subserve an intercellular communication role, although, as in other tissues, the evidence is circumstantial. With respect to ions (25), small metabolites (11), and microelectrode-injected dyes (24), the lens behaves as a syncitium, offering little resistance to the intercellular spread of current, or tracers of low molecular weight. However, even though these fiber cell junctions are said by some to resemble, by freeze fracture analysis, the gap junctions of other cell types such as the hepatocyte, they lack an easily demonstrable "gap;" thus the term "gap junction" seems inappropriate. Similarly, because the function of these interfiber cell junctions has not been directly demonstrated, the term "communicating junction" seems premature. Thus, to be conservative, we will refer to the interfiber cell junctions that appear in great numbers upon differentiation simply as "fiber cell junctions" (13).

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SDS PAGE of isolated and thoroughly washed lens membranes revealed that a single polypeptide with a relative molecular weight of ~27–28 kdaltons accounts for well over half of the total Coomassie Blue-staining protein (see lane *3*, Fig. 1). This protein has been called the Main Intrinsic Polypeptide (MIP[1]), or the 26-kdalton Membrane Protein (MP 26) (3, 5). The coincident abundance and developmental appearance of both this single polypeptide and the fiber cell junction led to speculation that the MIP may be a significant, or possibly the sole component of the fiber cell junctions. Subsequent investigation has lent credence to this belief: (*a*) density gradient centrifugation and selective detergent extraction appears to co-enrich for both MIP and fiber cell junctions (10, 33); (*b*) MIP has been immunocytochemically localized in isolated and purified fiber cell junctions (4; see, however 20); (*c*) MIP and fiber cell junctions appear at the same time and location developmentally (6, 10, 31).

Interestingly the dominant polypeptide of purified hepatocyte gap junctions is also in the same relative molecular weight range (27–29 kdalton (7, 12, 14), but, in addition to their suggested analogous roles in intercellular communication, the lens and liver proteins have few biochemical properties in common. Both readily undergo partial proteolysis to ~20–22 kdalton in situ, and both are inclined to aggregate with purification, resulting in the appearance of multimers in SDS PAGE (12). However, partial amino acid sequencing (19), peptide mapping (14), and immunologic analysis (14) all suggest that little or no sequence homology exists between the two proteins.

Little is known about the gap junctions between the lens epithelial cells, and between epithelial cells and fiber cells. Such junctions have not been purified from lens epithelium and, thus, biochemical information is not available. Immunofluorescence studies of lens, using anti-MIP, have been performed (6, 20, 31) and the results suggest that MIP is absent from, or present in very low quantity in the undifferentiated epithelium. MIP immunoreactivity appears with differentiation of the epithelial cell into a fiber cell, coincident with the proliferation of fiber cell junctions. However, the relative scarcity of interepithelial cell gap junctions, combined with their proximity to the fiber cells preclude the possibility of determining, by immunofluorescence, whether MIP is present in epithelial cell gap junctions.

Paul and Goodenough (20), in work that was performed concurrently with this, have used immunocytochemistry to localize MIP both by immunofluorescence in plastic sections, and at the electron microscope level on isolated and purified lens membranes. By immunofluorescence they find MIP to be distributed throughout the fiber cell plasma membrane in areas known to be junction rich ("ball and socket joints" between fiber cells), as well as in junction-poor regions (epithelial cell-fiber cell interface). In contrast, their ultrastructural localization in isolated junctions reveals MIP immunoreactivity only in what they define as nonjunctional areas. While this result would appear to contradict the findings of Bok, et al. (4), procedural and semantic differences may account for the apparent discrepancy. In both of these reports, however, as well as in most reports that study MIP, the data is drawn from a relatively small sample of lens membranes that has been purified from large quantities of starting material. Thus, small, and possibly unrepresentative classes of lens membranes have been selected for study. Further, the possibility exists that artifactual reorganization of membrane components has occurred as a result of the extensive use of chaotropes, detergents, etc.

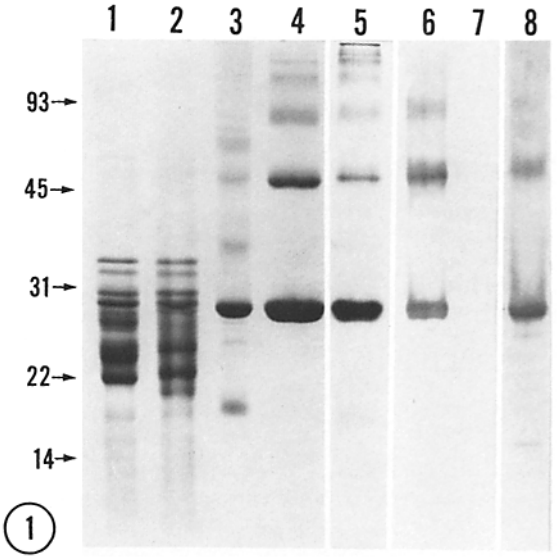


FIGURE 1 SDS polyacrylamide gel, 12.5%, prepared according to Laemmli. Lanes *1–4* are Coomassie-Blue stained. Numbers in the left-hand margin indicate the relative molecular weights of standards in kilodaltons; ~25 μg protein/lane. Lane *1*, "buffer wash" supernatant of the first centrifugation in the purification of MIP; lane *2*, "buffer wash" pellet of the first centrifugation in the purification of MIP. Lanes *1* and *2* combined represent total lens protein; lane *3*, "purified lens membranes" at the end of buffer, urea, and sodium hydroxide washes; lane *4*, "purified MIP" used in preparation of the affinity resin, and in preadsorption of the antibody for both immunocytochemistry and immunoreplica labeling. Note the appearance of higher order aggregates; lane *5*, "purified MIP" silver-stained, ~2.5 μg protein; lane *6*, immunoreplica of purified MIP; lane *7*, immunoreplica of "buffer wash supernatant" (comparable to lane *1*); lane *8*, immunoreplica of "buffer wash pellet" (comparable to lane *2*).

It is apparent, then, that the biological role and the in situ distribution of the MIP has not been unequivocally defined, nor has the homo- or heterogeneity of the lens epithelial and fiber cell junctions been established. We have chosen to examine these problems by immunocytochemically defining the distribution of the MIP in ultrathin frozen sections of rat lens and liver, and by comparing the immunocytochemical kinship of gap junctions and plasma membranes of lens fiber cells with those of both lens epithelium and hepatocytes. In this fashion we have sought to circumvent the selection of subclasses of membranes inherent in commonly used membrane isolation schemes.

MATERIALS AND METHODS

Purification of MIP and Isolation of Lens Membranes

MIP purification and characterization were described in an earlier report (4). Briefly, fresh or frozen bovine lenses were decapsulated, gently crushed, and the lens nuclei discarded. To remove soluble proteins the remaining fiber cell mass was "buffer washed" by repeated homogenization and centrifugation. Cytoskeletal and less soluble material was extracted in a similar fashion using 7 M urea followed by 0.1 N NaOH. For immunochemistry of membranes, the pellet from the last NaOH wash was resuspended in PBS (0.01 M phosphate buffer, 0.9% NaCl, pH 7.4). For the purification of MIP, the membranes were washed in distilled water, solubilized with SDS, and resolved by preparative-

[1] *Abbreviation used in this paper:* MIP, Main Intrinsic Polypeptide.

scale SDS PAGE. MIP isolated from the gel was further purified by hydroxylapatite chromatography.

Preparation of Affinity-purified Anti-MIP and Anti-MIP F(ab')$_2$

Antibody to MIP was raised as described in an earlier report (4). An affinity resin consisting of MIP immobilized on Sepharose 4-B was prepared using 1 mg of purified MIP and 1 g (dry weight) of cyanogen bromide-activated Sepharose 4-B (Pharmacia Fine Chemicals Inc., Piscataway NJ) according to the manufacturer's directions, but omitting the NaCl in the conjugation step to avoid precipitating the SDS-protein complex.

5 ml of rabbit antiserum to MIP was passed through the affinity resin, and the resin washed thoroughly with PBS. Bound antibody was harvested by inverting the column and eluting with aqueous 4 M $MgCl_2$. Fractions of 0.5 ml were collected in tubes containing 6 ml of 0.01 M phosphate buffer, pH 7.4. Fractions judged to contain protein by absorbance at 280 nm were pooled, dialyzed, and concentrated using a MicroProDiCon (Bio-Molecular Dynamics, Beaverton, OR). Antibody was stored at 1.0 mg/ml at −70°C. F(ab')$_2$ of anti-MIP was prepared essentially as described by Garvey et al. (8) using 1 mg of affinity-purified anti-MIP, and resolving the digest products by gel filtration on Sephadex G-150 in PBS. F(ab')$_2$ was concentrated to ~0.1 mg/ml and stored at −70°C.

Characterization of the Antibody

Detection and initial characterization of antisera were performed by immunodiffusion and crossed immunoelectrophoresis as described in an earlier report (4). Affinity-purified antibody was further characterized by immunologic analysis of nitrocellulose replicas of SDS polyacrylamide gels as described by Towbin et al. (30).

Purification of Ferritin-second Antibody

Some experiments were performed using ferritin conjugated to F(ab')$_2$ fragments of goat anti-rabbit F(ab')$_2$ (Cappel Laboratories, Cochranville, PA) that had been further purified by high performance liquid chromatography on a TSK/SW 4000 column (Beckman Instruments, Berkeley CA) to remove aggregated material.

Immunocytochemistry

Frozen Thin Sections from UCLA Colony: Frozen thin sectioning was performed essentially as described by Tokuyasu (29). In brief, young Fisher rats (100–150 g), anesthetized with sodium pentobarbital, were fixed by vascular perfusion for 5 min, and the eyes and liver removed. Fixation was continued by immersion at concentrations and times described in the figure legends. Tissue was infused with 1.2 M sucrose for 90 min prior to sectioning at −85°C. Sectioning was performed on the Sorvall MT-2B ultramicrotome modified with the FTS cryo-attachment (Ivan Sorvall, Wilmington, DE). Sections were harvested with a droplet of 2 M sucrose containing 2% BSA in 0.05 M phosphate buffer. All preconditioning, washing, and immunolabeling was performed in the presence of 4% BSA in PBS, on a rotary shaker. Primary antibody labeling was performed on 15 μl droplets using affinity-purified whole antibody or affinity-purified F(ab')$_2$. Controls consisted of section incubation on either anti-MIP preadsorbed with purified MIP, or on nonspecific rabbit IgG, at concentrations up to ten times greater than used in specific immunolabeling. Secondary antibody was F(ab')$_2$ fragment of goat anti-rabbit F(ab')$_2$, conjugated to ferritin (Cappel Laboratories, Cochranville, PA). Concentration and time of primary antibody labeling are contained in the figure legends. Concentrations of primary and secondary antibody are expressed in units of absorbance at 280 nm (A_{280}) in a 1-cm pathlength. Secondary antibody in all cases was employed at A_{280} = 1.0 for 30 min. Contrast for electron microscopy was achieved as described by Tokuyasu (28). Use of methylcellulose was omitted, with sections dried on the grids by adsorption of excess water with filter paper.

Isolated Membranes: Isolated, washed membranes were immunolabeled as described by Bok et al. (4), except that membranes were prepared as described earlier in this section.

RESULTS

A detailed characterization of the MIP and its purification have been described elsewhere (4). Two points must be born in mind in the evaluation of SDS PAGE and immunoreplica analysis of MIP and lens membranes: (*a*) MIP undergoes a physiologic posttranslational cleavage from ~27 to ~22 kdalton (MP 22), a process with a half-life on the order of years (15), and (*b*) both the MIP and the MP 22 are inclined to aggregate upon purification and concentration, or when heated in the presence of SDS, forming multimeric aggregates (32). Thus, an SDS PAGE profile of purified lens membranes may display bands with relative molecular weights of 22, 26, 44 (2 × 22), 52 (2 × 26), etc. that are all MIP-derived. Evidence that these non-26-kdalton bands are MIP-derived is threefold: (*b*) MIP is purified by cutting from a gel, thus eliminating higher and lower molecular weight contaminants. Yet this material, when re-electrophoresed, will exhibit multimeric aggregates (lanes *4* and *5*, Fig. 1, and reference 4). The degree of aggregation exhibited will be dependent on the degree to which the sample has been heated or concentrated, with boiling resulting in aggregation to the point where no protein enters the resolving gel. (*b*) Antibody that has been affinity-purified on a column of purified MIP labels the 26-kdalton band in nitrocellulose replicas, as well as the multimers. Preadsorption of the antibody with purified MIP suppresses such labeling. (*c*) Monoclonal antibodies to the MIP label these multimers, as well as some of the cleavage products that begin to appear in samples of purified MIP (26).

An example of the purified MIP that was used in antibody production and affinity purification is shown in Fig. 1, lanes *4* and *5*. To detect potential contaminants the gel is overloaded, a condition that results in multimer formation. It is apparent that no non-MIP protein is detectable, even when stained with the very sensitive silver staining method (lane *5*, Fig. 1).

It is, of course, possible that a contaminant exists that co-migrates with MIP in SDS PAGE. However, this potential contaminant would not only have to co-migrate in SDS PAGE, but co-purify on hydroxylapatite as well, and exhibit similar heat-induced aggregation to avoid detection (see reference 4). The chance for existence of a contaminant that possesses these identical characteristics seems remote.

Characterization of the Antibody to MIP

Whole antiserum to MIP was characterized in a previous report (4). That analysis showed antiserum to be free of detectable cross-reactivity with proteins other than the MIP and its derivatives. For immunocytochemistry of frozen thin sections the anti-MIP was affinity-purified, and characterized further by the nitrocellulose immunoreplica technique of Towbin et al. (30). Samples of the supernatant and pellet of the first buffer wash, as well as purified MIP, were characterized for immunoreactivity. The results of this immunolabeling, shown in Fig. 1, indicate that affinity-purified antibody reacts only with MIP and its derivatives. The first two samples represent total lens protein, soluble and insoluble, and thus contain all potential contaminants that purified antibody might recognize. No reactivity was observed in the soluble fraction (lane *7*, Fig. 1), and reactivity with the insoluble material occurs only at the anticipated molecular weight levels (lane *8*, Fig. 1). To insure that these bands were all MIP-derived the labeling was repeated but the antibody was preadsorbed with purified MIP prior to incubation. Such treatment eliminated all labeling (not shown).

Artifactual labeling of the nitrocellulose replica by nonspecific binding of either the primary or secondary antibody was assessed by the use of nonimmune rabbit IgG as a primary

antibody, or use of the secondary antibody alone, without primary antibody. No labeling was observed.

Immunocytochemistry

Frozen thin sections of lens exposed to anti-MIP consistently exhibited a heavy and uniform labeling of the fiber cell plasma membrane. The intensity of the labeling, and the signal-to-noise ratio improved steadily as modifications were made in the protocol and as conditions and reagents were optimized, but the pattern of labeling never changed. Although light labeling of the cytoplasm of fiber cells occurred in the early stages of the investigation, suggesting the localization of cytoplasmic MIP, this labeling was virtually eliminated after the secondary antibody-ferritin was purified by high performance liquid chromatography (compare Figs. 2 and 5). Thus, no pools of intracellular MIP were ever identified.

A key problem in the investigation of fiber cell junctions has been the unequivocal identification of the junction itself. By definition the "gap junction" possesses a 2-nm gap between the outer leaflets of the two plasma membranes involved, a gap that is readily visualized in tissues such as liver and heart. However, even under more conventional circumstances, that is, plastic sections (Fig. 4) of whole lens or isolated membranes, no gap is demonstrable in lens fiber junctions (4, 10, 14, 20, 33). In this report the problem of identification is further aggravated by the greater average thickness of frozen sections as opposed to plastic, and by the fact that uranyl acetate tends to downplay the "gap" by filling it with stain (see Fig. 12 of an hepatocyte gap junction). Finally, in sections where immunolabeling was optimized, the density of the ferritin was so great that details of the underlying membranes were obscured (see Fig. 5).

Fiber cell junctions that were sectioned obliquely were frequent and heavily labeled. Junctions that were sectioned perpendicular to the plane of the junctional membrane were less frequent, but occurred often enough to confirm that these were, in fact, immunoreactive (Fig. 3, *a* and *b*). These direct observations of labeled fiber cell junctions, combined with the facts that (*a*) lens junctions have been labeled in vitro with anti-MIP (4); (*b*) anti-MIP labeling is relatively constant and unchanged regardless of whether the region is junction rich or junction poor ("ball and socket joints" or epithelial cell-fiber cell interface respectively) and; (*c*) labeling is uninterrupted over the surface of a cell where junctions are said to occupy as much as 64% of the cell surface all lead to the conclusion that MIP is present in both junctional and nonjunctional domains of the plasma membrane.

Frozen thin sections of lens that included lens epithelium showed that MIP is not detectable in the basal or lateral regions of the lens epithelial cell membrane (Figs. 7 and 8). Particular attention was paid to the apico-lateral region where the inter-epithelial cell gap junctions occur. At no time did the labeling of the lateral epithelial cell membrane exceed background levels (Figs. 9 and 10). While label did appear on the apical surface (Fig. 8), the surface that abuts on the fiber cell, it was impossible to determine whether this labeling was due to the presence of MIP in the epithelial cell membrane, the fiber cell membrane, or both. The amount of label present at this fiber cell-epithelial cell interface was usually somewhat less than that at a fiber cell-fiber cell interface, suggesting that only one of the two membranes contained antigen, but this cannot be unequivocally demonstrated (see Fig. 8). These results, while inconclusive with respect to the apical surface of the epithelial cell, do demonstrate that MIP is absent from the basal and lateral surfaces, including the interepithelial cell gap junctions, or that it is present in an undetectable form or amount.

Frozen thin sections of hepatocytes, co-incubated with lens sections, also showed no sign of MIP immunoreactivity in either gap junctions or plasma membranes (Fig. 12).

Control sections of lens, incubated with either nonimmune rabbit IgG, or with antibody preadsorbed with MIP, showed no inclination to label in a specific fashion. Nonspecific sticking of label did occur at regions where tears occurred in the section. (Fig. 6).

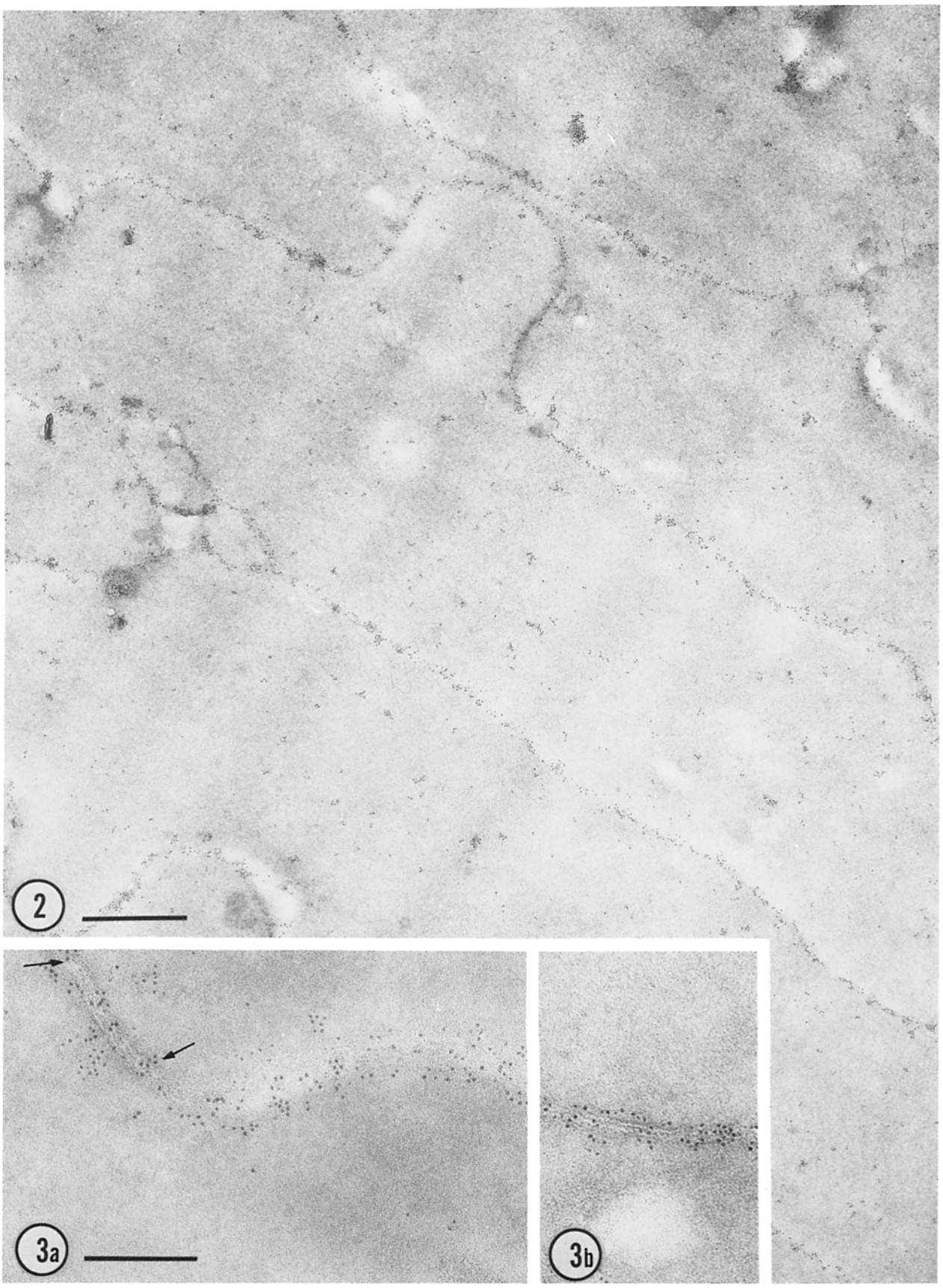
DISCUSSION

Earlier evidence has implicated MIP as a major, or possibly the sole component of the lens fiber cell junction, and many recent reports have centered around this speculation (4, 14, 20, 33). The evidence, in brief, is (*a*) fiber cell junctions and MIP co-exist in extraordinary quantity in the lens membranes. (*b*) MIP and fiber cell junctions appear simultaneously in development. (*c*) MIP and fiber cell junctions appear to co-purify. (*d*) MIP has been immunocytochemically demonstrated in isolated lens junctions.

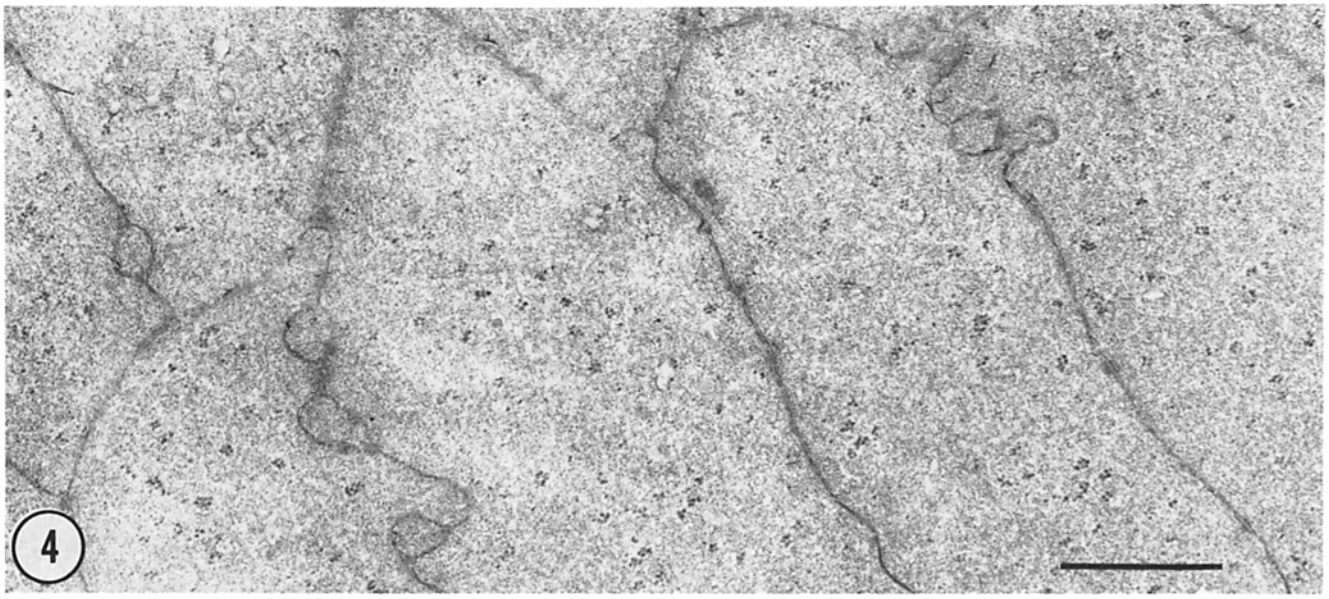
In this report we have demonstrated by immunolabeling of frozen thin sections that MIP is not confined to the domain of the fiber cell junction, but that it is distributed throughout the plasma membrane of the fiber cell. Thus, the MIP, by morphologic criteria, is not strictly a junctional protein. Functionally, however, MIP may well be the key junctional protein, with its extra-junctional presence representing an equilibrium between junctional and nonjunctional protein. Although casual examination of the distribution of ferritin grains suggests no obvious differences in the level of MIP immunoreactivity with respect to the fiber cell junction, no legitimate comparison of its concentration in junctional and nonjunctional domains can be made. The possibility exists that the MIP appears in different conformational states in these two domains, or that MIP concentration is so high that stoichiometric binding of the relatively large ferritin-antibody label cannot occur.

It has also been demonstrated here that the composition of the lens epithelial cell plasma membrane and interepithelial cell gap junction is immunocytochemically different from that of the lens fiber cell. Thus, if MIP represents the fiber cell junction protein, then the lens cell expresses at least two types of intercellular junction in the course of its development, an MIP-negative junction between epithelial cells, and an MIP-positive junction between fiber cells, a finding of considerable interest. In this light the nature of the junctions between epithelial cell and fiber cell becomes particularly interesting, but, as yet, unelucidated.

Data on the lens epithelial cell gap junctions is scarce. Freeze fracture studies on adult lens reveal some disimilarity in the morphology of junctions between epithelial cells and between fiber cells (1, 11, 22). The epithelial cell frequently displays the condensed, hexagonally packed morphology that is commonly found in conventionally fixed liver. In contrast, the fiber cell junctions remain uncondensed and disordered, even in the face of anoxia and other stimuli that induce particle condensation in other tissues (10). Scheutze and Goodenough (27), in a combined freeze fracture-dye transfer



FIGURES 2 and 3 Fig. 2: Frozen thin section of rat lens cortex immunolabeled with affinity-purified anti-MIP IgG at A_{280} = 0.05 for 30 min; fixed in 0.2% glutaraldehyde, 2.0% formaldehyde in 0.1 M phosphate buffer for 60 min. Bar, 4.75 μm. × 40,000. Fig. 3: (a) Immunolabeled frozen thin section of rat lens cortex, including a labeled gap junction (between arrows); fixed as in Fig. 2 and immunolabeled with affinity-purified anti-MIP IgG at A_{280} = 0.1 for 30 min. Bar, 0.16 μm. × 125,000. (*b*) Frozen thin section of rat lens cortex including labeled gap junction. Immunolabeled with affinity-purified anti-MIP F(ab')$_2$ at A_{280} = 0.05 and fixed in 0.2% glutaraldehyde, 2.0% formaldehyde. Bar, 0.16 μm. × 125,000.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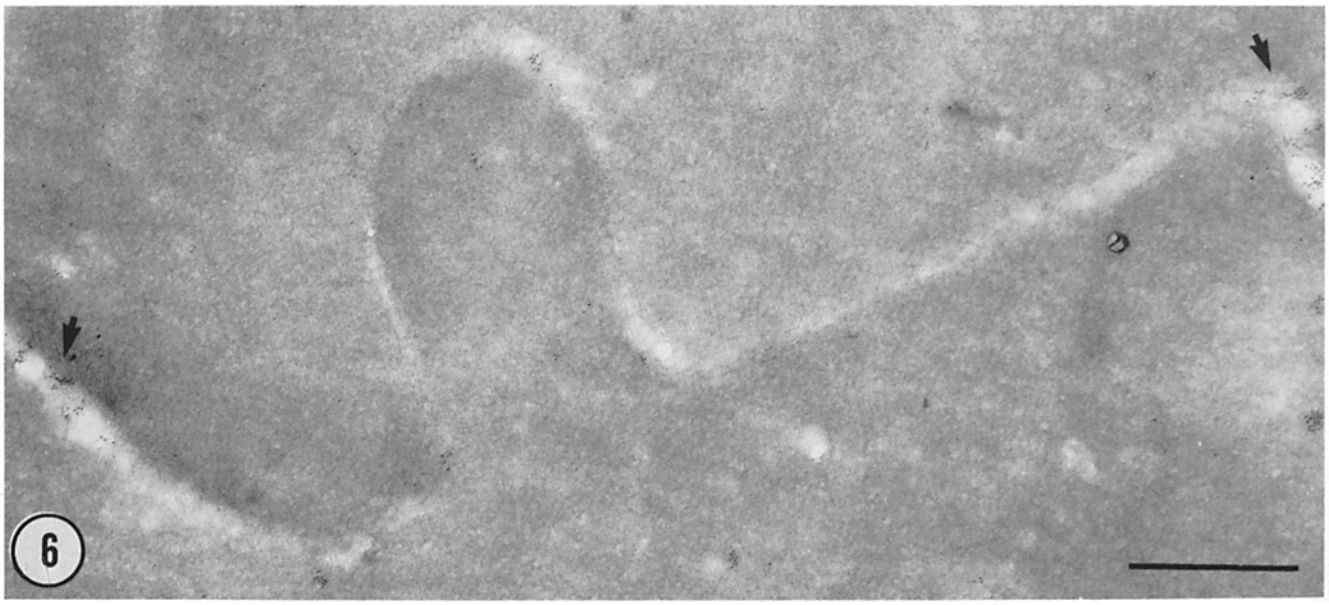
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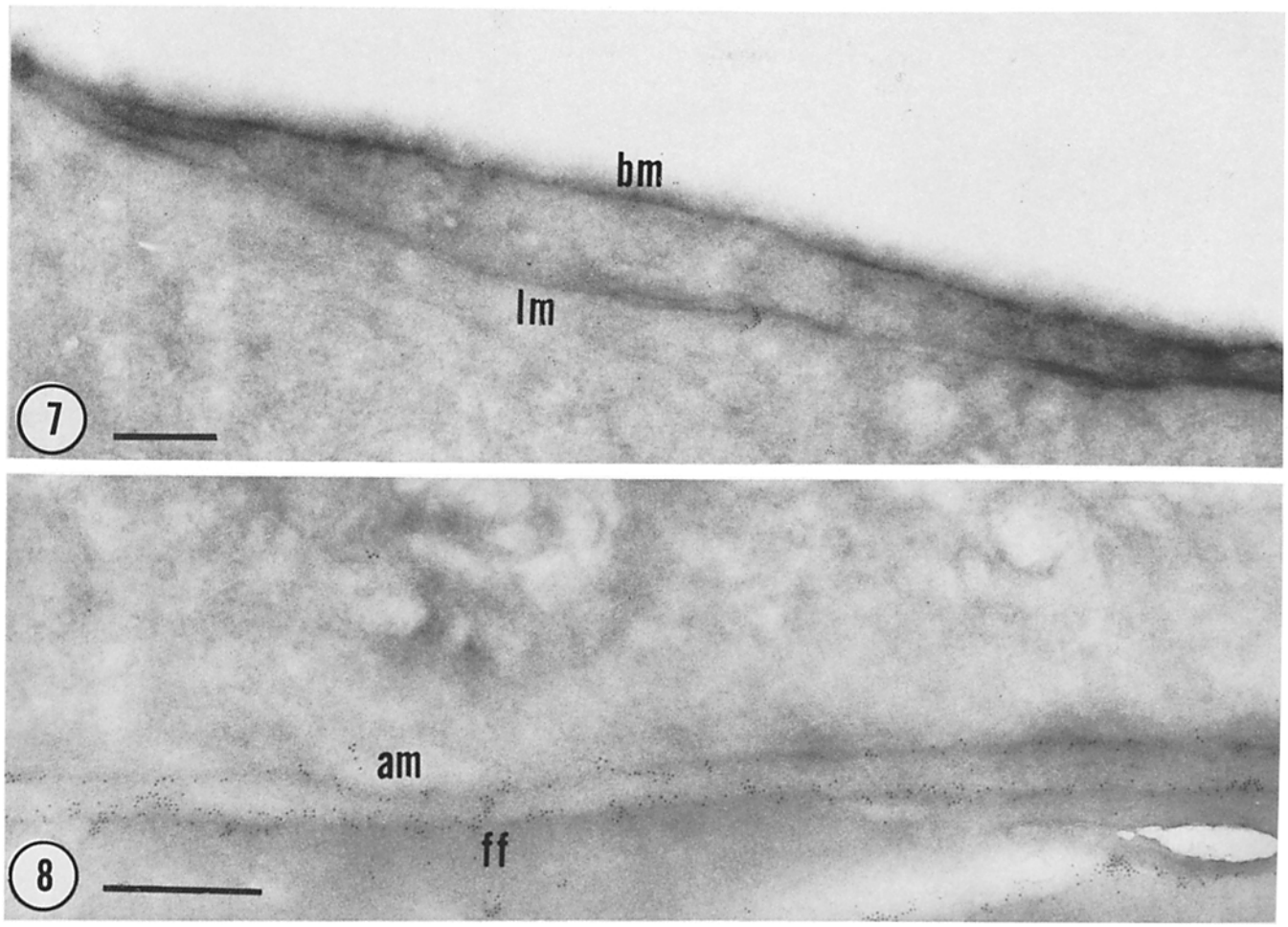
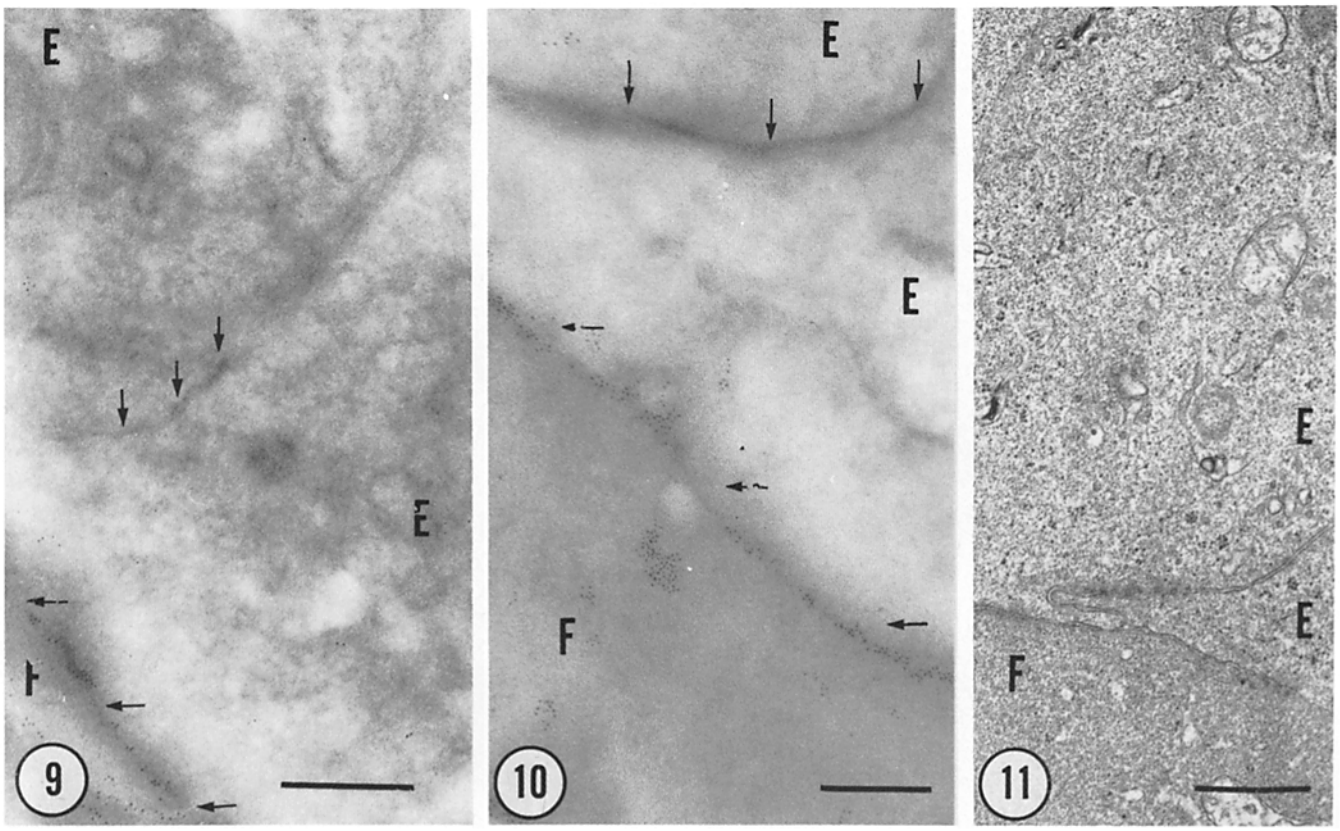


FIGURES 7 and 8 Fig. 7: Immunolabeled frozen thin section of rat lens epithelium, basal membrane (*bm*) and lateral membrane (*lm*); fixed as in Fig. 2. Primary antibody was anti-MIP F(ab')$_2$ at A_{280} = 0.05 for 30 min. Some nonspecific labeling occurs. Bar, 0.24 μm. × 62,000. Fig. 8: Same immunolabeling and fixation conditions as in Fig. 7, but view is of the apical surface/fiber cell interface. The epithelial cell apical border (*am*), and the first fiber cell-fiber cell border (*ff*) are running very close to each other and in parallel. Note that the epithelial apical membrane (*am*) that abuts on the fiber cells (*ff*) labels to a lesser degree than the fiber cell-fiber cell borders. *E*, epithelial cell. Bar, 0.24 μm. × 89,000.

study of embryonic chick lens fiber cells reported developmentally dependent changes in junctional sensitivity to CO_2. Coincident with this change in sensitivity was a change in junctional morphology from a condensing-type junction to a noncondensing type. Michalke and Lowenstein (18) reported that lens epithelial cells and hepatocytes could form low-resistance junctions in vitro. Such observations, combined with those presented in this report concerning the immunocytochemical disimilarity between lens epithelial and lens fiber cell junctions, might suggest a similarity between lens epithelial cell and hepatocyte gap junctions. However, this accumulated evidence, while suggestive, is circumstantial and incomplete at best.

The absence of MIP immunoreactivity in the hepatocyte plasma membrane and gap junction that was described in this report is not surprising in light of the biochemical and immunologic differences that have been reported between MIP and the purified 27-kdalton liver gap junction protein (14, 19). However, the observation that lens epithelial cells and hepatocytes can communicate in vitro places the lens epithelial cell in the interesting position of being able to communicate with at least two cell types, each with apparently different junctional proteins, the hepatocyte (in vitro), and the lens fiber cell (in vivo), in addition to communicating with other lens epithelial cells, whose junctional protein has not been described.

FIGURES 4–6 Fig. 4: Plastic section of rat lens cortex in a region comparable with that in Figs. 2, 3, 5, and 6. The relative paucity of membranous organelles is apparent in conventionally fixed and sectioned material; fixed overnight in 2.0% glutaraldehyde, 1.0% formaldehyde in 0.055 M cacodylate buffer. Bar, 0.65 μm. × 32,000. Fig. 5: Immunolabeled frozen thin section of rat lens cortex. Primary antibody at A_{280} = 0.01 for 30 min. Secondary antibody-ferritin was high performance liquid chromatography-purified (see text), and used at the same concentration. Note the increase in the density of the ferritin labeling, and the drop in background of cytoplasmic labeling when compared with Fig. 2; fixed as in Fig. 2, except 0.3% glutaraldehyde was used. Bar, 0.59 μm. × 54,000. Fig. 6: Frozen thin section of rat lens cortex. Primary antibody was nonspecific rabbit IgG at A_{280} = 0.1 for 30 min. Note that sites where the section has torn label nonspecifically (arrows). Fixed in 0.2% glutaraldehyde, 2.0% formaldehyde. Bar, 0.35 μm. × 63,000.



FIGURES 9–11 Fig. 9: Immunolabeled frozen thin section of two rat lens epithelial cells (*E*) and lens fiber cell (*F*) with an interepithelial cell border (vertical arrows) included in the view. Vertical arrows are pointing specifically to an interepithelial cell gap junction. Horizontal arrows point to the epithelial cell-fiber cell border. Fixed as in Fig. 2. Antibody labeling as in Fig. 7. Bar, 0.25 μm. × 71,000. Fig. 10: Region similar to Fig. 9. Some background labeling appears in the fiber cell (*F*) cytoplasm which is typical of sections that were exposed to second antibody-ferritin that had not been high performance liquid chromatography-purified. Immunolabeling as in Fig. 10. Bar, 0.17 μm. × 90,000. Fig. 11: Plastic section of rat lens fixed as in Fig. 3; field of view similar to Figs. 9 and 10. The difference in density of the epithelial cell cytoplasm and fiber cell cytoplasm is a result of the enormous synthesis of soluble crystallins in the fiber cell. The interdigitation of epithelial cell membranes at the apical surface is a common occurrence and is apparent in Figs. 9 and 10 as well. Bar, 0.60 μm. × 25,000.

It must be stressed that the biological role of the MIP has not been unequivocally defined. Evidence that has been used in the past to implicate this protein as a junctional vs. non-junctional protein must be evaluated with caution. These reports have presented data drawn from a relatively small sample of lens membrane that has been purified from large quantities of starting material, in a fashion that usually involves chaotropes, detergents, and high concentrations of sucrose (4, 10, 14, 33). Thus the possibility exists that selective purification of a small, unrepresentative subclass of lens membranes and/or extensive and artifactual redistribution of membrane components has occurred. Further limiting the conclusions that can be drawn from these reports are the significant variations in isolation protocol that have been employed by different investigators; differences that ultimately limit the confidence with which data from different laboratories can be compared.

It is possible that the MIP has no functional role in intercellular communication in the lens. MIP immunoreactivity in fiber cell junctions may be coincidental in that MIP is not actively excluded from the junctional domain. While such a coincidental presence may be difficult to imagine in the highly condensed junction of the hepatocyte, it is not difficult to imagine in the dispersed junctions of the lens fiber cell. This scenario, however, does not seem likely in light of the overwhelming dominance of MIP in SDS PAGE analysis of isolated and purified lens junctions (10).

Alternatively MIP may represent the principle component of a second class of membrane-membrane interactions that are specific to the lens, but whose function remains undefined, as suggested by Zampighi et al. (33). These interactions would exhibit a pentalaminar profile in transmission electron microscopy, as would any close apposition of plasma membranes, and thus closely resemble the gap junction. Indeed the homo- or heterogeneity of lens junctions remains unclear. This situation has not been clarified by freeze fracture analysis since images of hexagonally, tetramerically, and randomly packed particle have all been reported. Finally, transmission electron microscopy profiles of isolated junctions published by Zampighi et al. (33), and more recently Paul and Goodenough (20), reveal two distinct classes of membrane-membrane interaction in what has been thought to be relatively pure preparations of lens junctions. Both classes possess a pentalaminar profile, neither display an obvious "gap", but one is distinctly thicker than the other (~16 nm vs. 12 nm.). Paul and Goodenough (20) report MIP immunoreactivity in the thinner profiles and unit membranes, but not in the thicker profiles. It is not clear at this time what these thicker junctions represent. Surely they cannot be the structures that heretofore have been referred to as lens gap junctions (10),

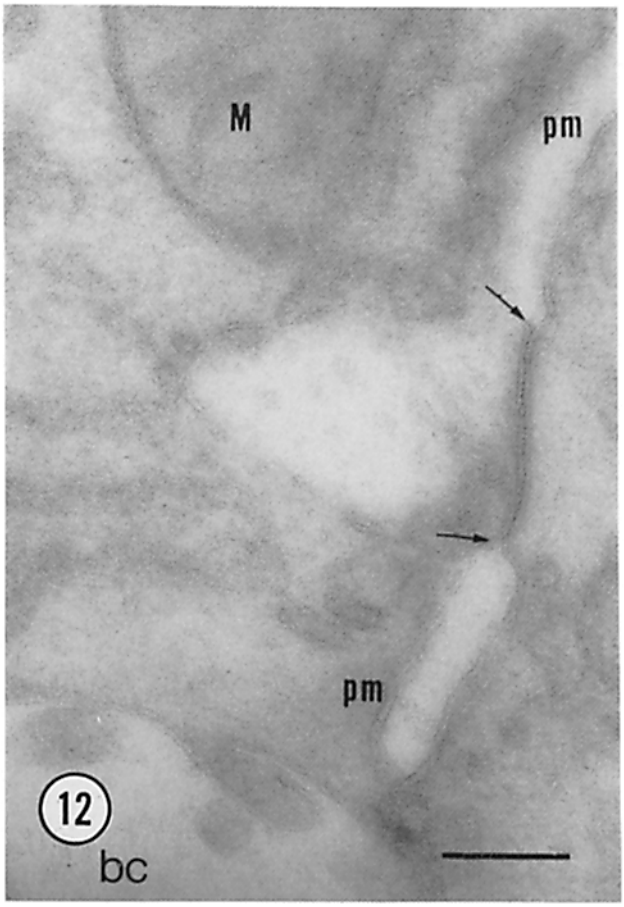


FIGURE 12 Immunolabeled frozen thin section of rat hepatocyte; fixed as in Fig. 4. Immunolabeled with anti-MIP IgG at A_{280} = 0.1 for 30 min. Gap junction (arrows), plasma membrane (*pm*), bile canaliculus (*bc*), mitochondrion (*M*). Bar, 0.14 μm. × 120,000.

since the thick junctions, as described by Zampighi et al. (33) are rare. Their scarcity can also be inferred from the work of Paul and Goodenough (20) since they are clearly a subpopulation of what was originally viewed as a highly enriched lens junction preparation, with MIP the predominant intrinsic protein (10). Whether these various structures that have all been called gap junctions represent different junctional types, different physiologic states of the same junctional type, or artifacts of preparation remains unclear. It is certain, however, from the data presented in this report that the lens fiber cell junctions are not only rich in MIP, but immunocytochemically distinct from those of the lens epithelial cell and the hepatocyte.

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