Identification of a 70,000-D Protein in Lens Membrane Junctional Domains

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ABSTRACT A 70,000-D membrane protein (MP70), which is restricted to the eye lens fibers and is present in immunologically homologous form in many vertebrate species, has been identified. By use of anti-MP70 monoclonal antibodies for immunofluorescence microscopy and electron microscopy, this polypeptide was localized in lens membrane junctional domains. Both immunofluorescence microscopy and SDS PAGE reveal an abundance of MP70 in the lens outer cortex that coincides with a high frequency of fiber gap junctions in the same region.

Intercellular membrane junctions in the vertebrate eye lens have attracted much research interest for several reasons. (*a*) Only young fiber cells at the lens periphery (outer cortex) display full metabolic activity, whereas protein synthesis in the older fibers in the lens nucleus is negligible (61). Extensive electrophysiological coupling (51) and metabolic cooperation (21) have been demonstrated in the lens, and it has been postulated that cortical fibers control homeostasis throughout the lens (54), presumably via intercellular communicating membrane junctions. (*b*) Membrane junctional domains appear important in maintaining the highly ordered arrangement of lens fibers and in minimizing extracellular space (37). (*c*) In many forms of lens cataract, the fiber membrane protein composition and membrane functions are altered (19, 54, 55, 58, 59). In fact, recent electron microscopy data suggest a pathogenic decrease of fiber gap junctions (39, 59) and reveal significant distortions of membrane-joining devices in cataractous lenses (13, 14).

Three different types of membrane junctional domains have been characterized in the vertebrate eye lens. (*a*) Fiber gap junctions are abundant in the lens cortex (3, 4, 7, 20, 36, 37, 40, 50) and have been isolated in many laboratories (3, 7, 16, 20, 26, 38, 46). These lens fiber gap junctions are believed to maintain extensive low resistance pathways in the eye lens (20, 21). Despite structural similarities between lens fiber gap junctions and the well studied liver gap junctions, the former show some distinct features. For example, connexons in isolated lens fiber gap junctions are normally arranged in a noncrystalline order (20, 26, 38, 46) but may be crystallized by in vitro manipulations (34, 48, 49). Fiber gap junctions often do not reveal a gap in thin-sectioned material (18, 26, 46, 47) and appear as pentalaminar membrane structures of 16–17-nm overall thickness. Despite this, we prefer to use the term gap junctions to describe this structure, because the term is well used and understood. The main intrinsic polypeptide (MIP)[1] with an apparent molecular weight of 26,000 is generally believed to be the major component of fiber gap junctions (4, 9, 20, 26, 31, 32, 38, 46, 53). However, no homology has been detected between MIP and the liver 28,000-D gap junction component (15, 25, 26, 46). More recently, by use of immunoelectron microscopy, MIP has also been localized in nonjunctional membrane regions (5, 18, 47). In one case, anti-MIP antibodies failed to bind to lens fiber gap junctions but did recognize MIP in single membrane areas (47). (*b*) A second type of lens plasma membrane junctional domain also appears pentalaminar but is significantly thinner (12–13 nm overall thickness) than fiber gap junctions in thin sections and has therefore been referred to as a thin junction or close membrane apposition (31, 44, 47, 64). Electron microscopy data demonstrate that thin junctions have a similar undulating appearance and a similar distribution to that of lens square arrays, which suggests they are the same structure (44, 64). However, these protein arrays are mostly found in only one of the closely apposed membranes (22, 44), and their communicating capability has been questioned (22, 33, 44). Furthermore, thin junctions have been described that labeled with anti-MIP antibodies on only one of the two opposed membranes (47). SDS PAGE analysis of purified bovine

[1] *Abbreviations used in this paper:* buffer A, a solution of 5 mM Tris pH 8.0 and 1 mM EDTA; buffer B, a solution of 100 mM Na cacodylate pH 7.4; buffer C, a solution of 10 mM Tris pH 8, 1 mM EDTA, and 100 mM NaCl; MIP, main intrinsic polypeptide; MP70, a 70,000-D membrane protein restricted to eye lens fibers.

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square arrays also revealed MIP as the principal component (64). (c) The flat, hexagonally shaped fibers in the lens cortex appear to be held together by so-called ball and socket membrane structures (13, 14, 36). These interdigitating membrane domains are predominantly located at the corners of the hexagonal fiber cross-section profile (13, 37). Ball and socket membranes contain MIP as demonstrated by immunofluorescence microscopy (47).

In a search for junctional membrane proteins other than the apparently ubiquitous MIP, we have made a library of monoclonal antibodies directed against proteins associated with urea-extracted lens cortical plasma membranes. We have isolated two hybridoma lines that secreted antibodies specific for lens membrane junctional domains. The antigen appears to be the same in both cases and its apparent molecular weight is 70,000 (MP70). The data suggest that MP70 is a component of the fiber gap junctions.

MATERIALS AND METHODS

Membrane Isolation: Sheep lenses were extracted from the eyes of animals that were less than 1 year old within minutes of death and were kept on ice. After decapsulation, they were dissected into a cortical and a nuclear fraction and stored frozen at −80°C. We designated as cortex the outer portion of the lens, which remained transparent upon chilling on ice, whereas the lens nucleus was more compact and became opaque when cooled (23).

For plasma membrane isolation, cortical and nuclear tissue from 100 lenses were homogenized separately (90 s full speed, VirTis Co., Inc., Gardiner, NY) in 200 ml 5 mM Tris pH 8.0, 1 mM EDTA, 5 mM β-mercaptoethanol. After pelleting for 30 min at 8,000 rpm in a Sorvall GSA rotor (Beckman Instruments, Inc., Fullerton, CA), the crude membranes were washed 3–5 times in 5 mM Tris pH 8.0, 1 mM EDTA (buffer A). To remove matrix proteins from this crude plasma membrane fraction, pellets were first extracted with 4 M urea, 5 mM Tris pH 9.5, 1 mM EDTA, followed by one or two extractions with 7 M urea in the same buffer. The urea-insoluble material was centrifuged for 60 min at 24,000 rpm in an SW27 Beckman rotor (Beckman Instruments, Inc.) and the pellet resuspended in 4 ml buffer A. To this, 12 ml 67% sucrose in buffer A was added, and 8 ml was underlayered in each of two sucrose density gradients with 45, 25, and 8% steps. The gradients were spun for 60 min at 24,000 rpm in a SW27 rotor. Lens membranes were collected at the 25/45% interface and washed free of sucrose with buffer A. Protein concentrations were measured according to Lowry et al. (45). To all solutions, aprotinin (Sigma Chemical Co., St. Louis, MO) at 1:1,000-fold dilution and 0.1 mM phenylmethylsulfonyl fluoride were added. All procedures were carried out at 4°C.

Antibody Production: Polyclonal antibodies against MIP were raised in rabbits. To purify MIP, 2–3 mg urea-insoluble membrane proteins were separated by preparative SDS PAGE (10%). MIP was identified by incubation of the gel with 0.5 M KCl, and the band was cut out and analyzed for purity by SDS PAGE. Rabbits were injected intramuscularly with ~0.5 mg MIP in crushed gel at 4-wk intervals (complete Freund's adjuvant first injection, thereafter in PBS). The rabbits were bled at days 12 and 20 after each boost. Antibodies were further selected for MIP specificity by affinity purification on a column of Sepharose 4B (Pharmacia Inc., Piscataway, NJ) conjugated with SDS PAGE purified MIP (47).

For monoclonal antibody production, 2-mo-old Balb/c mice were immunized with 0.5 mg urea-extracted cortical lens membranes in complete Freund's adjuvant and boosted 4 wk later with the same amount in incomplete adjuvant. 8 wk after initial immunization, the same amounts of antigen in PBS were injected on three consecutive days (57). All injections were intraperitoneal. 2 d later, spleen cells from immunized mice were fused with NS-1 myeloma cells and directly cloned by limiting dilution according to established methods (17, 56).

Screening for Lens Membrane Protein Specificity: Hybridomas were first screened for specificity against lens membrane proteins by radioimmunoassay. Briefly, urea-extracted membranes from lens cortex were adsorbed 50 μl/well to polystyrene microtiter dishes (Immunoplate IIF, Nunc, Roskilde, Denmark) at 0.2 mg/ml in phosphate-buffered saline (PBS) overnight at 4°C. Subsequent to blocking the wells with 10% calf serum and 2% bovine serum albumin (BSA) in PBS, 50-μl aliquots of hybridoma supernatants were reacted with the immobilized membranes for 1 h at 37°C. Unbound antibodies were removed by several rinses with PBS, and the plates then incubated with 50 μl/well 0.5 μCi/ml anti-mouse Ig ^{125}I(Fab')$_2$ (Amersham Corp., Arlington Heights, IL) in PBS for 1 h at 37°C. The plates were washed with PBS several times, dried, and exposed at −80°C onto Cronex 4 X-ray film (DuPont Co., Wilmington, DE) using intensifying screens (43).

Radioimmunoassay positive hybridomas were further screened by immunoblotting. Urea-insoluble lens membrane proteins were separated by SDS PAGE using 20-cm wide sample slots and transferred to BA 85 nitrocellulose sheets (Schleicher and Schuell, Inc., Keene, NH) according to Bowen et al. (10). 5-mm-wide strips thereof were used to test individual hybridoma supernatants as described in the section below. Those hybridomas that were found positive in both assays were once more subcloned by limiting dilution cloning to ensure their monoclonal character. From four fusions, we isolated 21 hybridoma lines that secrete antibodies against lens membrane proteins. Antibodies from this collection recognized 11 different proteins on immunoblots. Antibodies from two hybridomas (from separate fusions but recognizing the same protein) were found specific for fiber membrane junctional domains as assayed by immunofluorescence microscopy and immunoelectron microscopy.

SDS PAGE and Immunoblotting: SDS PAGE was performed according to established methods (42). Approximately 90 μg lens membrane proteins were solubilized in 50 μl sample buffer at room temperature immediately before loading the gels. Boiling the samples led to irreversible aggregation of MIP (12, 63). Immunoblotting was carried out according to Towbin et al. (60) and Renart et al. (52) with the modifications described in Kistler et al. (35).

Immunofluorescence Microscopy: The antigen distribution in lens plasma membranes and throughout the lens tissue was mapped by immunofluorescence microscopy. 10–18-μm thick cryostat sections of lens were dried on glass coverslips. Fixation with 2% formaldehyde (made fresh from paraformaldehyde) in PBS for 15 min was followed by several rinses in PBS. 100–150 μl hybridoma supernatant was applied to tissue sections for 1 h, unbound antibodies were removed by several PBS washes, and the bound antibodies were labeled with fluorescein isothiocyanate–conjugated sheep anti-mouse Ig antibodies (Serotec Ltd., Oxon, England) at 1:60 dilution in PBS in the dark for 1 h. Excess antibodies were removed by washing in PBS, sections were mounted in 50% glycerol in PBS, and 10 mM *p*-phenylenediamine was added as an antifading agent (30). All procedures were carried out at room temperature. Specimens were observed in either fluorescence or Nomarski mode, and micrographs were recorded on Kodak Tri-X Pan 400 film.

Electron Microscopy and Immunocytochemistry: Immunogold and immunoperoxidase labeling methods were used to visualize the distribution of antigens in lens plasma membranes. Urea-extracted cortical membranes were reacted with hybridoma supernatant for 2 h at room temperature followed by three washes with 10 mM Tris pH 8, 1 mM EDTA, 100 mM NaCl (buffer C). Bound antibodies were labeled with 5-nm colloidal gold coated with anti-mouse Ig antibodies (Janssen Pharmaceuticals, Schiphol, Netherlands) at 1:5 dilution in buffer C for 2 h. Before labeling, the gold was recycled as follows: larger gold aggregates were removed from the colloidal gold solution by pelleting (microfuge 10 min), and free antibodies were removed with the supernatant after centrifugation at 20,000 rpm for 2 h (Sorvall SS-34 rotor, Beckman Instruments, Inc.). Labeled membranes were washed free of unbound immunogold particles and fixed and embedded as below. Alternatively, bound antibodies were labeled with horseradish peroxidase–conjugated anti-mouse Ig (Fab')$_2$ (Amersham Corp.) at 1:200 dilution in PBS pH 7.2 for 2 h. After removing unbound immunoconjugates, the membranes were reacted for 20 min with substrate solution that was freshly prepared as follows: 35 mg 4-chloro-1-naphthol was dissolved in 300 μl ethanol and added to 100 ml PBS that contained 100 μl 30% H_2O_2. A white precipitate formed that was removed by filtration. After incubating the labeled membranes with substrate, membranes were washed twice in PBS, and further processed as below.

Antibody-labeled lens plasma membranes were washed in 100 mM Na cacodylate pH 7.4 (buffer B) and then treated in the following sequence (room temperature) with 3% glutaraldehyde in buffer B for 15 min, 3% glutaraldehyde, and 1% tannic acid in buffer B for 30 min, three washes in buffer B, 2% OsO_4 in buffer B for 30 min, three washes in buffer B, and two washes in H_2O. The fixed material was dehydrated and embedded in Epon according to established methods.

Specimens were viewed with a Philips 301 electron microscope (Philips Electronic Instruments, Inc., Mahwah, NJ) operating at 60 or 80 kV. A 50-μm objective aperture was used, and photographs were recorded on Kodak 4489 electron microscope film.

RESULTS

Antibody Labeling of Lens Fiber Membrane Domains

A collection of monoclonal antibodies that together recognized 11 different protein bands on immunoblots of urea-

extracted lens fiber membranes has been screened for membrane labeling by immunofluorescence microscopy on sheep lens cryosections. Labeling experiments with antibodies other than those from two particular hybridomas resulted in a continuous, fluorescent plasma membrane staining of lens fibers. Antibodies from hybridoma lines 6-4-B2-C6 and 1-B-B1-A5, however, were specific for distinct membrane domains. The staining patterns were identical for antibodies of both hybridomas, except that the membrane staining with antibodies from line 6-4-B2-C6 was more intense. The domain specificity of the latter antibodies is revealed in a gallery of fluorescence micrographs of sheep lens. Cross-sectioned fiber cells have a flat, hexagonal shape and are tightly ordered in a honeycomb pattern, each cell having two broad and four narrow sides (Fig. 1 *a*). Although not resolved in the light microscope, ball and socket structures at the corners interlock with adjacent cells (13). In fluorescence mode, 6-4-B2-C6 derived antibodies bind preferentially to the broad sides and less so to the narrow sides as seen in edge-on views in a direction parallel to the fiber long axis (Fig. 1, *a* and *b*). In a different tissue orientation, in which the broad sides are viewed edge-on and in a direction at right angles to the fiber long axis, elongated fluorescent spots are seen in rows corresponding to the fiber cell width (Fig. 1, *c* and *d*). When the broad sides are viewed face-on, the elongated fluorescent spots in Fig. 1, *b* and *d* are now resolved as macular fluorescent patches (Fig. 1, *e* and *f*).

Using 6-4-B2-C6 derived antibodies and immunofluorescence microscopy on mouse lens cryosections, the domain-specific membrane labeling was most prominant in the outer cortex (Fig. 2). Towards deeper lens regions the punctate fluorescence pattern converted to a more continuous and generally less intense membrane staining.

Domain-specific membrane staining was also observed in the lens outer cortical region of calf, chicken, and toad. No fluorescence signal was recorded for lens epithelium, brain, heart, and liver (tested for mouse and sheep). The domain specificity thus appears to be limited to lens fiber plasma membranes.

Identification of a Fiber Membrane Domain Protein

As the lens is avascular and fibers are devoid of cellular organelles, the washing of lens tissue homogenates with buffer directly gave a crude plasma membrane fraction. However, cytoskeletal proteins and some crystallins remained associated with the membranes and were removed by solubilization in urea (1, 8, 12, 20). SDS PAGE of urea-extracted lens plasma membranes revealed the relative enrichment of MIP and the urea-insoluble membrane-associated 34,000 and 36,000-D polypeptides. Also enriched and therefore representing integral or strongly bound peripheral membrane proteins were the polypeptides with apparent molecular weights of 18,000, 64,000, 70,000, 140,000, and 200,000 (Fig. 3, *b* and *c*). In contrast to these, several major proteins in buffer-washed membranes were reduced to trace amounts upon urea treatment and are presumably components of the membrane-adhering cytoskeletal material.

Changes in the protein pattern of fiber cell membranes between lens cortex and nucleus have been associated with the phenomena of fiber maturation and aging (27, 28). A comparison of gel lanes in Fig. 3, *c* and *d* reveals differences in Coomassie Blue–stained bands between urea-extracted membranes of cortex and nucleus. In particular, the membrane proteins with apparent molecular weights of 70,000 and 64,000 are significantly weaker in lens nucleus. The amounts of MIP are approximately equal in both tissue regions.

Protein patterns of lens membranes were sometimes complicated by MIP forming oligomers upon sample preparation for SDS PAGE. Immunoblotting analysis of gel lanes, as shown in Fig. 3, *b*, *c*, and *d* using affinity purified anti-MIP, showed that when samples had been solubilized at room temperature immediately before loading the gels, no MIP oligomers could be detected (Fig. 3, *e*, *f*, and *g*). Therefore, proteins like MP64 and MP70 that migrate to about the trimer position of MIP are unrelated to the lens fiber main intrinsic polypeptide. Monoclonal antibodies derived from hybridomas 6-4-B2-C6 (Fig. 3, *h*, *i*, and *j*) and 1-B-B1-A5 (data not shown) labeled both MP70 and MP64. The latter is most likely a limited proteolysis product of MP70, and we will refer to this antibody specificity simply as anti-MP70. Little or no MP70 was detected in urea-extracted fiber membranes from the lens nucleus. This is consistent with the immunofluorescence microscopy that revealed a gradual loss of membrane staining intensity towards deeper regions of the mouse lens (Fig. 2).

In summary, MP70 is an integral or strongly bound peripheral membrane protein of lens fibers. MP70 is localized in distinct, macular plasma membrane domains preferentially on the broad sides of fibers in the lens outer cortex.

MP70 Is a Component of Lens Membrane Junctions

Immunoelectron microscopy was used to characterize MP70-specific fiber plasma membrane domains. Urea-extracted membrane fractions from sheep lens cortex used for anti-MP70 labeling contained both single membranes and membrane junctions. The latter appeared as pentalaminar structures in thin sections. However, we have never observed thin and thick junction profiles in continuous lengths of the same pair of membranes. We have also isolated junction-rich membranes from total bovine lens by using the same procedure. With this material, we also failed to see continuous thin and thick pentalaminar structures. This contrasts with the work of Paul and Goodenough (47). Therefore, we cannot firmly assign these double membrane structures to either the thick fiber gap junctions or to the thin close membrane appositions. Anti-MP70 antibodies bound preferentially to these pentalaminar membrane regions, as can be shown after secondary labeling with immunogold (Fig. 4, *a* and *b*) or with peroxidase-conjugated anti–mouse Ig antibodies (Fig. 4, *d* and *e*). Unfortunately, much of the membrane-bound gold was lost during sample preparation for electron microscopy as has previously been reported by others (15, 29). However, a light fuzz due to bound antibodies was seen on both sides of pentalaminar structures, and often membrane junctions appeared cross-linked in small stacks via antibodies (Fig. 4 *b*).

Anti-MP70 antibodies bound to membrane junctional regions were better visualized by the immunoperoxidase method. Both sides of the pentalaminar profiles showed significant amounts of peroxidase-derived osmiophilic precipitate (Fig. 4, *d* and *e*), whereas single membranes appeared unlabeled.

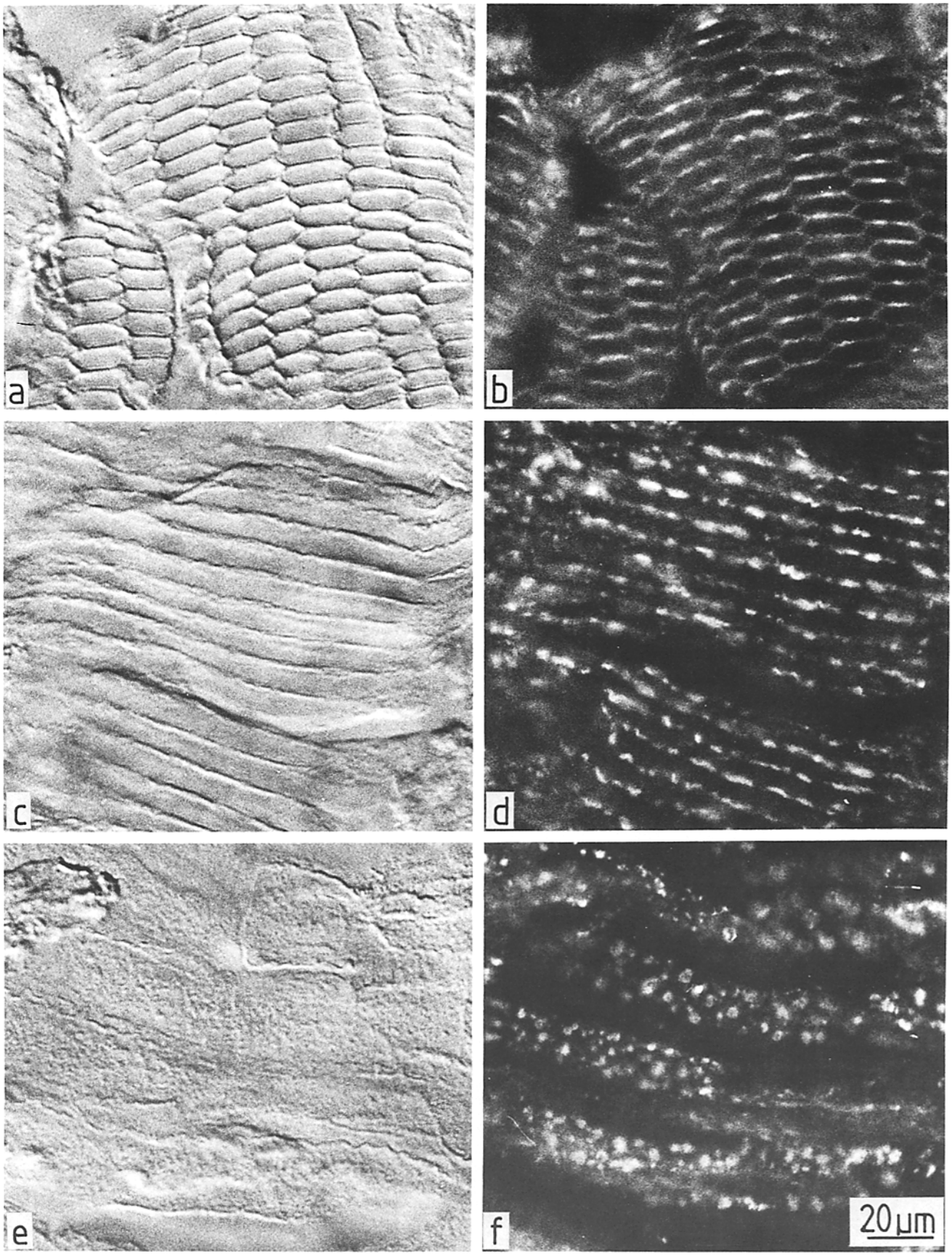

FIGURE 1 Nomarski (*a*, *c*, and *e*) and immunofluorescence (*b*, *d*, and *f*) microscopy of anti-MP70-labeled sheep lens cortical tissue. (*a* and *b*) Fiber cells cross-sectioned. The stronger fluorescence indicates preferentially labeled membrane domains on broad sides. (*c* and *d*) Fiber cells sectioned longitudinally, showing edge-on views of broad sides. (*e* and *f*) Fiber cells sectioned longitudinally, looking onto broad sides. Fluorescent macular patches represent preferentially labeled membrane domains on the broad sides. Bar, 20 µm. × 600.

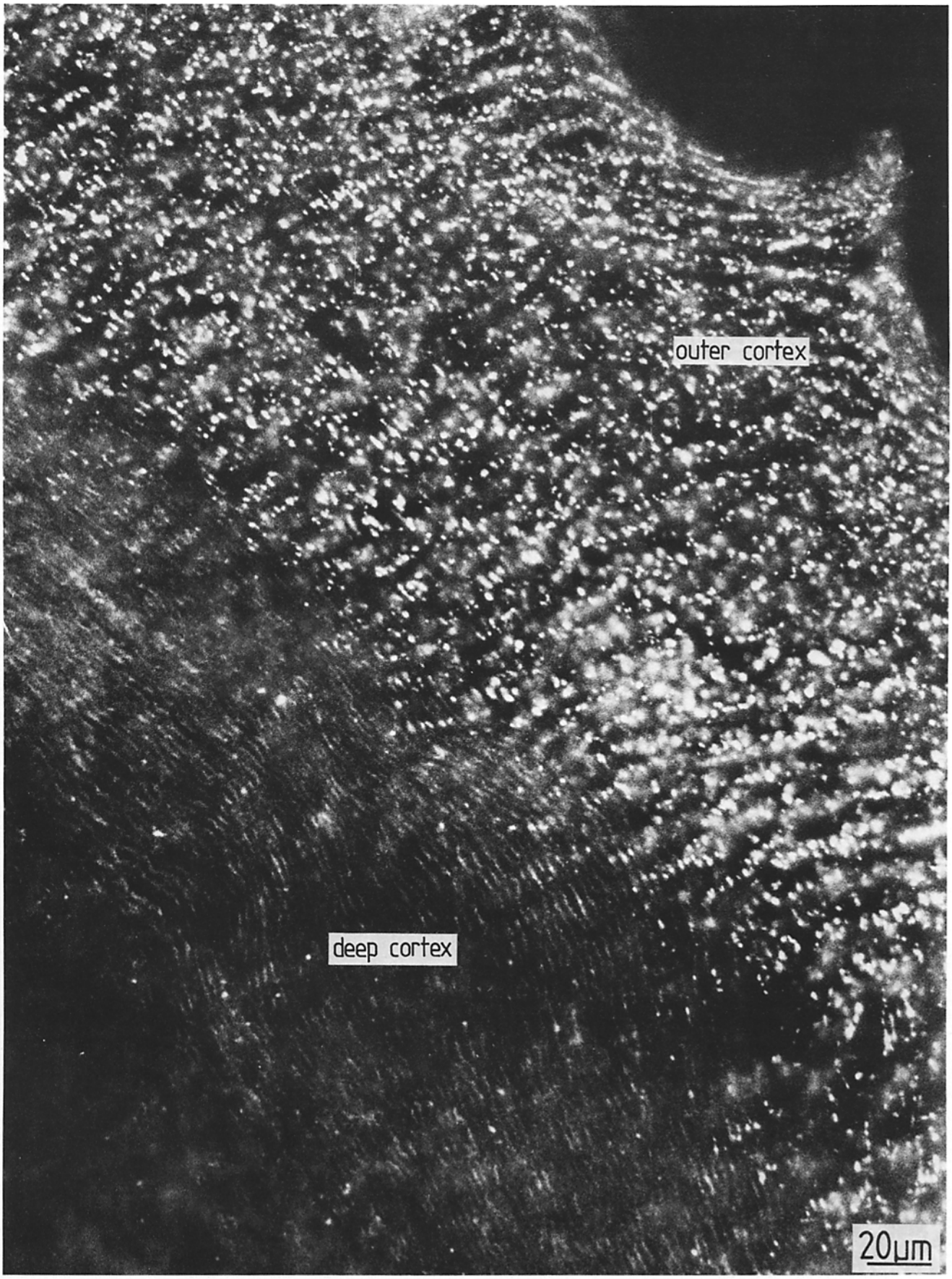

FIGURE 2 Immunofluorescence microscopy of mouse lens using anti-MP70 and fluorescein isothiocyanate-conjugated anti-mouse Ig antibodies. The strong punctate fluorescence pattern in the outer cortex converts to a generally weaker and more homogenous membrane labeling pattern in the deep cortex. The lens periphery has been distorted during cryosectioning. Bar, 20 μm. × 600.

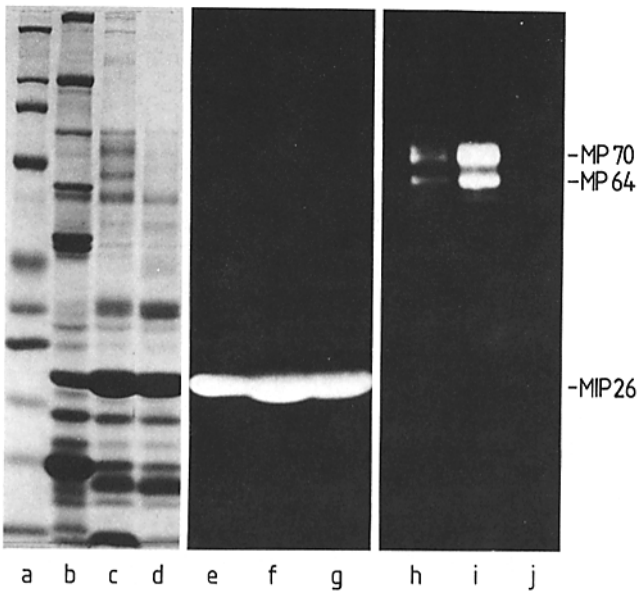

FIGURE 3 10% PAGE of sheep lens plasma membrane proteins and identification of MIP and MP70 by immunoblotting. (*a*) Markers from top, mol wt × 10^3: 205, Myosin; 116, β-galactosidase; 97, phosphorylase B; 66, BSA; 45, ovalbumin; 36, glyceraldehyde-3-phosphate dehydrogenase; 29, carbonic anhydrase; 24, trypsinogen; 20, soybean trypsin inhibitor; 14, α-lactalbumin. (*b*) Buffer-washed plasma membranes from lens cortex. (*c*) Urea-extracted plasma membranes from lens cortex. (*d*) Urea-extracted plasma membranes from lens nucleus. (*e*, *f*, and *g*) Gel lanes corresponding to *b*, *c*, and *d* were transferred to nitrocellulose by diffusion and reacted with anti-MIP antibodies. (*h*, *i*, and *j*) Complementary nitrocellulose sheet (exact replica of *e*, *f*, and *g*) reacted with hybridoma supernatant 6-4-B2-C6.

DISCUSSION

MP70 is a specific component of junctional domains in lens fiber membranes. Immunofluorescence microscopy reveals these domains as macular patches on the broad sides of cortical fiber cells. They are thus not likely to be part of the ball and socket structures that in sheep lens fibers are confined to the corners of the hexagonal profile (13). Most probably, MP70 is a component of either the close membrane appositions or the fiber gap junctions. The former, also referred to as thin junctions, is most likely identical with the undulating square array regions observed in freeze-fracture replicas of fiber plasma membranes (44, 64). These square arrays are frequent in the deep cortex and nucleus (5, 22, 44), but only very small square array patches (<0.1 μm^2) have been found in situ in the outer cortex (22, 44). In contrast, immunofluorescence microscopy revealed MP70-specific membrane domains highly abundant in the outer cortex and less so in the deeper lens regions. This renders a possible relationship of MP70 and thin junctions or square arrays unlikely. The fiber gap junctions are more likely to be identical with MP70-specific domains. In fact, freeze-fracture studies of lenses from various vertebrate species showed the fiber gap junctions to be highly abundant in the lens outer cortex region (references 2, 4, 20, 37, 41, and our own unpublished data), coinciding with the high frequency of MP70-specific macular domains in the same lens region. Also, the fluorescent macular patches are 0.5–10 μm^2 in size similar to that of freeze-fractured sheep fiber gap junctions. By analogy, a similar macular fluorescence pattern has been reported for various tissues labeled with antibodies against the liver 28,000-D gap junction component (15, 24, 25).

In dye transfer experiments using Procion yellow, radial rows of hexagonal fiber cells in the lens cortex became fluorescent after injury to the lens epithelium (see Fig. 12 in reference 21; see Fig. 1 *a* in reference 6). These images suggest that major pathways of intercellular communication are directed radially. This coincides with the preferential enrichment of MP70-specific junctional domains on the fiber broad sides. MP70-mediated intercellular communication in the eye lens thus appears plausible.

A membrane protein with an apparent molecular weight of 71,000, possibly identical with MP70, has previously been found in a study on polypeptide glycosylation in the mouse lens. This protein was however absent from fiber cell plasma membranes of cataractous lenses (19). Freeze-fracture electron microscopy revealed a sharp decrease of fiber gap junctions in cataractous lenses of mouse (59) and rat (39). These results could possibly be linked by mapping the junction distribution in normal and cataractous lenses using anti-MP70 immunofluorescence microscopy.

MIP, the most abundant membrane protein in lens, was initially believed to be domain specific. The question therefore arises as to whether the fiber membrane domains of MP70 and MIP are related. It has been claimed that MIP is contained in various membrane domains: fiber gap junctions (4, 9, 20, 26, 31, 32, 38, 46, 53), square arrays (64), and one membrane of close membrane appositions (47). Several laboratories now agree that MIP is also present in nonjunctional membrane regions throughout the lens (5, 18, 47). Using anti-MIP antibodies, one group failed to label fiber gap junctions, but the antibodies bound to nonjunctional MIP (47). We have found similar results to the latter by using polyclonal, affinity-purified anti-MIP antibodies for immunoelectron microscopy of isolated lens fiber membranes that showed single membranes predominantly labeled compared with fiber junctions. Also, immunofluorescence micrographs of anti-MIP labeled lens fibers published by Broekhuyse et al. (11) are complementary to our Fig. 1 *b*, in that fluorescence is very strong on the narrow sides but weak or absent in the middle of the broad sides. There are two possible scenarios: (*a*) MIP26 is excluded from communicating fiber junctions. This would be in conflict with the immunolabeling data of several laboratories (5, 9, 18, 31); and (*b*) MIP is present in both the junctional and the nonjunctional membranes. In this case, the antisera produced by Paul and Goodenough (47), Broekhuyse et al. (11), and by ourselves recognize epitopes on nonjunctional MIP but not on junctional MIP. Particular epitopes may have been masked during junction assembly. However, it seems that MIP could only be a junctional component through the interaction with another protein that separates junctional from nonjunctional MIP. In fact, MP70 may select MIP for lens fiber gap junction assembly.

Now that we have produced a monoclonal antibody that appears to be specific for a fiber gap junction protein, experiments of the type pioneered by Warner et al. (62) will now be feasible with the lens. This type of experiment would provide proof that fiber gap junctions are involved in intercellular communication in the lens.

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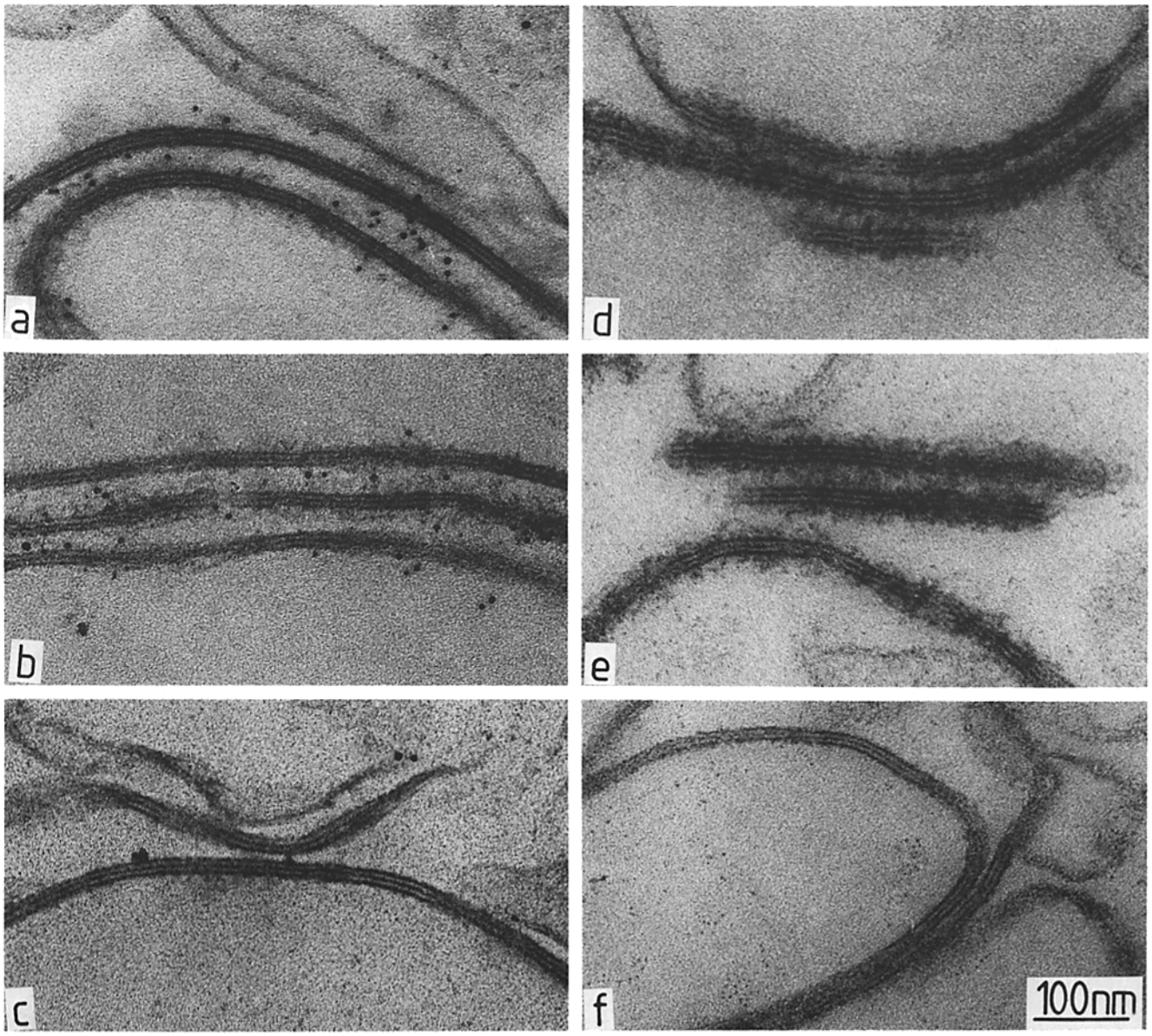

FIGURE 4 Immunogold (*a*, *b*, and *c*) and immunoperoxidase (*d*, *e*, and *f*) labeling of urea-extracted plasma membranes from sheep cortex. (*a* and *b*) Anti-MP70. Bound antibodies appear as faint fuzzy layers on both cytoplasmic faces of pentalaminar structures, but not generally on single membranes. Junctions are often found cross-linked by antibodies. Primary antibodies are further visualized by immunogold labeling, but much of the bound colloidal gold has been lost during dehydration and embedding (15, 29). (*c*) Same procedure as in *a* and *b*, but nonconditioned (i.e., without anti-MP70 monoclonal antibody) culture medium was used. (*d* and e) Anti-MP70. Antibodies are bound to both cytoplasmic faces of junctions and are enhanced by osmiophilic precipitates derived from the peroxidase-substrate reaction. Single membranes are generally not labeled. (*f*) Control with nonconditioned hybridoma culture medium. Bar, 100 nm. × 145,000.

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