STRUCTURAL AND MOLECULAR BIOLOGY OF THE EYE LENS MEMBRANES

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I. INTRODUCTION

Refractive power and transparency are both firmly associated with the unique design, tissue development, and architecture of the mammalian ocular lens.[1-4] Elongated cells, referred to as lens fibers, stretch between lens poles (Figure 1). Their plasma membrane profile is a flat hexagon, and fibers are arranged in such a way that they form radial stacks, with their broad sides always oriented parallel to the lens periphery. Anterior fibers are interfaced with the aqueous humor through an epithelial monolayer of cuboidal cells. In general, plasma membranes between neighboring cells interact closely so that extracellular space is minimal. The bulk portion of the lens consists of mature fibers which lack intracellular organelles such as nuclei and mitochondria. Hence, membrane systems are restricted to the plasma membranes enveloping the fiber cytoplasm and are designed such that the membrane-associated light scattering within the lens is minimal.

The lens refractive power is maintained by the unusually high concentrations of crystallins in the fiber cells. Crystallins are a somewhat heterogeneous population of low molecular weight, water-soluble proteins which must remain soluble for lens transparency. In aging and cataractous lenses, light scattering within the lens may increase because crystallins cross-link their free sulfhydryl groups to form higher molecular weight aggregates. In contrast to this, in a young and healthy lens, the cytoplasm maintains a slightly reducing milieu and thus keeps the crystallins soluble. This reducing power is directly linked to energy production through glycolysis. The lens is avascular, and epithelial and fiber plasma membranes contain pathways and transport mechanisms for the effective passage of metabolites and ions in order to ensure homeostatic control of the cytoplasmic components throughout the lens. For example, a nonequilibrium distribution of sodium and potassium is maintained by active pumping across the epithelial membranes, resulting in a negative electrical potential of the lens interior with respect to the aqueous humor. Inactivation of the Na-K-ATPase thus leads to an influx of sodium and water and hence to swelling of the lens, which, in turn, severely disrupts the ordered packing of fiber cells in the lens. This inevitably increases the amount of light scattered within the lens and limits transparency. Metabolites, such as sugars and amino acids, are also actively and selectively exchanged with the aqueous humor and then traverse membranes from cell to cell deeper in the lens. Thus, an extensive network of intercellular transmembrane pathways serves to maintain the transparency of the lens.[5,6]

The lens continues to grow during the individual's whole life span. Epithelial cells at the lens equator divide, and the daughter cells differentiate and elongate into fibers in the peripheral zone of the outer cortex. These young fibers are added onto the radial stacks of

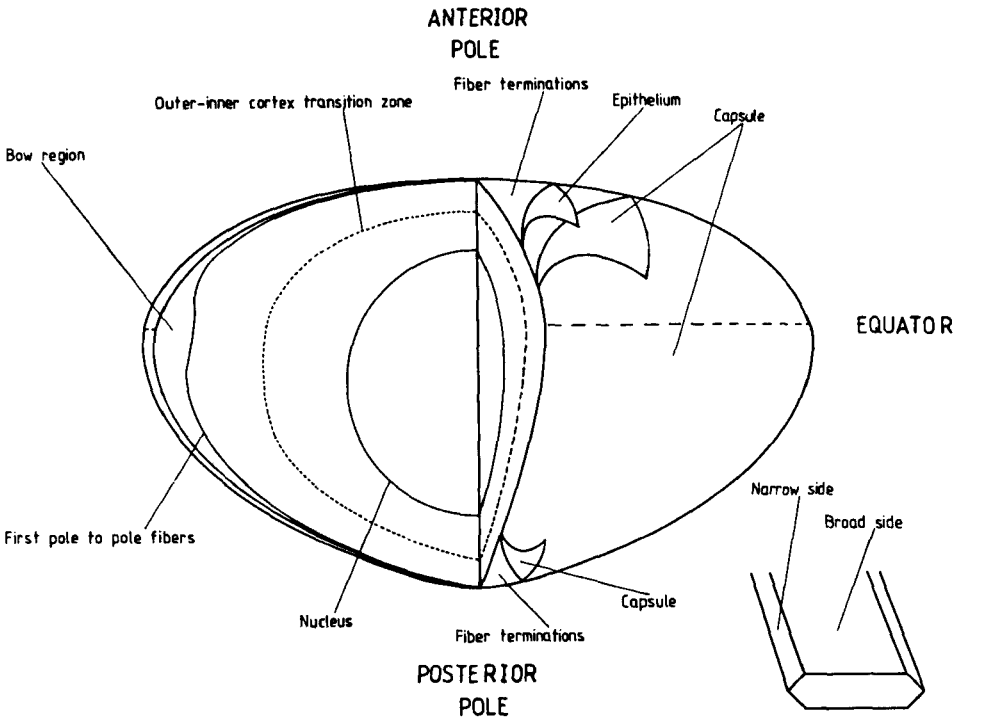

FIGURE 1. Schematic drawing of the structure of the lens, indicating fibers in the outer cortex, inner cortex, and nucleus. At right, broad and narrow sides are indicated in the diagram of a single fiber. (The figure was kindly provided by Mr. Terry Gruijters of our laboratory.)

fibers; thus, the older ones are located deeper in the lens. Fibers are never shed, and those in the lens center have the individual's present age. This differentiation of cuboidal epithelial cells into pole-pole spanning fibers involves massive *de novo* synthesis of membrane components. The outer cortex portion of the lens is therefore particularly interesting for the study of the structure and function of fiber plasma membranes.

This review focuses on the identification of distinct lens plasma membrane domains, in particular on the lens fiber junctions, their structure and protein composition, and their functional role for the maintenance of lens transparency. A wider perspective of membrane structure and function is emphasized, but all technical and biochemical aspects of membrane components are not covered. The reader is referred to reviews by Broekhuyse,[7] Benedetti et al.,[8] Bloemendal,[9] and Alcala and Maisel.[10]

II. COMMUNICATING CELL-TO-CELL CHANNELS AND LENS TRANSPARENCY

Lens cataract formation is often associated with osmotic fiber swelling and progressive tissue disorder. Cross-linking of the crystallins into large aggregates may also be involved. Transparency of the lens is linked with the permeability of plasma membranes to small molecules to maintain ion balance and homeostasis of cytoplasmic components in all regions of the lens. A study of lens transport and permeability using freeze substitution and electron microscopy elegantly demonstrated metabolic cooperation for lens cells:[6] incubated lenses selectively took up some radioactively labeled metabolites, but nonmetabolites did not enter the lens beyond the anterior epithelial cell monolayer. Transported molecules, e.g., ^{3}H-lysine and ^{3}H-glucose, rapidly spread throughout the lens cortex. Diffusion through the

narrow and highly tortuous intercellular spaces is too slow to account for the observed transport rates. From these results, it has been postulated that lens epithelial and fiber cells are joined between heterologous and homologous cells via an extensive network of cell-to-cell communicating channels. Electron microscopy has demonstrated the existence of such channels in the fiber plasma membrane and that these channels are clustered in plaque-like communicating junctions similar to the gap junctions characterized in other tissues (see Section III).

Communicating channels joining fiber cytoplasms have also been proposed from the results of electrical conductance measurements between different regions in the lens.[11,12] From these results, all lens fibers appear to be electrically coupled throughout the lens. Fibers may be reversibly uncoupled when the lens is exposed to certain "uncoupling" reagents. For example, in the frog lens, fibers in the outermost 200- to 300-μm-wide zone of the outer cortex could be reversibly uncoupled with 2-4-dinitrophenol.[13] Intercellular cells of fiber membranes deeper in the lens appeared to be unaffected by the treatment. This could be explained simply by restricted penetration of the reagent into the lens. Alternatively, it could be hypothesized that cell-to-cell channels in the outer cortex and deeper in the lens are somewhat different and that the former can open and close, whereas the latter always remain open.

While no direct evidence is available to support this latter hypothesis, we show in this review that intercellular junctions undergo age-related changes and that those located deeper in the lens are, indeed, biochemically and structurally distinct from their counterparts in the outer cortex. In Section VI, we review the evidence that junctional protein is cleaved *in vivo* and that the large junctional plaques in the outer cortex fragment to smaller sizes deeper in the lens. Such changes might have an effect on junctional permeability or on its regulation.

III. STRUCTURE OF LENS FIBER JUNCTIONS

Freeze-fracture electron microscopy of fiber plasma membranes in the lens outer cortex reveals abundant plaque-like clusters of intramembrane particles (Figure 2).[5,8,14-18] These plaques represent junctions involving neighboring plasma membranes and, in fact, are very reminiscent of the gap junctions found in a large variety of tissues. In the lens, they have been referred to as communicating junctions, intercellular junctions, fiber junctions, fiber gap junctions, or 16- to 17-nm fiber junctions. The latter reflects the overall thickness of these double membrane structures as determined by thin-section electron microscopy. By analogy with other gap junctions, lens fiber junctions appear to connect adjacent cells via large numbers of proteinaceous transmembrane channels which are believed to be permeable to small molecules. Strong indications for such channels have been obtained from electron micrographs of freeze-fractured and rotary-shadowed fiber junctions[16] and of negatively stained, isolated fiber junctions.[5] Typically for lens fiber junctions, junctional particles are generally unordered within the plaques, both *in situ* and after isolation (Figure 3a). However, crystallization of junctional particles into hexagonal arrays (Figure 3b) has been observed in a number of cases, such as after calcium treatment of crude lens membrane preparations,[19,20] after calcium-dependent proteolysis in lens outer cortex homogenates, after trypsinization of isolated fiber junctions, or in cataractous lenses.[20a] We believe that in all of these cases crystallization is induced by proteolytic cleavage of junctional proteins. A calcium-dependent lens protease has been identified for which junctional protein is, indeed, a primary substrate (see Section VI). The junctional membranes remain associated with each other even after extensive proteolysis.[21] Image analysis of trypsinized and negatively stained junctions reveals channel structures ordered in a hexagonal array (Figure 4) similar to those in liver gap junctions. In contrast to the latter, however, the lens junction lattice repeat of 13.3 nm is 50% greater linearly. There is additional stain-excluding protein between the

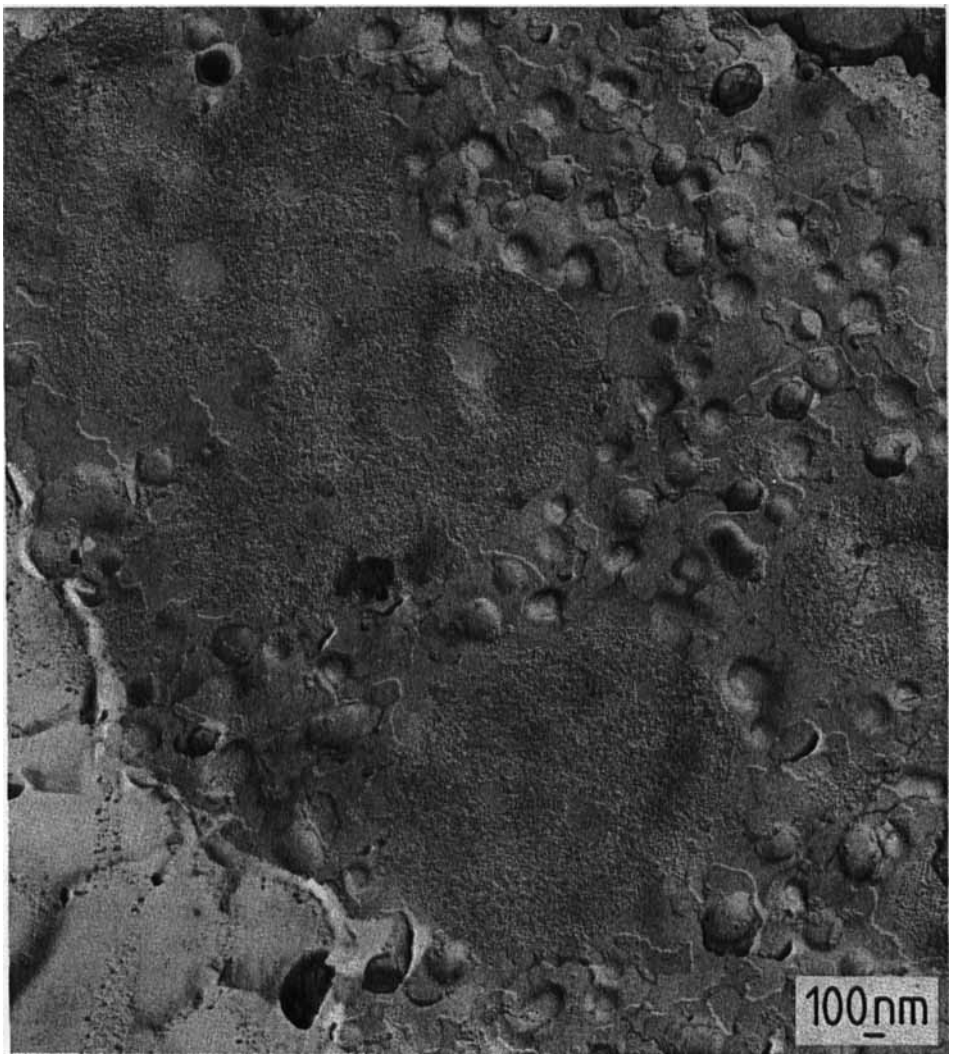

FIGURE 2. Freeze-fracture electron micrograph of fiber plasma membrane from sheep lens outer cortex revealing plaque-shaped intercellular junctions.

channel strucutres. This is consistent with the results from freeze-fracture electron microscopy of the lens hexagonal arrays (Figure 5): depressions on the membrane E-fracture face reveal a 7.5-nm repeat which is equal to 13.3 nm/$\sqrt{3}$ and which indicates that protein portions at both the six- and threefold symmetry positions in the lattice are embedded in the lipid bilayer. This feature of protein topology so far appears to be unique to the lens fiber junctions.

IV. MEMBRANE ISOLATION

The isolation protocol for fiber plasma membranes is greatly simplified by the fact that the lens is avascular and mature fibers are devoid of cellular organelles. Decapsulation of the lens also removes most of the epithelial cell monolayers. However, deeper in the lens, protein concentrations are very high (up to 50% in fish, 30% in humans) and thus it is not always easy to remove all cytoplasmic remnants from the membranes.[22] All precautions must be taken to minimize proteolysis postmortem. It is best to quick freeze lenses in liquid

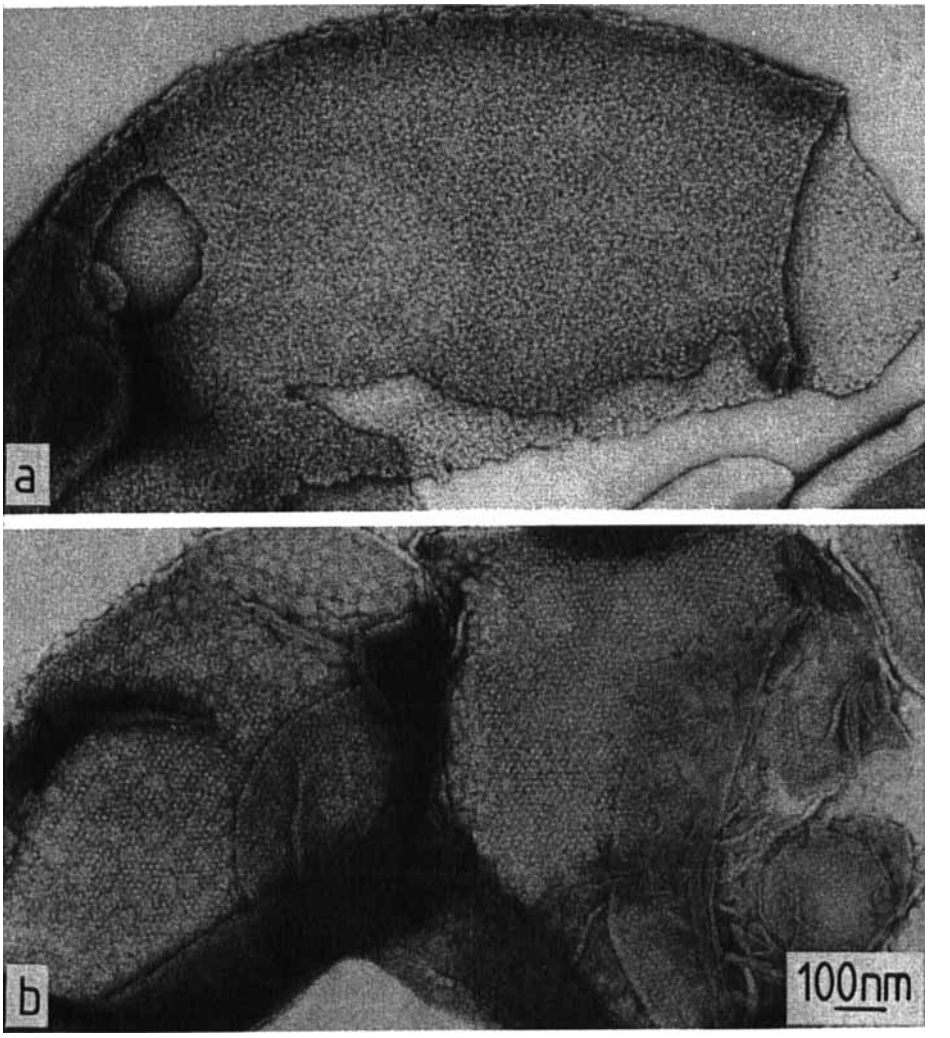

FIGURE 3. Electron micrographs of negatively stained sheep lens fiber junctions. (a) Urea/alkali-treated isolated fiber junction with unordered junctional protein; (b) 13.3-nm hexagonal lattice of junctional protein observed after trypsin treatment.

nitrogen seconds after extraction from the animal and store them at −90°C until use. A suitable set of protease inhibitors for tissue homogenization is, for example, 10 m*M* *N*-ethyl malemeide, 0.5 m*M* diisopropyl fluorophosphate, 1 m*M* EDTA, 1 m*M* EGTA, and 1/500 aprotinin.[23]

The evolution and improvements of the detailed isolation procedure for lens plasma membranes have been extensively reviewed previously.[8,10] We thus describe here a "consensus" protocol which is, with minor changes and with or without lens dissection, generally used by investigators keen on preserving a near-native membrane structure. For preliminary lens dissection, frozen lenses are kept at room temperature for a few minutes so that the capsule can be removed (also removing most epithelial cells). Immediately following this decapsulation, and while the lens is still frozen, the outermost region of the equatorial cortex is cut away with a razor blade and pooled as "outer cortex" fraction. For sheep lenses with an equatorial diameter of, e.g., 10 to 12 mm, 1.5- to 2-mm-thick slices are cut away and pooled. Lenses are thawed until the remainder of the cortex is transparent but the nucleus

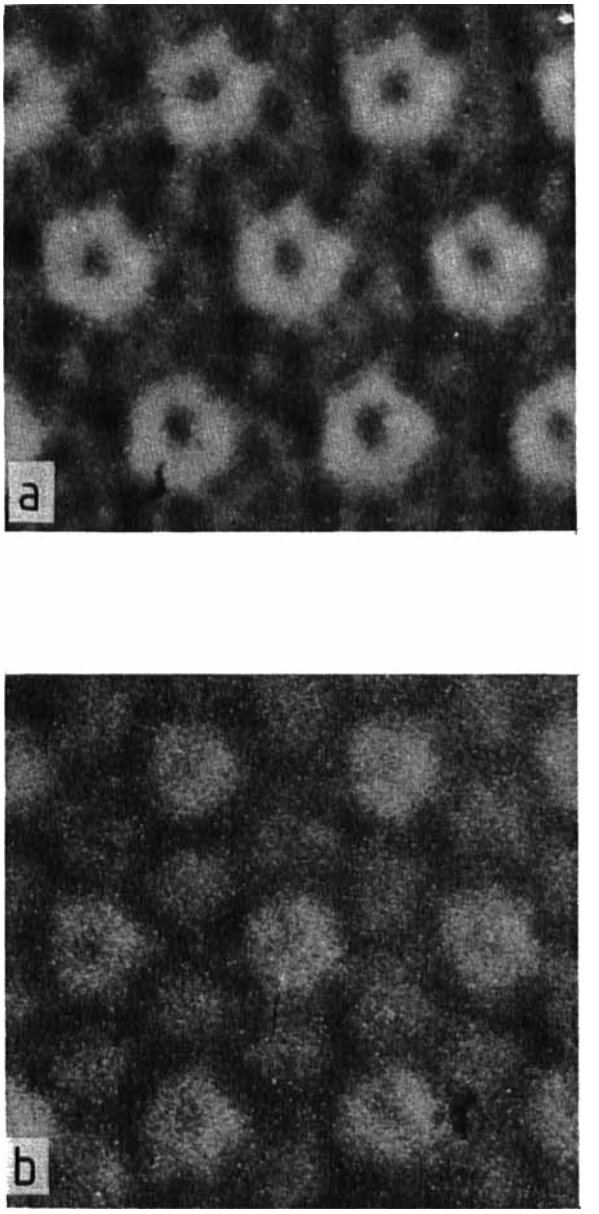

FIGURE 4. Image enhancement of negatively stained crystalline fiber junctions. (a) Projection view showing prominent channel structures; (b) under favorable staining conditions, stain-excluding protein is also revealed at the threefold symmetry positions between the channel structures. The repeat between channel structures is 13.3 nm.

remains opaque. This remaining cortex can easily be peeled off the lens nucleus and is pooled as "inner cortex" fraction. The remainder is collected and labeled "nucleus". All three fractions are henceforth treated equally but separately. The tissues are homogenized on ice in 5 m*M* Tris (pH 8) containing the protease inhibitors as detailed earlier. A water-insoluble fraction consisting mostly of crude plasma membranes is obtained after several cycles of pelleting and resuspending in the same buffer, and the bulk of the crystallins and

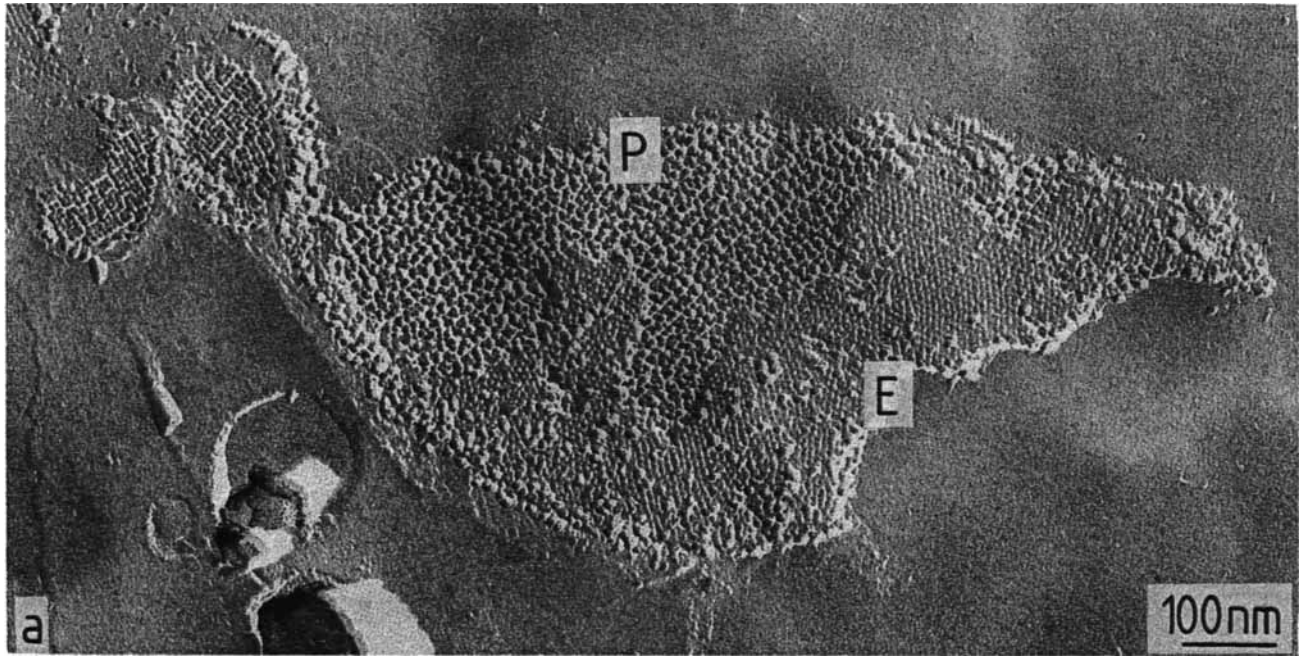

FIGURE 5. Freeze-fracture electron microscopy of crystalline fiber junction and optical diffraction analysis. (a) Micrograph shows particulate P-fracture face and E-fracture face with depressions on a 7.5-nm hexagonal lattice. Shadows are white. Square array (see Section VII) with 6.6-nm repeat for calibration at left in the micrograph. (b) Optical diffraction pattern of fiber junction P-face. Asymmetry is due to unidirectional shadowing. (c) Diffraction pattern of negatively stained fiber junction showing the same lattice dimensions as those derived from freeze-fractured fiber junctions. (d) Diffraction pattern of junction E-face.

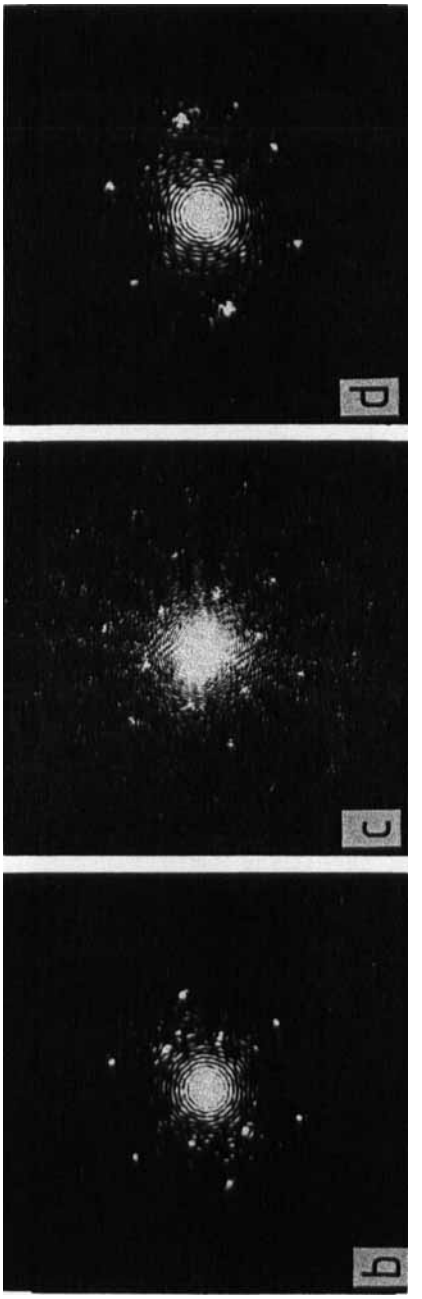

FIGURE 5.

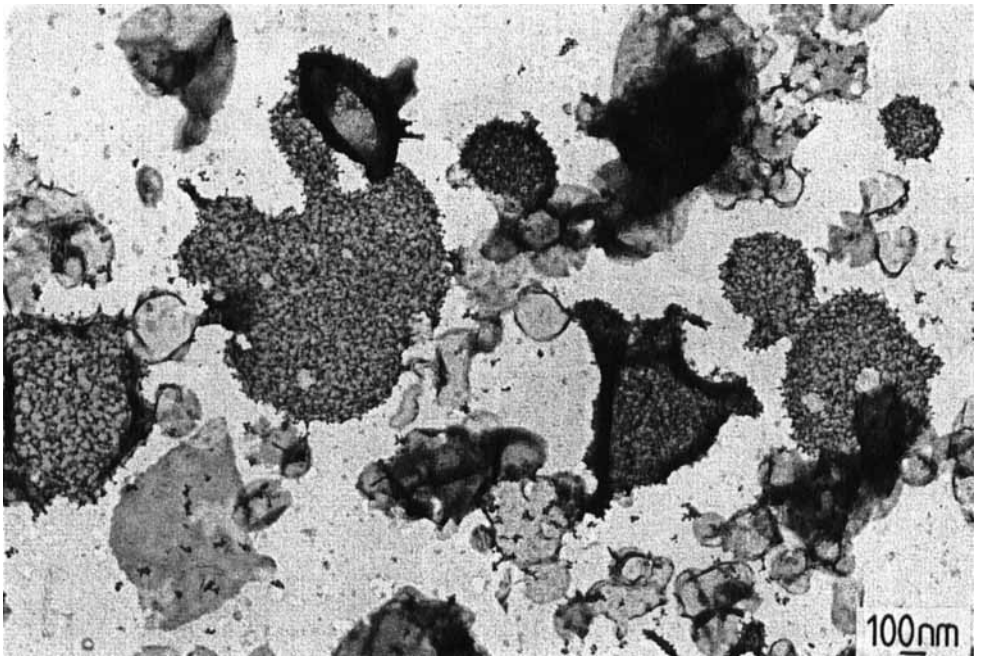

FIGURE 6. Negatively stained preparation of membranes isolated from sheep lens outer cortex. Junctional membranes have been labeled with fiber junction specific anti-MP70 immunogold (see Section V). Unlabeled membranes are from nonjunctional regions of the fiber plasma membranes.

other water-soluble components is discarded. In the electron microscope, crude membranes appear aggregated and heavily contaminated with fibrous cytoskeletal remnants.[22,24] The latter can be removed by resuspending the membranes in 5 m*M* Tris (pH 9.5), 7 *M* urea, 1 m*M* EDTA/EGTA.[5,15,25-27] This material, after pelleting out of the urea solution, is often referred to as the urea-insoluble fraction in the literature and, when analyzed by negative stain electron microscopy, contains fiber junctions and nonjunctional membranes (Figure 6). Fiber junctions are abundant in urea-insoluble material derived from the outer cortex and reduced in that from deeper regions. While not altering the general appearance of these membranes at the electron microscope level, further protein can be removed from the urea-insoluble material by solubilization in 20 m*M* NaOH (approximately pH 12). It is thought that this alkaline treatment strips fiber plasma membranes of peripheral proteins, which adhere to the membranes more strongly than those solubilized by urea.[23,28,29] Total protein yields for urea and alkaline isolated membranes from less than 1-year-old sheep lens, for example, average 494 ± 85 μg per lens for whole lens, 78 ± 15 μg per lens for outer cortex, 212 ± 44 μg per lens for inner cortex, and 204 ± 60 μg per lens for nucleus.[23]

V. MEMBRANE COMPOSITION

A. Junctional and Nonjunctional Membrane Proteins

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of lens fiber membranes separates proteins ranging in apparent molecular weight from 10,000 to 250,000. Urea- and alkaline-treated preparations from whole sheep lens, sheep lens outer cortex, inner cortex, and nucleus are compared to each other in Figure 7. Major bands are consistently observed at positions on the gel corresponding to apparent molecular weights of 200,000, 140,000, 70,000, 64,000, 38,000, 26,000, 24,000, and 18,000.[23] Because these proteins

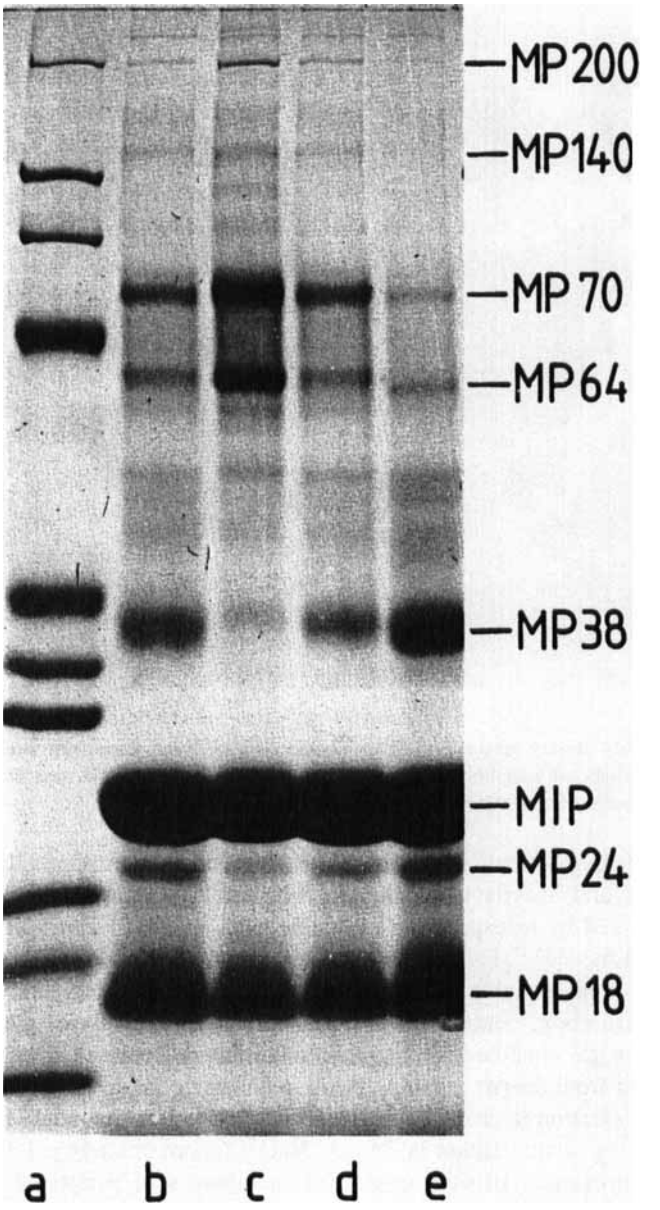

FIGURE 7. SDS-PAGE (10%) of urea- and alkali-treated isolated fiber plasma membranes. (a) Markers: from top, mol wt × 10^3: 205, myosin; 116, β-galactosidase; 97, phosphorylase B; 66, BSA; 45, ovalbumin; 36, glyceraldehyde-3-phosphate dehydrogenase; 29, carbonic anhydrase; 24, trypsinogen; 20, soybean trypsin inhibitor; 14, α-lactalbumin. Membranes were isolated from whole sheep lens (b), outer cortex (c), inner cortex (d), and nucleus (e).

resist alkaline stripping and are detergent soluble, they are likely to be intrinsic membrane proteins, i.e., some peptide portions of these proteins are embedded in the lipid bilayer.

Proteins with similar molecular weights have been identified in fiber plasma membranes purified from bovine lens cortex: 200,000, 68,000, 43,000, 35,000, 27,000, 22,000, and 20,000.[30] Membrane proteins in the chicken lens are 75,000, 56,000, 54,000, 48,000, 34,000, 32,000, 25,000, and 22,000.[31] It is, however, often difficult to compare the lens

protein patterns from different animals on the basis of what has been reported in the literature. This is because gel patterns are often shown without molecular weight standards and, furthermore, because protein patterns vary considerably with variable removal of proteins adhering to the membrane surface and with the age of the lens.[31-35] In fact, even in a single lens, the protein pattern changes dramatically between different regions (Figure 7). These changes in the protein pattern reflect the fiber maturation and aging gradient in the lens.

Most research on lens plasma membrane biology concerns the characterization of the major intrinsic polypeptide (MIP) with an apparent molecular weight of 26,000. The data on MIP have been extensively reviewed recently,[10] and only a few aspects are highlighted here. Most striking is the unusual electrophoretic behavior of MIP: heating in sample buffer containing SDS, normally done to solubilize proteins for electrophoresis, results in aggregation of MIP such that it does not enter the gel.[26,32,36] Warming the sample mildly before electrophoresis often results in oligomerization of MIP, and dimers and trimers appear on the stained gel. It is therefore best for SDS-PAGE of lens fiber membranes to solubilize them in cold sample buffer immediately before application to the gel. MIP is highly conserved among vertebrate species.[37] Membrane-bound MIP can be phosphorylated.[38-41] A cDNA has been isolated which represents the entire coding region of the mRNA for MIP of bovine fiber plasma membranes.[42] The molecular weight calculated for the amino acid sequence, which has been deduced from DNA sequencing, is 28,000. The predicted structure for MIP is that it transverses the lipid bilayer six times with both amino and carboxy termini on the cytoplasmic side.

MIP has been generally referred to as the major component of the fiber junctions.[43-45] Initially, this was proposed on the basis of the apparently excellent correlation between the large amount of fiber junctions and the large amount of MIP in lens membrane preparations. Immunoelectron microscopy with anti-MIP antibodies, indeed, localized MIP in the fiber junction membranes.[46-48] More recently, MIP has been reconstituted in membrane vesicles[49-51] and in planar lipid bilayers[52,53] and found to have channel activity. Some doubt, however, remains on the purity of the reconstituted material. MIP was also found to bind calmodulin[54,55] in accordance with the proposed role of calcium as a gap junction regulator.

The enthusiasm for MIP as the junctional protein has been diminished by a number of observations. Improved electron microscopy of lens membranes has revealed a considerably higher proportion of nonjunctional membranes in samples previously assumed to be predominantly fiber junctions. The correlation of fiber junctions and MIP may, therefore, be invalid.[56] Also, anti-MIP immunofluorescence microscopy of lens fibers does not produce a macular or punctate staining as one might expect for junction-specific staining. In fact, several groups agree that MIP is also localized in nonjunctional membranes.[14,57-59] In one case, anti-MIP antibodies did not label the 16- to 17-nm fiber junctions but did bind to nonjunctional membrane regions.[59] Furthermore, failure to bind calmodulin to MIP was reported.[60,61] Comparison of MIP and the liver 27-kDa gap junction protein did not reveal detectable sequence homology,[42,62,63] peptide homology,[64,65] or immunological cross-reactivity.[66,67]

All this casts some doubt on the "true" role of MIP in lens fiber plasma membranes. If MIP forms junctional channels, it is surprising to find so much of this protein in nonjunctional membrane regions, in sharp contrast to the specific localization of gap junction proteins in other tissues.[66,67] Alternatively, if MIP is predominantly nonjunctional, the sheer abundance of it may allow it to diffuse into the somewhat loose junctional protein clusters, perhaps explaining some of the immunolocalization data.[68]

Other bands on gels from lens fiber membranes correspond to proteins with apparent molecular weights of 24,000 and 18,000. Similarly to MIP, these polypeptides also aggregate when heated in SDS. They are more prominent in the lens nucleus and in lenses from older

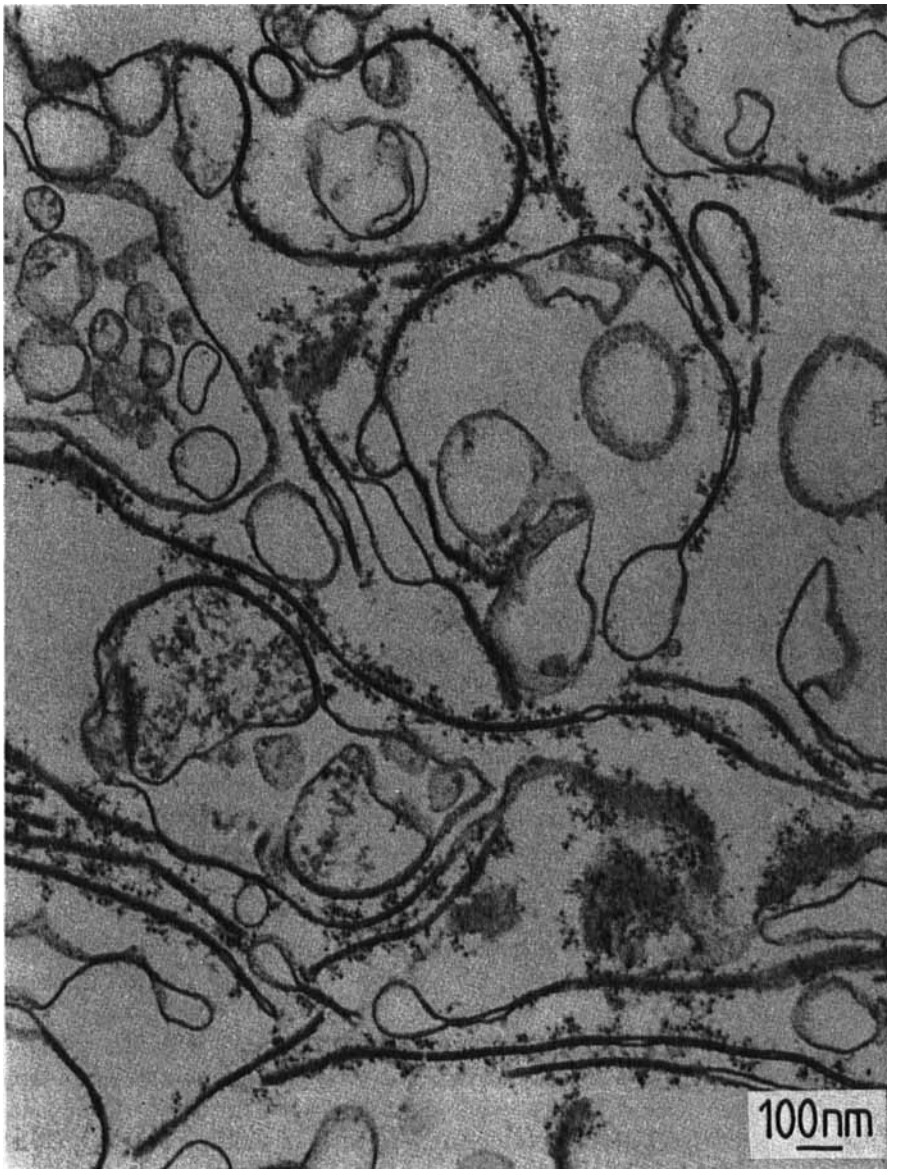

FIGURE 8. Thin-section electron micrograph of isolated bovine cortical fiber membranes. Only the 16- to 17-nm fiber junctions are labeled with anti-MP70 immunogold; single membranes are not labeled. (Courtesy of Dr. Daniel Goodenough, Harvard Medical School.)

animals. Their precise arrangement in the fiber cell plasma membrane and their function are unknown.

The gel bands at position 70,000, 64,000, and 38,000 represent proteins which are related to each other by cleavage. The 70,000-Da membrane protein (MP70) has been identified as a component of the 16- to 17-nm fiber junctions.[69,70] By immunoelectron microscopy, monoclonal anti-MP70 antibodies bind specifically to the junctional plaques and do not label nonjunctional membrane regions (Figure 8). Using these antibodies for immunofluorescence microscopy, a macular staining pattern on the fiber broad faces reveals the arrangement of the fiber junctions (Figure 9). The 64,000-Da polypeptide (MP64) is a proteolytic breakdown

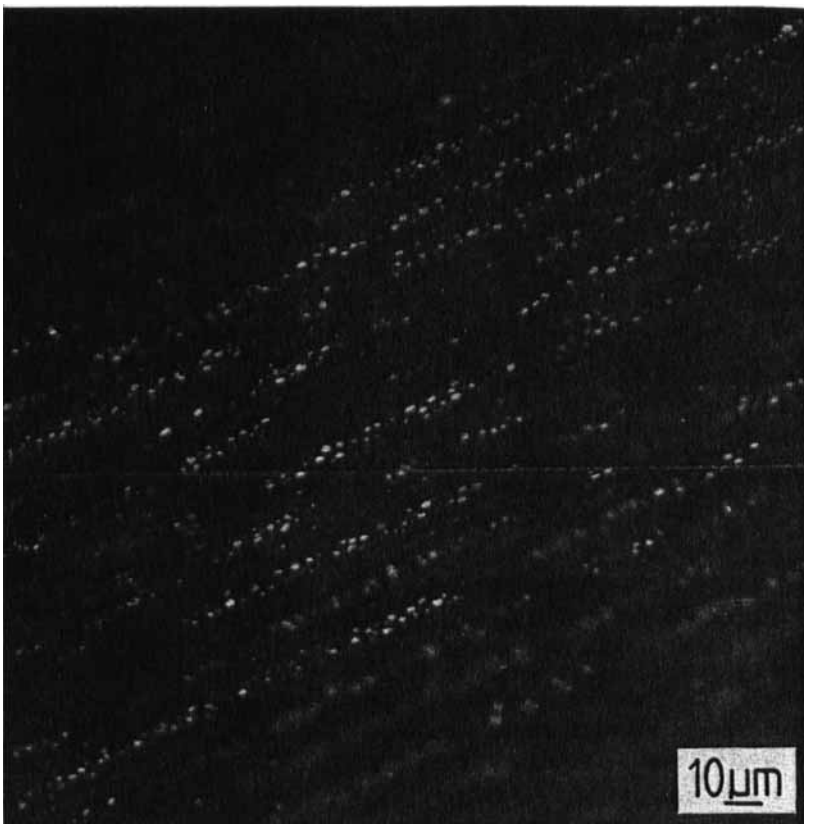

FIGURE 9. Anti-MP70 immunofluorescence microscopy of tangential section of sheep lens outer cortex shows macular staining pattern on the fiber broad sides, revealing the arrangement of the fiber junctions.

product of MP70 and also binds monoclonal anti-MP70 antibodies. The ratio of MP70 to MP64 is variable between preparations and is in favor of MP70 when the protease inhibitor mixture described earlier is used for homogenization of lens tissue.[23] MP70/64 are predominant in the outer cortex and are sharply reduced deeper in the lens. This is in agreement with the results from anti-MP70 immunofluorescence microscopy of freshly fixed lenses which show a sudden reduction of punctate staining at the outer/inner cortex transition (Figure 10). MP70/64 are cleaved to MP38 *in vivo*, thereby losing the epitope for anti-MP70 monoclonals.[23] Aspects of this processing will be discussed in Section VI.

The 140,000-Da protein (MP140) appears as a faint but consistent band on Coomassie-stained gels of the sheep lens outer cortex, but only traces are usually seen in the nucleus. MP140 is glycosylated and has been labeled on nitrocellulose blots with iodinated wheat germ agglutinin. MP140 is preferentially localized in the ball and sockets,[71] and this is reviewed in more detail in Section VII. Heslip et al.[72] have identified a 130,000-Da glycoprotein in bovine lens which has similar characteristics and a similar distribution in the fiber membrane to MP140.

Furthermore, a protein with an apparent molecular weight of 200,000 (MP200), which is consistently contained in membrane preparations from the outer cortex band, is present in reduced amounts deeper in the lens. Its function is presently unknown.

B. Alkaline Soluble Proteins

A number of proteins are fully or partially urea insoluble but are solubilized in 20 m*M* NaOH. In the sheep lens, such proteins have apparent molecular weights of 56,000, 57,000,

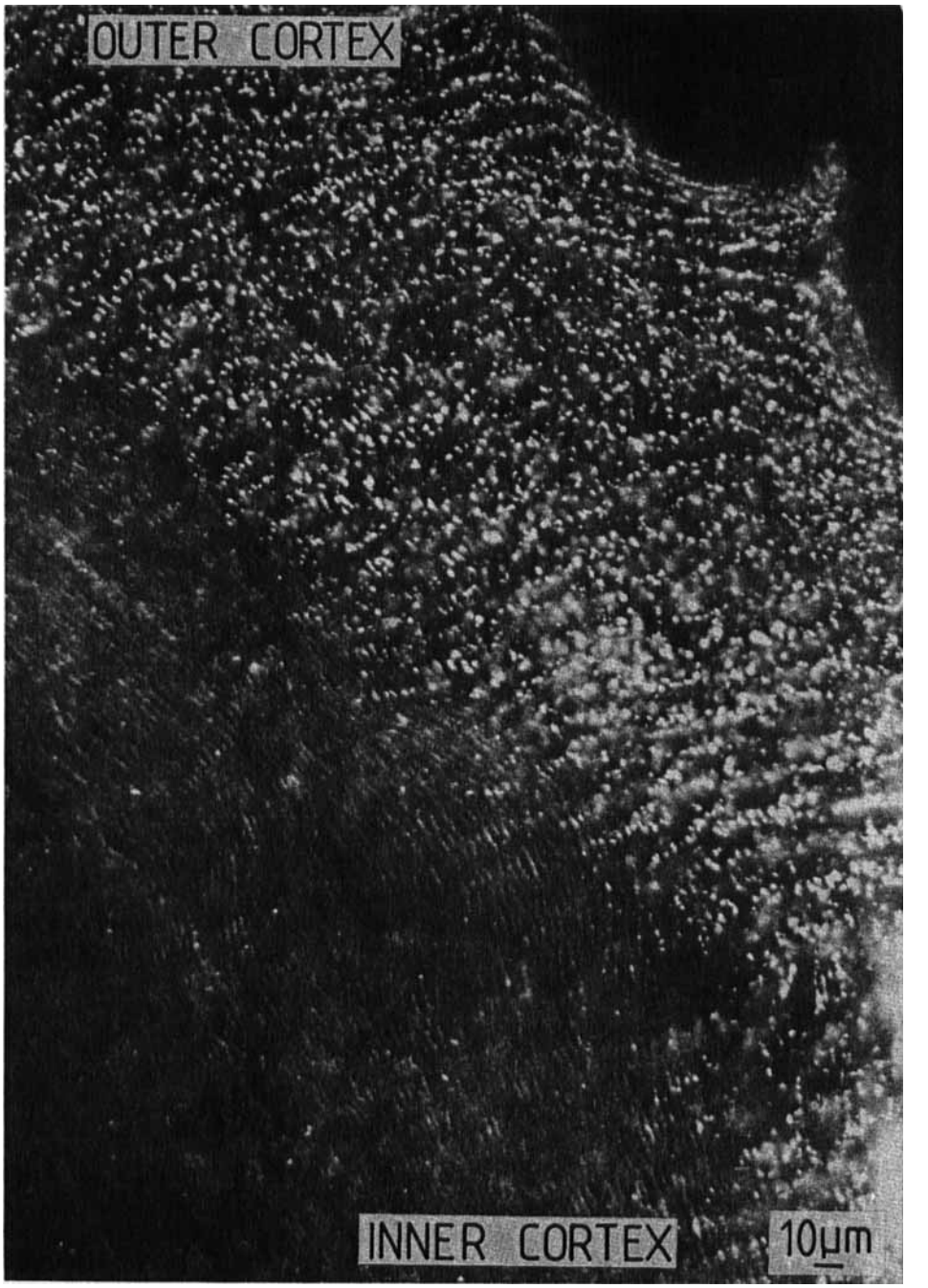

FIGURE 10. Immunofluorescence microscopy of mouse lens cryosection using monoclonal antibodies against the junctional component MP70. The strong punctate staining is sharply reduced at the outer/inner cortex transition.

and 82,000 (Figure 11). The 56,000-Da protein has been identified immunologically as vimentin[20a] and is sharply reduced in the lens nucleus, in agreement with the reported degradation of intermediate filaments in this lens region.[73] The other two polypeptides appear to be predominantly associated with the fiber plasma membranes, as suggested by the results of immunofluorescence microscopy using monoclonal antibodies.[35] The 82,000-Da poly-

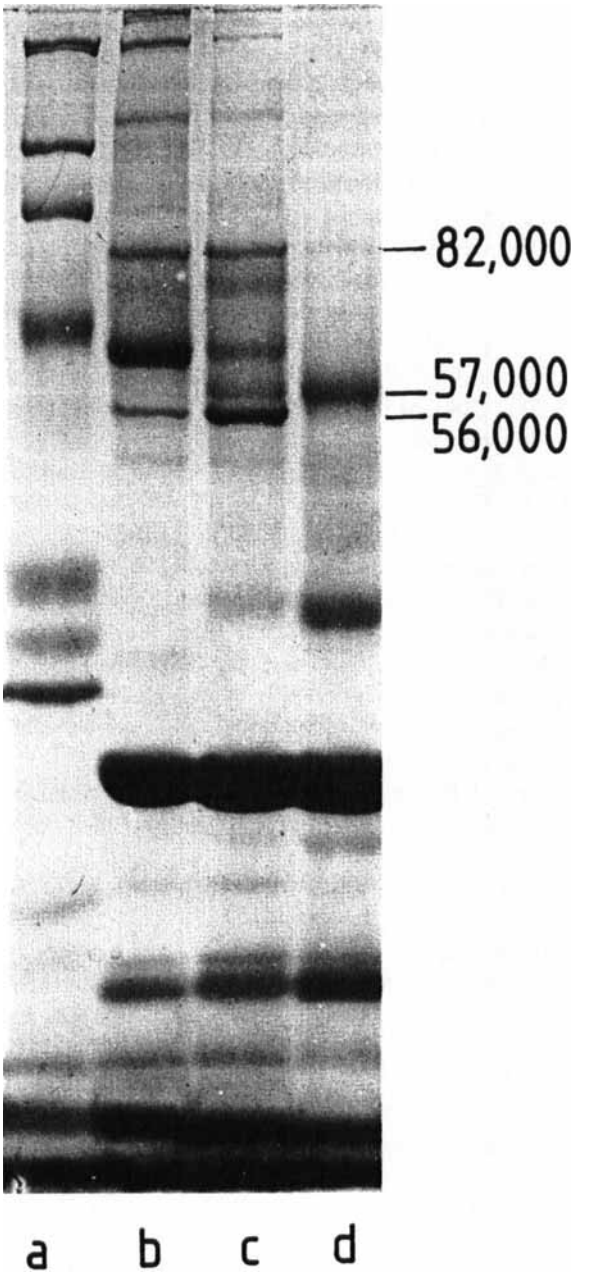

FIGURE 11. SDS-PAGE (10%) of urea-treated (but not alkali-treated) isolated sheep lens fiber plasma membranes. (a) Markers are as in Figure 7, (b) membranes from outer cortex, (c) inner cortex, and (d) nucleus. Only alkaline-soluble proteins are indicated.

peptide undergoes age-related cleavage deeper in the lens. In contrast to this, the 57,000-Da protein is enhanced in the nucleus and virtually absent in the outer cortex. Instead, monoclonal antibodies against the 57,000-Da polypeptide detect a urea soluble protein with an apparent 115,000 mol wt in the outer cortex. It is possible that the two proteins are related in a precursor-product manner as a result of age-associated proteolysis.

C. Glycoproteins

The overall carbohydrate content in the lens fiber plasma membrane fraction is 3 to 4%.[7] Major residues (for bovine lens) are galactose, *N*-acetylglucosamine, glucose, *N*-acetylgalactosamine, and mannose, listed in decreasing order. Data on which membrane proteins are glycosylated are sparse and difficult to compare due to species differences and variations in membrane isolation protocol. Gas chromatography of purified MIP showed no sugar residues.[25] Periodic acid Schiff (PAS) staining of polyacrylamide gels revealed a glycosylated 68,000-Da component in bovine lens fiber membranes.[30] In our experience, PAS staining of gels, such as that shown in Figure 7c (sheep outer cortex membranes), detects only MP140 as a well-stained band. More sensitive tests to identify galactose-containing glycoproteins, using radioactive markers, revealed membrane proteins with apparent molecular weights of 22,000, 26,000, 32,000, 50,000, 69,000, 103,000 in rabbit lenses and 22,000, 35,000, 71,000, 82,000, 103,000, 128,000 in mouse lenses.[74,75] In another sensitive assay, which detects lectin binding to lens membrane proteins on nitrocellulose blots, the following polypeptides were labeled: wheat germ agglutinin bound to a protein with an apparent molecular weight of 140,000, concanavalin A to 67,000 and 63,000, *Ricinus communis* agglutinin to 90,000, 67,000, and 63,000, and *Pisum sativum* agglutinin to 36,000.[76] Iodinated wheat germ agglutinin, which binds preferentially to *N*-acetylglucosamine-linked carbohydrate, labeled MP140 in the sheep lens cortex.[71] Similarly, in the bovine lens, a 130,000-Da protein was found to be glycosylated.[72] Both these proteins have been localized predominantly in the ball and socket regions of the fibers (see Section VII). Cultured lens epithelial cells synthesize a major glycoprotein with a apparent molecular weight of 120,000 to 130,000 which incorporates tritiated glucosamine and may be related to MP140 and/or the 130,000-Da glycoprotein.[77,78]

The lipid biochemistry of lens plasma membranes has been exhaustively reviewed by Broekhuyse,[7] and little new information on this subject has since been reported.

VI. JUNCTIONAL PROTEIN PROCESSING AND MAPPING

A. Cleavage of MP70 and MP38

The identification of MP70 as a lens fiber junction protein has been met with criticism on the basis that MP70 appears to be too minor a protein in whole lens membrane preparations to account for the large number of fiber junctions in the cortex.[44,79] It is now clear that overall MP70 levels are low because this protein is abundant only in the outer cortex and is cleaved to MP38 deeper in the lens[23] (Figure 7 in Section V). This cleavage can be demonstrated by incubating sheep lens outer cortex homogenate with 2 m*M* $CaCl_2$ at 37°C: MP70 (and MP64) is gradually decreased in amount and converted to MP38 (Figure 12). This limited proteolysis of MP70 can be inhibited with the addition of EDTA/EGTA. These results indicate that the lens contains a calcium- or divalent cation-activated protease. An enzyme with similar characteristics, calpain II, has been identified in rat lens.[80] A membrane protein of apparently 66,000 mol wt, possibly related to the sheep MP70, is also a primary substrate for this calcium-activated protease.[81] Because MP38 accumulates *in vivo* in the lens nucleus, it appears likely that this protease cleaves MP70 as part of the fiber maturation and/or aging process. On a whole lens basis, amounts of MP38 are equal or greater than MP70, but presumably this ratio depends on the age of the lens. In earlier attempts to purify fiber plasma membranes and junctions,[82,83] a major membrane protein MP34 was identified, which now appears likely to be related to MP38, the cleavage product of the fiber junction MP70.

B. Junction Mapping in the Outer Cortex

The mammalian lens provides a unique model to study the formation of intercellular junctions. Epithelial cells at the lens equator divide during the whole life span of an animal,

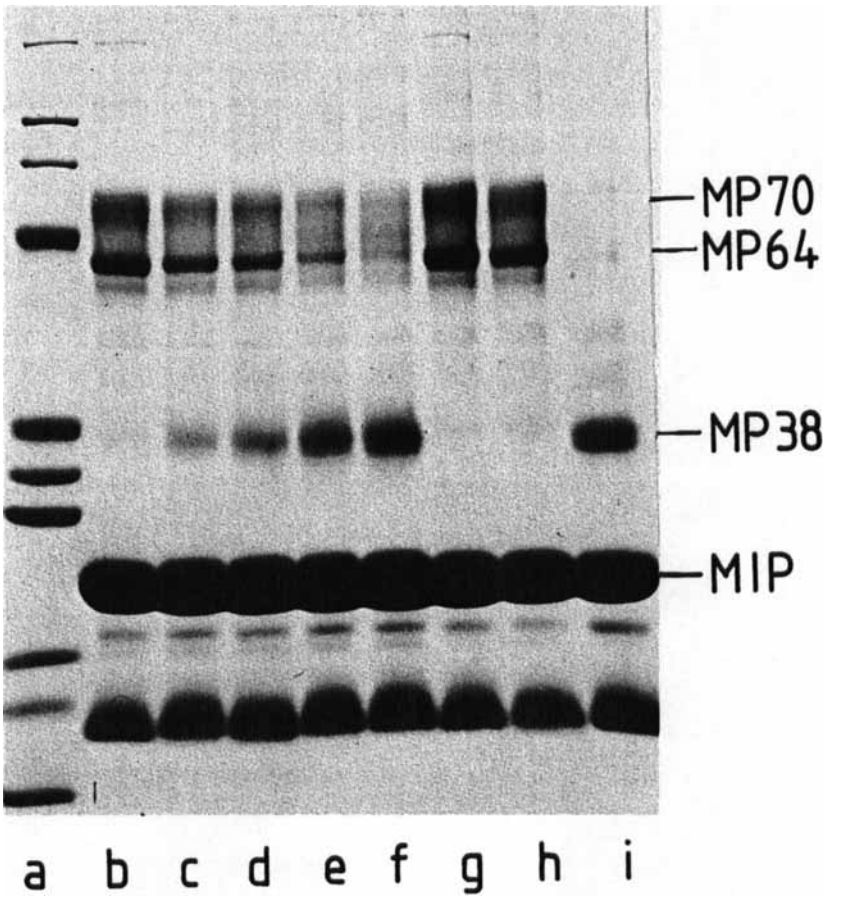

FIGURE 12. *In vitro* cleavage of MP70/64 to MP38. SDS-PAGE (10%) of urea- and alkali-treated isolated membranes from sheep lens. (a) Markers are as in Figure 7. Aliquots of outer cortex homogenate were treated as follows prior to membrane isolation: (b) added 5 m*M* EDTA/EGTA, kept on ice 40 min; (c) added 2 m*M* $CaCl_2$, incubated at 37°C 5, min; (d) 2 m*M* $CaCl_2$, 10 min; (e) 2 m*M* $CaCl_2$, 20 min; (f) 2 m*M* $CaCl_2$, 40 min; (g) 5 m*M* EDTA/EGTA, 37°C, 40 min; (h) no additives 37°C, 40 min; (i) urea- and alkali-treated isolated membranes from lens nucleus for comparison.

and daughter cells elongate to fibers stretching between anterior and posterior poles. *De novo* membrane synthesis and assembly of fiber junctions accompany this elongation. From an earlier freeze-fracture electron microscopy study of plasma membranes in the elongation zone, the onset of junctional assembly appeared to correlate with the appearance of a new class of intramembrane particles with a homogeneous size distribution of around 9 nm. These particles formed either short linear rows or small clusters.[84] The small particle clusters were found to grow to larger junctional plaques in parallel with fiber elongation.

More recently, lens fiber junctions have been mapped using anti-MP70 monoclonal antibodies and immunofluorescence microscopy. This approach offers the advantage of studying an immunologically defined set of junctions in sections through the whole lens[69] as well as along the entire length of one fiber cell or of a narrow fiber bundle.[85] Anti-MP70 antibodies were chosen for this as they are specific for the 16- to 17-nm intercellular junctions and are thus superior to immunocytochemistry with anti-MIP. Although a candidate for a junctional protein, MIP is also present in nonjunctional membranes. Therefore, anti-MIP immunoflu-

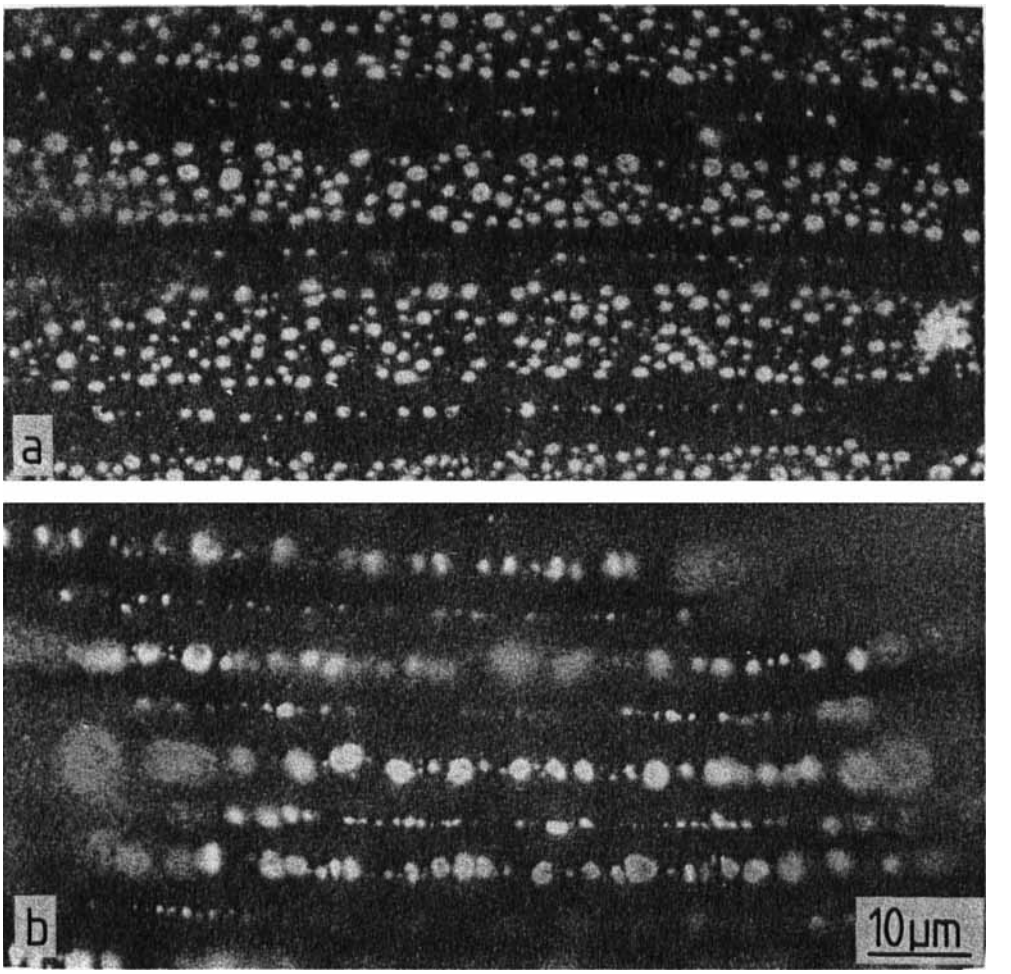

FIGURE 13. Anti-MP70 immunofluorescence microscopy of dissected outer cortex fibers from sheep lens. (a) Junctions are dispersed on fiber broad sides in equatorial region; (b) near the lens poles, large and small junctional plaques line up in rows. (Courtesy of Mr. Terry Gruijters.)

orescence staining on fiber membranes is rather homogeneous and does not produce a macular staining pattern as is characteristic for anti-MP70. Using the latter in the peripheral region of the outer cortex, the fine, spotty fluorescence staining reveals small junctional plaques dispersed on the freshly elongating cells (Figure 13a). Somewhat deeper in the lens, in a zone where fibers have elongated to reach both lens poles, junction shape and distribution change along the length of the fiber. Enlarged oval-shaped junctional plaques are still dispersed on the fiber broad sides in the equatorial plane of the lens, whereas near the poles, junctional plaques line up in the middle of the fiber broad side (Figure 13b). As both smaller and larger junctions line up, they cannot be positioned simply by the restricted width of the fiber broad side, and it appears likely that membrane peripheral or cytoskeletal proteins are involved.

Further into the lens, near the outer/inner cortex transition and MP70 cleavage zone, the large junctional plaques dissociate into diffuse clusters of smaller junctional entities (Figure 14). This fragmentation of fiber junctions is observed along the whole fiber length towards the poles.

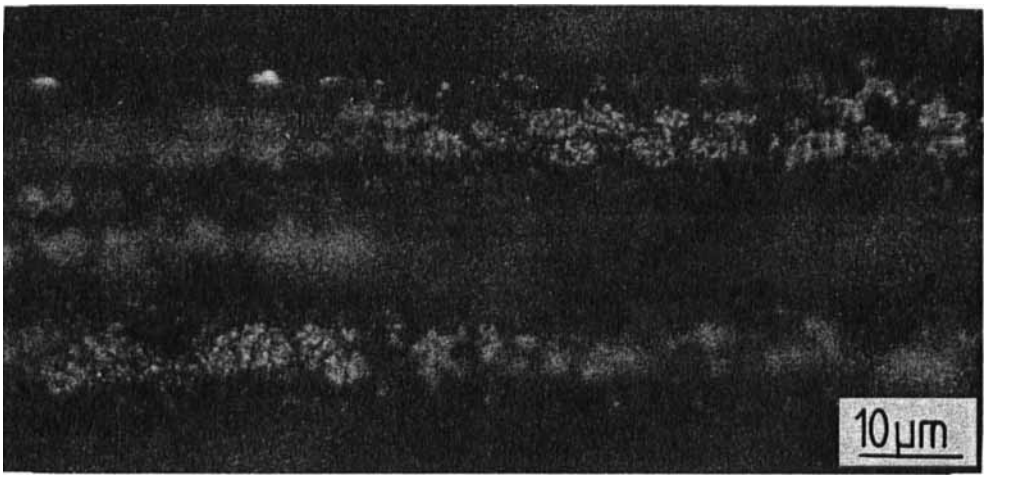

FIGURE 14. Anti-MP70 immunofluorescence microscopy of region near the outer/inner cortex transition shows large fiber junctions fragmenting into smaller sizes. (Courtesy of Mr. Terry Gruijters.)

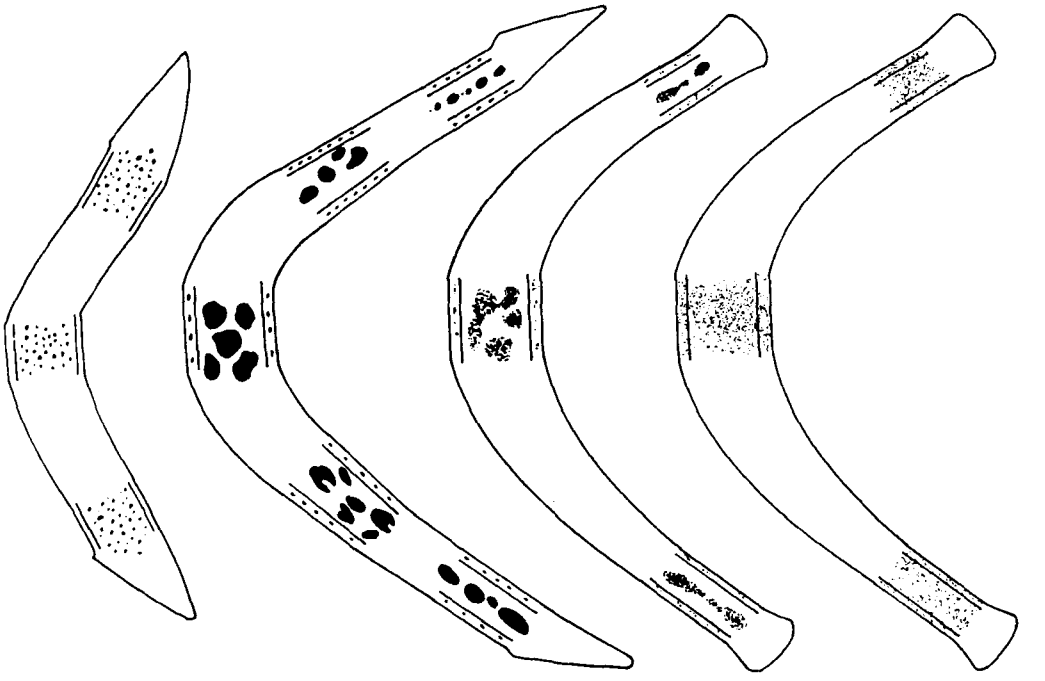

FIGURE 15. Temporal and spatial chronology of formation, distribution, and dissociation of fiber junctions. Fiber age increases toward the right of the scheme. (Courtesy of Mr. Terry Gruijters.)

The various stages of fiber junction formation and dissociation are summarized in Figure 15. The results suggest that lens fiber junctions are dynamic structures which change in size, shape, and position according to a prescribed schedule which is dictated by the processes associated with fiber differentiation. Some possible functional implications of this were discussed in Section II.

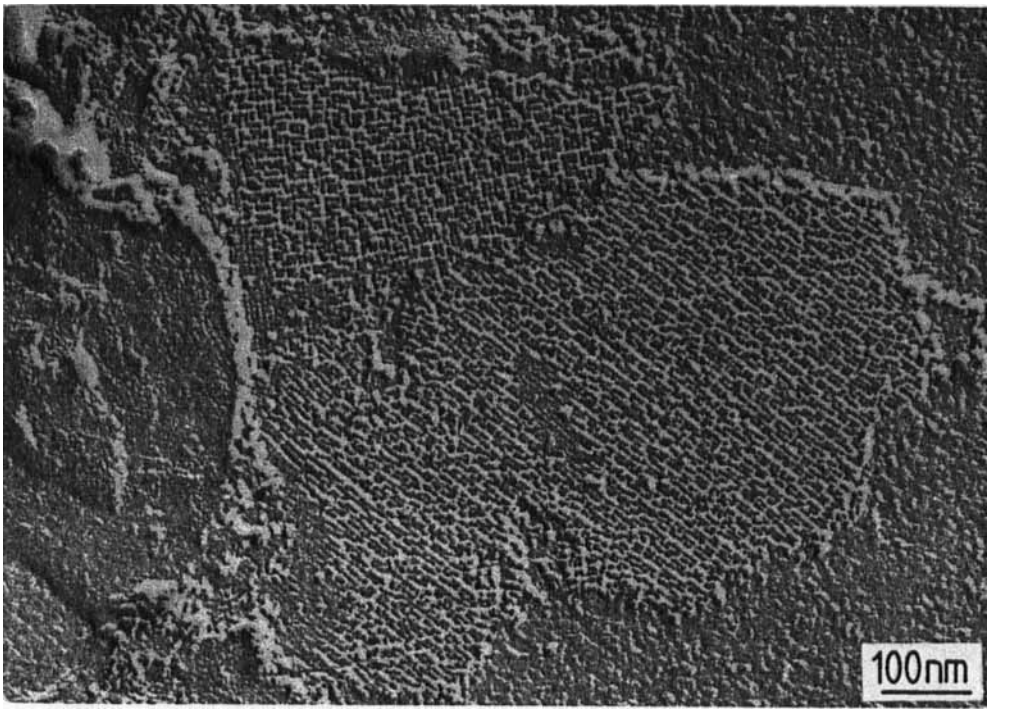

FIGURE 16. Monolayer freeze-fractured preparation of square arrays, formed after trypsin treatment of urea- and alkali-treated isolated sheep lens cortical fiber membranes.

VII. SQUARE ARRAYS

Membrane regions with intramembrane particles arranged on a square lattice of a 6- to 6.5-nm repeat (Figure 16) have been visualized by freeze-fracture electron microscopy.[86-90] Initially, square arrays were believed to represent a low pH uncoupled form of the lens fiber junctions.[87] Inconsistent with this view, square arrays were shown to be a product of membrane proteolysis, and detergent-isolated square arrays consisted of only one membrane, casting doubt on the relationship with the fiber junctions.[86] *In situ*, square arrays rarely coincided in the two adjoining membranes and hence could not be fiber junctions.[91] Furthermore, Lo and Harding[90] showed that square arrays were most abundant in the undulating membrane regions of the aged fibers in the lens nucleus. The square arrays were generally present in one membrane in the valleys and in the other in the ridges, so that they did not overlap and again could not be junctions. In thin sections, these undulating membranes revealed regions of close membrane appositions of 12 nm thickness, significantly less than the 16- to 17-nm overall thickness of lens fiber junctions.[88,90] These 12-nm close membrane appositions, sometimes referred to as thin junctions and most likely containing the square arrays, have been labeled with anti-MIP antibodies asymmetrically to one membrane[59] and they do not bind anti-MP70 antibodies.[70] In summary, the square arrays are immunologically and structurally distinct from the 16- to 17-nm lens fiber junctions, and hence these two membrane interactions appear not to be related to each other. The complete molecular composition of the square arrays has yet to be determined, and the functional role of the protein which forms them is presently unknown.

VIII. BALL-AND-SOCKET INTERACTIONS

Ball-and-socket-like membrane structures are regularly spaced along the corners of the flat hexagonal fibers (Figure 17a) and firmly interlock neighboring cells.[2,4] Although the

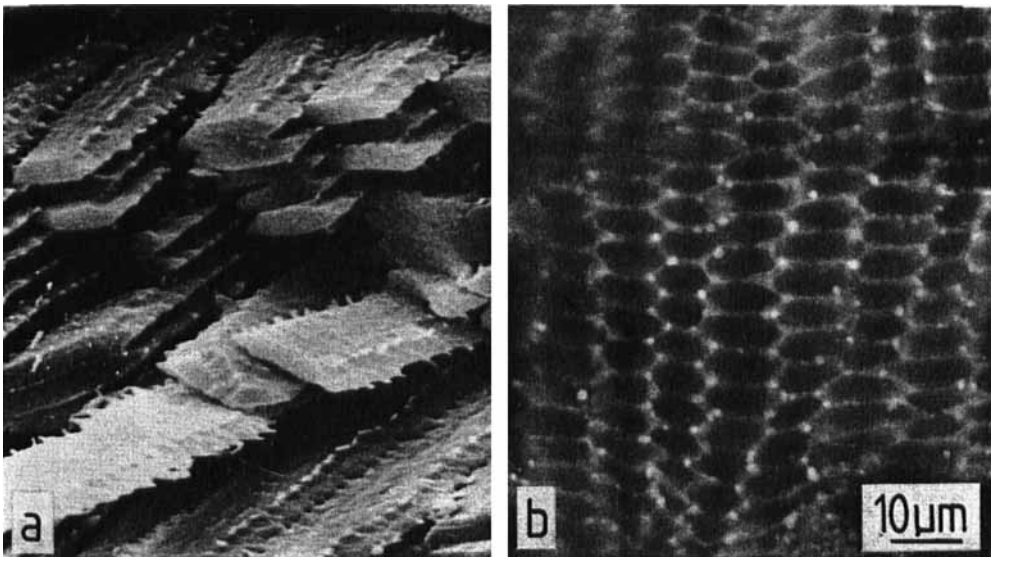

FIGURE 17. Ball-and-socket membrane interactions interlock adjacent lens fibers. (a) Scanning electron microscopy of cortical fibers; (b) wheat germ agglutinin binds predominantly to the ball and sockets as revealed by fluorescence microscopy of sections of the cortex.

ball and sockets are clearly demarcated membrane domains, it has not yet been possible to purify these interlocking structures. Data on their protein composition can, however, be obtained from immunocytochemical-labeling studies of lens fibers. MIP, ubiquitous in fiber plasma membranes, has also been localized in the ball and sockets.[59,92] Ball and sockets also bind anti-MP70 antibodies,[71] which is in agreement with an earlier report that junctional plaques were often observed at the base of ball and sockets.[5]

Wheat germ agglutinin preferentially binds to the ball and sockets (Figure 17b) and more weakly to other plasma membrane regions.[71] This suggests that MP140 (see Section V) and/or a 32,000-Da polypeptide are accumulated in the ball and sockets. The relative contributions of these two proteins to the ball and sockets are unknown. The 32,000-Da protein binds to fibronectin, a major constituent of the extracellular matrix in tissues. However, while fibronectin has been localized in the lens capsule, its presence in the bulk of the lens is controversial.[93,94] As for the shape and spacing of ball and sockets, it is worth mentioning that anti-actin immunofluorescence microscopy also shows strong staining of these interdigitating structures.[95-97]

Ball and sockets are most frequent in the lens cortex and reduced deeper in the lens. By interlocking fibers with each other, they prevent sliding of fibers during the lens shape changes associated with visual accommodation. This is important as sliding would disrupt the transmembrane channels in the fiber junctions and thus impair lens homeostatic control.

IX. PLASMA MEMBRANES OF THE LENS EPITHELIUM

Few data are available on the biochemistry of lens epithelial membranes as most research concerns the fibers in the bulk of the lens. The Na-K-ATPase is presently the best-characterized protein complex in the plasma membranes of the lens anterior epithelial cell monolayer. This enzyme has been localized histochemically by light and electron microscopy in the lateral membranes interfacing adjacent epithelial cells.[98,99] Some controversy exists on the further localization of Na-K-ATPase in the membrane portion at the epithelial-fiber interface as well as minor amounts in the membranes of the anterior cortical fibers.[100,101]

The epithelial plasma membrane region in contact with the (noncellular) capsule appears to be devoid of Na-K-ATPase, suggesting that Na and K pumping occurs to and from the extracellular space between epithelial cells rather than directly at the membrane surface interfacing the aqueous humor.

The lens Na-K-ATPase has been partially purified in membrane-bound form after deoxycholate treatment and density gradient centrifugation.[102] Polypeptides with 93 kDa (α subunit) and 51 kDa (β subunit, glycosylated) have been identified.

Gap junctions have been identified in the plasma membranes between epithelial cells as well as at the epithelial-fiber interface. Gap junctions from these two membrane regions appear to have physiologically distinct permeability characteristics. For example, high carbon dioxide treatment blocked the spread of microinjected fluorescent dye within the epithelial cell monolayer, but did not impair dye transfer into the underlying fibers.[103] Gap junction proteins of the lens epithelial cells have not yet been identified.

In one case, epithelial membranes have been isolated from the remnants adhering to the peeled-off lens capsule. SDS-PAGE analysis revealed a prominent polypeptide MP34 and no MIP.[104] This result is in agreement with those from immunohistochemistry which show anti-MIP binding to fiber membranes but not to epithelial membranes.[59,105,106] Our preliminary data suggest that MP70 is also absent from epithelial membranes. It appears that protein synthesis for MIP and MP70 is not switched on until epithelial cells differentiate into fibers at the lens equator.

X. MEMBRANE ABNORMALITIES IN LENS CATARACTS

While cataract formation can be initiated by a variety of insults to the lens, the later opacification generally results from membrane dysfunction, structural changes and breakdown, and from precipitation of the fiber cytoplasmic proteins. Gross morphological changes at the cellular and membrane levels have been described for a number of different cataract models in a recent review by Harding et al.,[107] which also contains excellent illustrations. In the present review, we focus on aspects of cataract-associated membrane protein dysfunction, processing, and redistribution.

In one model, human senile cataract, oxidative stress due to increased levels of hydrogen peroxide in the aqueous humor gradually inactivates the Na-K-ATPase in the anterior lens membranes.[108] This, in turn, causes a gross imbalance of lens interior and exterior ion concentrations, resulting in osmotic fiber swelling. Because the swollen fibers have a round rather than flat hexagonal cross-profile, fiber order in the lens is disrupted, and internal light scattering increases. Membranes may break by further osmotic swelling, thus exposing cytoplasmic proteins to oxidative conditions. Water-soluble proteins, such as the crystallins, become increasingly insoluble as cross-linking of sulfhydryls progresses. Large molecular weight aggregates appear to attach to the inner plasma membrane surface via a 43-kDa extrinsic membrane protein.[109-111] As the mechanical stress on the membranes increases, they disrupt further and form abundant globular and multilaminar structures, ultimately leading to total lens opacification.

Na-K-ATPase dysfunction is also a feature of two other cataract models: inhibitors of this enzyme have been isolated from inherited cataractous lenses from Cac mouse[112] and Nakano mouse.[113] Other membrane proteins in the Nakano lens appear to be modified as well. For example, levels of MIP are greatly reduced in Nakano lenses when compared with wild type of similar age. Furthermore, comparative electron microscopy of normal and cataractous lenses reveals significantly reduced levels of gap junctions in some cases.[114-116]

In yet another osmotic-type cataract model inherited in a New Zealand Romney sheep strain, fiber swelling is associated with degeneration and loss of the ball-and-socket structures.[117] The redistribution of the putative ball-and-socket proteins MP140 and 32,000 (see

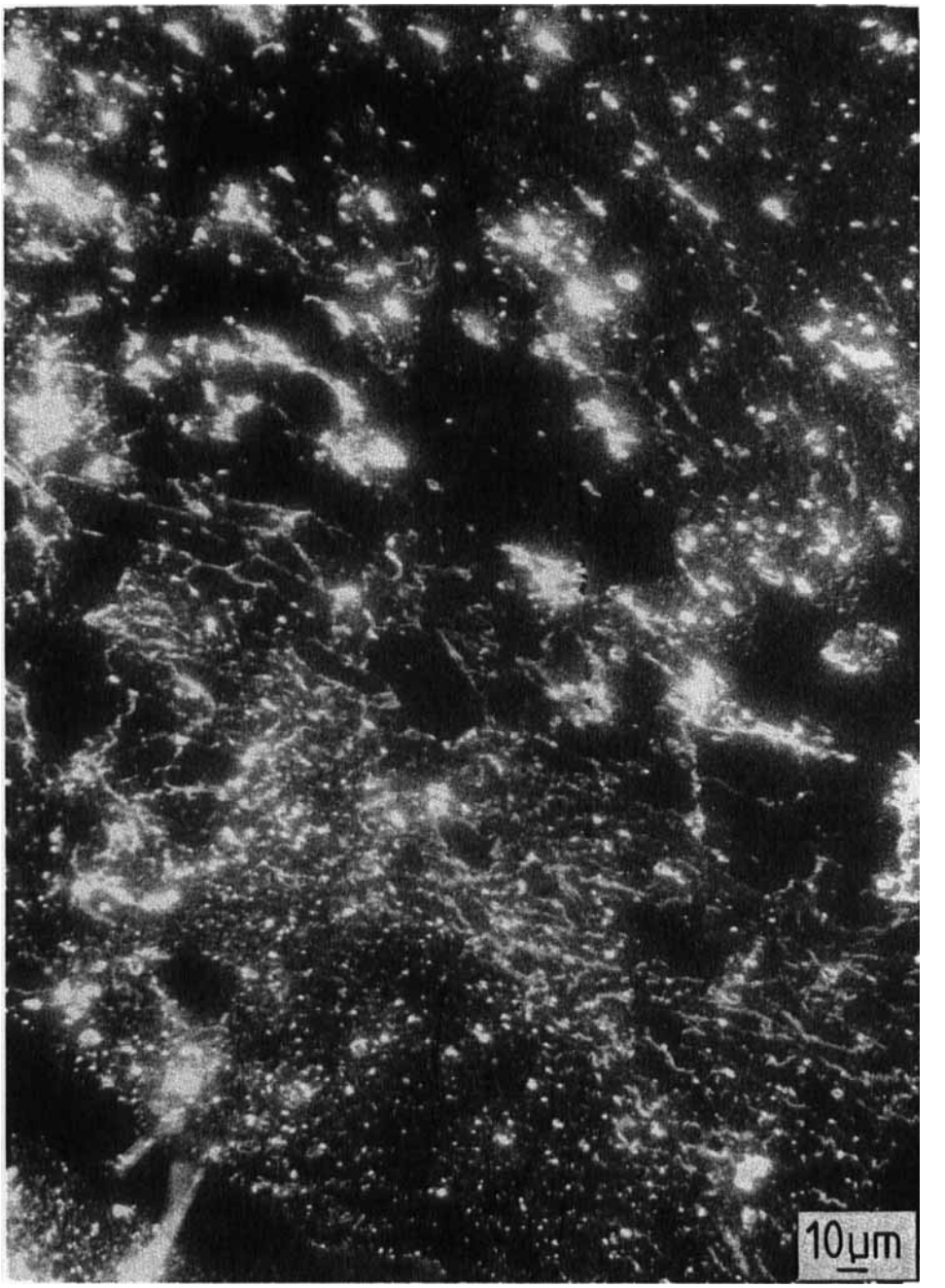

FIGURE 18. Wheat germ agglutination fluorescence microscopy of inherited sheep lens cataract showing the disintegration of fiber plasma membranes and aggregation of membrane protein in globular bodies in the upper half of the picture. (Courtesy of Miss Kim Gilbert of our laboratory.)

Section VIII) has been monitored by wheat germ agglutinin-fluorescence microscopy of cataractous lenses. While some lens regions may still reveal the spotty staining characteristic for the ball and sockets, most areas show a large globular fluorescence pattern (Figure 18). It appears that ball-and-socket membranes coalesce into globular structures which may be multilaminar in nature, as judged from their very strong staining intensity.[71] SDS-PAGE of

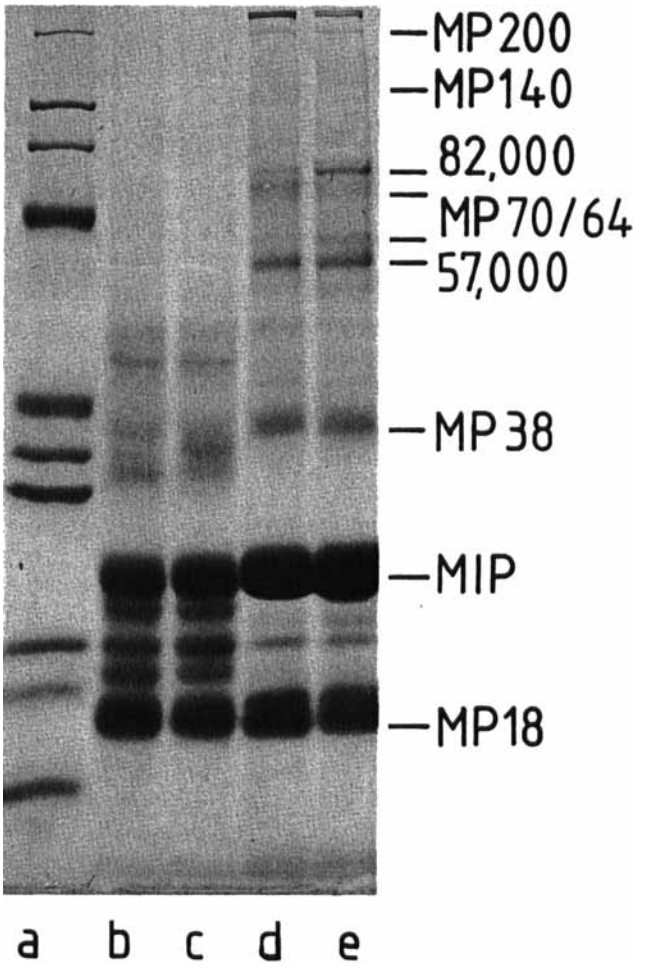

FIGURE 19. SDS-PAGE (10%) of fiber plasma membranes isolated by urea (but not alkaline) extraction of sheep cataractous and normal lenses. (a) Markers are as in Figure 7; (b) membranes from two cataractous lenses no. 86-19/25; (c) cataractous lenses no. 86-49/70; (d) partial cataractous lenses no. 86-15/36; (e) normal lenses.

plasma membranes isolated from mature cataractous sheep lenses reveals the loss of most higher molecular weight membrane proteins, in particular, MP200, MP140, and MP70 (Figure 19). The alkaline-soluble, membrane-associated proteins with apparent molecular weights of 82,000 and 57,000 are also reduced. None of these changes in membrane protein composition has been observed in sheep lenses at early stages of opacification. Hence, the loss of these proteins is a consequence of cataract formation rather than its cause.

Loss of membrane proteins has also been found to be associated with selenite-induced cataracts in rat lenses.[81] SDS-PAGE of control lenses reveals MP18, MIP, and MP66. The former two are heat aggregatable, whereas MP66 is not. This latter protein is therefore not an oligomer of MIP and is likely to be the rat lenses analog of the sheep MP70 junctional component. In cataractous lens, MP66 is no longer detectable by SDS-PAGE, MIP is strongly reduced, and MP18 remains at similar levels. There is evidence that a calcium-dependent protease is activated during selenite cataractogenesis. More recently, such a protease, calpain II, has been purified from normal rat lenses and shown to cleave fiber plasma membrane proteins.[80]

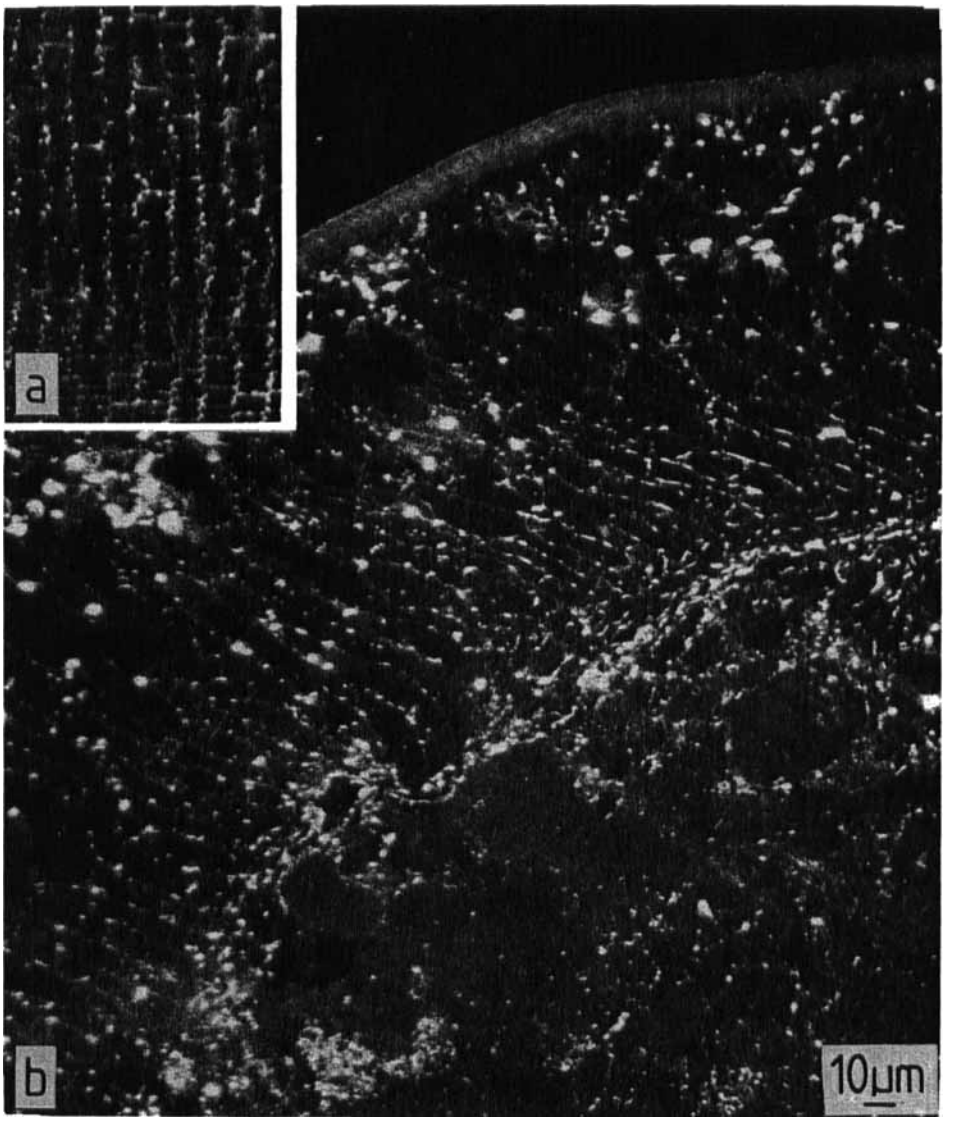

FIGURE 20. Wheat germ agglutination fluorescence microscopy of normal (a) and galactosaemic (b) rat lens. The latter shows general membrane disintegration and the preferential accumulation of globular bodies at the outer/inner cortex transition. (Courtesy of Miss Kim Gilbert.)

The breakdown of fiber membranes and the accumulation of globular membrane bodies have been reported for several cataract types.[118-121] In galactosaemic rat cataract, such globular bodies (detected by wheat germ agglutination) occur with increasing frequency in a narrow zone approximately 200 µm from the lens periphery (Figure 20). This region overlaps with the transition zone in the normal rat lens where cleavage of fiber junction MP70 to MP38 takes place. It has yet to be determined whether the two phenomena are related to each other.

XI. CONCLUDING REMARKS

Although this review covered the current knowledge on lens membranes more generally, we put a stronger emphasis on the biochemistry and structure of the lens fiber junctions.

We believe understanding their function is a central issue for many ongoing research interests. For those researchers studying the function of communicating junctions in general, lens fiber junctions are relevant because they appear to represent an excellent example of tissue adaptation of these intercellular channels. Although fiber junctions are in many ways similar to the gap junctions of other tissues, fiber junction proteins — MIP and/or MP70 (and/or others?) — differ from those in other classes of gap junctions. No sequence homologies between the 26K MIP and the liver 27K gap junction protein have been detected. A lot more work is however needed for a complete molecular description of lens fiber junctions. The sequence of MP70 has yet to be determined. No direct data on the opening and closing of the channels of lens fiber junctions have been obtained. Some attempts have been made to purify and reconstitute MIP, and channel activity has, indeed, been observed. The data on the purity of the reconstituted protein are, however, not convincing. In many cases, minor proteins (i.e., in comparison with the ubiquitous nature of MIP) such as MP70, MP38, and others have simply been ignored. This neglect is unfortunate as it is not yet known how much (or how little) protein is needed to reconstitute the observed channel activity. The development of unambiguous functional assays to screen for potential channel-forming proteins is therefore still open to innovative approaches.

Other researchers may be more interested in the molecular mechanisms which lead to lens cataract. Many forms of cataract involve ion imbalances and osmotic swelling of fibers. Intercellular channels are thus likely to play a role in maintaining lens transparency. Furthermore, the limited proteolysis of junctional MP70 to MP38 deeper in the lens may have an effect on channel regulation. It also reinforces earlier results from lens biochemistry, namely, that the lens does not consist of a homogeneous tissue, but that it is divided into biochemically distinct regions. Similarly, cataract formation often appears to progress in an unhomogeneous fashion. It is likely that these two phenomena are related to each other. Therefore, the functional aspects of the processing of membrane proteins in deeper lens regions deserve greater attention among cataract researchers.

ADDENDUM

Amino-terminal sequence analysis has revealed extensive homologies between lens MP70 and the 28- and 47-kDa gap junction components from liver and heart, respectively. No homologies have been found between these proteins and MIP. Hence, MP70 is thus far the largest component in a divergent family of gap junction proteins.[122]

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