Apparent Coordination of Plasma Membrane Component Synthesis in the Lens

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Purpose. This study explores the order of assembly of the lens fiber cell plasma membrane. Because the lens must synthesize most of its membrane components, our approach was to map directly the spatial distribution of cholesterol, fatty acid (reflecting phospholipid), and the main intrinsic protein, MP26, synthesis in the lens and, thereby, determine the extent to which membrane component synthesis was coordinated during fiber cell elongation.

Methods. Young rat lenses were incubated with either tritiated water as the substrate for cholesterol and fatty acid synthesis or tritiated leucine as the substrate for MP26 synthesis. We developed a simple technique for uniformly dissolving the decapsulated lens into small fractions by incubating the lens with gentle stirring in sodium dodecyl sulfate-containing buffer. Based on the protein content of each fraction and available information on the radial distribution of protein in the young rat lens, each fraction was equated to a specific percentage of the lens radius. Cholesterol was precipitated from each fraction by digitonin; fatty acids were extracted and isolated by thin-layer chromatography. The MP26 was recovered both by immunoprecipitation from each fraction with anti-MP26 polyclonal antibody and from sodium dodecyl sulfate polyacrylamide gel electrophoresis gels of intact crude membrane, which was isolated from lens fractions by dissolving the lens in a urea-containing buffer.

Results. The spatial distribution of incorporation of cholesterol, fatty acid, and MP26 was virtually superimposable, with essentially all the incorporation occurring in the outer 10% of the lens radius and peak incorporation occurring in approximately the outer 3–6% of the radius.

Conclusions. These results indicate that the synthesis of lens membrane components is highly coordinated and imply that the plasma membrane accumulates constant proportions of cholesterol, phospholipid, and MP26 throughout the course of fiber cell elongation. Invest Ophthalmol Vis Sci. 1993;34:2186–2194.
posed of cholesterol, phospholipid, and a principal 26-kilodalton protein called the main intrinsic protein (MP26).\textsuperscript{3,4} We believed that determining the spatial distribution in the lens of the synthesis of these membrane components could provide insight into the order of membrane assembly because the lens likely synthesizes most, if not all, of its membrane components (ie, cholesterol,\textsuperscript{7} fatty acids for phospholipids,\textsuperscript{8} and obviously, its proteins) and because it appeared reasonable to assume that, once synthesized, these components would be rapidly incorporated into the membrane rather than entering a static, nonmembrane pool.

Tritiated water was used to measure the synthesis by intact rat lenses of cholesterol and fatty acid (taken to reflect phospholipid synthesis), and tritiated leucine was used as the substrate for the membrane protein synthesis. A simple technique based on the gradual dissolution of the lens in sodium dodecyl sulfate (SDS)-containing buffer provided the opportunity to "dissolve" the lens uniformly into small fractions. Each fraction was then equated to a precise segment of the lens radius, based on the percent of total lens protein in each fraction and the available knowledge about the spatial distribution of protein in the young rat lens.\textsuperscript{9} The results of this study indicate that synthesis of all major components of the lens fiber cell plasma membrane is highly coordinated. A precedent for such coordinated synthesis of membrane components was provided previously.\textsuperscript{10} The earlier report demonstrated that cholesterol and phospholipid synthesis was coordinated in proliferating L\textsubscript{6} myoblasts.

**MATERIALS AND METHODS**

**Radial Fractionation of Lenses**

Lenses (n = 10–16) from 20–25-day-old Sprague-Dawley rats (male and female), which had been incubated as described subsequently, were carefully decapsulated and placed into a 5-cm plastic culture dish, which was partitioned into a large (3.5 cm) and small (1.5 cm) compartment by a nylon mesh screen (1 mm\textsuperscript{2}) glued across the dish. The lenses were placed into the larger compartment and suspended in 3 ml of either 5 mmol/l Tris buffer (pH 8) containing 1 mmol/l ethylenediaminetetraacetic acid, 5 mmol/l 2-mercaptoethanol (Buffer A), and 0.2% SDS (wt/vol) or in the 10 mmol/l phosphate cell lysis buffer (pH 7.5) described earlier,\textsuperscript{11} which contained 1% Triton X-100 and 0.1% SDS. The lenses were immediately agitated at room temperature on a gyrorotatory shaker (Model G76, New Brunswick Scientific, Edison, NJ) at approximately 80 oscillations/min. The buffer was quickly replaced at 1, 2, 4, 6, 8, 10, 12, 15, 20, and 30 min. The lens region remaining after 30 min (the nucleus) was homogenized in 3 ml of the buffer. The volume of each fraction was estimated by weight, and each was assayed for protein content by a modified Lowry assay.\textsuperscript{12}

This procedure provided a simple method for the uniform and rapid dissolution of the lens (Fig. 1A) at a rate which appeared to follow first-order kinetics (Fig. 1B). Because no particulate matter was seen by low-power microscopy in the buffer or at the lens periphery during dissolution, we assume that the plasma membrane was being "solubilized" along with the other components of the fiber cell. Furthermore, ultracentrifugation of the solubilized lens fractions yielded no pellet. Each fraction obtained (0–1 min, 1–2 min, and so forth) was eventually equated to a specific percentage of the lens radius, based on the percent of the lens total protein removed with each fraction. Using published data for the protein distribution versus lens radius in the 30-day-old rat,\textsuperscript{9} we constructed a plot of the cumulative percent of the lens protein removed, "solubilized" versus the percent of lens radius remaining (Fig. 1C). The calculated relationship was well approximated by direct experimental observation (Fig. 1C). Although this method can fractionate the lens into small arcs of radius, it cannot distinguish between equatorial, anterior, or posterior regions of the lens.

The animals were killed by carbon dioxide inhalation, and all procedures adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The lenses were collected and used immediately after death.

**Measurement of Lens Sterol and Fatty Acid Synthesis**

Intact lenses (n = 10–14) from 24–25-day-old rats were incubated for 3 hr in Dulbecco’s modified Eagle’s medium (DMEM, minus calf serum) containing either 8 or 16 mCi/ml of tritiated water (25 mCi/g, New England Nuclear, Boston, MA) at either 0°C or 37°C. The lenses were incubated in 5-cm culture dishes, which were sealed with Parafilm (American Can Co., Greenwich, CT) to prevent contamination of the incubator. The lenses were then rinsed several times with phosphate-buffered saline (pH 7.4), carefully decapsulated, and dissociated in 3-mi aliquots of Buffer A plus 0.2% SDS as described. Each 3-ml fraction was lyophilized and saponified in 2 ml of 0.67 N KOH in 67% ethanol (100°C for 2 hr). Carrier cholesterol (1 mg) and triolein (0.2 mg) were added before saponification. The carriers were also added to the blank samples, which were identically treated. After dilution with 2 ml of water, the cholesterol was ex-
FIGURE 1. Dissolution of the rat lens in SDS-containing buffer. (A) Decapsulated lens (21-day-old rat) was incubated with stirring for varying times (minutes) in 3.0 ml of Buffer A containing 0.2% SDS. The lens was photographed at the indicated times, and aliquots of the suspended solution were removed for protein assay (approximately 10 sec required per time). Bar = 1 mm. (B) First-order plot of time-dependent changes in lens radius. (C) Relationship between lens protein distribution and radius (calculated from published data, solid line, and experimentally determined from the lens in A and B).

Measurement of the Lens Regional Distribution of Cholesterol and Phospholipid Mass

Decapsulated lenses (n = 16) from 24-day-old rats were fractionated by dissociation in Buffer A with 0.2% SDS as described. After removing small aliquots for protein determination, the fractions were lyophilized, and the total lipids were extracted into two 3-ml aliquots of chloroform–methanol (2:1, vol/vol). The lipid residue remaining after solvent evaporation was dissolved in chloroform–methanol (2:1, vol/vol) and divided into two portions. One was used for measurement of total phospholipid phosphorus by colorimetric assay after ashing the samples at 500°C, and the other was used for total cholesterol determination by gas-liquid chromatography (after saponification) as described previously. The molar cholesterol–phospholipid ratios for the individual lens fractions were calculated and related to specific percentages of the lens radius based on the protein distribution.

Measurement of the Distribution of Lens Membrane Protein Synthesis

Immunoprecipitation of MP26

Sixteen rat lenses (from 20-day-old rats) were incubated at 37°C in 3 ml of leucine-deficient DMEM containing 50 μCi/ml of added tritiated 3,4,5-leucine (158 Ci/mmol, New England Nuclear). The lenses were decapsulated and dissolved in 3 ml of the SDS cell lysis buffer described earlier, which additionally contained 0.1 mol/l of unlabeled L-leucine. Dithiothreitol was omitted. The 26-kilodalton main intrinsic protein (MP26) of the lens membrane was immunoprecipitated using rabbit anti-bovine lens MP26 antiserum (furnished by Dr. Isaac Bekhor, University of Southern California, Los Angeles, CA) by a procedure similar to one recently described for the immunoprecipitation of 3-hydroxy-3-methylglutaryl coenzyme A reductase, an intrinsic protein of the endoplasmic reticulum membrane. The anti-bovine lens MP26 antibody had a high reactivity toward rat lens MP26,
even when tested at a dilution of 1:50,000 by western blot (data not shown). Others also measured the synthesis of lens MP26 by quantitative immunoprecipitation of sulfur-35-labeled MP26 using a polyclonal antibody to this protein.14

The dissolved lens fractions (each equated to a specific percent of the lens radius, based on its protein content) were incubated for 30 min at 37°C and then sonicated for 10 sec on ice (Probe Sonifier-Cell Disruptor 200, Branson Sonic Power, Danbury, CT). Both steps were done to ensure solubilization of MP26. We added 1 ml of each 3-ml fraction to a 1.5-ml Microfuge tube (Beckman Microfuge B, Fullerton, CA) and precleared it by adding 40 µl of a 25% suspension of agarose-bound Protein A (Sigma, St. Louis, MO). The samples were spun for 2 min, and 40 µl of anti-MP26 antisera was added to the recovered supernatant and incubated with rotatory mixing for 90 min at room temperature. We added 80 µl of the Protein A suspension, and the incubation continued for an additional 90 min at room temperature with mixing. The antigen–antibody–Protein A complex was sedimented by centrifugation, and the resulting pellet was washed five times with 1-ml aliquots of the SDS-containing lysis buffer with 0.1 mol/l unlabeled L-leucine. The antigen was released from Protein A by adding 45 µl of Laemmli15 sample buffer containing 8 mol/l urea. The proteins were separated by SDS polyacrylamide gel electrophoresis (SDS-PAGE) using 12% gels by the Laemmli method and subjected to fluorography using EN 3HANCE (New England Nuclear), according to manufacturer's instructions. An estimate of the relative contribution of each lens fraction to total MP26 synthesis was obtained by densitometric scanning of the gel lanes in the exposed film (GS 300 scanning densitometer, San Francisco, CA) and comparing the area under the absorbance curves for MP26 in each fraction to the total absorbance of MP26.

We attempted to test the completeness of the immunoprecipitation of MP26 by western blotting aliquots of the supernatant recovered from centrifugation of the antigen–antibody–Protein A complex using the anti-MP26 antisera. However, the presence of trace amounts of unprecipitated immunoglobulin G in this supernatant resulted in immunostain appearing at molecular weights corresponding both to immunoglobulin G heavy and light chains (approximately 25 kilodaltons). Although the concentrations of MP26 in the dissolved lens fractions were similarly low (estimated at 2–4 µg for each of the first ten fractions) and the concentration of antisera was high, we could not ensure that the newly synthesized MP26 was quantitatively precipitated from each fraction. Thus, we sought to confirm the distribution of MP26 synthesis in the lens using an additional approach.

Direct Estimation of MP26 Synthesis

Because the lens membrane is insoluble in 8 mol/l urea,14 we recovered MP26 from the lens fractions by dissolving the lenses in Buffer A containing 8 mol/l urea. Inclusion of SDS in the dissolution buffer would solubilize the plasma membrane and, thereby, prevent the separation of intrinsic membrane protein from the great concentration of the lens's soluble protein. Although the urea-containing buffer is useful in separating the decapsulated lens into numerous fractions, dissolution by stirring in such a buffer was a less precise method for fractionating the lens than dissolution in SDS-containing buffers. The lens fiber cells unravel in bundles and clumps in the urea-containing buffer. Also, the rate of dissolution was very rapid, with 60% or more of the total lens protein "solubilized" in 20 min.

Between 14–16 lenses from 24-25-day-old rats were incubated for 3 hr at either 0°C or 37°C in 5-cm culture dishes containing 3 ml of leucine-deficient DMEM with 50 µCi/ml of tritiated leucine. The lenses were then washed with phosphate-buffered saline, decapsulated, and dissociated in 3-ml aliquots of Buffer A containing 8 mol/l urea. After removing small aliquots for protein determination, each fraction was centrifuged (100,000 × g × 60 min, SW 60 Beckman rotor), and the pellet was washed once with 2.5 ml of the urea-containing buffer. The urea-insoluble fraction (crude lens membrane) was washed once with Buffer A (without urea), and the pellet was dissolved in Laemmli sample buffer. The proteins were separated by SDS-PAGE and stained; the bands corresponding to MP26 were excised. The protein in the excised bands was solubilized according to a published method,16 and the radioactivity was measured. Specifically, the bands were homogenized in 1.0 ml of 0.1% SDS, 50 mmol/l NH4HCO3, and 5% 2-mercaptoethanol (vol/vol); heated for 5 min at 100°C; and incubated for 18 hr at 37°C with trypsin (250 µg/ml, TLCK free, Sigma). We added 15 ml of aqueous counting solution (Ready Solv HP, Beckman), and the tritium content was measured by scintillation counting.

Lens Distribution of Tritiated Leucine

The distribution of substrate for MP26 synthesis was examined in lenses from 19-day-old rats, which were incubated at 37°C for up to 18 hr in leucine-deficient DMEM containing 50 µCi/ml of tritiated 3,4,5-leucine. Lenses, in groups of two or four, were removed at 3, 8, and 18 hr; quickly washed twice with Buffer A containing 10 mmol/l unlabeled L-leucine; blotted dry; decapsulated; and physically separated by teasing into the cortex and nucleus. Here the cortex ac-
RESULTS

The spatial distribution of de novo cholesterol and fatty acid synthesis in the lens appeared identical, with 90% of the total of each occurring in the outer 10% of the lens radius (100% to 90% of the radius) and peak synthesis occurring in approximately the outer 3–6% of the radius (97% to 94% of the radius, Fig. 2). Fatty acid synthesis was equated with phospholipid synthesis occurring in approximately the outer 3–6% of the radius (97% to 94% of the radius, Fig. 2). Fatty acid synthesis was equated with phospholipid synthesis because essentially all lenticular fatty acid is present in phospholipids. Between 90–95% of the carbon-14-labeled acetate incorporated into fatty acids by the lens was recovered in phospholipids.8,21

The molar ratio of cholesterol to phospholipid throughout the outer 25% of the young rat lens radius tended to be uniform (Fig. 2). The ratio was approximately 0.36 from 97% to 64% of the radius and slightly less, approximately 0.26–0.29 from 100% to 97% of the radius. The inner 64% of the lens radius corresponds to the nucleus and possessed a cholesterol-to-phospholipid molar ratio of approximately 0.83, a value very similar to that seen earlier.52 The high cholesterol to phospholipid ratio in the nucleus reflects a loss of phospholipid from this region, rather than an increase in cholesterol.22,23 The constancy of the cholesterol-to-phospholipid ratio throughout most of the cortex supports the possibility that the lens sites for cholesterol and phospholipid synthesis are similar. The apparent slightly lower cholesterol-to-phospholipid ratio in the extremely superficial cortex (outer 1–2% of the radius) might reflect a carry over of phospholipids from epithelial cells during differentiation. The epithelial cells from the embryonic chicken lens possess a lower cholesterol-to-phospholipid ratio than that of its fiber cells (Fleschner CR, unpublished observations, 1992).

Rabbit anti-bovine MP26 antibody precipitated four tritiated leucine-labeled proteins of approximately 20–28 kilodaltons from the solubilized rat lens fractions (Fig. 3A). The principal protein was 26 kilodaltons and, therefore, taken as MP26. The identity of the other immunoprecipitated proteins is unknown. However, because MP26 is degraded to lower molecular weight forms,24 including a 19.5-kilodalton form,25 it appeared possible that proteolysis of MP26 occurred during the isolation procedures. In fact, others also observed some proteolysis of MP26 during its isolation by immunoprecipitation.14

TABLE I. Distribution of $^3$H-Leucine Substrate (TCA-Soluble and Nonvolatile Radiolabel) in the Lens

<table>
<thead>
<tr>
<th>Sample</th>
<th>Incubation Time (hr)</th>
<th>No. of Lens</th>
<th>Protein (mg)</th>
<th>Estimated Water (μl)</th>
<th>dpm × 10^{-4}/μl Water</th>
<th>dpm × 10^{-5}/Sample</th>
<th>Nucleus/Cortex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortex</td>
<td>3</td>
<td>2</td>
<td>5.14</td>
<td>5.60</td>
<td>1.831</td>
<td>3.270</td>
<td>0.37</td>
</tr>
<tr>
<td>Nucleus</td>
<td>3</td>
<td>3</td>
<td>8.97</td>
<td>7.66</td>
<td>0.982</td>
<td>1.217</td>
<td></td>
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<tr>
<td>Cortex</td>
<td>8</td>
<td>4</td>
<td>10.62</td>
<td>11.58</td>
<td>3.277</td>
<td>2.830</td>
<td>0.47</td>
</tr>
<tr>
<td>Nucleus</td>
<td>8</td>
<td>4</td>
<td>16.50</td>
<td>14.09</td>
<td>1.869</td>
<td>1.326</td>
<td></td>
</tr>
<tr>
<td>Cortex</td>
<td>18</td>
<td>4</td>
<td>17.41</td>
<td>14.61</td>
<td>2.702</td>
<td>1.890</td>
<td>0.55</td>
</tr>
<tr>
<td>Nucleus</td>
<td>18</td>
<td>4</td>
<td>17.41</td>
<td>14.61</td>
<td>2.702</td>
<td>1.890</td>
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</tbody>
</table>

a Lenses (19-day-old rats) were incubated in DMEM + 50 μg/ml $^3$H-leucine for the indicated times. Lenses were divided into cortex and nucleus and the TCA-soluble and nonvolatile radiolabel ($^3$H-leucine) recovered and measured.

* The water content of 21–to-22-day-old rat lens was previously determined to be 52% in cortex and 46% in nucleus.
The intensity of MP26 labeling was greatest in fractions 1–2 min to 6–8 min (Fig. 3A). These fractions collectively accounted for 9% of the lens total protein and corresponded to approximately the outer 6% of the lens radius (estimated from Fig. 1C). Only trace labeling of MP26 was seen in fraction 30–45 min and in the nucleus that represented 85% to 78% and the inner 78% of the radius. The increase in MP26 labeling in fraction 20–30 min is partially the result of the greater protein content of this fraction (5.8% of total lens protein) compared with the early fractions (1.4–2.2% of total protein). The selective precipitation of MP26 by the anti-MP26 antiserum from the total lens protein is made obvious by comparison of the fluorographs of the immunoprecipitated MP26 (34-day film exposure) with the intense labeling of total protein from an equivalent fraction of the lens (3-day film exposure, Fig. 3B).

We attempted to quantify the spatial distribution of MP26 synthesis in the lens by (1) estimating the intensity of MP26 labeling in each fraction by densitometric scanning of the exposed film and (2) relating the area under the scan curves of MP26 to its synthesis. With the exception of one point, 85% of the outer 10% of the lens radius accounted for approximately 90% of the total synthesis, with the peak synthesis occurring in the outer 5% of the radius. When the relative MP26 synthesis was expressed per milligram of protein, the major synthetic activity was confined to the outer 3–5% of the radius (97% to 95% of the total radius, Fig. 4B).

Because of uncertainty about the extent of quantitative immunoprecipitation of the newly synthesized MP26, a second approach was used to measure the distribution of tritiated MP26 in lenses incubated with tritiated leucine. Intact crude lens membrane was recovered from lenses dissolved into several fractions by stirring in urea-containing buffer. The proteins extracted from the 26-kilodalton band of the SDS-PAGE gels of the crude membrane were assayed for tritium content. Although the MP26 band was intensely immunostained by the anti-MP26 antiserum (data not shown), we cannot exclude the presence of other labeled proteins underlying these bands. However, others recently demonstrated that only one polypeptide from lens membrane migrated at 26 kilodaltons, MP26.5 Direct measurement of the tritium content of the 26-kilodalton intrinsic membrane protein indicated that membrane protein synthesis was largely confined to the outer 10% of the lens radius (Fig. 5).
The somewhat broader spatial distribution of membrane protein synthesis seen with the urea-dissociated lenses likely reflects the lesser capacity of the urea-containing buffer to dissolve the lens uniformly. However, by permitting recovery of the intact membrane, the urea-dissociation approach enabled us to show that the spatial distribution of MP26 synthesis essentially paralleled that seen when radiolabeled MP26 was isolated by immunoprecipitation.

![Figure 3](https://iovs.arvojournals.org//pdfaccess.ashx?url=/data/journals/iovs/933173/)

**FIGURE 3.** Distribution of MP26 synthesis in the lens: fluorographic analysis. Sixteen intact lenses from 20-day-old rats were incubated for 18 hr at 37°C in 5.0 ml of leucine-deficient DMEM containing 50 μCi/ml of tritiated leucine. (A) Decapsulated lenses were dissolved in 5.0-ml portions of Buffer A plus 0.2% SDS. The protein distribution in the fractions was determined. The MP26 was immunoprecipitated from 1-ml aliquots using antiserum to MP26, and the recovered protein was subjected to SDS-PAGE with detection of the radiolabel by fluorography. The gels were exposed to film for 34 days. The upper numbers in the figure (0-1, 1-2, and so forth) are the lens dissolution intervals (fractions) in minutes. The lower numbers in parentheses show the percent of the total lens protein that each interval (fraction) contained. (B) Fluorography (F) of an aliquot of total protein from the 0-1-min sample, 3-day film exposure.

![Figure 4](https://iovs.arvojournals.org//pdfaccess.ashx?url=/data/journals/iovs/933173/)

**FIGURE 4.** Quantitative estimation of the distribution of MP26 synthesis in the lens: fluorographic analysis. An optical density scan of the fluorographs in Figure 3 was made, and the areas under the MP26 absorbance curves were determined. (A) The area under each MP26 band (Fig. 3, sample 0-1 to nucleus) was expressed as a percent of the total synthesis (total area) and related to a specific percentage of the lens radius on the basis of protein distribution. (B) The distribution of synthesis was expressed on the basis of the protein content per fraction (the area under MP26 absorbance curve per fraction divided by the milligrams of protein per fraction).
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FIGURE 5. The distribution of MP26 synthesis in the lens: direct analysis. Fourteen to sixteen intact lenses from 24–25-day-old rats were incubated for 3 hr at 0°C or 37°C in 3.0 ml of leucine deficient DMEM containing 50 μCi/ml of tritiated leucine. The results of two experiments are shown. Decapsulated lenses were dissociated in Buffer A plus 8 mol/l urea. The crude membrane was recovered by ultracentrifugation of each fraction, and the membrane protein was separated by SDS-PAGE. The polypeptide band at 26 kilodaltons was excised, and the protein was solubilized and counted. Exp 1: MP26 total counts above background (37°C) = 16,980 dpm. Incorporation at 0°C = 0 dpm. Exp 2: MP26 total counts above background (37°C) = 16,650 dpm.

DISCUSSION

Tritiated water is an excellent substrate for measuring the distribution of lipid synthesis in the lens because it provides hydrogen atoms for the synthesis of both cholesterol and fatty acids7 and because it rapidly equilibrates with the total lens water. Thus, the specific activity of the substrate remains constant throughout the lens. Because we were unable to detect incorporation of tritium from tritiated water into MP26 in preliminary studies, a radiolabeled amino acid (tritiated leucine) was used as the substrate. This represents a compromise because, after 3-, 8-, and 18-hr incubations of intact lenses with tritiated leucine, the concentration of labeled leucine in the lens nuclear water was only 37%, 47%, and 55%, respectively, of that in the cortex. However, because incorporation of tritiated leucine into MP26 was shown to be confined to approximately the outer 10–15% of the lens radius (and largely to the outer 6–7%), it appears reasonable to assume that the specific activity of the substrate was uniform in the outer cortex, particularly in the longer term incubation.

Measuring the relative distribution of the synthesis of cholesterol, fatty acid, and MP26 along the lens radius should identify the sites of synthesis of these components and assembly into the fiber cell membrane. We assume that membrane components are assembled into membrane at the place where the components are synthesized. Our measurements indicate that the distribution of synthesis for all major components of the lens membrane is virtually identical and confined to a small region, one which approximately occupies the outer 2–8% of the lens radius. Peak synthesis appeared to occur in a narrow band representing the outer 3–6% of the lens radius. If the average cross-sectional diameter of a lens fiber cell is taken to be 1.0 μm27 and the radius of the 21-day-old rat lens to be 1.5 mm, the region of maximum membrane synthesis (the outer 3–6% of the radius) would span fiber cell layers 45–90 below the capsule. Dr. Kuszak (Rush Presbyterian, Chicago, IL) estimated that rat lens fiber cells are fully elongated, extending from suture to suture, by approximately 100–150 cell layers below the capsule (personal communication). This would correspond to approximately the outer 10% of the lens radius, the site at which we observed synthesis of all membrane components to be essentially complete.

Earlier work located lens cholesterol and fatty acid synthesis to the cortex, but no more specifically. Detailed measurements of the lens distribution of the messenger RNA for MP26, the other major membrane component, provided information on the distribution of MP26 synthesis in the lens if we assume that all of the messenger RNA identified was translationally active. Using in situ hybridization with an antisense messenger RNA, the distribution of MP26 messenger RNA was mapped in young rat lenses. The messenger RNA was immediately identified in elongating fiber cells, found in the highest concentration in the bow region, and distributed throughout the cortex. It was present also in low concentrations in the lens nucleus. Thus, the messenger RNA for MP26 seemed more broadly distributed in the lens than did the MP26 synthesis observed in the current study.

In conclusion, this study identified a narrow shell of the lens radius where the bulk of lens membrane synthesis occurred. Within this discrete region, which reflects the zone of rapid cellular elongation, the synthesis of all membrane components appeared to be highly coordinated. This finding implies that, as a fiber cell elongates, its membrane accumulates constant proportions of cholesterol, phospholipid, and MP26 throughout this process.

Key Words

lens, plasma membrane, cholesterol, fatty acid, main intrinsic protein
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References