In vitro synthesis of the major lens membrane protein
(lens polyosomes/cytoskeleton)

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ABSTRACT The biosynthetic activity of a polyribosomal fraction isolated from the lens fiber plasma membrane-cytoskeleton complex by DNase I treatment has been assayed. After translation of these polyribosomes in a reticuocyte cell-free system and analysis of the products by electrophoresis in sodium dodecyl sulfate gels, the preferential synthesis of a protein with an apparent molecular weight of 25,000 was observed. By means of immunoochemical characterization we showed that this protein, which seems not to be synthesized by "free" polyribosomes, is identical with the major intrinsic plasma membrane protein MP26 of lens fibers. Upon storage, the molecular weight of the newly synthesized protein decreases to about 22,000, a phenomenon that has been observed for MP26 in isolated plasma membranes and that may be caused by the presence of a specific proteolytic cleaving site in the protein.

The mammalian eye lens is a very suitable system for the study of terminal differentiation. During this process, great amounts of plasma membrane constituents are newly synthesized, among which is an intrinsic plasma membrane protein designated MP26 (1). Almost 90% of the lens protein is contributed by the so-called crystallins (for review, see ref. 2). The remaining 10% consists of quantitatively minor lens proteins of which a number participate in essential structures such as plasma membranes (3) and the cytoskeletal matrix (4). In a recent study, Kibbe et al. (5) have proved that one of these proteins (MP42) is identical with actin. Previous observations provided evidence that the lens cytoskeleton consists of 7-nm and 10-nm filaments that end at the cytoplasmic surface of the plasma membrane (4). Furthermore, it was shown that ribosomes and crystallin aggregates were associated with the filaments. Upon incubation with DNase I, a fraction of polyribosomes could selectively be released from the cytoskeleton (4, 6).

Although MP26 represents the major intrinsic protein in fiber plasma membranes, no lens fiber polyribosome population carrying the mRNA coding for this protein could be detected after application of the standard procedure (7). This is rather striking because polyribosomes that direct the synthesis of the other characteristic intrinsic membrane protein, MP34, are obtained by this method (8). In the present paper we demonstrate that this failure was due to the fact that the polyribosomes fraction preferentially synthesizing MP26 is not "free" but seems to be associated with the plasma membrane-cytoskeleton complex in lens fibers.

MATERIALS AND METHODS

Calf eyes were obtained on ice from the slaughterhouse and used immediately. They were washed with distilled water and opened at the lateral side, so that the lenses could be removed without adherent iris material. Also, the capsule and epithelial cells were removed. L-[35S]Methionine (specific activity, 400 Ci/mmol; Ci = 3.7 × 109 becquerels) was purchased from the Radiobiological Centre (Amersham, England). DNase I was obtained from Sigma and, under the conditions used, did not show RNase or protease activity.

Preparation of Plasma Membranes for Gel Electrophoresis. Lens fiber plasma membranes were isolated at room temperature in either 1 mM NaHCO3 buffer or in 50 mM Tris-HCl, pH 7.4/25 mM KCl/5 mM Mg(OAc)2 (TKM buffer) according to the procedure described (9), with discontinuous sucrose gradients. After exhaustive washing in the respective buffers, the fractions were analyzed directly by NaDodSO4 gel electrophoresis.

Preparation of Free- and Membrane-Bound Polyribosomes. Lens cortices were dissected out and homogenized in TKM buffer. The 10,000 × g supernatant of the homogenate was used for the isolation of free polyribosomes as described (7). Membrane-bound polyribosomes were isolated from the remaining plasma membrane pellet after exhaustive washing with TKM buffer at room temperature. In these steps, heparin (1 mg/ml) was added to the buffer. The release of polyribosomes could be achieved by incubating the membranes for 1 hr at room temperature in either deoxycholate at 1.5% or DNase I at 0.5 mg/ml. In the DNase I incubation, 2 mM Mn(OAc)2 was added. Incidentally, phenylmethylsulfonyl fluoride at a final concentration of 0.1 mM was used in the incubation mixture. For a blank incubation, only TKM buffer was used.

The 10,000 × g supernatants of the incubations were layered on a 2 M sucrose cushion in TKM buffer and centrifuged for 12 hr at 100,000 × g. The pellets were further purified either by 0.25% deoxycholate treatment and recentrifugation on a 2 M sucrose cushion in TKM buffer or by direct centrifugation on a 15–35% linear sucrose gradient and subsequent concentration in a Ti50 rotor. The purified polyribosomes were diluted in twice-distilled water to a concentration of 1 mg/ml (determined spectrophotometrically, assuming an extinction coefficient of 13 at 260 nm).

Electron Microscopy. Electron microscopy was performed as described (9).

Synthesis of Lens Protein in a Reticuocyte Lysate. Rabbit reticuocytes were prepared as described by Evans and Lingrel (10) and lysed by addition of water. A 50,000 × g supernatant fraction of the lysed cells was used as a cell-free system, and incubations were performed at 30°C for 1 hr. The reaction mixture contained (per ml): 0.6 ml of reticuocyte cell-free extract, 1 μmol of ATP, 0.2 μmol of GTP, 1 μmol of dithioerythritol, 10 μmol of creatine kinase, 20 μmol of Tris-HCl (pH 7.4), 100 μmol of KCl, 1 μmol of Mg(OAc)2, and 0.1 μmol of each of the naturally occurring amino acids (40 μCi of [35S]-methionine). Polyribosomes were added at 20–200 μg/ml. The incubations were either used directly for gel electrophoretic

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analysis or immunoprecipitation or were stored at −20°C for 3 weeks and thereafter analyzed.

**Immunoprecipitation of MP26.** The MP26 was cut out of a preparative NaDodSO₄/polyacrylamide gel, and antibodies against this protein were raised in a rabbit by the method of Alcalá and Maisel (11). MP26 in reticulocyte lysates was detected by using this antiserum according to the procedure described by Van Zaane et al. (12). Immunoprecipitates were analyzed by NaDodSO₄/polyacrylamide gel electrophoresis.

**Polyacrylamide Gel Electrophoresis.** The translation products were analyzed by NaDodSO₄ gel electrophoresis according to Laemmli (13) with the modification that a slab gel instead of rods was used. The slab was 12 cm long and contained 13% acrylamide, 0.35% methylenebisacrylamide, and 0.1% NaDodSO₄. In this method, a stacking gel was applied. Staining and destaining were performed as described by Weber and Osborn (14). For detection of the labeled proteins, the procedure of Bonner and Laskey (15) was used in combination with the drying procedure described by Berns and Bloemendal (16).
RESULTS

Isolation of Plasma Membranes Under Conditions that Stabilize Cytoskeletal Structures. Plasma membranes from lens fiber cells were isolated at room temperature under ionic conditions that stabilize both polyribosomes and the cytoskeletal structure. Fig. 1 shows a NaDodSO₄ gel electrophoretic protein pattern of membrane fractions isolated either under cytoskeleton-destabilizing (NaHCO₃) or stabilizing (TKM buffer) conditions. NaHCO₃-isolated plasma membranes showed MP26 as main protein constituent, as well as α-crystallin and other components including MP34, MP45, and MP55. After isolation of membranes in TKM buffer, the cytoskeleton was still attached (compare Fig. 1, lane d); two polypeptide fractions in the MP45 and MP55 region were greatly enriched in these preparations. One of the polypeptides with an apparent molecular weight of 45,000 has been identified as actin (5). MP55, previously designated by us as a desmin-like protein, has now been characterized and appears to be of the so-called vimentin type, a major constituent of intermediate filaments (unpublished data). The cytoskeleton was released from the membranes when neither magnesium nor potassium ions were present in the isolation buffer. In this case only traces of actin and vimentin were observed in the electrophoretic pattern (Fig. 1, lanes b and c).

Isolation of Polyribosomes Associated with the Plasma Membrane–Cytoskeleton Complex. The polyribosomes released from the plasma membrane–cytoskeleton complex by deoxycholate or DNase I treatment were put on a continuous sucrose gradient and centrifuged. The profiles shown in Fig. 2 were obtained. After a control incubation containing neither deoxycholate nor DNase I, no polysomal pattern was observed. Sucrose gradient profiles of polyribosomes isolated after deoxycholate or DNase I treatment were virtually identical. A slightly higher 80S peak was seen when the polyribosomes were isolated by deoxycholate treatment. In both cases the ratio A₂₆₀/A₂₈₀ increased up to 1.70 during isolation. Electron microscopy of the pellet obtained after DNase I treatment showed the presence of arrays of monosomes attached to a thin strand. Morphologically, these polyribosomes were indistinguishable from "free" lens polyribosomes (Fig. 3).

The amount of polyribosomes that could be isolated from the purified membrane–cytoskeletal complex by the methods described was about 1% (0.5 μg/g of tissue) of the total amount of polyribosomes isolated from the cytoplasm of lens fiber cells (60 μg/g of tissue). That at least part of the actin from the cytoskeletal complex was depolymerized by DNase I treatment is shown in Fig. 4. After this treatment, the supernatant fraction

![Figure 5](image1.png)

**Fig. 5.** NaDodSO₄ gel electrophoretic patterns of polypeptides synthesized de novo in a reticulocyte cell-free system under the direction of lens fiber polyribosomes. Lanes: a, "free" polyribosomes; b–d, polyribosomes isolated after DNase I treatment (50, 100, and 200 μg/ml, respectively); and e, blank incubation.

![Figure 6](image2.png)

**Fig. 6.** NaDodSO₄ gel electrophoretic patterns of MP26 immunoprecipitate of a reticulocyte lysate incubated with DNase I-isolated polyribosomes (lane a), with "free" polyribosomes (lane b), or with deoxycholate isolated polyribosomes (lane c) and polypeptides synthesized in a reticulocyte lysate under the direction of "free" lens fiber polyribosomes (lane d).
Immunoprecipitation of the translation products obtained after incubation with "free" lens polyribosomes was always negative, indicating that no mRNA coding for MP26 is present in the latter fraction.

DISCUSSION

Isolated lens fiber plasma membranes are characterized by two major constituents designated MP26 and MP34, respectively (1). Evidence has been provided that these polypeptides are intrinsic constituents of the communicating junctions connecting lens fibers (2, 17). It is remarkable that the MP26 is absent in lenticular epithelial plasma membranes, whereas it becomes a major component in the stage of terminal differentiation of lens cells. When lens polyribosomes prepared according to the standard procedure (7) were translated in a heterologous cell-free system, only MP34 could be detected among the newly synthesized lens polypeptides. We wondered why a marker of cell differentiation as important as MP26 was not found as a translation product. One of the explanations was that we might have lost a specific population of polyribosomes as a consequence of the isolation procedure applied. In most other systems, polyribosomes occur either free or as membrane-bound structures, each class being involved in the biosynthesis of specific proteins (18). Although the endoplasmic reticulum is not present in lens fibers, a certain class of polyribosomes seems to be associated with the plasma membrane–cytoskeleton complex (4, 6).

The present study demonstrates, by means of biochemical techniques, that under proper conditions these polyribosomes are isolated with the plasma membrane–cytoskeleton complex and can be released after DNase I treatment. Because DNase I has been shown to specifically depolymerize actin filaments (19), our finding that treatment of the cytoskeletal complex with this enzyme releases polyribosomes strongly supports the idea that part of the polyribosomes in lens fibers is attached to the actin-containing microfilaments. Interaction between (polypeptide) ribosomes and cytofilaments has also been postulated by other investigators in HeLa cells (20, 21), acrosomes (22), ascites cells (23), and cultured fibroblasts and kidney cells (24), mainly on the basis of electron microscopy. These studies did not show that the polyribosomes were involved in the synthesis of a specific protein.

From all these observations and from the results presented in this paper, the most intriguing question that remains to be answered is the nature of the interaction between filaments and ribosomes. Our finding that the main intrinsic lens fiber plasma membrane protein, MP26, is synthesized exclusively on polyribosomes that are attached to the membrane, presumably via microfilaments, suggests an important role of these structures in lens cell differentiation. The interaction may be necessary to ensure proper and efficient incorporation of newly synthesized MP26 into lens fiber plasma membranes.

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