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The Major Polypeptide (MIP) of Lens Fiber Junctions and its Synthesis in Cultured Differentiating Lens Epithelial Cells*

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Abstract. Lens and liver contain many gap junctions, which for a long time have been considered to be very similar. Recent results, however, point to differences on morphological and biochemical levels, especially when the liver gap junction polypeptide (26,000 Daltons) is compared with the main intrinsic polypeptide (MIP) from lens junctions. The lens fiber specific MIP, which represents a marker molecule for lens cell differentiation could be detected by indirect immunofluorescence as well as by immunodiffusion in lens epithelial cells, which differentiated in vitro under distinct culture conditions. The fine structure of these differentiated cells is presented.

Key words: Lens – Communicating junctions – Polypeptide – Synthesis – In-vitro-differentiation

The eye lens, forming an essential part of the optical system, has some peculiarities which possibly might be linked to the hypothetical functions of lens fiber junctions. Its wet weight and size increase with age. This is a result of both, the continuing process of fiber formation by epithelial cell differentiation and the absence of cell degradation or cell shedding. Lens cells formed in the embryonic period of life are later-on found in the center of the lens, known as the nucleus, whereas the newer cells are laid down one after and above the other forming concentric layers, the lens cortex. This appositional mode of growth originates from a monolayer of kuboidal epithelial cells which cover the anterior surface of the lens. The epithelial cells of the preequatorial (germinative) region undergo mitoses. In the equatorial region they start to elongate and finally differentiate into long lens fibers (factor 1,500) that extend from the anterior pole of the lens to the posterior pole. These new fiber cells synthesize a diverse class of proteins, the lens specific proteins, called crystallins, along with their membrane proteins.

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Shortly after elongation the fiber cells of the cortex lose mitochondria, cytoplasmic membranes and polysomes, even the cell nucleus decays some time later. Older fiber cells are incapable of macromolecular synthesis (Kuwabara 1975).

The energy metabolism of the fiber cells which form the mass of the organ lens decreases to about $^1/_{10}$ of that in lens epithelial cells. Thus energy and substrate supply including the active transport of electrolytes for the encapsulated, avascular organ, has to be arranged mainly by the lens epithelium (Hockwin 1971).

A prerequisite of normal vision is the transparency of the lens. It depends on the highly ordered arrangement of long hexagonal fibers which are free of intracellular particles but contain large amounts of crystallins. The high cell density causes specific transport problems within this organ, which contains minimal amounts of extracellular water (Rink 1978). Thus very high numbers of communicating junctions are found which presumably mediate metabolic coupling. Metabolic cooperation is suggested to be an essential feature for cells in avascular structures (Goodenough et al. 1980).

The lens fiber junctions have been compared with the well investigated gap junctions of the liver. In fact there are many apparent similiarities when their morphology is examined by thin section electron microscopoy (Henderson et al. 1979; Rafferty and Esson 1974) and when their electrophysiological properties are compared (Rae 1979). The molecular structure of the communicating junctions in both systems, liver and lens, is apparently formed by only one major polypeptide. The major polypeptide associated with liver gap junctions has a molecular weight of 26,000 (Henderson et al. 1979). Lens fiber junctions also contain a predominant polypeptide of 26,000 (Broekhuyse et al. 1976) which has been called "main intrinsic polypeptide" (MIP) and was also referred to as MIP 26, MP 26 or 26 K (Broekhuyse et al. 1976; Bloemendal et al. 1977; Broekhuyse et al. 1979). Although many of similiarities have been described in morphology and structure/function relationship, there are surprising differences. Both these polypeptides are quite dissimilar in several respects: Both polypeptides give raise to different peptide maps (Hertzberg et al. 1982; Horwitz and Wong 1980) when analyzed with four distinct proteolytic enzymes. On the other hand the peptide maps of MIP from mammals, birds, reptiles and amphibians are similar (Takemoto et al. 1981). The NH_2 terminal amino acid sequence differs for MIP and the corresponding liver polypeptide (Nicholson et al. 1980). Recently differences in the molecular weights of the two polypeptides in question have been reported: liver gap junction polypeptide: 27,000 Daltons and MIP: 25,000 Daltons (Hertzberg et al. 1982; Zampighi et al. 1982). Another difference has been found by X-ray crystallography: lens fiber connexons appear to consist of tetrameric subunits rather than of hexamers, which had been demonstrated in liver gap junctions (Peracchia and Peracchia 1980; Zampighi et al. 1982). Finally, antibodies raised against MIP from bovine lens do not crossreact with the corresponding polypeptide from liver gap junctions (Hertzberg 1980; Zigler and Horwitz 1981).

The MIP of the lens occurs intra- and extrajunctionally in the membranes of the fiber cells as visualized by immunofluorescence studies (Bok et al. 1982).

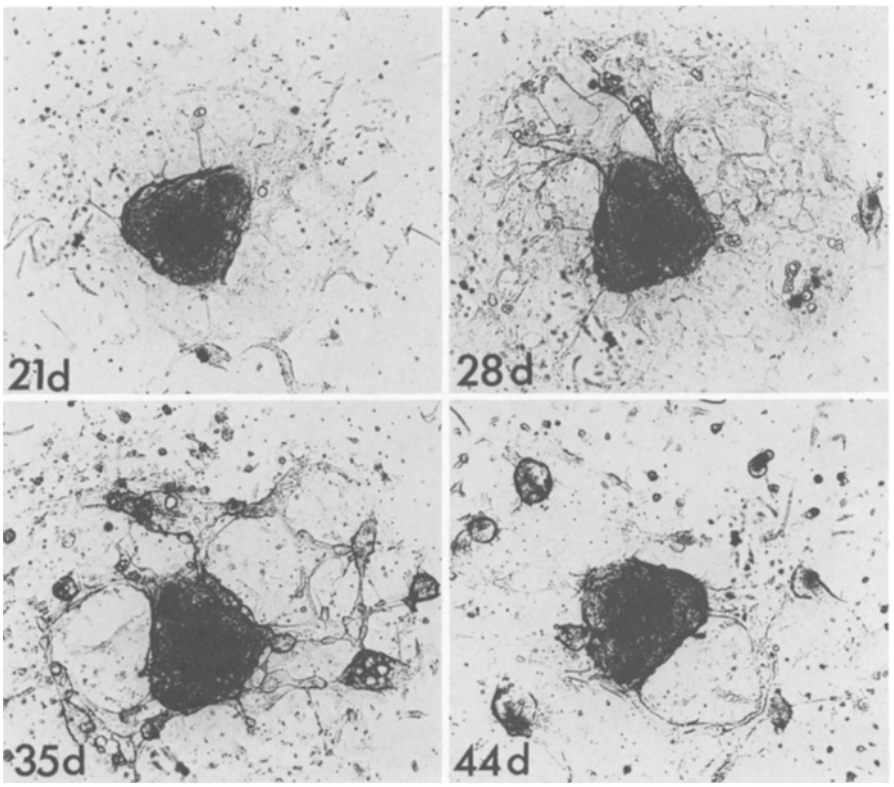
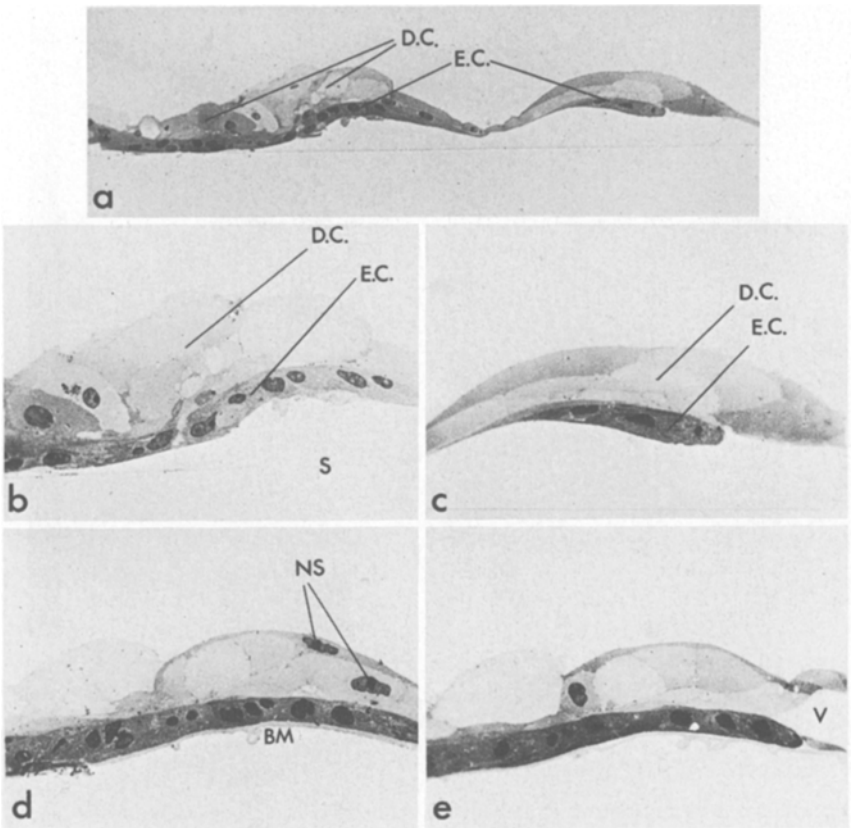


Fig. 1. Time dependent aggregation of cultured rat lens epithelial cells, differentiating in vitro (35×). Lens capsules from newborn rats, 4–6 days of age, with adherent epithelial cells have been cultured in DMEM supplemented with 20% of fetal calf serum

Epithelial cells however which also contain communicating junctions do not react with an antiserum directed against MIP (Broekhuyse et al. 1979). Thus, the lens junction protein appears to be a unique gene product which is formed during the elongation (differentiation) process and which differs from the 26,000 Dalton polypeptide from liver gap junctions. In other words MIP is a fiber-specific polypeptide which may be used like γ-crystallin as a marker molecule for the process of lens cell differentiation.

Working on the aging and differentiation of lens epithelial cells in tissue culture, we looked for experimental conditions which allow the differentiation of lens epithelial cells together with the expression of MIP (Vornhagen et al. 1982). Under normal growth conditions we obtained, like many other authors did, well growing but rapidly dedifferentiating cells, having lost the capacity to synthesize lens specific proteins. Under conditions, however, which do not allow rapid proliferation (arrested cultures, according to Dell'Orco 1975), cells regained the capacity to express crystallins after a lag period (Vornhagen and Rink 1981). In order to get a more complete pattern of biochemical differentiation we investigated cultured lens epithelial cells which were derived from very young



Figs. 2a–e. Sagittal semi-thin sections (0.5–1.0 μm) of rat lens epithelial cells, differentiation in vitro (**a** = 125×, **b–e** = 540×; *EC* = epithelial cells, *DC* = differentiated cells, *NC* = nucleolus, *V* = vacuoles, *BM* = basal membrane. Cells were cultured for 30 days (DMEM, 20% f.c.s.), fixed by glutaraldehyde (2.5%) and OsO_4 (1.5%) in 0.1 M sodiumcacodylate buffer, pH 7.2; dehydrated, contrasted with uranylacetate (1.5%) and phosphowolframate (1.5%) in 70% ethanol and finally embedded in an araldite: dodecencylsuccinate mixture (26 : 24) with 2,4,6 *Tris* (dimethylaminomethylphenole) 3%. Semi-thin sections were stained with azur II (0.5%), methylenblue (0.5%) and sodiumtetraborate (0.5%)

donor animals and which were inocculated densely in primary cultures. Under these conditions translucent cell aggregates, socalled lentoid bodies, appear after a period of 4–6 weeks. These aggregates contain terminally differentiated lens epithelial cells which may be compared with lens fiber cells in situ. Both systems are able to synthesize the marker molecules γ-crystallin as well as MIP.

The morphology of the developing cell aggregates involving biochemically differentiated cells is represented in Figs. 1a–d. The aggregates increase in size and are located above the dense monolayer of undifferentiated epithelial cells. Microscopy of semi-thin sections (Fig. 2) shows the fine structure of these aggregates and gives the following results:

a) aggregates are located on the top of the epithelial monolayer,

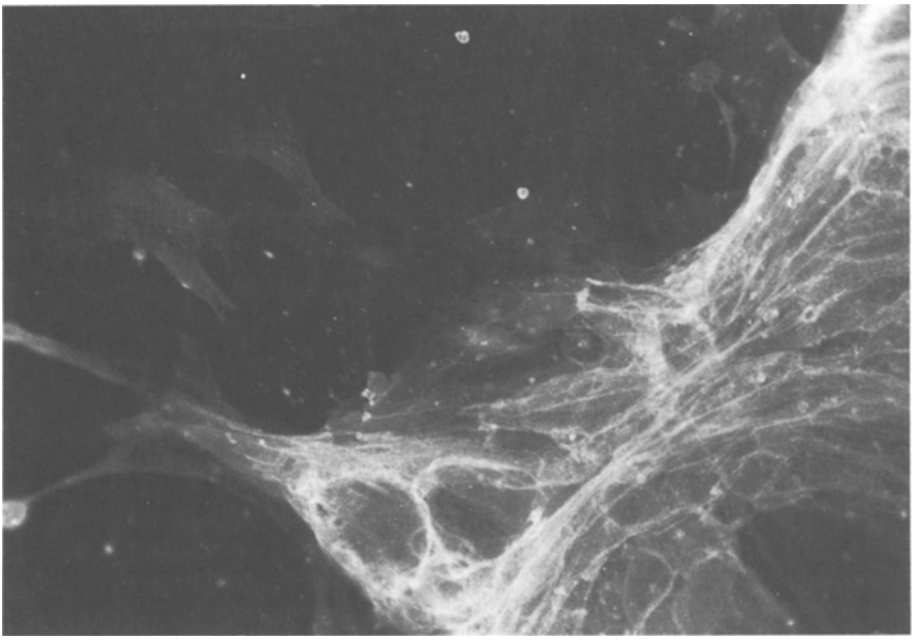


Fig. 3. Aggregates of rat lens epithelial cells (30 days in culture) stained with anti-bovine MIP antiserum (140×). Cells were fixed with ethanol (20 min) and incubated for 30 min with antiserum diluted 1 : 10 in a moist chamber. Afterwards cells were washed thoroughly and incubated for 30 min with 1 : 20 diluted FITC anti-rabbit IgG serum. Photographs were taken with an ortholux II microscopoe (Leitz) at 450–490 nm

b) the cytoplasm of aggregated cells exhibits no intracellular structures and is uniformly stained,
c) nuclei of aggregated cells show enlarged nucleoli,
d) cell volume increases,
e) vacuoles accumulate and enlarge.

Electron microscopic investigations corroborate these findings and additionally show numerous polysomes in the aggregated cells. Communicating (gap) junctions, however, could not be detected. An antiserum raised in rabbits against chloroform-methanol extracted MIP from bovine lenses (kindly provided by Dr. Broekhuyse, Nijmegen) showed crossreactivity with MIP from rat lenses. It was used to detect the MIP containing cells by indirect immunofluorescence. Figure 3 illustrates cell aggregates containing differentiated cells which react positively when stained with anti-bovine MIP antiserum. Whereas the immunofluorescence with antisera directed against crystallins is found in the cytoplasm (not shown here), the anti-MIP fluorescence is mainly found on membranes. When water-insoluble proteins of the aggregates were solubilized and analyzed by immunodiffusion against anti-bovine MIP antiserum a single precipitin line was formed, representing the MIP antibody complex (Fig. 4). No reaction occured, however, with an antiserum against gap junction plaques from mouse liver or with an antiserum against the SDS denatured 26,000

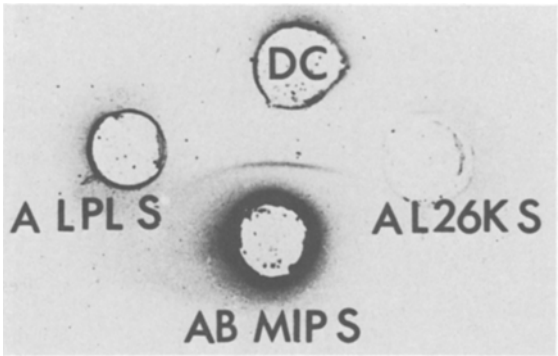


Fig. 4. Immunodiffusion test of water-insoluble proteins from in vitro differentiating lens epithelial cells (*DC*) against 3 different antisera. Agar-gel as well as sample buffer contained Triton X-100 (1%). *AB MIP S* = anti-bovine MIP antiserum; *A LPL S* = anti-mouse liver gap junction plaques antiserum; *A L26K S* = anti-mouse SDS denatured liver gap junctions protein (26K) antiserum

Dalton polypeptide from mouse liver gap junctions. Both these antisera were kindly provided by Dr. Willecke (Essen) and were characterized by Traub et al. (1982).

The most interesting question whether or not the MIP-synthesizing cells in our cell aggregates are coupled to each other by communicating junctions remains open. In preliminary experiments using the rather insensitive method of fluorescent dye injection, no transfer of Lucifer Yellow could be monitored [U. Frixen (Essen) and R. Vornhagen (Bonn), unpublished results]. Thus at present we know that lens epithelial cells in vitro have the capacity to synthesize MIP, but we do not know whether these cells are also able to arrange MIP into communicating junctions. To elucidate this question further measurements of electrical cell-cell coupling and cocultivations with heterologous communicationcompetent cells are in progress. The cell-culture system described above should be useful to study the synthesis of MIP as well as its arrangement and function in communicating junctions.

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