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LETTER TO THE EDITORS

Protein Synthesis in Bovine and Human Nuclear Fiber Cells

When lens epithelial cells elongate to fiber cells, they lose their nuclei and ribosomes (Kuwabara, 1975). Nevertheless, the protein synthesizing machinery remains active in the outer cortex of the lens (Bloemendal, Schoenmakers, Zweers, Matze and Benedetti, 1966; Vermorken, Hilderink, Dunia, Benedetti and Bloemendal, 1977). A low level of incorporation of labeled amino acid into protein may also occur in the inner core region comprising about 50% of the lens (Spector and Kinoshita, 1964). However, essentially no activity was detected in the protein of the core region of the lens (Dilley and Van Heyningen, 1976; Wannemacher and Spector, 1968).

That little or no protein synthesis takes place in the nucleus seemed to be a general concept (Kleef, De Jong and Hoenders, 1975; Harding and Dilley, 1976; Bloemendal, 1977; Maisel, Harding, Alcalá, Kuszak and Bradley, 1981) and on this basis the following hypotheses have been put forward: (*a*) proteins present in the nucleus of adult lens have been synthesized during fetal life; (*b*) any change of protein observed in the nuclear part of the lens or inner cortex reflects either postsynthetic alterations, or, less likely, different biosynthetic activity in the early stage of lens development.

In the terminally differentiated lens cortical fiber cells, most organelles have disappeared with the exception of the plasma membranes, polyribosomes (Bloemendal, Zweers, Vermorken, Dunia and Benedetti, 1972) and cytoskeleton (Benedetti, Dunia, Ramaekers and Kibbelaar, 1981). Moreover, for protein synthesis a complex assembly system and a variety of specific components should be present. Since the existence of polyribosomes and ribosomes isolated from the nuclear fiber cells (NFC) was found electron-microscopically by the authors, the activity of these ribosomes in protein biosynthesis had to be elucidated. On the other hand, upon incubation of decapsulated lenses with labeled amino acid, radioactivity was detected also in the nuclear part of the lens.

In this report a link between the ultrastructural observation and the biosynthetic activity was made by *in vitro* experiments using adult bovine and human lens nuclear material which represented less than 25% wet weight of these lenses.

Decapsulated lenses were incubated and a reticulocyte cell-free system supplemented with isolated lens polysomes was used. Incubation of adult bovine and human (1 month to 85 years of age) individual decapsulated lenses was performed for 24 hr at 37 °C in 5 ml of Minimum Essential Medium (Eagle) containing Earle's salts, 0·2% sodium bicarbonate, without glutamine and without methionine; glutamine (to a final concentration of 2 mM) and 20 μCi ml^{-1} or 50 μCi ml^{-1} of [^{35}S]-methionine (specific activity 1016 Ci mM^{-1}, Radiochemical Centre) per lens were added. After the incubation, lens nuclei were punched by a plastic tube with a diameter less than half that of the lens. By removal of both ends of the punch composed of the anterior and the posterior parts of the lens, the nucleus was obtained, and the remaining lens was taken as the cortex (bovine $n = 5$; 13% ± 1·3; human, $n = 7$; 21% ± 1·7 of decapsulated lens, respectively). Forceps for removal of the cortical parts were rinsed each time in acetone and milli-Q water to avoid contamination. The wet weight of cortical and nuclear parts of the lenses was measured. Fractionation of lenses was performed in

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TABLE I

Incorporation of (^{35}S)-methionine into proteins of human lens fiber cells during 24 hr in tissue culture

Fraction	Sample	Age (years)	cpm per mg protein		
			Cortex (C)	Nucleus (N)	C/N
Water-soluble	1	1 month	1417	167	8·5
	2	20	1295	245	5·3
	3	62	213	82	2·6
	4	85	124	89	1·4
Water-insoluble	1	8	1824	869	2·1
	2	38	2474	1134	2·2
	3	59	1309	574	2·3
	4	67	2156	552	3·9

Twenty μCi ml^{-1} of (^{35}S)-methionine was added to the culture medium.

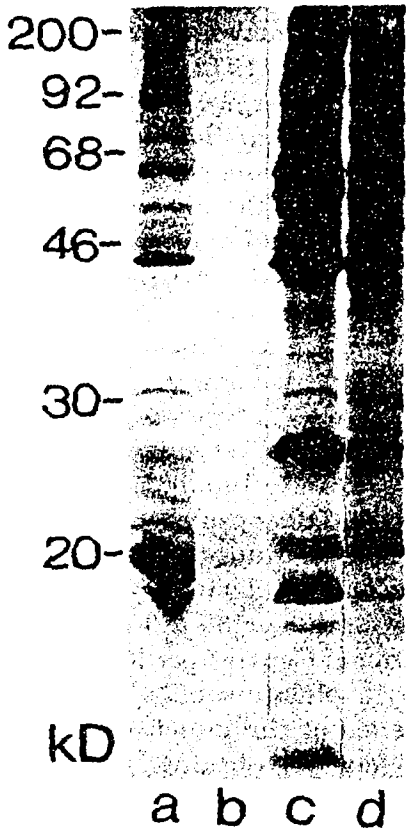

FIG. 1. Autoradiograph of 1-D SDS-polyacrylamide gel electrophoretic patterns of proteins from fractionated human lens (7 years) after 24 hr culture. Incorporation of [^{35}S]-methionine into lens fiber cells is shown. Water-soluble fractions (added 160 μg of sample): a = cortex; b = nucleus. Water-insoluble fractions (added 400 μg of sample): c = cortex; d = nucleus (4-week exposure, 50 μCi ml^{-1} of labeled amino acid was added to tissue culture medium).

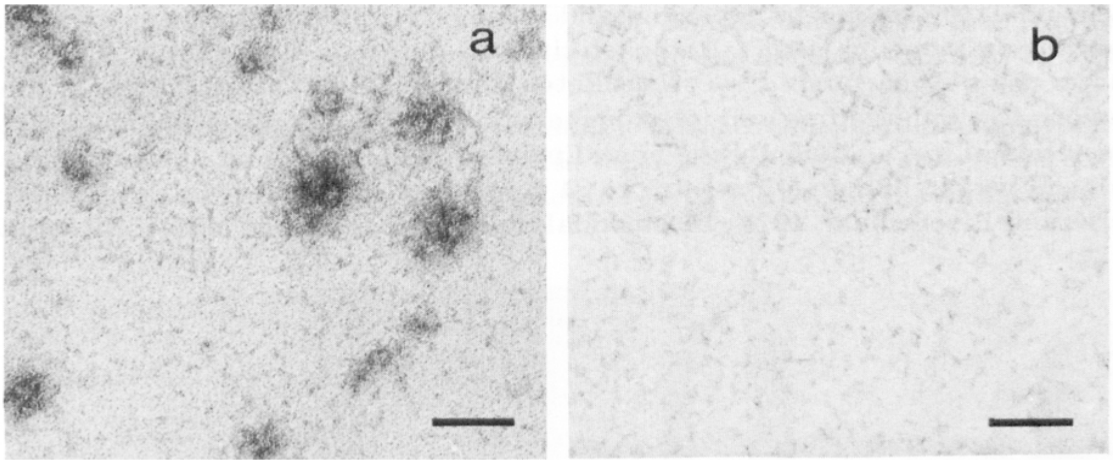

FIG. 2. (a). *Electron micrograph of isolated free polysomes from human lens nuclear fiber cells.* (b) Electron micrograph of the same preparation after treatment with RNase. The remaining structures are due to background staining. (*Negative staining; bar indicates 50 nm*)

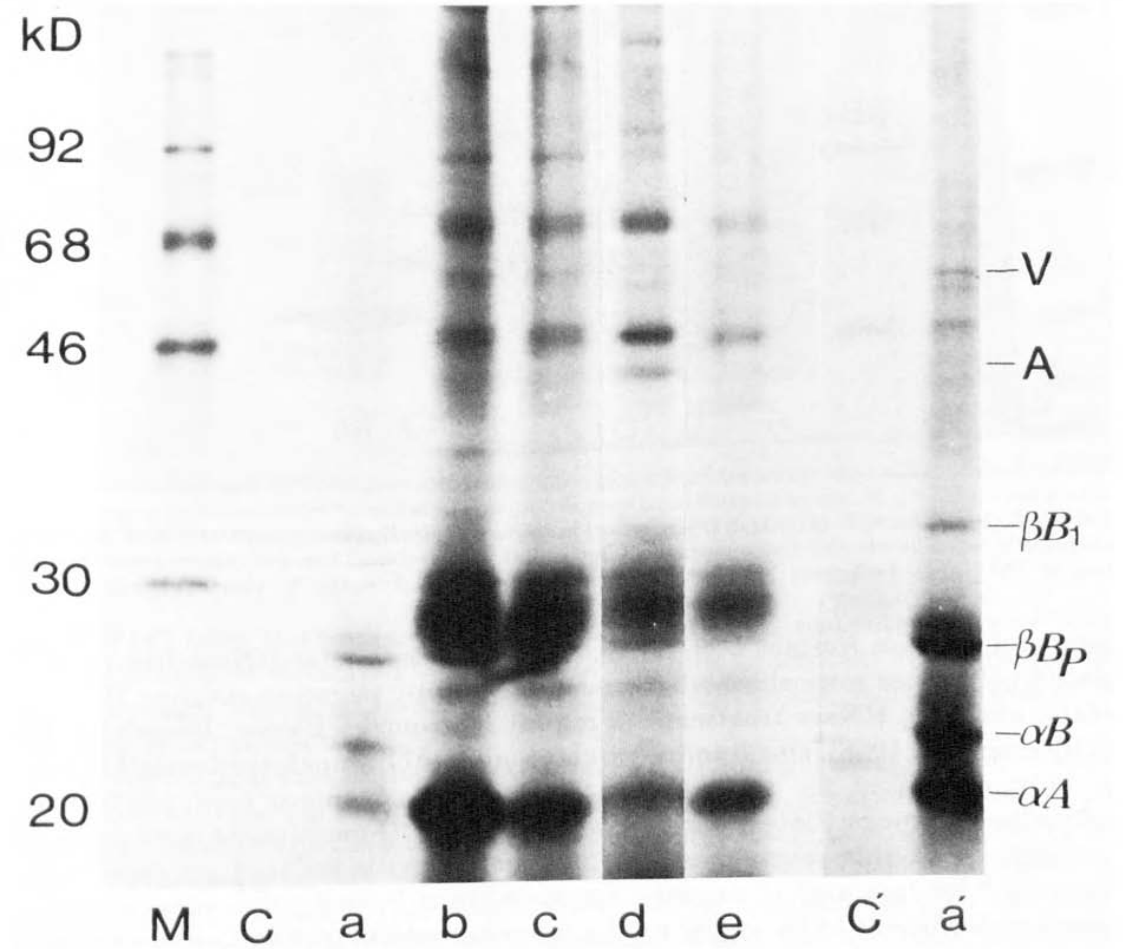

FIG. 3. Autoradiograph of SDS gel-electrophoretic patterns of newly synthesized polypetides in a reticulocyte cell-free system supplemented with isolated polysomes from bovine lenses. M: ^{14}C-labeled markers; C, C', controls (C': longer exposure); a, a', calf, for comparison (a': longer exposure); b, c, adult bovine-free polysomes: b, cortex; c, nucleus; d, e, adult bovine membrane-bound polysomes: d, cortex; e, nucleus.

TMK buffer: 50 mM Tris-HCl, pH 7·6, 10 mM $MgCl_2$, 25 mM KCl (Kibbelaar and Bloemendal, 1977; Ringens, Hoenders and Bloemendal, 1982). Protein assay was done according to Peterson (1977). Radioactivity was determined in a liquid scintillation counter using Aqua Luma Plus® scintillator (Lumac 3M bv) after dialysis of the lens fractions to eliminate free amino acids including [^{35}S]-methionine and electrolytes.

Isolation of free and membrane-bound polysomes of cortex and nucleus from adult bovine (4 years), human (15 months to 81 years) and calf (as control) was performed (Berns and Bloemendal, 1974; Bloemendal, Benedetti and Bont, 1974). All solutions,

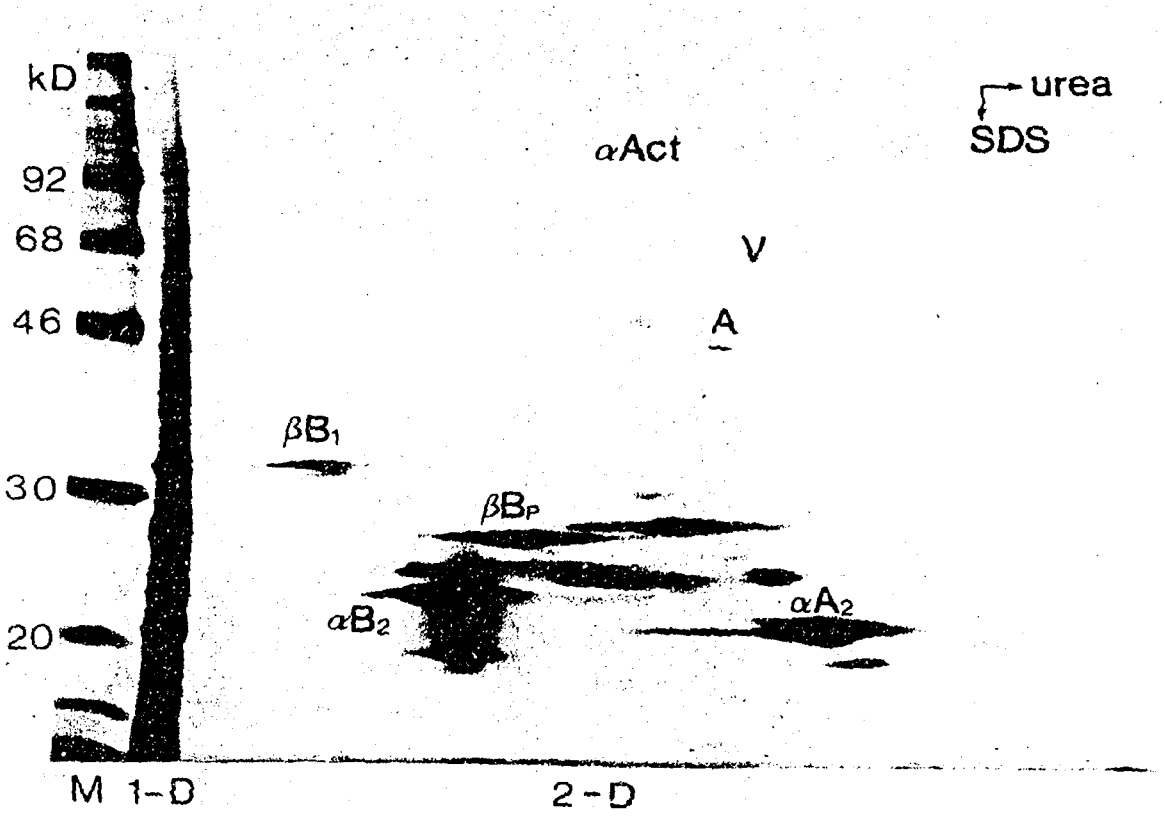

FIG. 4. Autoradiograph of 2-D SDS-polyacrylamide gel electrophoretic patterns of translational products in a reticulocyte cell-free system supplemented with isolated free polysomes from the calf lens cortex. M: ^{14}C-labeled markers; αA_2, αB_2, βB_p, βB_1: crystallins; A: actin; V: vimentin; αAct: α-actinin.

plastic and glass, in contact with polysomes were used under RNase-free conditions. Isolated polysomes were checked ultrastructurally by negative staining (1% uranyl acetate) and with RNase treatment (2 mg ml^{-1}, Promega Biotec; Benedetti, Zweers and Bloemendal, 1968). One hundred and ten μl of rabbit nuclease-treated lysate that had been optimized for mRNA translation, 4 μl of amino acid mixture minus methionine (Promega Biotec) and 16·5 μCi ml^{-1} of [^{35}S]-methionine were used. Ten μl of an aliquot of this mixture was supplemented with isolated polysomes (1–2μl, 1·5 mg ml^{-1} for free and 15 mg ml^{-1} for membrane-bound polysomes, respectively, extinction coefficient of 25 at 260 nm by spectrophotometry) and incubated at 30 °C for 90 min. Two μl of RNase (2 mg ml^{-1}) was added and incubated at 37 °C for 20 min to stop the translation.

Analysis of newly synthesized proteins was performed by one- and two-dimensional

(2-D) gel electrophoresis (Laemmli, 1970): 13% of polyacrylamide with 0·1% SDS for one- and second-dimensional gels and 8% polyacrylamide in 8 M urea for one-dimensional slab gel. Autoradiography after drying was done as described by Berns and Bloemendal (1974).

The results of incorporation of [^{35}S]-methionine into proteins of human lens nuclear fiber cells are shown in Table I and Fig. 1. The mean incorporation ratios of the cortex to the nucleus are 4·6 in the water-soluble fractions (WSF) and 2·2 in the water-insoluble

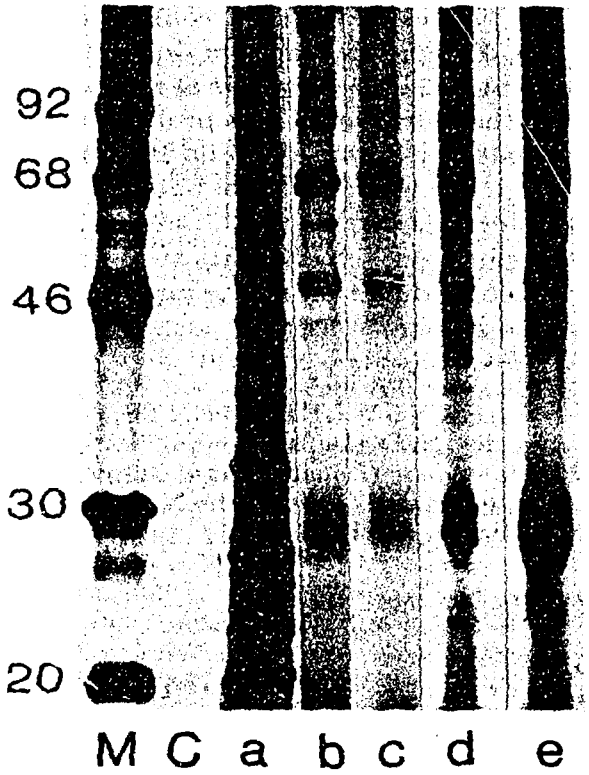

FIG. 5. Autoradiograph of SDS gel-electrophoretic patterns of translation products in a reticulocyte cell-free system supplemented with isolated free polysomes from human lenses. M: ^{14}C - labeled markers; C: control; a, calf (for comparison); b–c, 15 months; b, cortex, c, nucleus; d–e, 58 years: d, cortex: e, nucleus.

fractions (WIF) from the human lens, whereas 8·2 and 2·2, respectively, were found for bovine lenses (not shown). The activity in the human cortical WSF from young lenses is very high compared to aged cortical WSF and to the young as well as the aged nuclear WSF, respectively.

As far as the cortical part of the lens is concerned, in younger cultured lens (7 years) there is protein synthesis in the WSF but only negligible incorporation of radioactivity in the nuclear part. In the nuclear WIF a remarkable protein synthesis activity was observed albeit quantitatively less than in the cortical WIF (Fig. 1). This result can also be deduced from Table I. Our results are not easily comparable with the studies of Dilley and van Heyningen (1976) and Wannemacher and Spector (1968), since those workers only considered total protein without discriminating between WSF and WIF. However, all the results might be similar if one remembers that the calf WIF comprises about 3% of the total proteins (Bours, Doepfmer and Hockwin, 1976). Dilley and van

Heyningen (1976) estimated the weight of human lenses without providing the exact protein determination.

The nature of polysomes isolated from the nuclear part of human lens was verified by electron microscopy before and after RNase treatment (Fig. 2). In Fig. 3, in the α-, β-crystallin, actin and vimentin regions, newly synthesized polypeptide bands are also recognizable after translation of polysomes derived from the nucleus. Relatively much actin is produced by cortical membrane-bound polysomes. These results are confirmed by 2-D gel electrophoresis of newly synthesized proteins from an incubation of the lysate supplemented with isolated calf lens polysomes. However, even with the 2-D procedure no γ-crystallin synthesized de novo could be detected (Fig. 4). Previous results of Berbers, Boerman, Bloemendal and De Jong (1982) also revealed only a negligible amount of newly synthesized γ-crystallin as compared to α- and β-crystallin. We have stressed elsewhere that for human lens proteins the 2-D method (for some unknown reason) is less satisfactory (Ozaki, Jap and Bloemendal, 1985). As far as the translation of polysomes from human lens nuclei and cortices is concerned, the 1-D pattern clearly shows the synthesis of β-crystallin and other proteins such as actin and vimentin (Fig. 5). Therefore, our experiments suggest that polysomes from the inner part of the lens are able to direct de novo synthesis of proteins both in vitro and in a reticulocyte cell-free system.

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