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αB-Crystallin is expressed in kidney epithelial cell lines and not in fibroblasts

Chandrasekharam N. Nagineni and Suraj P. Bhat

Jules Stein Eye Institute, University of California, Los Angeles, School of Medicine, Los Angeles, CA 90024-1771, USA

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We have recently shown the presence of αB-crystallin in non-ocular tissues of diverse embryological origins such as the heart, brain, spinal cord, kidney, retina, etc. Using an αB-crystallin-specific antiserum and immunofluorescence, immunoblotting, immunoprecipitation and peptide mapping with *Staphylococcus aureus* protease, we demonstrate differential expression of αB-crystallin in epithelial and fibroblast cell lines. αB-Crystallin was detectable only in epithelial cell lines such as MDBK, MDCK, LLCPK$_1$ and JTC-12, and was not observed in two kidney fibroblast cell lines, one skin fibroblast cell line, and one corneal fibroblast cell line. Differential expression of the αB-crystallin gene was also confirmed by Northern blot analysis of the RNAs isolated from these cell lines. These data suggest a cell-type-specific role for αB.

Epithelial cell; Fibroblast; Crystallin, αB-

1. INTRODUCTION

Mammalian lens crystallins (α,β and γ) act as structural proteins which bring about the transparency of the lens (reviews [1,2]). The lens contains an epithelium, present as a single layer of cells on the anterior surface [3]. Through division and differentiation, this single layer of cells gives rise to fiber cells which constitute the main mass of the lens. Among the mammalian crystallins, α-crystallins are major structural proteins of the ocular lens. Only α-crystallins are present in significant quantities in the epithelium whereas other crystallins are produced upon differentiation of the lens epithelial cells into fibers [4,5]. α-Crystallin is known to be composed of two subunits, αA and αB. The relative distribution of these subunits is very lopsided in favor of one or the other subunit with respect to its location within the lens. αB is the predominant form of α-crystallin in the epithelial cell layer, while αA predominates in the fiber cells [1,2].

We have recently reported the presence of αB-crystallin (αB) in several non-lenticular and non-ocular tissues, such as heart, brain, spinal cord, lungs, skin and kidney [6], thereby suggesting a possible extra-lenticular role for this protein. Expression of αB in the rat involves a developmental component in that the protein increases in content in most of the adult tissues except in the heart where it appears very early in fetal life [6]. Expression of the αB gene has also been described in murine tissues by Northern blot analysis [7]. Since morphologically and functionally distinct cell types are present in different tissues, it is important to determine whether αB is expressed in all or only some cell types. Here, we thus selected epithelial cells and fibroblasts, the two major cell types established in culture. We also extended this study to an ocular and a skin fibroblast cell line established in our laboratory. We present data demonstrating that αB, in the cell lines examined, is expressed in epithelial cells and not in the fibroblasts, suggesting a particular role for this protein.

Correspondence address: C.N. Nagineni, Jules Stein Eye Institute, Rm B118, UCLA School of Medicine, Los Angeles, CA 90024-1771, USA

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2. MATERIALS AND METHODS

2.1. *Cell cultures*

The cell lines investigated here and their passage numbers are given in table 1. Cell lines were obtained from Dr A. Jabbar (MDBK,MDCK, CV_1), Dr E. Nord ($LLCPK_1$, JTC-12) and Dr G. Krishna (NRK_1). HFLEC, HFCF and HFSF were cultured from primary explants of the respective tissues of human fetuses of age 18–24 weeks. All cultures were grown in minimum essential medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin, at 37°C in an atmosphere of 95% air and 5% CO_2. Only confluent cultures were used.

2.2. *Immunofluorescence*

Cells were grown on glass coverslips. They were fixed in an acetone:methanol (1:1) mixture at -20°C for 5 min, air-dried and incubated at 37°C for 30 min in a humidified atmosphere in the presence of αB antiserum (10-fold diluted with 1% BSA). This rabbit antiserum was raised against the C-terminal decapeptide of αB. It reacts specifically with αB and not αA [6,8]. Coverslips were rinsed in PBS and further incubated with 100-fold diluted FITC-conjugated goat-anti-rabbit IgG (Miles Labs). After rinsing in PBS, coverslips were mounted and photographs of fluorescent cells taken on a Zeiss (Max ERB) photomicroscope, fitted with an epi-illuminator.

2.3. *Preparation of cell extracts and immunoblotting*

Cultures were scraped into extraction buffer (50 mM Tris, pH 7.4, 25mM KCl, 5mM $MgCl_2$ 1mM EDTA, 0.2 mM PMSF), lysed by freeze-thawing on solid CO_2, then homogenized. The lysate was centrifuged at 17 000 $\times g$ for 20 min and the supernate collected (the soluble fraction). Protein was estimated with BCA reagent (Pierce). Immunoblotting with this fraction was performed essentially as in [8].

2.4. *Immunoprecipitation*

Cultures were labeled with [^{35}S]methionine (Trans Label, 1CN, CA) for 3 h and a 17 000 $\times g$ supernate prepared as described [8]. To the supernatant fraction 2× volume of the lysis buffer (10 mM Tris, pH 7.4, 100 mM NaCl, 2mM EDTA, 1% NP-40, 0.5% deoxycholate, 1% aprotinin), and 3 μl of either αB antiserum or preimmune serum were added and incubated for 2 h at 4°C with constant shaking. 25 μl protein A-agarose beads (Repligen, MA), washed and suspended in lysis buffer, were added to each sample and incubation continued for a further 2 h at 4°C . Samples were washed with 0.5 M NaCl containing 5 mg/ml BSA, 0.5 M NaCl, and finally with lysis buffer, suspended in sample loading buffer containing SDS and β-mercaptoethanol, boiled for 3 min and analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (15%) according to Laemmli [9]. After staining and enhancer treatment, (RPI, IL), the gels were dried and autoradiographed.

2.5. *Peptide mapping*

The 17 000 $\times g$ supernatant fractions of HF lens (8 μg), HFLEC (8 μg), and MDBK (150 μg) or MDCK (150 μg) were digested with *Staphylococcus aureus* protease (Millipore, NJ) for 1–2 min at 37°C as in [6]. Briefly, the reaction mixture in a final volume of 8 μl contained 20 mM Tris-HCl (pH 8.0), 0.1% SDS, 0.4 μg protease and sample proteins. The reaction was terminated by adding protein sample buffer and boiling for 3 min. Peptides were detected by immunoblotting [8].

2.6. *Northern blot analysis of RNA*

RNA was isolated as described [10]. Northern (RNA) blot analysis was performed according to Maniatis et al. [11]. After 4 h prehybridization (5× SSPE, 5× Denhardt, 0.1% SDS, 50% formamide and 200 μg/ml denatured salmon sperm DNA), membranes were incubated with a random-primed [12], ^{32}P-labelled αB probe in prehybridization buffer at 42°C for 18 h. Blots were washed sequentially in 3 × SSC, 0.5% SDS (2×15 min at 25°C), 1× SSC, 0.5% SDS (2× 30 min at 25°C), 1× SSC, 1% SDS (2×60 min at 65°C) and 1× SSC (2×10 min at 65°C) and exposed to X-ray film at -70°C with intensifying screens.

3. RESULTS

Table 1 lists the cell lines used and their passage numbers at the time of analysis. The fluorescence micrographs shown in fig.1 indicate predominant cytoplasmic staining for αB in cultured epithelial cells. No specific reaction was seen in CV_1 (fig.1f) and other fibroblast cultures (not shown). Staining intensity was uniform in MDBK and MDCK cells (fig.1b,c), similar to that seen in HFLEC (fig.1a). Exclusive localization of αB to a specific area in the cells was not observed. In $LLCPK_1$ and JTC-12 cultures, differential fluorescence is noticeable among the cells examined (fig.1d,e). This could be related to the passage number of the cell lines and/or be due to altered levels of αB in the cells during the cell cycle or the differentiation state. In this regard, it is also important to note that MDBK, MDCK, $LLCPK_1$, JTC-12 cultures originated from distal and proximal tubules of the kidney, respectively. The variable distribution of αB may thus reflect the known diversified in vivo functions of these nephron segments [13]. We should point out that we have found no differences in the syn-

Table 1

Cell lines used

Cell line	Origin of culture	Passage no.
HFLEC	human fetal lens epithelial cells	3
MDBK	bovine kidney epithelial cells	134
MDCK	dog kidney epithelial cells	72
$LLCPK_1$	pig kidney epithelial cells	13
JTC-12	monkey kidney epithelial cells	13
CV_1	monkey kidney fibroblasts	42
NRK	rat kidney fibroblasts	100
HFCF	human fetal corneal fibroblasts	4
HFSF	human fetal skin fibroblasts	4

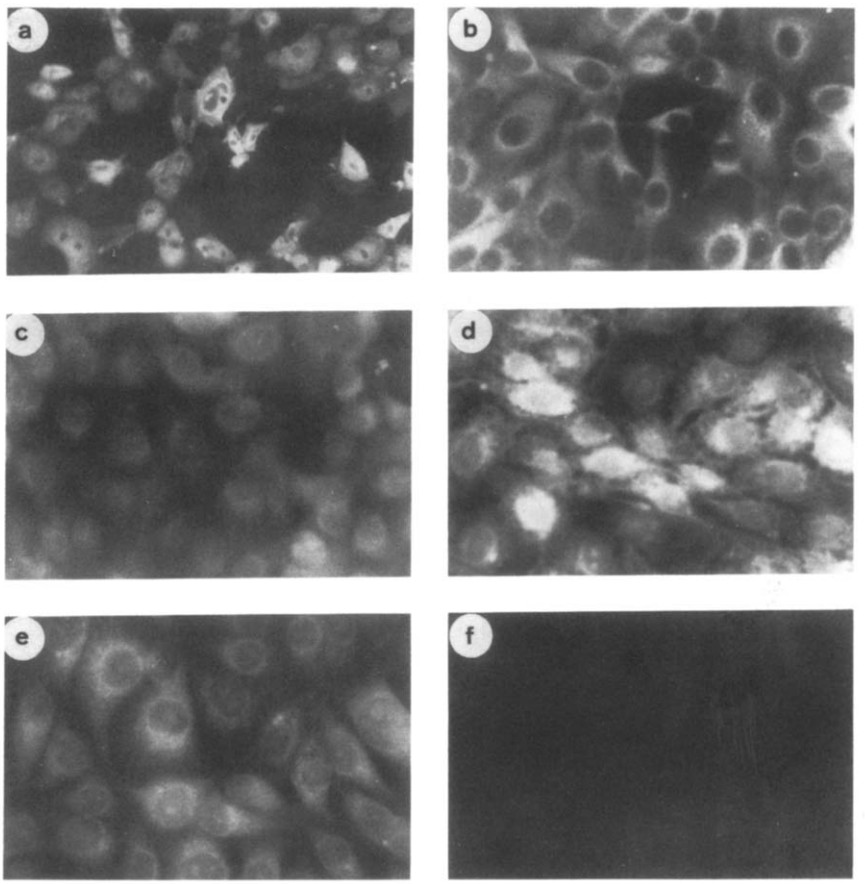

Fig.1. Detection of αB in cultured epithelial cells by indirect immunofluorescence. (a) HFLEC, (b) MDBK, (c) MDCK, (d) $LLCPK_1$ (e) JTC-12, (f) CV_1. No reaction was observed with preimmune serum. See table 1 for details of cell lines.

thesis or content of αB in MDBK cells as a function of age and confluency (unpublished).

Immunoblot analyses of the supernatant proteins indicate the presence of an intense band reacting with αB antiserum in all epithelial cells (fig.2). This band exhibits electrophoretic mobility similar to that of αB in human fetal lens and HFLEC. However, a band of much lower intensity with a molecular mass of about 40 kDa was also observed. The intensity of this band in JTC cells is almost equivalent to that of the αB band. This higher molecular mass band may represent either an undissociable dimer of αB or a related peptide. This remains to be investigated. No immunoreactive band was detected in fibroblast protein extracts.

In order to increase the detectability of αB, cells were labeled with [^{35}S]methionine and the soluble protein extracts were analysed by immuno-

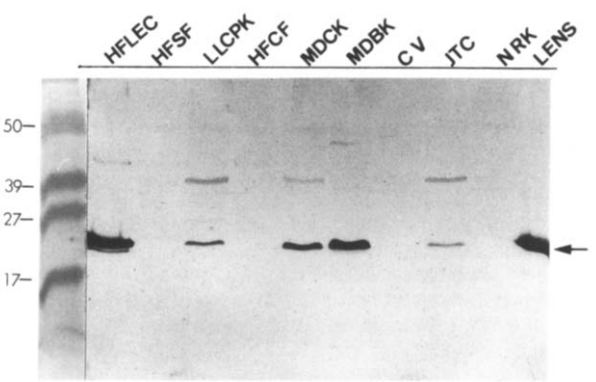

Fig.2. Immunoblot analysis of protein extracts of different cultured epithelial cells and fibroblasts. The amount of protein applied to each lane was 25 μg ($LLCPK_1$, MDCK, MDBK, JTC-12), 50 μg (HFSF, HFCF, CV_1, NRK) and 5 μg (HFLEC, HF lens).

precipitation using αB antibody. The results are shown in the autoradiographs in fig.3. A band with an apparent molecular mass of 22 kDa similar to that of αB as detected in the lens epithelial cells was observed in kidney epithelial cells (MDBK, MDCK) but not in fibroblasts (HFCF) (fig.3). Although there is a high level of background in the panels depicting MDBK and MDCK, this protein band was clearly precipitable only with αB antiserum, and was not observed when preimmune serum was used (see fig.3, lanes C). It should be noted that greater amounts of radioactivity needed to be analysed for non-lens cells. While this must raise the relative concentration of αB, it also concomitantly increases the non-specific label. Under similar experimental conditions, however, no protein band comparable to αB was seen in fibroblasts (fig.3, HFCF).

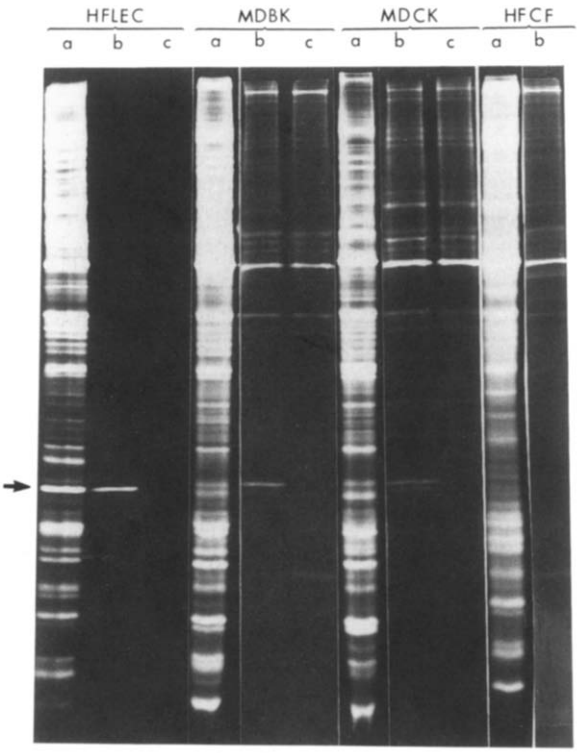

Fig.3. Immunoprecipitation of αB from [^{35}S]methionine-labeled cell extracts. About 1×10^7 cpm of MDBK, MDCK, HFC and 5×10^5 cpm of HFLEC were used for immunoprecipitation. 1.5×10^6 and 1.5×10^5 cpm of MDBK, MDCK, HFCF, and HFLEC, respectively, were applied per lane for unprecipitated samples. Unprecipitated sample (lane a), immunoprecipitated with anti αB (lane b), and preimmune serum control (lane c). The position of the immunoprecipitated αB band is indicated by the arrow. All samples were run on the same gel. Different lanes were exposed for variable time periods.

The presence of αB and its identity were further confirmed by peptide mapping with *S. aureus* protease [14]. After limited proteolysis, three prominent bands which exhibit an immune reaction with αB antiserum were detected in HF lens, HFLEC, MDBK and MDCK (fig.4). Since the antiserum was raised against the C-terminal decapeptide of αB, only those cleaved peptides carrying the C-terminal within them would react. The approximate molecular masses of the peptides were found to be 17 and 12 kDa. The similarity of the peptide maps for these samples strongly indicates that the amino acid sequence of immunoreactive protein in MDBK and MDCK cells is identical to or highly homologous to that in HF lens and HFLEC.

We have previously isolated αB cDNA clones from rat heart [6]. These clones have been characterized by sequence analysis and found to be homologous to αB [15]. We used these cDNA clones to assess expression of the αB gene in different cell lines. Northern blot analysis of RNA (fig.5) indicated a positive hybridization signal in HFLEC and MDBK cultures. The size of the mRNA in HFLEC and MDBK (fig.5c,e), which is about 0.8 kb, is the same as that in bovine and human lens RNA (fig.5a,b). No hybridization signals were detected in fibroblast cell lines, HFSF, HFCF and CV1 (fig.5d,f,g). These results indicate the presence of αB mRNA in MDBK cells and pro-

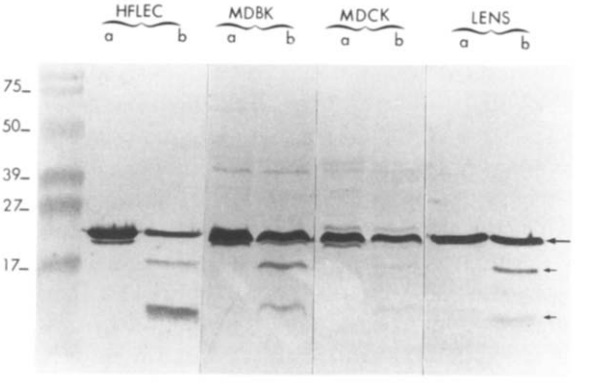

Fig.4. Peptide mapping by limited proteolytic digestion with *S. aureus* protease. Control and protease-treated samples were applied to lane a and b, respectively, Bands 1-3 (arrows) have apparent molecular masses of 22, 17 and 12 kDa, respectively, peptides were detected by immunoblotting (see text).

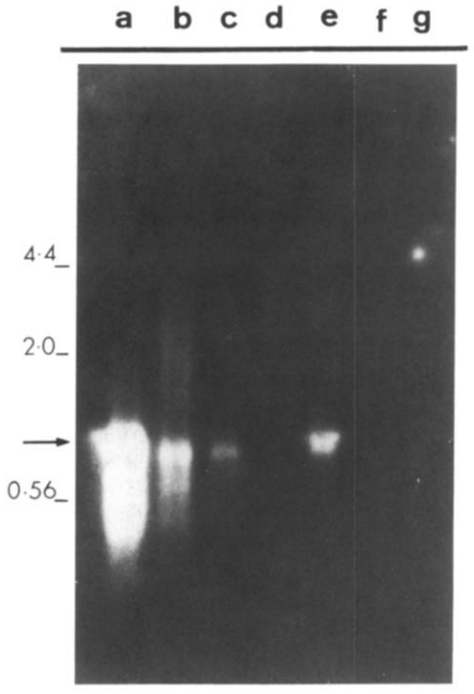

Fig.5. Detection of αB RNA in different cell cultures. αB cDNA clone from rat heart [6] was used as the probe in Northern blot analysis. Lanes: (a) fetal bovine lens (2 μg), (b) fetal human lens (10 μg), (c) HFLEC (1 μg), (d) HFSF (35 μg), (e) MDBK (35 μg), (f) HFCF (35 μg), (g) CV_1 (35 μg). Poly(A)$^+$ RNA (lane a) and total RNA (lanes b–g) were used. λDNA/*Hin*dIII standards (Bethesda Research Labs) (kb) are indicated at the side. Arrow indicates the position of αB mRNA.

vide further support for the data obtained from the protein studies.

4. DISCUSSION

The present data clearly indicate that, within the limits of detection ot the techniques employed, for the cell lines examined, the expression of ocular lens αB was detected only in epithelial cell lines and not in fibroblast cell lines. This conclusion is based (at the protein level) on immunological characterization by different techniques and (at the RNA level) by Northern blot analysis with a well characterized αB cDNA [6]. It is important to underscore the fact that the αB antiserum used in these studies is a highly specific antiserum, raised against the C-terminal decapeptide of αB. The C-terminal decapeptide of αB is highly conserved between such diversified species as hamster, cow and man [1,15,16]. Neither αA nor other crystallins react with this antiserum [6,8]. The Northern blot analysis further confirms the presence of αB in epithelial cells and its absence in fibroblasts, as indicated by the lack of hybridization in fibroblasts under conditions of low stringency (blots washed in 1 × SSC at 65°C).

The failure to detect αB in four different fibroblast cell lines indicates that fibroblasts in culture do not express the αB gene and therefore do not need this protein. Alternatively, it is possible that they do contain this protein but in extremely low amounts. While these data point to a specific role for αB, it is obvious that fibroblasts can be exploited for understanding possible functions of this protein by using these cells as potential vehicles for the introduction and expression of αB gene sequences. Also, some of the epithelial cell lines studied here can be used in lieu of lens epithelial cells for investigating this protein.

The distribution of αB within a tissue, with respect to specific cell-type(s), remains to be investigated. Its function in the heart, for example, is of particular interest since this organ mainly contains myocardial(muscle) cells. We have also found αB in retinal pigment epithelium and neurosensory retina (unpublished). It would be of interest to ascertain whether αB is present in epithelial cells of other tissues such as intestine, lung and skin and if it takes part in functions such as cellular development, differentiation and polarization [17]. It is interesting to note that fibroblasts have been shown to be more prone to oxidative attack by H_2O_2 as opposed to epithelial cells [18]. It is worthy of note that αB is a member of the family of heat-shock proteins [19,20], and therefore, its presence in epithelial cells may be one of the factors responsible for the greater resistance to oxidative stress. However, the data presented here do not establish a direct link between susceptibility to H_2O_2 and the lack of αB in fibroblasts.

In recent studies [21–23], considerable interest has been focused on taxon-specific crystallins which, in some cases, have been shown to be related to metabolic enzymes. In each taxon, because of the high concentration of the protein (enzyme in question) within the lens, it has been suggested [21] that these enzymes have been recruited for structural functions. The evolutionary advantages of such a taxon-specific phenomenon

are unclear. In contrast to these taxon-specific crystallins, α-crystallin is found in the lenses of all species. The possibility remains that αB is a housekeeping enzyme, but seems unlikely in the light of our present findings.

αB is a member of the family of heat-shock proteins [19,20]. While most of the stress proteins are synthesized in response to environmental stress signals, some of the proteins are expressed transiently during different developmental programs [19,20]. It remains to be determined whether αB plays a specific developmental role. In this regard it is important to point out that the synthesis of αB is known to be altered under different experimental conditions including dedifferentiation of lens epithelial cells in vitro [24,25]. Based on the present findings and the diversity of tissues in which αB is present, it is conceivable that this polypeptide has important lenticular as well as extralenticular function(s).

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