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BASP1 in the Lens

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ABSTRACT

BASP1 was detected in the embryonic and adult chicken lens, using immunological methods and by peptide sequence analysis. The protein was predominantly expressed in fiber cells and only faintly detected in annular pad cells. Localization of the protein was along the plasma membrane of fiber cells often in discrete areas. The role of BASP1 in the lens requires further study. J. Cell. Biochem. 105: 699–702, 2008.

KEY WORDS: LENS; CHICKEN; BASP1; FIBER CELLS

Brain acid soluble protein (BASP1) is a cytoskeleton-associated calmodulin-binding protein that is abundant in the brain, in nerve endings and also present in nerve–muscle junctions [Widmer and Caroni, 1990; Mosevitsky, 2005]. *N*-myristolation of the protein is thought to be important for binding to a lipid membrane [Mosevitsky et al., 1997]. This protein, also known as CAP-23 [Widmer and Caroni, 1990], and NAP-22 [Maekawa et al., 1994], when bound to the plasma membrane attracts cholesterol molecules thus depleting adjoining membrane areas [Maekawa et al., 2003].

BASP1 is functionally related to growth associated protein-43 (GAP-43) and plays a critical role to regulate nerve sprouting and the actin cytoskeleton [Frey et al., 2000]. BASP1 together with GAP 43 promote F-actin accumulation.

The presence of GAP 43 in the lens has been reported by Stupp et al. [2007]. In this study, we report the presence of BASP1 in the chicken lens.

MATERIALS AND METHODS

LENSES

Lenses from freshly killed 3- to 4-month-old White Leghorn chickens were decapsulated and annular pad cells collected. The fiber mass was dissected into annular pad cortex and nucleus. Each fraction was homogenized and the water-insoluble fraction (WIF), and water-soluble fraction (WSF) collected after centrifugation at 32,000*g* for 20 min [Katar et al., 1993]. The buffer used in the isolation process consisted of 50 mM Tris–HCl ph 7.4, 0.9% NaCl, 1 mM EDTA, 10 mM mercaptoethanol and 20 mM phenylmethyl-sulforyl fluoride. The WIF was dissolved in 1% SDS-buffer [Katar et al., 1993].

SDS–POLYACRYLAMIDE GEL ELECTROPHORESIS

One-dimensional electrophoresis (PAGE) was performed according to Laemli [1970] with 7.5%, 10%, and 15% (w/v) gels used for final resolution. Two-dimensional PAGE was done according to O'Farrel [1975].

IMMUNOBLOTTING

Protein was transferred from gels onto nitrocellulose paper according to Towbin et al. [1979]. Immunoblots were developed according to Ireland and Maisel [1984] with the secondary antibody conjugated to alkaline phosphatase. Rabbit antibody to chick BASP1 was a gift of Dr. Irina Korshunova (University of Copenhagen, Denmark) prepared according to Mosevitsky et al. [1997].

IMMUNOFLUORESCENCE ANALYSIS

Immunofluorescent studies on sections of 12-day chick embryo lenses, and 3-month-old human fetal lenses were done according to Waggoner and Maisel [1978].

PEPTIDE ANALYSIS

Peptide analysis was done on gel fragments cut from 2-D electrophoresis of lens samples. Analysis were at by the Proteomics Facility, Michigan State University (Lansing, MI). Gel bands were subjected to in-gel tryptic digestion according to Jensen et al. (Methods in Molecular Biology, Vol. 112, 1999) based on Shevchenko (Anal Chem 1996 Mar 1; 68(5):850–858). The extracted

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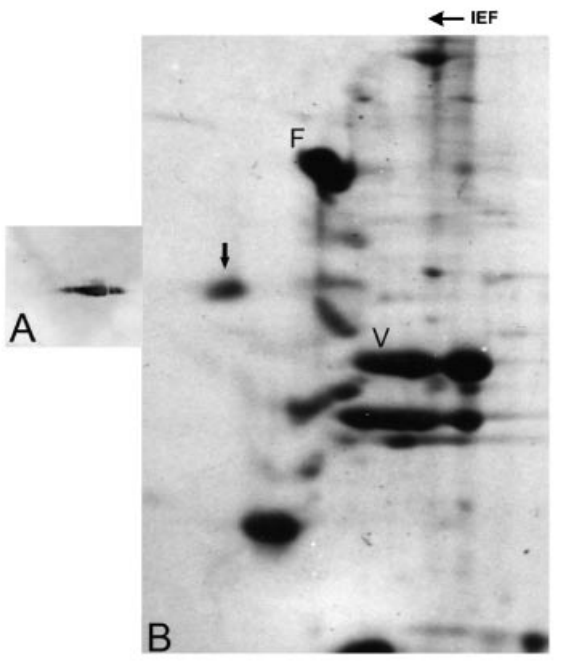

Fig. 1. A: Immunoblot of acidic part of 2-D gel of chicken lens WIF shown in B. The antibody to chicken BASP1 reacted with the protein indicated by the vertical arrow in B. V, vimentin; F, filensin.

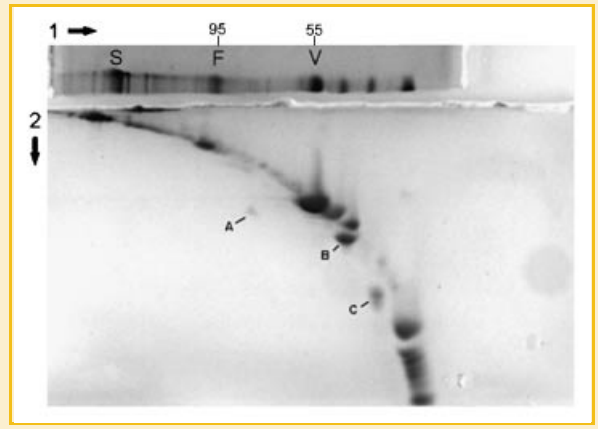

Fig. 2. Gel migration behavior of chick lens WIF. Protein was first electrophoresed in a 7.5% gel (horizontal arrow), and then into 15% gel in the second dimension (vertical arrow). Three proteins indicated by arrows migrated faster in the 15% gel.

peptides were then automatically injected by a Michrom Paradigm Endurance Bio-Cool Autosampler (www.michrom.com) onto a Michrom Paradigm Platinum Peptide Nanotrap (C18, 0.15 mm × 50 mm) and washed for 5 min. The bound peptides are then eluted onto a 10 cm × 75 μm New Objective Picofrit column (www.newobjective.com) packed with Michrom Magic C18 AQ packing material and eluted over 30 min with a gradient of 5% B to 90% B, with constant 10% C in 24 min using a Michrom Paradigm MDLC (Buffer A = 100% Water, Buffer B = 100% Acetonitrile, Buffer C = 1% formic acid) into a ThermoFisher LTQ Linear Ion trap mass spectrometer with a flow rate of ~250 n1/min. The top five ions in each survey scan are then subjected to data-dependent zoom scans followed by low energy collision induced dissociation (CID) and the resulting MS/MS spectra are converted to peak lists using Bio Works Browser v 3.2 (ThermoFisher). All animal protein entries were downloaded from NCBI (31 March, 2006) and the data were search against this database using the Mascot searching algorithm v2.2 (www.matrixscience.com). Spectral assignments are then validated using Scaffold (www.proteomesoftware.com). Matches are considered correct if the confidence level of the Scaffold identification is greater than 95%.

RESULTS

A 2-D gel pattern of chicken nuclear WIF is shown in Figure 1B. The protein spot (arrow in Fig. 1B) and acidic to vimentin was cut from the gel and its peptide sequence determined. The sequences obtained covered 45% of the protein (Table I). Seven unique peptides were identified which passed both the Mascot 95% confidence filter and the Scaffold 95% confidence filter. This protein also reacted with the antibody to chicken BASP1 (Fig. 1A). It was thus identified as BASP1. In a separate experiment, the WIF was first electrophoresed in 7.5% gel, and then in 15% gel. Several proteins showed anomalous migration (arrows Fig. 2). Protein A was identified BASP1 on the basis of peptide sequences (data not shown). Spot B contained β-actin, and spot C contained beta β_1 crystallin. These

TABLE I. Amino Acid Sequence of Chicken Brain BASP1 (NCBI, NP989447)

MGGKLSKKKK	GYSVNDEKAK	DKDKKAEGAA	TEEEETPKEA
EDAQQTTETT	EVKENNKEEK	VEKDAQVSAN	KTEEKEGEKE
KTVTQEEAQK	AEPEKSEAVV	DAKVEPQKNN	EQAPKQEEPA
AASAPAASSE	APKTSEPSSD	AKASQPSEAT	APSKADDKSK
EEGEAKKTEA	PATPAAQETK	SEVAPASDSK	PSSSEAAPSS
KETVAATAAP	SSTAKASDPS	APPEEAKPSE	APATNSDQTI
AVQD			

The lens amino acids are in bold.
The highlighted areas show sequences identified from the 2-D gel fragment of lens WIF.

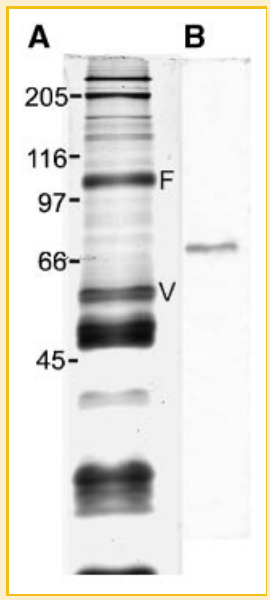

Fig. 3. A: 10% gel pattern of whole lens WIF, (B) immunoblot with anti BASP1. Only one band was observed. F, filensin; V, vimentin.

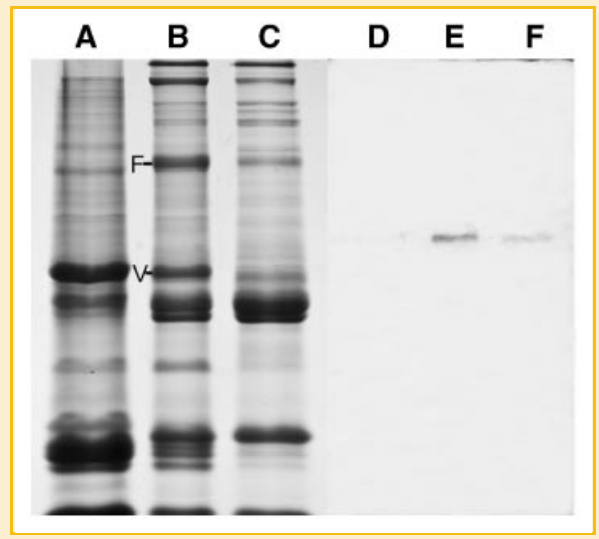

Fig. 4. One-dimensional 10% gel showing the presence of BASP1 in all regions of the chicken lens. A: Annular pad WIF; (B) cortical fiber WIF; (C) nuclear fiber WIF; (D–F) corresponding immunoblot. There is only a faint reaction for annular pad WIF.

three proteins migrated faster and to a lower mol. wt. position in the 15% gel.

By immunoblot, the antibody to chick BASP1 identified the protein in all regions of the chicken lens, but predominantly in the cortical fiber cells (Figs. 3 and 4). Only one band was detected in the whole lens WIF (Fig. 3) and in the regional WIF. Immunoreactivity was faint in the annular pad, intense in the cortex and less in the nucleus (Fig. 4). Immunofluorescent studies showed the protein localized to the fiber cell membranes, with discrete patchy concentrations (Fig. 5B,C). It was only faintly noted in the anterior epithelium, and more prominent in the annular pad cells (arrow c in Fig. 5A). The junction between the anterior epithelium and fiber cells was intense (arrow d in Fig. 5A). BASP was also detected in the epithelium and endothelium of the cornea (a,b in Fig. 5A).

DISCUSSION

The findings in this study document the presence of BASP1 in the chicken lens concentrated predominantly along fiber cell membranes. The identification of lens BASP1 was based on peptide sequence, immunological reactivity and its anomalous migration in SDS–polyacrylamide gels of different concentration. Only one band was detected on immunoblot, and the BASP immunologically related proteins reported by Zakharov et al. [2003] was not observed in the lens study.

BASP1 a member of growth associated proteins which include GAP-43 and MARCKS, is abundant in neurons during brain development and persists in some regions into adult life [Mosevitsky, 2005]. Its expression is upregulated during nerve

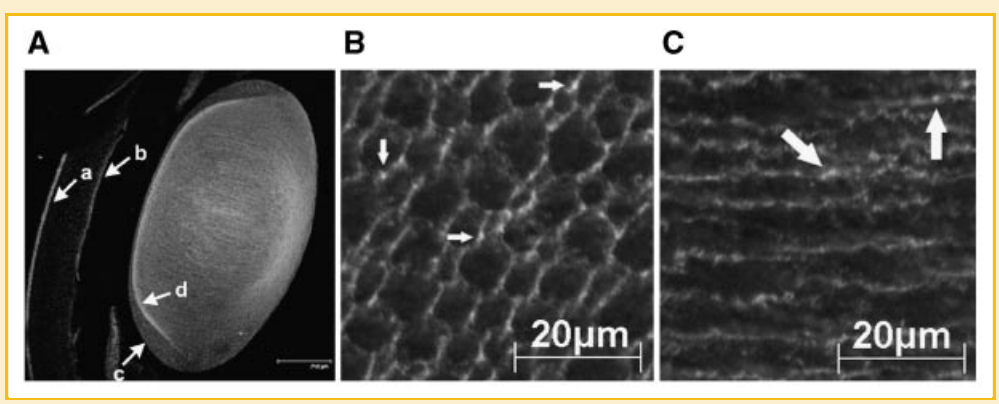

Fig. 5. Immunofluorescent study of 12-day-old chick embryo lens. A: Equatorial section showing intense fluorescence in the fiber cell mass. Arrows a–d point to cornea epithelium, cornea endothelium, annular pad, and interface between fiber mass, and anterior epithelium. B: Localization of BASP1 to fiber cell membranes is evident with punctate distribution in cross-section of cells (arrows). C: Fiber cells in longitudinal section, showing punctate distribution (arrows).

regeneration [Frey et al., 2000], and overexpression of BASP1 promotes nerve sprouting [Caroni et al., 1997].

In recent studies, Korshunova et al. [2008] concluded that the main function of BASP1 is to regulate the organization and morphology of the plasma membrane. This is relevant to the localization of BASP1 in the lens. The protein is mainly localized at the plasma membranes of the fiber cells with frequent discrete enhanced concentrations. Such localization could represent sites of lipid rafts where BASP1 is known to be present [Laux et al., 2000], and/or sites where membrane projections of interlocking devices are found. Definitive resolution of this question requires further study.

BASP1 has been detected in testis, kidney, and lymphoid tissue [Zakharov et al., 2003]. It is functionally related to GAP-43, and both proteins promote the accumulation of subplasma leminal actin. GAP-43 has been detected primate (including human) lenses and in rat lenses. This protein was highly expressed in cells of the anterior epithelium, the germinative zone and young fibers of the transibicual zone. Whereas GAP-43 was detected in young and adult primate lenses, its presence in rat lenses declined markedly with age of the animal. In contrast to the localization of GAP-43, chicken lens BASP1 was only faintly present in the anterior epithelial, and annular pad cells, but highly expressed in cortical fiber cells, and a lesser extent in nuclear fibers. These differences in localization of BASP1 and GAP-43 require further study. They may be related to species different or may reflect different functional roles for these proteins in epithelial and fiber cells.

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