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Paralemnin of the Lens

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Abstract Paralemmin was identified in the chicken lens as a protein with mol. wt 65 kDa and a splice variant of 60 kDa, both soluble in Triton X-100. Paralemmin is localized to the plasma membrane of fiber cells, and was not detected in the annular pad cells. Thus in the chick lens it is another feature of fiber cell differentiation. Its localization to the short side of the fiber cell and the sites of fiber cell interlocking suggests that paralemmin may play a role in the development of such interdigitating processes. J. Cell. Biochem. 89: 917–921, 2003.

Key words: lens; paralemmin; fiber cells

Paralemmin, a prenyl-palmitoyl-anchored phosphoprotein anchored to the plasma membrane was first described by Kutzleb et al. [1998], and implicated in plasma membrane dynamics and cell process formation. The gene for this protein in the lens was identified by Chauhan et al. [2002, 2003] in a cDNA microarray analysis of genes regulated by PAX 6 in the mouse lens. These authors also detected the protein in the mouse lens by Western blot and Immunocytochemistry.

In this study, we characterize paralemmin of the chicken lens, and show its specificity for fiber cell differentiation.

MATERIALS AND METHODS

Lenses from freshly killed 3–4 month old White Leghorn chickens were decapsulated and the annular pad cells collected. The fiber mass was dissected into 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 was 5 mM Tris-HCl, 1 mM EDTA, 0.5 mM PMSF, 2 μg/ml pepstatin A, and 1 mM DTT, pH 7.5. Aliquots of the WSF and WIF were dissolved in 1% SDS-buffer as previously described [Katar et al., 1993]. Samples were also taken for 2-D electrophoresis. The remainder of the WIF was extracted with 8 M urea in buffer, and centrifuged. The urea-soluble fraction was retained and the urea-insoluble fraction extracted with 1% Triton X-100 in a ratio of 100 mg wet wt/ml Triton. After centrifugation the soluble and insoluble fractions were dissolved in 1% SDS.

SDS–Polyacrylamide Gel Electrophoresis

One dimensional SDS–polyacrylamide gel electrophoresis (PAGE) was performed according to Laemli [1970] with 10% (w/v) gels used for final resolution. Two-dimensional PAGE was done according to O'Farrrel [1975].

Immunoblotting

Protein was transferred from polyacrylamide gels onto nitrocellulose paper according to Towbin et al. [1979]. Immunoblots were developed as previously described [Ireland and Maisel, 1984] with the secondary antibody conjugated to alkaline phosphatase. Antibodies to paralemmin were produced in rabbits by injection of the protein cut from 2-D gel slabs.

Immunofluorescence Analysis

Immunofluorescent studies on 10–12 day old chick embryo lenses were done as previously

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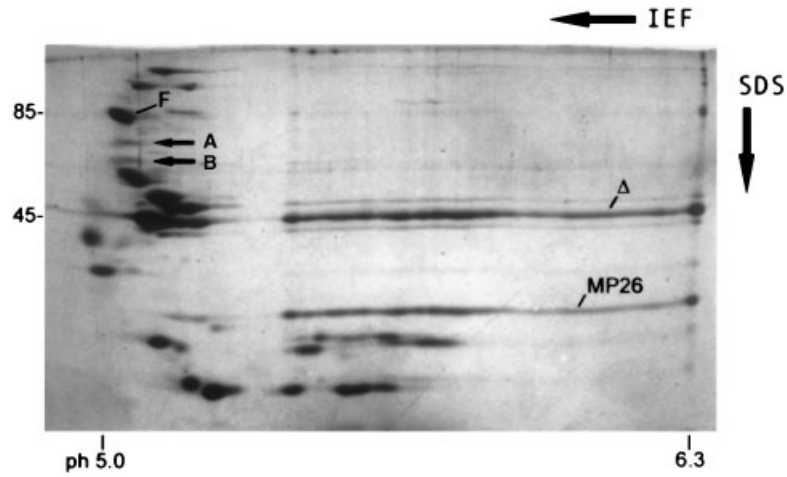

Fig. 1. Two Dimensional SDS–PAGE gel pattern of the chick nuclear fiber cell water-insoluble fraction (NWIF). The bands labeled A and B were found below filensin (F) with molecular weight of 65 and 60 kDa, and at pI 5.0. Δ, delta crystallin; MP26, major intrinsic membrane protein.

described [Waggoner and Maisel, 1978], and on the rat lens according to Lo et al. [1996].

Peptide Analysis

After 2-D electrophoresis of a lens sample, the gel was stained with Coomassie blue and the appropriate gel fragments excised and sent to the Proteomics Facility, Michigan State University, Lansing, MI. After in-gel digestion, extracted peptides were desalted and separated using a LC Packing Ultimate HPLC utilizing a Michrom Cap Trap cartridge for online desalting. Peptides were separated on a 75 μm picofrit column packed with Michrom Magic C18AQ packing material and eluted directly into a LCQ Deca XP ion trap mass spectrometer through a Picoview nanospray source. The top four co-eluting peptide ions were subjected to automated MS/MS fragmentation and analysis. Un-interpreted product ion spectra were searched against the NCB non-redundant protein database using the Mascot search program. Scores above the significance level determined by the Mascot algorithm were considered correct identifications.

RESULTS

The 2-D electrophoretic pattern of the chick lens nuclear WIF is shown in Figure 1. Two bands (A and B) of molecular weight 65 and 60 kDa respectively were noted at the acidic end of the gel (pH 5), below filensin. Delta crystallin and the main intrinsic membrane protein MP26 are indicated for reference purposes. The proteins (A and B) were more prominent in the nuclear urea-insoluble membrane fraction (UIMF) (Fig. 2A), and readily solubilized in 1% Triton X-100 in contrast to the cytoskeletal protein filensin which remained Triton-insoluble (Fig. 2B). The position of the two bands is also shown in a 1-D gel of the

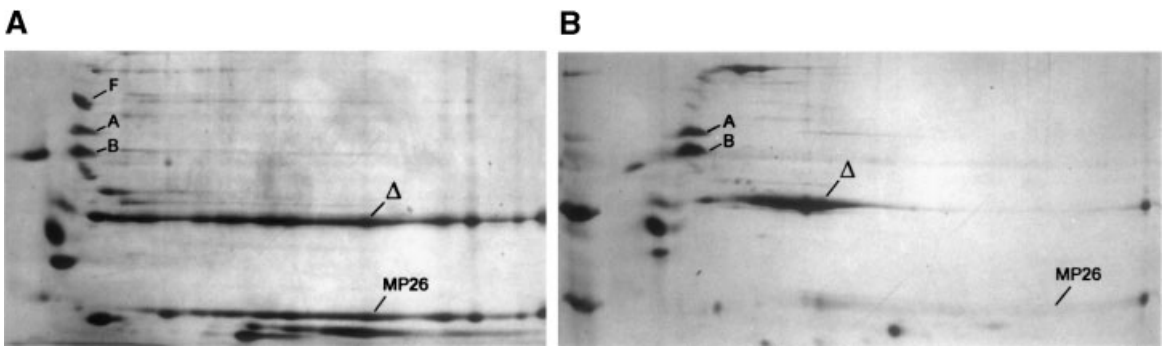

Fig. 2. **A**: Two Dimensional gel pattern of the nuclear fiber cell urea-insoluble membrane fraction (NWIF) (**B**) the Triton X-100 extract of this fraction. Bands A and B are Triton-soluble, while other proteins such as MP-26 and filensin (F) are insoluble.

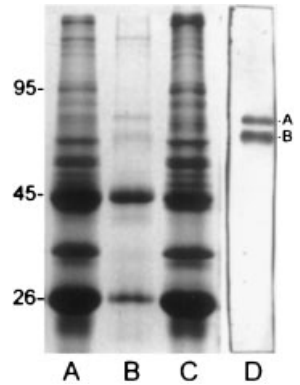

Fig. 3. One Dimensional gel showing two bands of 65 and 60 kDa in the Triton-soluble fraction of nuclear WIF. A, nuclear WIF; B, Triton soluble extract of WIF; C, Triton-insoluble fraction of WIF. D, Western blot with antibody to the 65 kDa protein.

Triton-soluble fraction (Fig. 3). Bands A and B were separately excised from a 2-D gel corresponding to Figure 2B, homogenized, and injected into different rabbits to prepare antisera specific for each protein.

Bands A and B cut from a stained gel were also sent for peptide analysis. An excellent match with the known sequence of chick paralemmin was obtained for band A (Table I). Analysis of the 65 kDa protein generated 24 matching peptides (consisting of 240 amino acids). Analysis of the 60 kDa protein yielded 27 matching peptides (160 amino acids) and is interpreted as a splice variant.

Antisera prepared against individual bands were highly specific and reacted only and with both the 65 and 60 kDa proteins irrespective of which band was used to generate the antiserum (Fig. 3). This specificity is illustrated in Figures 4 and 5. The 2D gel pattern of a nuclear WIF is shown in 4A, and the Western blot developed with the antiserum in 4B. The only reactive bands corresponded in position to the 65 and 60 kDa proteins. The Western blot was then reacted with an antiserum to total lens protein to confirm the localization of the reactions found in B.

Paralemmin was only detected in the fiber cell of the chick lens cortex and nucleus, and not found in the annular pad cells (Fig. 5). Immunoreactive bands at different molecular weights were also detected in other vertebrate lenses (Fig. 6).

Immunofluorescent studies on the embryonic chick lens revealed a localization on the short sides and angles of the fibers, but not in the annular pad cells or anterior epithelial cells (Fig. 7). In the rat lens labeling was seen as both the long and short side of the fiber cells. In addition, a dotted pattern was frequently seen on the long sides of the cells (Fig. 8).

DISCUSSION

Chick lens proteins of 65 and 60 kDa were identified as paralemmin based on the extensive

TABLE I. The Amino Acid Sequence of Chick Paralemmin (Swiss-Prot: Q9YGL6) and That of Lens Peptides Obtained From Gel Fragments A and B

PARALEMMIN_CHICK	MEAVEANTLQQERLQAIAEKRKRQTEIENKRRQLEDDRRQLQHLKSKALRERWLLEG-AP	59
LENSA_CHICK	----------WLLEG-AP	7
LENSB_CHICK	----------WLLEGSAP	8
	***** **	
PARALEMMIN_CHICK	SSASEEDEAMKKQMQEDEVKTKELEETIQRLERELESLENSSSVTSTKENLAEAAAPAKE	119
LENSA_CHICK	SSASEEDEAMK-------KTKELEETIQ---RELESLENSSSVTSTKENLAEAAAPAKE	56
LENSB_CHICK	SSASEED-AMK--------KTKELEETIQ---RELESLENSSSVTSTKENLAEAAAPAKE	56
	********* *** ********** **********	
PARALEMMIN_CHICK	EKKENIPSVQKSPLGTAIAEKKVSSSPMKAVQGTDMMKAAMYSVEITVEKDRVTGETKVL	179
LENSA_CHICK	EKKENIPSVQKSPLGTAIAEKK----------MYSVEITVEKD--------	89
LENSB_CHICK	EKKENIPSVQKSPLGTAIAEKK----------	78
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PARALEMMIN_CHICK	SSTTLLPQNHCVQGIKVYEDELKVVHAVSAEDGALQNGAQPLSSSEVDELLHKADEVTLG	239
LENSA_CHICK	----------VYEDELKVVHAVSAEDGALQNGAQPLSSSEVDELLHKADEVTLG	133
LENSB_CHICK	----------ADEVTLG	85
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PARALEMMIN_CHICK	EATASGDAPGSATSSQKATPRREITGLQAKPRENSTEGAEPSREQPVTMIFMGYQNVEDE	299
LENSA_CHICK	EATASGDAPGSATSSQ---KREITGLQAKPRE--------QPVTMIFMGYQNVEDE	178
LENSB_CHICK	EATASGDAPGSATSSQ----KREITGLQAKPRP----------VTMIFMGYQNVEDE	128
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PARALEMMIN_CHICK	NETKKVLGLEGTIKAELVVIEDAESKAEPEGKDHAPPNGTALEPAAAPLQGDEVPGGQKP	359
LENSA_CHICK	NETKKVLGLEGTIKAELVVIEDAESK----------	204
LENSB_CHICK	NETKKVLGLEGTIKAELVVIEDAESKAEPEGK----------	160
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PARALEMMIN_CHICK	GTNATEAKEAEPDMDAKKQRCKCCTVM	386
LENSA_CHICK	----------	
LENSB_CHICK	----------	

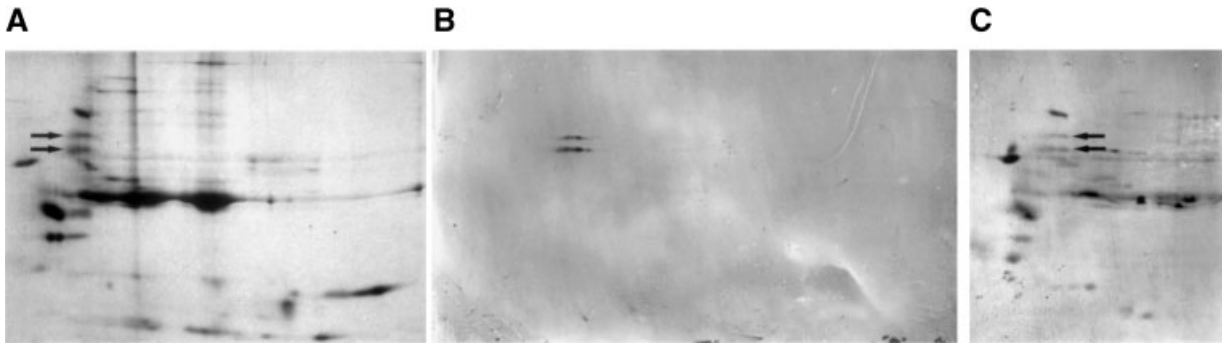

Fig. 4. **A**: Two Dimensional SDS–PAGE gel pattern of the chick nuclear WIF; (**B**) Western blot of A; (**C**) shows the blot in B, subsequently reacted with the antiserum to total lens proteins. The arrows point to proteins A and B in part A. Part C has arrows pointing to the immunoreaction bands.

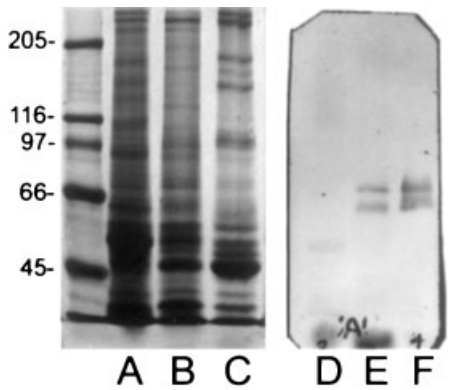

Fig. 5. SDS–PAGE gel pattern of (**A**) WIF of adult chicken annular pad cells, (**B**) WIF of cortical fiber cells, (**C**) nuclear fiber cell WIF. The corresponding Western blot is shown in **D–E**. Molecular weight markers are on the left. A 7.5% (w/v) gel was used.

identity of peptide sequences with the known amino acid sequence [Kutzleb et al., 1998, Swiss-Protein Q9YGL6]. Furthermore, the presence of upper and lower bands and a calculated iso-electric point of pI 5.0 agree with the findings of Kutzleb et al. [1998], as does the size heterogeneity between bird and mammals.

Several features of the regulation of paralemmin expression and its localization suggest that this protein may play a key role in the

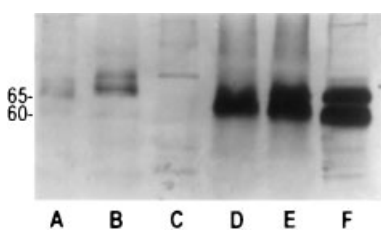

Fig. 6. Western blot of the lens WIF of human, rat, frog, duck, goose, and chicken (**A–F**).

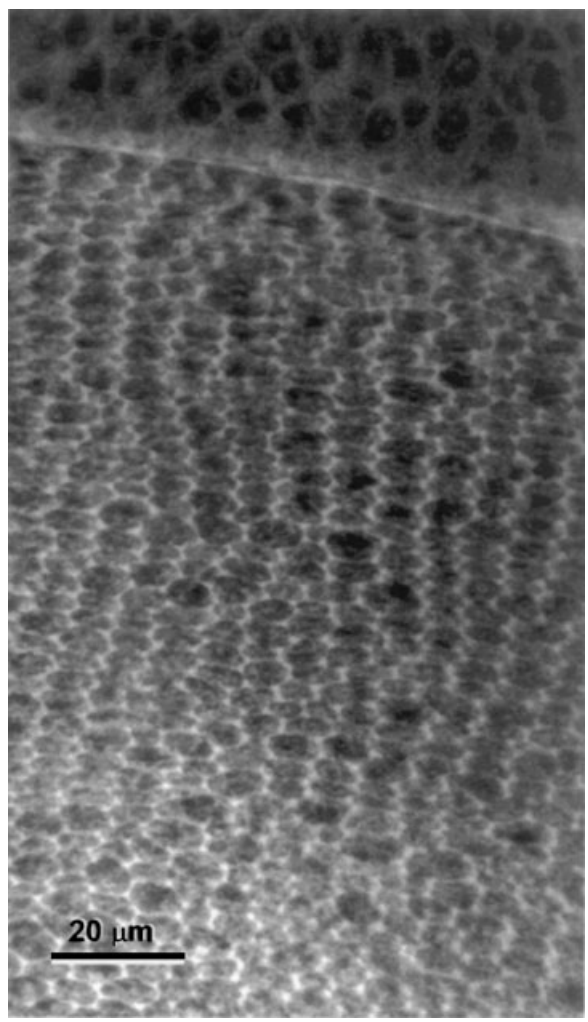

Fig. 7. Immunofluorescence pattern of paralemmin in a 12 day chick embryo lens. Note the concentration at the short sides of the fiber cells and their angles. The annular pad cells (top) are negative.

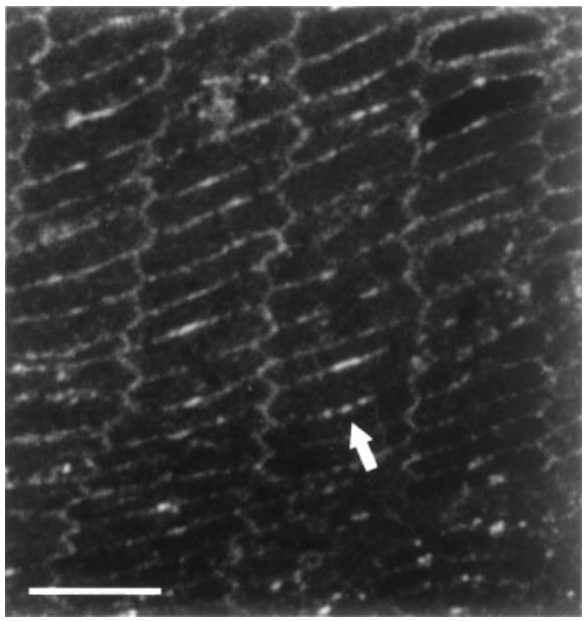

Fig. 8. Immunofluorescence pattern of paralemmin in a 5-week-old rat lens. The labeling is seen along both short and long sides of cortical fiber cell membranes. A dotted pattern of labeling is often observed along the long sides of the fiber cells (arrow). Bar, 10 μm.

differentiation of fiber cells. Thus paralemmin was found in high levels in the normal adult mouse lens and plasma membranes, but was down-regulated in PAX 6(5a)-transgenic lenses [Chauhan et al., 2002]. These authors suggested that PAX 6 directly regulates paralemmin expression in the lens.

Studies by Kutzleb et al. [1998] indicate that paralemmin is concentrated at sites of plasma membrane activity such as filopodia and microspikes, and induces cell expansion and process formation. These authors proposed that paralemmin functions in the control of cell shape.

In this study, paralemmin was detected by Western blot in the membrane fraction of chick fiber cells and not in the initial elongating annular pad cells. Further development of annular pad cells into cortical fiber cells is characterized by elongation and the elaboration of complex inter locking processes between the cells [Kuszak et al., 1981]. The appearance of paralemmin in the fiber cells coincides with the appearance of the latter process. Indeed there is localization of this protein at the angles and short sides of the chick fiber cells where such processes are found. Similar localization was evident in the rat lens. In addition in the rat lens there was also a clear spot-like localization on the long sides of the fiber cells. These could be sites of interlocking processes. The detailed localization of paralemmin must await electron microscopic analysis, and its role in lens plasma membrane development requires further experimentation.

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