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Associated Proteins of Lens Adherens Junction

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Abstract Cytoplasmic proteins associated with adherens junctions were identified in the chicken ocular lens. The catenins, α, β, and γ, were present in epithelial and fiber cells, although their pattern of distribution changed with fiber cell differentiation. The sharp decline in α-catenin with fiber cell formation and the increasing Triton-insolubility of N-cadherin suggests that another subtype of α-catenin exists in the lens. J. Cell. Biochem. 86: 700–703, 2002.

Key words: lens; catenins; adherens junctions

The presence of adherens junctions in the ocular lens is well established [Volk and Geiger, 1984; Geiger et al., 1985]. Lo et al. [2000] proposed that the belt-like zonulae adherens play a role in the early stages of lens development, while the macular fasciae adherens serve to maintain the structural stability of the epithelium and fiber cells during lens cell development and maturation. Cadherins are the transmembrane proteins of these junctions, and both N-cadherin and B-cadherin are present in the chicken lens [Leong et al., 2000].

This study was undertaken to determine the presence and distribution of cytoplasmic proteins which in other tissues are known to associate with cadherins and mediate their adhesive properties. The lens is particularly suited for the analysis of biochemical changes associated with development and aging, since it consists of epithelial cells at sequential stages of differentiation. The anterior epithelium elongates at the equator of the chicken lens as the annular pad cells which then grow markedly in length as fiber cells. The latter constitute the bulk of the lens.

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MATERIALS AND METHODS

Lenses

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* [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 mecaptoethanol, and 20 mM phenylmethylsulphonyl fluoride. The WIF was dissolved in 1% SDS-buffer as previously described [Katar et al., 1993].

In separate experiments, the WIF was extracted in 1% Triton $\times -100$ in a ratio of 100 mg wet weight per 1 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.

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. Monoclonal antibodies to α-, β-, and δ-catenin were obtained from Dr. M. Wheelock. A polyclonal antibody to N-cadherin, (Zymed Lab, San Francisco, CA), and antibodies to total chick lens proteins [Ireland and Maisel, 1984] were also used. The polyclonal antibody to α-catenin was produced against an amino acid sequence common to both α-N and α-E catenin.

RESULTS

The SDS–PAGE pattern of the WIF of annular pad cells, cortical and nuclear fiber cells is shown in Figure 1. The intermediate filament protein (vimentin) is a major component of the annular pad cells, while the beaded filament protein filensin is unique to fiber cells. Figure 2 shows the immunoblots of the WIF after reaction with monoclonal antibodies to catenins and N-cadherin. Alpha catenin decreased markedly with differentiation of annular pad cells into cortical fiber cells. The same pattern was obtained with both monoclonal and polyclonal antibodies to α-catenin. In contrast, β- and δ-catenins increased in amount with cortical

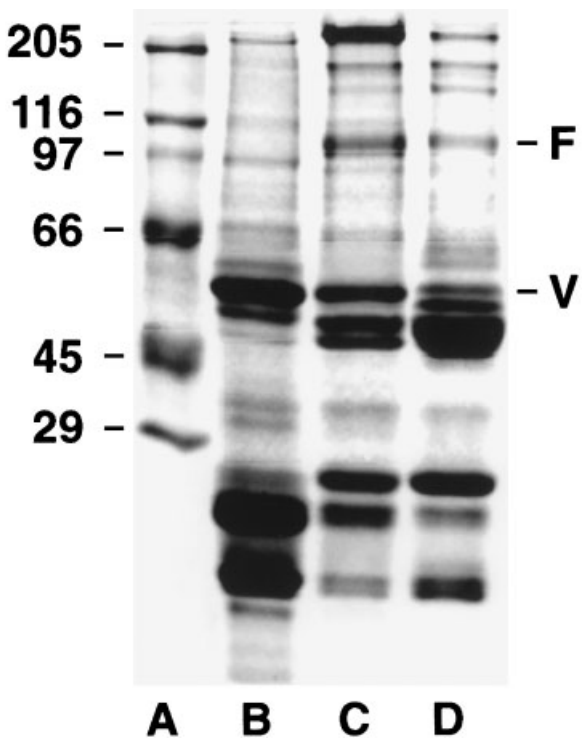

Fig. 1. (**A**) SDS–PAGE pattern of chick lens WIF; (**B**) annular pad cells; (**C**) cortical fiber cells and (**D**) nuclear fiber cells. F, filensin, V, vimentin, A, molecular weight markers.

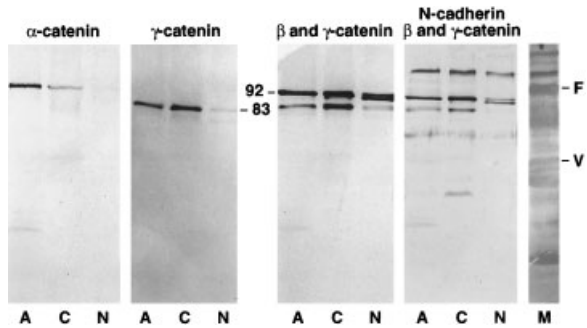

Fig. 2. Immunoblot of chick lens WIF as shown in Figure 1, after reaction with antibodies to catenins and N-cadherin. A, C, N, WIF of annular pad cells, cortical, and nuclear fiber cells, respectively. M, Immunoblot of immediately preceding immunoblot, after reaction with antibody to total lens proteins. F, filensin, V, vimentin.

fiber formation and then declined markedly in the nuclear fiber cells, especially δ-catenin. In the nuclear fiber cell fraction β-catenin formed a doublet. The distribution of N-cadherin corresponded to that of the β- and δ-catenins, increasing with cortical fiber formation and then declining in the nuclear WIF.

The Triton solubility of these proteins changed with fiber cell differentiation and maturation. Thus N-cadherin was mainly Triton soluble in the annular pad cells. However, there was increasing insolubility of this protein in cortical and nuclear fiber cells (Fig. 3). The same pattern pertained to the catenins (Fig. 4, α-catenin not shown). In this experiment a β-catenin doublet was not formed.

DISCUSSION

Cadherins are transmembrane proteins located primarily within adherens junctions, which confer calcium-dependent cell–cell adhesion [Angst et al., 2001]. The extracellular regions make head-to-head contacts across the intercellular gap to form an adhesive zipper

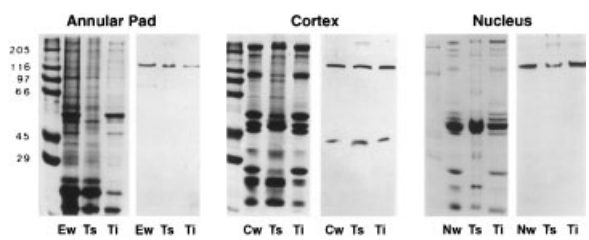

Fig. 3. SDS–PAGE patterns and corresponding N-cadherin immunoblots of Triton X-100 soluble (Ts) and insoluble (Ti) fractions of annular pad cells, and cortical and nuclear fiber cells. EW, CW, and NW = WIF before extraction with Triton.

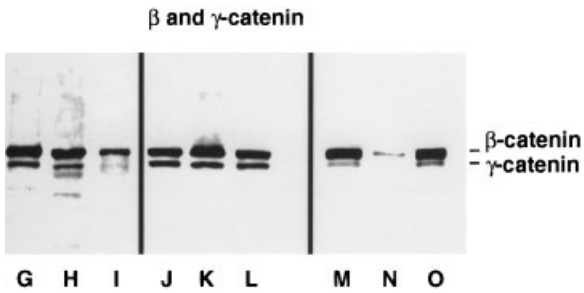

Fig. 4. Immunoblots of Triton-soluble and insoluble fractions reacted with antibody to β- and δ-catenin. Epithelium; G, H, and I, corresponding to beginning WIF, Triton-soluble fraction and Triton-insoluble fraction. J, K, L = cortex fractions, M, N, O = nuclear fractions.

[Shapiro et al., 1995]. The intracellular regions link the cadherins with their cytoplasmic partners, β- or γ-catenin (plakoglobin) which in turn binds directly to α-catenin [Yap et al., 1997]. α-Catenin interacts directly or indirectly (via α-actinin or vinculin) with the actin filament network [Knudsen et al., 1995; Rimm et al., 1995; Watabe-Uchida et al., 1998]. β-Catenin, when not associated with the adherens junction complex, can act as transcriptional activator in the Wnt growth factor signaling pathway that controls cell fate determination [Polakis, 2000]. γ-Catenin, which can substitute for β-catenin in the adherens junction, can also function as a transcription factor. However, its function in cellular signaling appears to differ from that of β-catenin [Simcha et al., 1998; Zhurinsky et al., 2000].

The results of this study demonstrate that cadherin associated cytoplasmic proteins are present in the lens and that changes in distribution accompany fiber cell differentiation. The major changes are represented by an increase in N-cadherin and β- and γ-catenin with cortical fiber formation and a subsequent decrease in the older nuclear fiber cells. By contrast, α-catenin declined markedly with epithelial cell differentiation.

The significance of these observations remains to be elucidated. One question that arises concerns the presence of both β- and γ-catenins in the lens and the subsequent decline in expression of these proteins with increasing age of the lens fibers. Both of these catenins are found in adherens junctions linked to the cytoplasmic domain of N-cadherin. However, γ-catenin, but not β-catenin is also found linked to the cytoplasmic domain of the desmosomal cadherins, desmoglein, and desmocollin [Koch and Franke, 1994]. Desmosomes have been reported between lens epithelial cells and elongating fibers but have not been found between mature fibers [Kuszak, 1995]. Interestingly, decreases in γ-catenin expression by epithelial cells have been shown to correlate with loss of desmosome formation [Lewis et al., 1997]. In light of this, the marked decline in γ-catenin in the nuclear fiber fraction (Fig. 1) is consistent with the loss of desmosomes in the mature fibers.

A second question concerns the distribution of α-catenin, expression of which declined markedly with fiber cell fraction. This finding suggests that a change occurred in the linkage of cadherin to the actin cytoskeleton in cortical fiber cells. The precise nature of such a change remains to be elucidated, but is consistent with observations that N-cadherin becomes increasingly Triton-insoluble with fiber cell formation as previously noted by Leong et al. [2000]. It is possible that another isoform of α-catenin exists in the cortical and nuclear fiber cells which the antibodies used in this study cannot detect. Two subtypes of α-catenin have already been identified; αE-catenin expressed predominantly in epithelial cells and αN-catenin expressed predominantly in neuronal cells [Hirano et al., 1992]. In addition two isoforms of αN-catenin have also been identified [Uchida et al., 1994]. In the present study, a monoclonal antibody that recognizes only αE-catenin and a polyclonal antibody that is likely to recognize both αE- and αN-catenin were used. While loss of α-catenin expression is not unprecedented in different cell types, it is usually associated with development of carcinomas [Ewing et al., 1995; Kadowaki et al., 1994; Morton et al., 1993; Shimoyama et al., 1992] and not cellular maturation. In addition the increasing Triton-insolubility of N-cadherin in the nuclear fiber fraction would suggest an increased association of N-cadherin with the actin cytoskeleton. However, the decrease of α-catenin in this fraction contradicts this idea since it is a necessary link between N-cadherin and the cytoskeleton. This is further support for the possibility that another subtype of α-catenin exists in these cells.

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