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Immunohistochemical Studies of Lens Crystallins in the Dysgenetic Lens (*dyl*) Mutant Mice

SAMIR K. BRAHMA AND SOMES SANYAL*

*Department of Medical Anatomy and Embryology, The State University, Janskerkhof 3A, 3512 BK Utrecht, and *Department of Anatomy, Erasmus University, Rotterdam, The Netherlands*

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The lens in the *dyl* mutant mice shows a persistent lens–ectodermal connection as well as degeneration and extrusion of lens materials after the initial differentiation of the fibres. Immunohistochemical investigation of the ontogeny of the lens crystallins in this developing mutant lens has been carried out using the indirect immunofluorescence staining method with antiserum to adult mouse lens total soluble proteins. The results have been compared with those for coisogenic normal lens used as a control. In both, the first positive reaction was detectable at identical stages of lens development. A rapid increase in the intensity of fluorescence, most marked in the elongating fibre progressing through the equatorial region to the epithelium, was recorded in the mutant as well as in the normal lens. However, the stalk leading to the lens epithelium did not show any reaction. Appearance of vacuoles in the lens nucleus and cortex marked the beginning of degeneration of fibres which otherwise showed strong fluorescence. This was followed by extrusion of lens crystallin materials through the stalk. As a result, the lens became increasingly reduced and malformed but the surviving cells making up the vestigeal lens in the adult showed positive immunofluorescence. The results demonstrate that despite a failure of lens–ectoderm separation in the mutant mice, the ontogeny of the lens crystallins and differentiation of the lens up to a certain stage of development follow an apparently normal course before the commencement of cataractous degeneration.

Key words: mouse; lens; *dyl* mutant; lens crystallins; immunofluorescence; cataract; anterior chamber defect; Peter's anomaly.

1. Introduction

The crystallins which comprise 90% of the soluble lens proteins in mammals are grouped on the basis of their net charge and molecular weight into the α-, β-, and γ-crystallin classes. Of these, the α- and β- are heterogeneous oligomeric proteins while the γ-crystallins are monomers (Harding and Dilley, 1976). The onset of γ-crystallin synthesis has been associated with lens fibre differentiation (Papaconstantinou, 1967). This has also been reported in the non-mammalian lens where γ-crystallin is present (Clayton, 1974; McDevitt and Brahma, 1982). Bloemendal (1979) reported that fibrogenesis is accompanied by a change in the subunit composition of α- and β-crystallins. Distributionwise, α- and β- are found in the epithelium as well as in the fibres, while γ- could be detected only in the fibres.

In the mouse, as in other mammals, all of the three crystallin classes are present in the lens, and in this article we present results on the ontogeny of the lens crystallins in a mutant, compared with those in a coisogenic normal mouse via the indirect immunofluorescence staining method (Coons, 1956) using antibodies against adult mouse lens total soluble proteins raised in young rabbits. The mutant, a new autosomal recessive gene, *dysgenetic lens* symbol *dyl* (Sanyal and Hawkins, 1979), located in the chromosome 4 (Van Nie, Sanyal, de Veld and Hawkins, in prep.)

Please address correspondence to: Dr S. K. Brahma, Department of Medical Anatomy and Embryology, The State University, Janskerkhof 3A, 3512 BK Utrecht, The Netherlands.

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has already been described. It is characterized by a persistent lens–ectoderm connection, small eye and corneal opacity. The lens appears normal (histologically) during the early stages of development, but cataractous degeneration starts soon and the lens materials are extruded through the stalk. As a result, the lens becomes reduced in size and disorganized. Other anterior chamber abnormalities appear during later development.

2. Materials and Methods

The *dyl* mutation arose spontaneously in the Balb/c strain of albino mice and the mutant and the normal genotypes are being maintained as coisogenic lines. Matings were set to produce homozygous mutant and homozygous normal embryos. Pregnant females were killed at known intervals after mating. The day the vaginal plug was detected was counted as Day 0. For early embryonic stages the entire heads, and for late embryonic and postnatal stages isolated eyes, were fixed in cold Carnoy's fluid and processed according to methods described earlier (Brahma and van Doorenmaalen, 1971). Paraffin sections were cut at 5 μm thickness through the eye lens region and were used for staining. Developmental stages of the lens were determined from histological sections according to Pei and Rhodin (1970).

The γ-globulin fraction from the antiserum against adult mouse lens total soluble proteins raised in young rabbits was isolated by the saturated ammonium sulphate solution and was used in combination with fluoresceine (FITC)-conjugated goat anti-rabbit γ-globulin as the secondary antibody.

Similarly prepared γ-globulin from non-immunized rabbit serum was used as a control.

The sections were stained according to Brahma and van Doorenmaalen (1971) and examined with a Leitz fluorescence microscope under dark field illumination. Photomicrographs were taken with Tri-X Pan film (Kodak).

3. Results

The immunofluorescence patterns in the mutant and normal lens at various stages of development are shown in Figs 1 and 2. The first positive reaction in both mutant and normal lens was detected on day 11 (mid-stage 2). The activity was localized in a few scattered cells on the inner wall of the lens vesicle [Fig. 1(A) and (B)]. Normally, at this stage, the lens pit disappears and the lens takes the form of a closed vesicle but in the mutant, the margin of the pit remains open. However, no difference was observed in the intensity of fluorescence between mutant and normal lens. At Day 12 (stage 3) cells of the inner wall of the vesicle in both mutant and normal started to elongate and the entire lens showed strong positive reaction [Fig. 1(C) and (D)]. In the normal mice the vesicle is completely detached from the cranial ectoderm, while in the mutant, the connection is maintained and the lens is slightly smaller in size. In both, the most intense reaction was observed in the cytoplasm facing the retina. At Day 13 (stage 4) the primary fibres obliterated the lens cavity. In the normal lens the cortical fibres appear elongated and aligned closely parallel to each other while in the mutant lens, the fibres appear to be less elongated and less regularly aligned. However, the overall pattern and intensity of fluorescence appeared to be the same in mutant and normal lens [Fig. 2(A) and (B)]. Normally, at this stage the future cornea is well distinguishable, covering the lens frontally while in the mutant it is not well defined and the stalk (connection between the lens and the cranial ectoderm) does not show any fluorescence. It is observed that the lens epithelium marges on either side with the cranial ectoderm and boundary between the two can be distinguished from the immunofluorescence reaction [Fig. 2(A)]. By Day 16 (stage 6) secondary fibres developed and the normal lens acquired adult architecture showing strong fluorescence

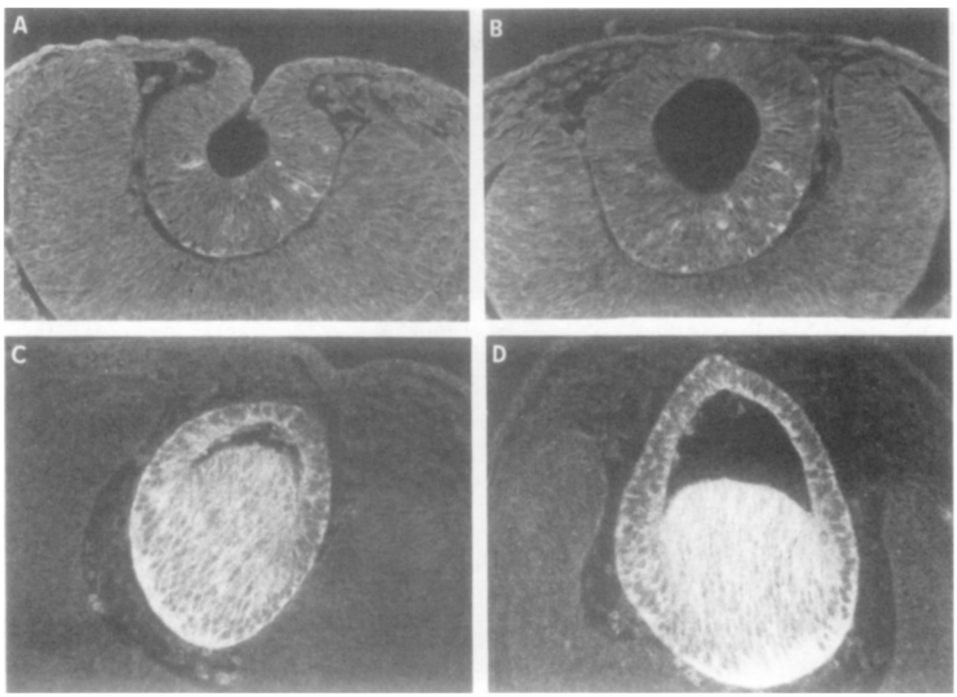

FIG. 1. Dark-field photomicrographs of developmental stages of lenses from dysgenetic lens (*dyl*) mutant and normal mice treated with the γ-globulin fraction from mouse lens total protein antiserum in combination with FITC-conjugated goat anti-rabbit γ-globulin used as the secondary antibody. A. Day 11 mutant lens (mid-stage 2). The lens vesicle remains open. Few cells in the inner wall of the vesicle show positive reaction. $\times 75$. B. Day 11 normal lens (mid-stage 2). The lens vesicle is closed and the immunofluorescent pattern is similar to the mutant lens, but the lens appears bigger in size. $\times 75$. C. Day 12 mutant lens (stage 3). The lens remains connected with the cranial ectoderm but the section illustrated here is taken at a distance. The lens appears normal histologically but smaller in size. Immunofluorescent reaction is seen all over the lens with increased intensity. $\times 75$. D. Day 12 normal lens (stage 3). Immunofluorescent pattern is similar to the mutant at this stage but the lens is bigger. $\times 75$.

in the epithelium, cortex and nucleus [Fig. 2(D)]. However, in the mutant lens at this stage vacuoles appear all over the cortex and nucleus as a result of cataractous degeneration. Nevertheless the epithelium and non-vacuolated area of the cortex and nucleus showed reaction comparable to that of the normal lens. At this stage, the future cornea is visible and its continuity appears to be interrupted by the lens epithelium and disorganized fibres bulging through it and discharging the lens contents outside [Fig. 2(C)].

At postnatal stage, the mutant lens becomes very much reduced and distorted but still shows weak positive immunofluorescence reaction [Fig. 2(E)].

Sections treated with the γ-globulin fraction from non-immunized rabbit did not show any activity.

4. Discussion

The failure of separation of the lens from the cranial ectoderm in the *dyl* mutant does not seem to have any immediate effect on morphogenesis or on the synthesis of

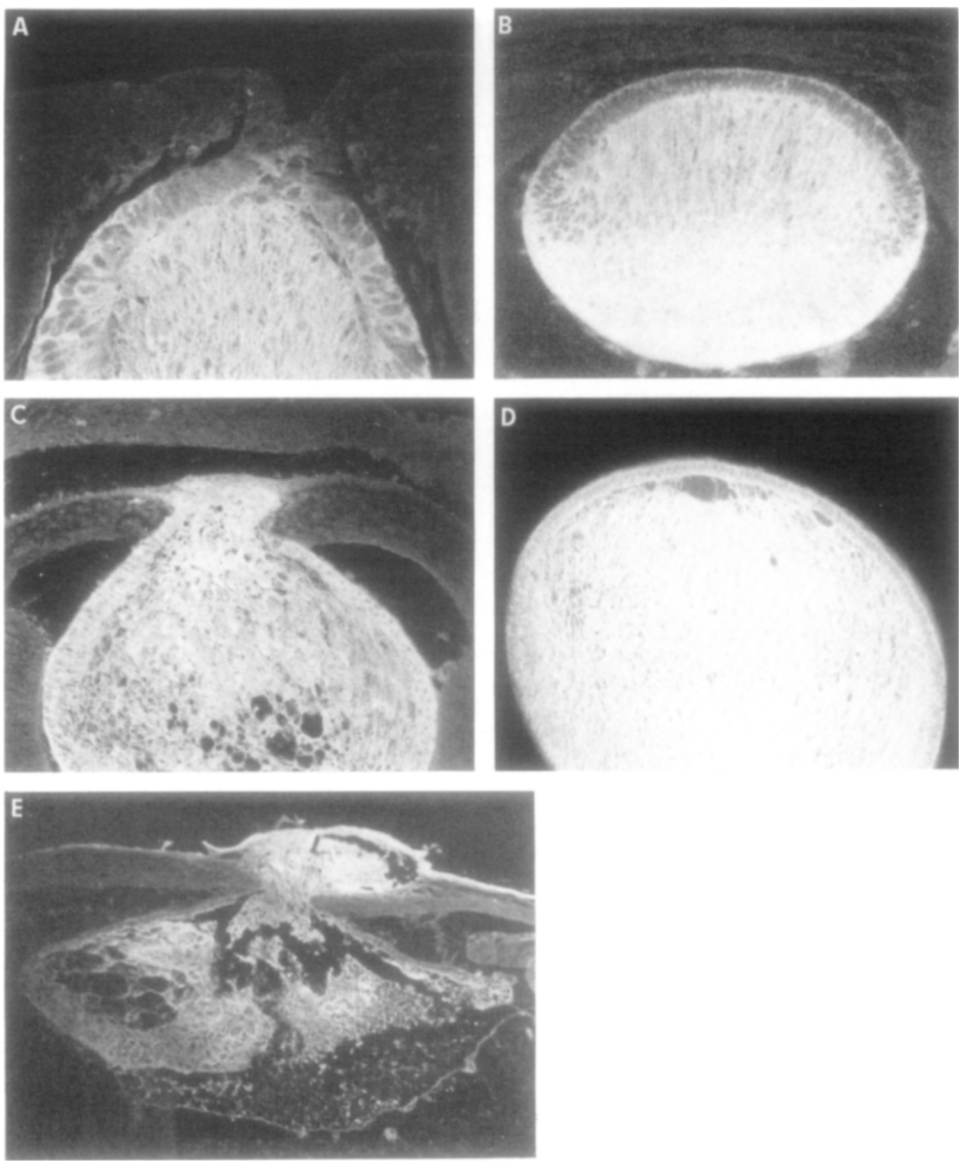

FIG. 2. Dark-field photomicrographs of developmental stages of lenses from dysgenetic lens (*dyl*) mutant and normal mice treated with the γ-globulin fraction from mouse lens total protein antiserum in combination with FITC-conjugated goat anti-rabbit γ-globulin used as the secondary antibody. A. Day 13 mutant lens (stage 4). The entire lens shows positive reaction but the cells of the stalk showing the connection between the future cornea and the lens epithelium do not show any reaction. At the retinal end, on either side, the epithelium is seen marging into the retina. ×88. B. Day 13 normal lens (stage 4). Intensity of immunofluorescence appears to be similar to the mutant lens. The future cornea is clearly visible. ×35. C. Day 16 mutant lens (stage 6). Part of the epithelium and fibre cell mass are seen protruding through the cornea. Vacuoles are observed in the cortex as well as in the nuclear area. Strong positive reaction is seen all over the epithelium and non-vacuolated area of the fibre cells. ×56. D. Day 16 normal lens (stage 6). The lens has acquired the basic adult architecture. The secondary fibres are developed at this stage. The staining pattern and its intensity resemble the mutant lens. ×35. E. Newborn mutant lens. It is disorganized and vacuolated but the epithelium and remains of the fibre cells show weak but positive reaction. Lens materials coming out through the stalk also exhibit positive immunofluorescence indicating that lens crystallin/s is/are leaching out whose nature could not be determined from the antiserum used. ×35.

lens crystallins as compared with the normally developing lens at identical stages. The lens merely appears to be smaller in size.

Our results with the antiserum used in the present experiment are similar to those of van de Kamp and Zwaan (1973) with Swiss Webster mice. We also observed the first positive reaction in some of the cells of the inner wall of the lens vesicle and by the time the epithelium became positive the lens was detached from the cranial ectoderm in the normally developing mice. In the *dyl* mutant lens, despite the persistence of the stalk, the intensity of immunofluorescence in the lens epithelium is comparable with that in the normal lens epithelium but it disappeared near the base of the connecting stalk, even though the cells making up the two structures are contiguous.

In contrast with the normal, the *dyl* mutant lens showed cataractous degeneration of the primary and secondary fibres which resulted in the vacuolation and apparent extrusion of lens materials synthesized in the fibres.

From the positive immunofluorescence reaction it appears that the extruded material contains crystallin/s but the nature of these could not be determined from the antiserum used. We also could not decide which of the crystallin classes appeared first in the *dyl* and normal lenses. There are, however, reports showing the α- to be the first of the crystallins to appear during lens development in the mice (Barabanov, 1966; Konyukhov, Malinina, Platonov and Yakovelv, 1978; Ikeda, Seki, Yoshi and Mishima, 1981; Zwaan, 1983).

Various forms of pre- and postnatal hereditary cataracts have been reported in mice. In mice afflicted by gene Cat^{Fraser} swelling and degeneration of the lens fibres starts at Day 14 of embryonic life (Zwaan and Williams, 1968; Hamai and Kuwabara, 1975) and according to Garber, Stirk and Gold (1983) this results in an increase in α-crystallin, decrease in γ-crystallin and the absence of β-high crystallin aggregates. Also in mice afflicted by *Elo* (*Eye lens obsolence*) gene degradation of central fibres starts at 14 days of embryonic life and γ-crystallin is detected in most of the necrotic fibres (Watanabe, Fujisawa, Oda and Kameyama, 1980).

Similarly, a leakage of γ-crystallin (Nakano mice) and γ- and β-crystallin (Philly mice) in the anterior chamber of the eye have been reported during cataractogenesis at 40 and 30 days after birth respectively (Russel, Smith, Carper and Kinoshita, 1979; Zigler, Carper and Kinoshita, 1981; Iwata and Kinoshita, 1971; Kador, Fukui, Fukushi, Jernigan and Kinoshita, 1980). Selective loss of a β-crystallin polypeptide having a molecular mass nearly 27 kdalton has been reported in the Philly mice (Carper, Shinohara, Piatigorsky and Kinoshita, 1982).

Using specific anti α- from duck and chick and anti γ-crystallin antisera from bovine calf lens, with which mouse lens total soluble proteins cross-react, we found that the extruded fibre cell materials contained both α- and γ-crystallins (unpubl.). Using the same antisera we also observed that in the *dyl* as well as in the normal genotype α-crystallin appears first followed by the γ (unpubl.) supporting the results of Barabanov (1966); Konyukhov et al. (1978); Ikeda et al. (1981) and Zwaan (1983).

A number of genes are known to cause a failure of lens ectoderm separation. The two semidominant genes *Coloboma* (*Cm*) and Dickey's small eye (*Dey*), which are likely to be the allelic and are both lethal in homozygote, produce lens abnormalities similar to *dyl* (Theiler, Varnum and Stevens, 1978; Theiler and Varnum, 1981).

Breeding (Sanyal and Hawkins, 1979) and linkage (Van Nie, Sanyal, De Veld and Hawkins, in prep.) data show that the *dyl* mutation is fully viable and the locus is non-allelic to any known gene affecting the eye. Immunohistochemical observations

show a fair degree of normal development suggesting that the failure of lens–ectoderm separation is the specific and primary effect of the gene. Since lens detachment is dependent on the formation of anterior lens capsule (Berman and Pierro, 1969) and since maintenance of lens cells depends on nutritional support from the aqueous humour (Raviola, 1977), cataractous degeneration could follow from this initial defect leading to gross morphological changes in the anterior chamber. In this respect, the sequence of changes in the *dyl* mice, following normal histological differentiation, closely resembles the etiology of Peter's anomaly which is a category of anterior chamber defect (Waring, Rodrigues and Laibson, 1975) marked by kerato-lenticular adhesion and cantral leukoma (Townsend, 1974).

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