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Deficiency of Functional Messenger RNA for a Developmentally Regulated β-Crystallin Polypeptide in a Hereditary Cataract

Abstract. *The messenger RNA for a β-crystallin polypeptide with a molecular size of 27 kilodaltons, first detected 5 to 10 days after birth in the normal mouse lens and the Nakano mouse cataract, was not detected in the Philly mouse cataract with translation in vitro. The heterozygous Philly lens had intermediate levels of the 27-kilodalton β-crystallin polypeptide and exhibited delayed onset of the cataract. The deficiency of functional 27-kilodalton β-crystallin messenger RNA is the earliest lesion reported yet for the Philly lens and points to a transcriptional or posttranscriptional developmental defect in this hereditary cataract.*

Development of the ocular lens is characterized by differential synthesis of the crystallins (structural proteins) and is consequently a favorable system for the study of differential gene expression in eukaryotic cells (*1*). Normally the lens is transparent. In certain strains of mice, however, the lens becomes opaque—cataractous—after birth. The Philly mouse, a derivative of the Swiss-Webster strain, develops an osmotic cataract during the fourth postnatal week (*2*). The Philly cataract progresses from an initial faint subcapsular opacity to a dense nuclear cataract in about 1 month (*3*). Crystallin synthesis is severely depressed in the fiber cells of the Philly cataract (*4*). This appears to be caused, at least in part, by ionic changes within the lens that interfere with the translation of crystallin messenger RNA's (mRNA's) (*5*). Prior to the general reduction in crystallin synthesis, a β-crystallin polypeptide with a molecular size near 27 kilodaltons (27 K) is selectively missing from the Philly cataract (*6*). We now report that this β-crystallin polypeptide is a developmentally regulated protein whose mRNA cannot be detected by translation in vitro until the second week after birth for the normal Swiss-Webster mouse and cannot be detected at all by translation in vitro for the Philly mouse.

The polypeptide compositions of normal Swiss-Webster and Philly mouse lenses were examined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Fig. 1). The arrows in Fig. 1 point to the 27 K β-crystallin polypeptide band. This band of protein was not present in the normal Swiss-Webster lens at day 1 or day 5 but was easily visible by day 10 and accumulated thereafter. In contrast, the 27 K β-crystallin polypeptide appeared to be missing from the Philly lens at all stages examined. Experiments were not performed on older mice because β-crystallins degrade during the process of opacification (*4*, *6*).

A trace of the 27 K β-crystallin polypeptide was observed as early as days 1 and 5 after birth, and considerable amounts were evident by the tenth postnatal day in the BALB/c and Nakano lenses (Fig. 1). Nakano mice were derived originally from BALB/c mice. The Nakano mouse develops a hereditary osmotic cataract associated with ionic imbalances (*7*) caused by the production of an inhibitor of the cellular Na,K–adenosine triphosphatase (*8*). Thus the time of appearance for the 27 K β-crystallin polypeptide may vary slightly with the strain of mouse. Moreover, the deficiency in the 27 K β-crystallin polypeptide is not due to general osmotic imbalances.

Total RNA's extracted from the lenses of Philly and control mice were tested by translation in a reticulocyte lysate to determine whether the Philly lens lacks a functional mRNA for the 27 K β-crystallin polypeptide. Autoradiograms of sodi-

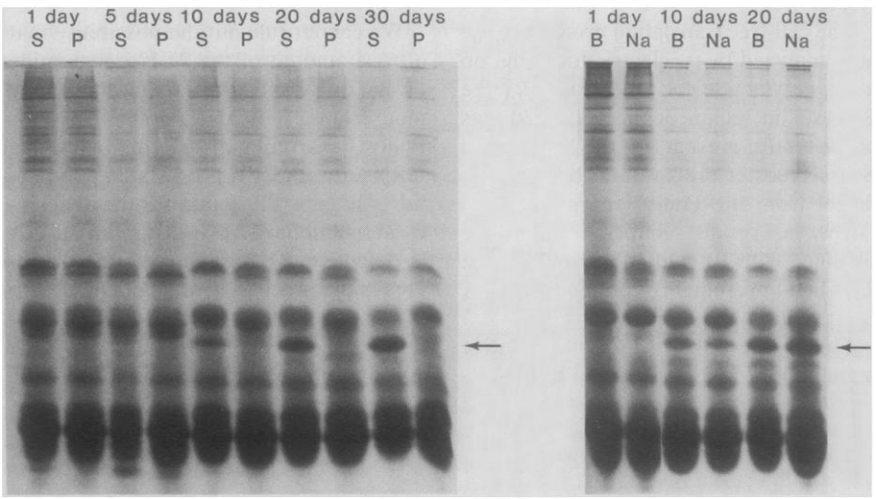

Fig. 1. Sodium dodecyl sulfate–polyacrylamide gels of lens proteins from Swiss-Webster control (*S*), Philly (*P*), BALB/c (*B*), and Nakano (*Na*) lenses. The lenses were removed from the eyes and homogenized in 10 m*M* 2-mercaptoethanol, 1 m*M* EDTA, and 1 percent sodium dodecyl sulfate. Samples of total lens protein (35 μg) were subjected to electrophoresis in a 15 percent polyacrylamide slab gel (*13*). Gels were stained with Coomassie brilliant blue R (Bio-Rad). The arrows point to the 27 K β-crystallin polypeptide; other small differences between the control and Philly lens proteins were not reproducible.

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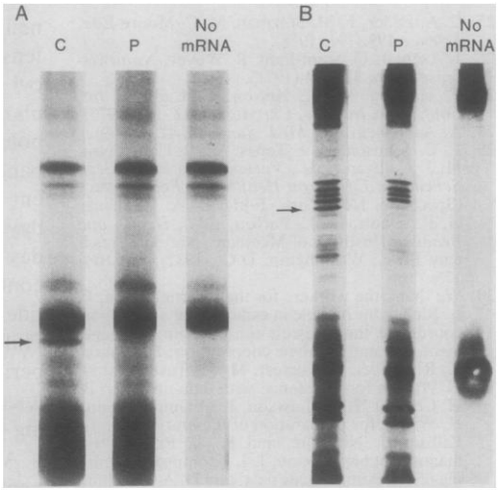

Fig. 2. (A) Autoradiograms of sodium dodecyl sulfate–polyacrylamide gels of proteins derived by translation of total RNA's from 20-day-old Swiss-Webster control (*C*) and Philly (*P*) lenses. The RNA's were extracted from batches of ten lenses with a mixture of phenol, choloroform, and isoamyl alcohol (50:50:1) (*14*), precipitated with ethanol, and dissolved in 350 μl of sterile water; 10 μl of the RNA were translated in 33 μl of a rabbit reticulocyte kit (New England Nuclear) containing 50 μCi of [^{35}S]methionine (1000 Ci/mmole). The autoradiograms represent 15 μl of translation products. The molecular size of the translation product indicated by the arrow as determined by molecular size markers was approximately 27,000. (B) Autoradiograms of an isoelectrofocusing gel showing translation products of lens RNA's from 20-day-old Swiss-Webster control (*C*) and Philly (*P*) mice. Each lane represents 5 μl of translation product. The arrow indicates a β-crystallin product present in control lenses but severely deficient, if not absent, in Philly lenses. Other differences noted on this gel between the products of control and Philly lenses were not reproducible in preparations derived from 1-, 10-, and 30-day-old lenses.

um dodecyl sulfate–polyacrylamide gels show that the RNA from the 20-day-old Philly lens, unlike that from the control lens, did not direct the synthesis of the 27 K β-crystallin polypeptide (Fig. 2A). In additional tests (not shown), the 27 K β-crystallin polypeptide was not synthesized in reticulocyte lysates supplemented with RNA from the Swiss-Webster control lens until the mice were 10 days of age. The 27 K β-crystallin polypeptide was not synthesized in vitro from RNA preparations of the Philly mouse lens even at 30 days of age. As was expected, RNA from both BALB/c and Nakano mouse lenses between days 5 and 30 stimulated the synthesis of the 27 K β-crystallin polypeptide in a reticulocyte lysate.

The cell-free translation products were also analyzed by isoelectric focusing, on a polyacrylamide gel. One product in the β-crystallin region of the gel (*6*, *9*) was consistently present in the 20-day-old Swiss-Webster preparation, but was deficient from the Philly lens preparation (arrow in Fig. 2B). Similar results were obtained with 10- and 30-day-old lenses. This band presumably contains the 27 K β-crystallin polypeptide.

Finally we investigated the relative amount of 27 K β-crystallin in F_1 hybrids between normal and Philly parents. The F_1 progeny had approximately half as much 27 K β-crystallin as did the normal mice (not shown). Ninety-five percent of the F_1 (120 mice) developed bilateral opacities between 45 and 53 days of age, which was 10 to 15 days later than in the Philly homozygotes. Fifty backcross progeny (normal × F_1) were maintained for 110 days and had a 1:1 ratio between clear and opaque lenses; 18 percent of the F_2 progeny (60 mice) were still clear-eyed at 100 days of age. These data indicate that the Philly cataract is a dominantly inherited characteristic and that an intermediate level of the 27 K β-crystallin polypeptide in the heterozygote is associated with delayed onset of the opacity.

We cannot rule out the possibility that a trace amount of the 27 K polypeptide or its mRNA is present in the Philly mouse lens. It is also possible that the Philly lens contains a nonfunctional mRNA for the 27 K β-crystallin polypeptide. One possible cause for the deficiency in functional 27 K β-crystallin mRNA is defective RNA splicing, such as occurs in the processing of globin mRNA in β^+-thalassemia patients (*10*) or of albumin mRNA in analbuminemic rats (*11*). An alternative possibility is a mutation or deletion of the β-crystallin gene or its regulatory elements, such as occurs in Gr^B mutations of the chorion genes in *Bombyx mori* (*12*). We do not know whether the deficiency in the 27 K β-crystallin polypeptide is causally related to the ionic imbalance (*2*), ultrastructural alterations (*3*), or opacity in the Philly lens. It may be significant, however, that the selective deficiency in the 27 K β-crystallin polypeptide is the earliest defect observed yet in this cataract.

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