numbers, as expected under conditions of adenosine starvation. In contrast, protozoa from cultures containing 100 μM adenosine showed no increase in 3′-nucleotidase activity (Fig. 1B), although they continued to multiply. In a similar experiment, cycloheximide (at concentrations as low as 10 μM) completely inhibited the increase in 3′-nucleotidase activity in *C. fasciculata* transferred to purine-deficient medium. This result indicates a protein-synthesis requirement for the observed increase in 3′-nucleotidase levels. The increased expression of 3′-nucleotidase activity in *C. fasciculata* was not observed under other growth-limiting conditions, including the depletion of such essential nutrients as inorganic phosphate and hemin, which indicates the specificity of this regulatory phenomenon.

Loss of the 3′-nucleotidase activity was observed after transfer of *C. fasciculata* from a purine-deficient medium to one containing 100 μM adenosine. The activity of 3′-nucleotidase increased before cell multiplication resumed (Table 2). The data suggest that the loss of enzyme activity was caused by degradation or inactivation and not simply by a cessation of new enzyme synthesis. In contrast, protozoa from control cultures lacking adenosine showed a further increase in 3′-nucleotidase activity. Changes in 5′-nucleotidase and acid phosphatase activities paralleled those in 3′-nucleotidase activity but were much smaller. Cycloheximide prevented the loss of 3′-nucleotidase activity from purine-starved cells, which were expressing high levels of this activity, upon transfer to purine-replete medium.

These results support a role of trypanosomatid 3′-nucleotidase in the salvage of exogenous preformed purines. Under specific conditions of purine starvation, *C. fasciculata* contains increased levels of 3′-nucleotidase activity that would enable the organism to acquire purine nucleosides after hydrolysis of available extracellular 3′-nucleotides. Alternatively, if sufficient quantities of adenosine are present for growth, the 3′-nucleotidase activity is not required and therefore the level of its activity may be reduced. Purine starvation of *C. fasciculata* is accompanied by a five- to sixfold increase in adenine transport (*10*), and increases of this magnitude were observed in the levels of activity of two other crithidial membrane enzymes, 5′-nucleotidase and acid phosphatase. However, such increases are small in comparison to the increase in the level of 3′-nucleotidase activity. Initial results have shown that purine-starved cells which were 300-fold more active with respect to 3′-nucleotidase activity than purine-replete cells were 40-fold more active in the uptake of isotopically labeled adenosine. Further studies should help to determine the regulation of the 3′-nucleotidase, nucleoside transport, and intracellular metabolism of purines in *C. fasciculata*. These data, however, do not establish a mechanism by which 3′-nucleotidase activity levels are controlled in *C. fasciculata*, and it is not known whether they apply to other trypanosomatids, although initial results indicate that promastigotes of *L. donovani* do show increased levels of 3′-nucleotidase activity upon transfer to purine-depleted medium.

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Selective Loss of a Family of Gene Transcripts in a Hereditary Murine Cataract

Abstract. *The eye lens of the Fraser mouse contains a dominantly inherited cataract with reduced amounts of seven distinct but homologous γ crystallins encoded by a family of γ-crystallin genes. The results of experiments with cultured lenses, cell-free RNA translation, and Northern blot hybridization indicated a specific loss of the family of γ-crystallin messenger RNA's in the Fraser mouse lens. Southern blot hybridization of genomic DNA's from normal and Fraser mice showed no differences in γ-crystallin coding sequences.*

The ocular lens is a transparent tissue consisting primarily of elongated fiber cells containing specialized proteins called crystallins (*1*). The crystallins account for approximately 90 percent of the soluble protein of the mammalian lens and comprise three antigenically distinct families (α, β, and γ) of related polypeptides with molecular weights between 18,000 and 32,000 (*2*). Hereditary cataracts (lens opacities) are useful models for investigating possible connections among defects in crystallin gene expression, lens opacification, and spatial distributions of different crystallins within the lens. For example, the Philly mouse, which bears a hereditary cataract (*3*), develops an opacity approximately 4 weeks after birth and has a selective deficiency in a β-crystallin polypeptide (β27) preceding cataract formation (*4*). The Fraser cataract (*5*) (Cat^{Fr}), another murine hereditary cataract, begins to show lens deterioration between days 10 and 14 of the mouse's intrauterine life (*6*). Studies have indicated that the Cat^{Fr} lens is deficient in γ crystallin (*7*), and two-dimensional (2D) electrophoresis (*8*) has revealed that this deficiency involves every member of this family of gene products (*9*). Our investigation shows that, in the Cat^{Fr} lens, there is a loss of γ-crystallin synthesis accompanied by a selective loss of the family of messenger RNA's (mRNA's) that encodes them.

Our experiments were designed to determine whether the deficiency of γ crystallins is caused by a nonrecurring event early in development or whether it results from an ongoing process. We compared the net incorporation of [^{35}S]methionine into γ crystallins in cultured normal (+/+), heterozygote (c/+), and homozygote (c/c) Cat^{Fr} lenses of neonatal and 40-day-old mice. Total soluble lens proteins were fractionated by 2D electrophoresis on polyacrylamide gels (*9*) and then subjected to fluorography. There was a specific decrease in label incorporation into γ crystallins in the lenses of both neonatal and 40-day-old mutant mice (Fig. 1). The c/+ lenses showed a smaller reduction in labeled γ crystallin than did the c/c lenses. All γ crystallins

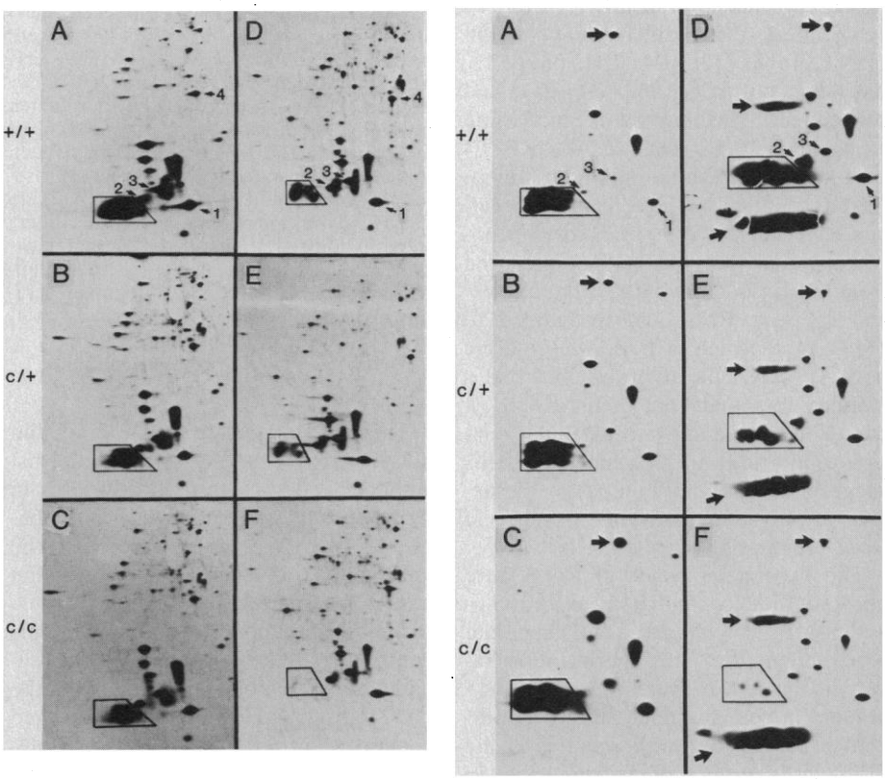
in normal lenses could be detected in the fluorograph of mutant lens culture products. There were no other reproducible differences in the fluorographs among the genotypes at either age.

We confirmed the visual impression gained from the gels by quantitative methods. After alignment of the gels with their fluorographs, the γ crystallins, α crystallins (αA_2, $\alpha\beta_2$, and αA^{ins}), and an unidentified noncrystallin protein (Fig. 1) were eluted from the gels overnight at 37°C in 90 percent (by volume) NCS solubilizer (Amersham) and assayed for radioactivity by scintillation counting. The ratio of label in the total α crystallins to that in the noncrystallin protein was similar in the +/+ and c/c lenses from the neonatal and 40-day-old mice. In contrast, for both ages examined, the amount of label incorporated into γ crystallins from c/c lenses was one-third of that incorporated into γ crystallins from +/+ lenses (expressed as the ratio of label in the γ crystallins to that in the α crystallin or in the noncrystallin protein).

To test the possibility that the reduced amounts of labeled γ crystallin in the mutant lenses were due to preferential leakage of these proteins, we analyzed the culture media by 2D electrophoresis and fluorography. There was no evidence of leakage of any labeled crystallin from the cultured lenses, although several high molecular weight noncrystallin lens proteins common to all genotypes were detected in the media.

We next determined whether the deficiency in net accumulation of γ crystallins in the mutant lenses was caused by depressed translation of γ-crystallin mRNA's. Cell-free translation experiments were conducted with rabbit reticulocyte lysate and total RNA prepared from +/+, c/+, and c/c lenses. The proteins synthesized were examined as before by 2D electrophoresis and fluorography (Fig. 2). In contrast to the results from cultured neonatal lenses, the lens RNA from the three genotypes of neonatal mice directed the synthesis of similar amounts of γ crystallin (Fig. 2, A to C). The RNA preparations from 40-day-old c/+ and c/c lenses, however, synthesized less γ crystallin than did that from the 40-day-old +/+ lens (Fig. 2, D to F) and showed dose dependence; the c/c lens RNA synthesized less γ crystallin than did the c/+ lens RNA. The results were measured by elution and scintillation counting. In neonatal mice, the ratio of synthesis of the γ-crystallin to the α-crystallin polypeptides was only 10 percent lower in the c/+ and c/c lenses than in the +/+ lenses. In contrast, in 40-day-old mice the γ:α ratio was 35 percent lower in the c/+ lenses and 67 percent lower in the c/c lenses than in the +/+ lenses.

We next determined the relative amounts of crystallin mRNA's in the lenses of the three genotypes by Northern blot hybridization with cloned murine complementary DNA's (cDNA's) for αA_2 (*10*), β23 (*11*), and γ (*12*) crystallin. The αA_2 cDNA (pMαACr1) hybridizes to the αA_2 and αA^{ins} mRNA's, and the β23 (pMβCr1) cDNA hybridizes specifically to its own mRNA. The γ cDNA ($pM\gamma_2Cr1$) cross-hybridizes with at least four other γ-crystallin mRNA's at 60°C in 0.1× standard saline citrate (SSC) (*12*). The cDNA's hybridized to RNA's of the proper size, as determined previously (*10–12*), thus showing their specificity (Fig. 3). The 40-day-old c/c lenses had less γ-crystallin mRNA than did the +/+ lenses for an equivalent amount of total RNA examined (Fig. 3A). An inter-



Figs. 1 and 2. Fluorographs of the 2D gel separations of total soluble proteins in lens explant cultures (Fig. 1) and of the products of translation of total RNA (Fig. 2) from +/+, c/+, and c/c lenses from neonatal (A, B, and C) and 40-day-old mice (D, E, and F). The boxed areas delimit the γ crystallins. The arrows in (A) and (D) indicate αA_2 (1), $\alpha\beta_2$ (2), αA^{ins} (3), and an unidentified noncrystallin protein (4). The acidic region of the gels is to the right of each panel; the basic region is to the left. Fig. 1 (left). Approximately 100,000 count/min was subjected to electrophoresis. In (E), a slight cathodic drift occurred on the acidic side of the gel; however, all proteins are present despite their slightly altered positions. Lenses were dissected free of adhering material and transferred to culture media under aseptic conditions. Dulbecco's α–minimum essential medium (*24*) lacking unlabeled methionine and supplemented with Hepes buffer (25 m*M*, *p*H 7.4) and [^{35}S]methionine (50 μCi/ml) was used to culture the lenses. Single lenses were cultured for 24 hours in 400 μl of medium at 37°C with gentle shaking. After incubation, the medium was removed from the lenses and retained. The lenses were extracted in lens-solubilizing buffer [50 m*M* tris-HCl (*p*H 7.0), 100 m*M* KCl, 5 m*M* EDTA, and 10 m*M* β-mercaptoethanol] as described (*9*). Two dimensional electrophoresis was performed as described (*9*). Gels were prepared for fluorography (*25*) with SB-5 film (Eastman Kodak). Fig. 2 (right). Approximately 50,000 count/min was subjected to electrophoresis. Endogenous protein synthesis of the reticulocyte lysates is indicated by large arrows. Only those regions of the fluorographs showing labeled proteins are depicted. Total lens RNA was prepared from 4 to 10 lenses of each genotype. These were homogenized in 400 μl of a solution containing equal volumes of 0.1 m*M* tris-HCl (*p*H 8.8) and buffer-saturated phenol. The aqueous phase was collected after centrifugation; the phenol phase was extracted twice more with 200 μl of fresh buffer. The RNA was precipitated at −20°C overnight by addition of 0.1 volume of 2.0*M* potassium acetate and 2 volumes of 95 percent ethanol. The nucleic acid centrifugate was dissolved in 100 μl of water, and an equal volume of 5*M* NaCl was added. After refrigeration overnight, the fraction that was insoluble in 2.5*M* NaCl was subjected to another water-solubilization and salt-precipitation. Cell-free translation was performed in micrococcal nuclease–treated rabbit reticulocyte lysates prepared as described (*26*). Various amounts (2 to 12.5 μg) of total lens RNA were translated in a final volume of 25 μl. These samples were incubated at 37°C for 90 minutes and then subjected to electrophoresis as described above.

mediate reduction in γ-crystallin mRNA was evident in the 40-day-old c/+ lenses. Hybridization of RNA to the αA_2 and β23 cDNA probes showed no differences among genotypes (Fig. 3, B and C).

Neonatal lens RNA's were also examined. Mixed α-crystallin and γ-crystallin probes were hybridized simultaneously to the blots (Fig. 3D). Samples of 40-day-old lens mRNA's were placed in the adjacent lanes. The α-crystallin and γ-crystallin mRNA contents of neonatal lenses appeared similar for all genotypes. Hybridization of mRNA's on rep licate filters to five different γ-crystallin cDNA probes (*12*) (pMγ$_1$Cr1, pMγ$_1$Cr2, pMγ$_2$Cr1, pMγ$_3$Cr1, and pMγ$_4$Cr1) and subsequent washing with increasing stringencies (0.1× SSC at 50° to 70°C) gave the same results (data not shown). Results identical to those from the previous experiment were obtained with polyadenylated [poly(A)$^+$] RNA prepared from equivalent amounts of total +/+ and c/c lens RNA and hybridized to pMγ$_2$Cr1. A trace of both +/+ and c/c poly(A)$^-$ RNA did hybridize but had a mobility less than that of full-length γ-crystallin mRNA and was in similar relative proportions in all genotypes. This suggests no gross differences in the degree of polyadenylation in +/+ and c/c RNA preparations.

The hybridized bands of RNA were cut from the blots and assayed for radioactivity. In an average of three separate experiments, the γ:α hybridization ratios in c/c lenses were reduced by 16 percent in neonatal mice and by 51 percent in 40-day-old mice relative to the +/+ lenses in mice of the same age. In one experiment the γ:α hybridization ratio was 41 percent lower in the c/c lenses than in the +/+ lenses of 20-day-old mice. These results agree well with the cell-free translation experiments, indicating selective and progressive loss of γ-crystallin mRNA during cataract formation in the Cat^{Fr} mouse.

Finally, Southern blot analyses of genomic DNA were performed to search for possible abnormalities in the γ-crystallin genes of the Cat^{Fr} mouse. No differences were detected in blots when liver DNA's of 40-day-old normal Swiss Webster and Cat^{Fr} mice were digested with Eco RI, Bam HI, or Hind III and hybridized with αA_2-crystallin, β23-crystallin, or γ-crystallin (pMγ$_2$Cr1) cDNA probes (data not shown). A com parison of Hpa II and Msp I digests (*13*) of liver and lens DNA of both +/+ and c/c mice indicated that the γ-crystallin genes are hypomethylated in the lens. However, DNA from normal and mutant mice showed no differences.

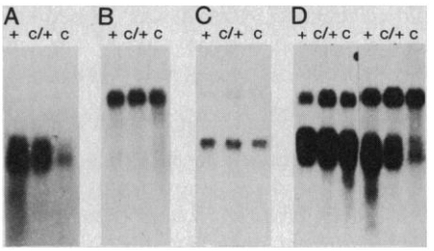


Fig. 3. Northern blot analyses of RNA preparations from lenses from neonatal and 40-day-old mice of each genotype. The RNA's from 40-day-old lenses were probed with pMγ$_2$Cr1 (A), pMαACr1 (B), and pMβCr1 (C). (D) The RNA's from 1-day-old lenses (three left lanes) and 40-day-old lenses (three right lanes) were probed with both pMαACr1 and pMγ$_2$Cr1. The autoradiographs were washed at 60°C. Total RNA was prepared from four to ten pooled lenses of each genotype as described (see legend to Fig. 2). A portion (2 μg) of each sample was separated on 1.5 percent agarose-formaldehyde gels (*27*) and transferred (*28*) to Pall Biodyne membrane. Replicate filters were hybridized to purified (*29*) and nick-translated (*30*) cDNA inserts (see text). Filters were washed in 2× SSC at room temperature and again in 0.1× SSC at either 50°, 60°, or 70°C before autoradiography.

These data indicate that γ-crystallin mRNA is present in nearly normal amounts and in a translatable form in lenses of Cat^{Fr} mice at birth. It is probable that the Cat^{Fr} mutation leads to the inefficient translation of γ-crystallin mRNA's and ultimately, in older lenses, to their degradation. We assume that this contributes to the deficiency in net accumulation of soluble γ crystallins in the Cat^{Fr} lens (*7*). However, an increased conversion of γ crystallins to an insoluble form could also contribute to this deficiency.

Inefficient translation has been proposed as a cause for the initial reduction in crystallin synthesis in the osmotic cataracts of the Philly and Nakano mice (*14*) and of the galactosemic rat (*15*). Experiments indicate that ionic imbalance (*16*) and loss of free amino acids (*17*) contribute to the reduced translation of crystallin mRNA's in these osmotic cataracts. Although the Cat^{Fr} lens has not been established as an osmotic cataract, the edema and swelling of the embryonic lens fiber cells as the cataract develops suggest this possibility (*6*). Loss of the γ-crystallin mRNA correlates in time with destruction of the central lens fiber cells in the Cat^{Fr} mouse (*7*). Because γ crystallin is the principal protein accumulated in the central fiber cells (*15, 18*), it is reasonable to expect that γ-crystallin mRNA would be degraded as these cells are destroyed. The more limited degeneration of central lens fibers in the lens of the Cat^{Fr} heterozygote showed an intermediate amount of γ-crystallin mRNA in the 40-day-old mouse.

Synthesis of γ crystallin appears to be lost more selectively in the Cat^{Fr} lens than in the Philly (*3, 14*), Nakano (*14*), and galactose (*15*) cataracts. This is probably explained by the preferential destruction of the central fiber cells of the Cat^{Fr} lens (*6, 7*). The longer retention of inactive crystallin mRNA's in the Philly and Nakano lenses (*14*) is probably due to the later onset and slower progression of these hereditary cataracts (*6, 19*). Synthesis of γ crystallin is selectively reduced in a rat nuclear hypocholesteremic cataract that is associated with ionic imbalances, as are osmotic cataracts (*20*).

Another mechanism we cannot rule out is that the Cat^{Fr} mutation affects the transcription of the γ-crystallin genes or the processing of their transcripts (or both). Because it appears that the γ-crystallin genes are closely linked (*21*), there is a possibility of some regulatory mutation affecting γ-crystallin gene expression. Another dominantly inherited cataract, Elo (eye lens obsolescence), is associated with decreased γ-crystallin content of the developing lens and a cataract (*18, 22*). This mutation has been shown to be closely linked to the γ-crystallin genes (*23*).

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Behavioral Facilitation of Reproduction in Sexual and Parthenogenetic *Drosophila*

Abstract. *In a normal bisexual laboratory strain of* Drosophila mercatorum, *females housed with either fertile or sterile males lay more eggs than do females housed in pairs or as isolates. Females of a derived parthenogenetic strain have suffered genetic loss of this behavioral facilitation of egg production, a loss comparable to the loss of sexual receptivity. Despite these losses there has been a large increase in fecundity in the parthenogenetic strain. These findings are compared with those in a parthenogenetic lizard.*

Morphological, physiological, and behavioral components of reproduction coevolve under different selection pressures. In vertebrates, the presence and behavior of the male facilitates ovarian growth in the conspecific female. In the green anole lizard, *Anolis carolinensis*, courtship behavior of the male is critical for normal pituitary gonadotropin secretion in the female (*1*). Behavioral facilitation of reproduction is also seen in unisexual lizards. The whiptail lizard, *Cnemidophorus uniparens*, reproduces parthenogenetically and consists only of female individuals. Individuals perform both complementary male-like and femalelike displays similar to the displays seen during courtship and copulation in closely related sexual species (*2*). The presence and behavior of conspecifics have been experimentally shown to facilitate reproduction in this unisexual lizard (*3*).

We studied the effect of different "social" conditions on reproduction in both sexual and parthenogenetic strains of *Drosophila mercatorum*. We present evidence for a measurable behavioral facilitation of egg-laying in sexual *D. mercatorum* and show that females of this strain of parthenogenetic flies have lost the behavioral facilitation of oviposition characteristic of their bisexual ancestor. There is, however, an increased oviposition rate that does not depend on social factors.

Individual virgin females of a sexual strain of *D. mercatorum*, denoted S (H62.60 LaPalma, El Salvador), and a parthenogenetic strain derived from it by artificial selection in 1961 (S-1) were separately exposed to one of the following social conditions (*4*): housing in isolation, housing with another female, housing with one sterile (XO) male, housing with two sterile males, housing with one fertile (XY) male, and housing with two fertile males. Eggs produced by each female were counted on a daily basis for four consecutive days (*5*).

In the sexual strain, virgin females housed with males laid the most eggs ($\chi^2(9) = 28.01$, $P < 0.001$); females housed with males produced 13 times more eggs than isolated females and 6 times more than females housed in pairs (Table 1). Although isolated virgin females had fully developed ovaries, they deposited eggs only sporadically (*6*). Females housed together laid more eggs per female than isolated females ($\chi^2(7) = 47.71$, $P < 0.01$) (Fig. 1). Courtship behaviors, not copulatory stimuli, from the male may be responsible for this facilitation of reproduction, but this is not yet known. Analysis with 2 by 2 contingency tables shows that egg production in females with sperm in the seminal receptacle was not statistically different from that of unfertilized females with males ($t(18) = 0.64$, $P > 0.05$) (*7*). Further, there was no statistically significant difference among females housed with sterile males compared with fertile males ($t(38) = 0.25$, $P > 0.05$) (*8*). The average daily egg production of females housed with two males, fertile or sterile, was less than that of females housed with one male (44.65 ± 2.19 and 53.55 ± 2.92, respectively; $t(38) = 2.44$, $P < 0.02$). Aggression between males may inhibit ovarian activity in the female, as has been shown with the green anole lizard (*9*).

There was no effect of social condition on egg production in the parthenogenetic strain ($F(5, 231) = 0.19$, $P > 0.05$) (Table 1 and Fig. 1). Parthenogenetic female isolates laid approximately 15 times more eggs than sexual female isolates (mean, 55.9 eggs per day compared with 3.8 eggs). Mean egg production by females of the parthenogenetic strain S-1 when first reported in 1962, 6 months after the origin of the strain, was 6.1 eggs per day; about 1 year later it was 10.4 eggs per day (*10*). Thus, the current rate is more than five times greater than the rate observed in 1963. This is a significant change in egg production, presumably resulting from natural selection for fecundity in the stock over the 23 years of its existence. This change is especially noteworthy in view of the fact that the parthenogenetic rate of the S-1 stock (number of impaternate females per unabout 2 percent since the strain was established (*11*). Accordingly, the S-1 stock has increased its egg production but suffered a loss in two behavioral traits, facilitation of egg production (our data) and sexual receptivity (*12*).

Carson *et al.* (*12*) have observed that individuals of the S-1 parthenogenetic strain of *D. mercatorum* are less likely to mate with males than are females of the sexual strain from which it arose. This loss of sexual receptivity was interpreted as evidence for the disappearance of a genetic trait (sexual receptivity) due to the absence of a selective force (the need for males for reproduction). The difference between parthenogenetic whiptail lizards, which continue to exhibit both sexual behavior and behavioral facilitation of reproduction, and parthenogenetic *D. mercatorum* may lie in the nature of the chromosomal origin of the respective species. *Cnemidophorus uniparens* is a