Central Role for the Lens in Cave Fish Eye Degeneration

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Astyanax mexicanus is a teleost with eyed surface-dwelling and eyeless cave-dwelling forms. Eye formation is initiated in cave fish embryos, but the eye subsequently arrests and degenerates. The surface fish lens stimulates growth and development after transplantation into the cave fish optic cup, restoring optic tissues lost during cave fish evolution. Conversely, eye growth and development are retarded following transplantation of a surface fish lens into a cave fish optic cup or lens extirpation. These results show that evolutionary changes in an inductive signal from the lens are involved in cave fish eye degeneration.

The evolution of development is usually studied in distantly related species (*1, 2*). This approach has revealed conserved genes with universal developmental roles, but large evolutionary distances have obscured the mechanisms that generate morphological diversity. The evolution of development in the teleost *Astyanax mexicanus*, a single species with eyed surface-dwelling (surface fish) and eyeless cave-dwelling (cave fish) forms, has also been studied (*3, 4*). The eyed and eyeless forms of *Astyanax* probably diverged from a common ancestor within the past million years (*5, 6*). Here, we show that evolutionary changes in an inductive signal from the lens are involved in cave fish eye degeneration.

Although adult cave fish lack functional eyes, eye formation is initiated during embryogenesis (Fig. 1, A and B). The lens vesicle is formed but later degenerates, and the cornea, iris, and other optic tissues are absent or rudimentary (Fig. 1, C and D) (*7, 8*). The optic cup and neural retina are formed in cave fish, but the retinal layers are disorganized, growth is retarded, and photoreceptor cells do not differentiate. The degenerate eye sinks into the orbit and is covered by a flap of skin. Constructive changes have also evolved in cave fish, including enhanced lateral line and gustatory systems (*4, 9*).

Cave fish lens cells undergo apoptosis before the arrest of eye development (*3*). Because extensive apoptosis has not been detected in other eye tissues at this time, lens apoptosis may be an important factor in eye degeneration. Alternatively, lens apoptosis and eye degeneration could be controlled by a signal from the optic cup, which is diminished in cave fish (Fig. 1, A and B). To determine the control of lens apoptosis, we transplanted a surface fish lens vesicle into a cave fish optic cup and vice versa (Fig. 2A) (*10*) and assayed for apoptosis by TUNEL (terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick-end labeling) (*11*). The lens vesicle of a donor embryo was transplanted into the optic cup on one side (transplant side) of a host embryo after removal of the host lens vesicle. The eye primordium on the other side (control side) served as a control. In another control, a surface fish or cave fish lens vesicle was transplanted into a surface fish or cave fish optic cup, respectively. Eyes typical of the respective hosts were formed in the latter control, showing that the operations do not affect optic development. The cave fish lens underwent apoptosis on schedule in the surface fish host (Fig. 2, F and G). Conversely, the surface fish lens vesicle did not undergo apoptosis and formed a differentiated lens in the cave fish host (Fig. 2, D and E). Thus, apoptosis is controlled autonomously within the cave fish lens vesicle.

Eye development was stimulated on the transplant side of the cave fish host (Fig. 3, A to D) (*12*). Although no differences were apparent after 48 hours (Fig. 3A), a larger eye was detected on the transplant side after 8 days of development. After 2 months, a large eye (restored eye) with a distinct pupil was present on the transplant side, whereas the eye had degenerated and sunk into the orbit on the control side (Fig. 3, B and C). Sections of the restored eye showed an anterior chamber, cornea, iris, and lens (Fig. 3D). The epithelial cells of the cornea and lens expressed the transcription factor Pax6 (*13*), which is absent in the area of the cave fish eye that corresponds to the cornea (Fig. 4, A to C) (*14*). The following experiment was performed to be certain that the rescued eye parts were derived from host rather than donor tissues. A surface fish lens vesicle labeled with fluoresceinated lysine-fixable dextran (FLDX) was transplanted into an unlabeled cave fish optic cup, and the host was allowed to develop until a large eye formed on the transplant side. Sectioning showed that FLDX labeling was restricted to the lens, indicating that the restored eye parts were derived from the cave fish (Fig. 2, B and C). The results show that a surface fish lens can induce the development of anterior eye parts that have been lost during cave fish evolution.

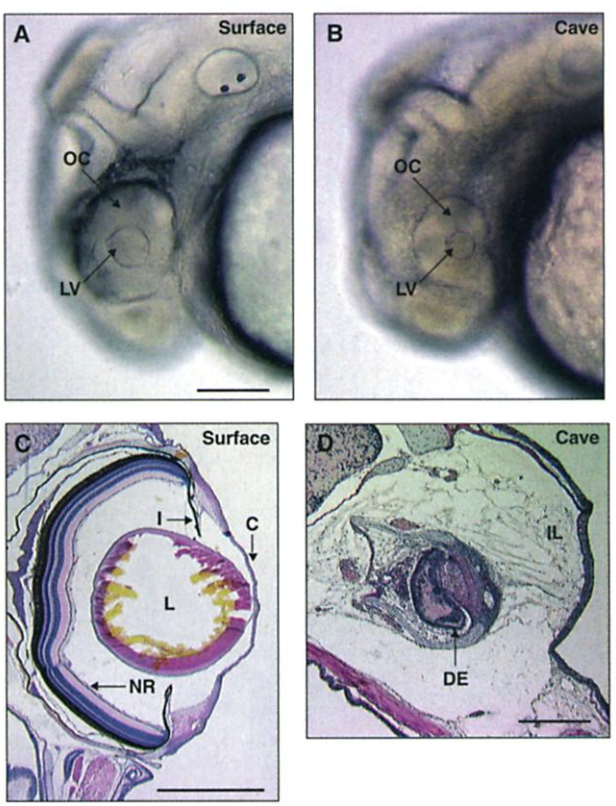

Fig. 1. Eye development and degeneration. Developing eye primordia of 24-hour-old (**A**) surface fish and (**B**) cave fish embryos. Scale bar, 150 μm; same magnification in (A) and (B). Sections of (**C**) adult surface fish and (**D**) cave fish eyes. Scale bars in (C) and (D) are 500 and 180 μm, respectively. C, cornea; DE, degenerate eye; I, iris; IL, integument layer covering degenerate eye; L, lens; LV, lens vesicle; NR, neural retina; OC, optic cup.

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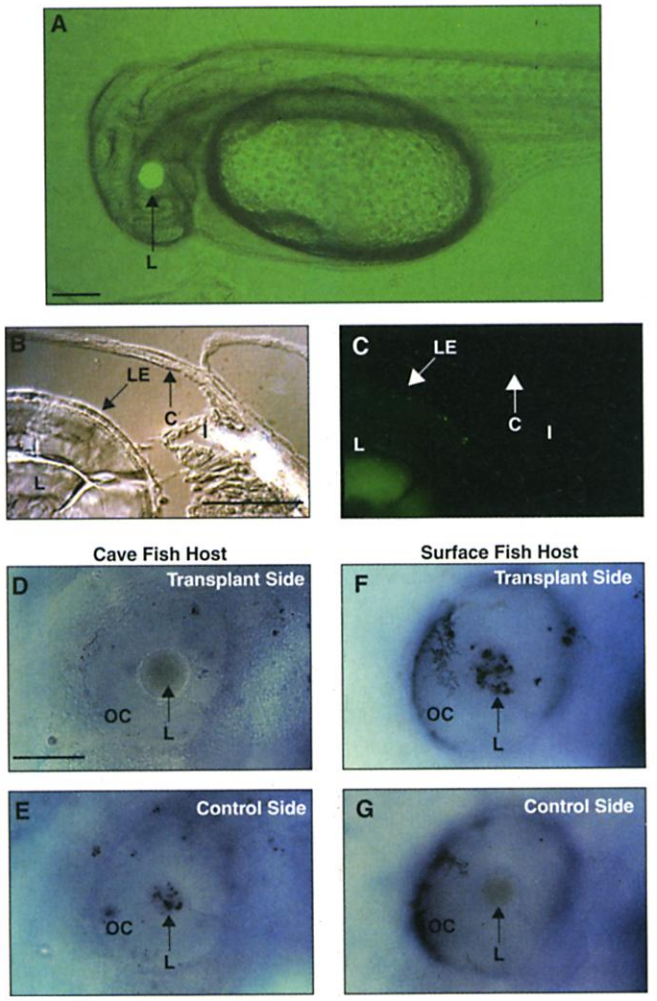

Fig. 2. Lens vesicle transplantation and apoptosis. (A to C) Lens transplantation. (**A**) One-day-old cave fish embryo containing an FLDX-labeled surface fish lens. Scale bar, 150 μm. (**B**) Bright and (**C**) dark field images of sections showing FLDX restricted to the lens. Scale bar, 40 μm; same magnification in (B) and (C). A one-cell surface fish embryo was microinjected with FLDX (molecular weight of 10,000) (Molecular Probes, Eugene, Oregon), and the FLDX labeled lens was transplanted into the optic cup of an unlabeled cave fish embryo and allowed to develop for 10 days. The cave fish host was fixed in 4% paraformaldehyde and viewed by fluorescence microscopy. (**D** to **G**) Apoptosis. TUNEL of the optic primordia of a 48-hour-old cave fish host showing lens apoptosis on (E) the control but not on (D) the transplant side. TUNEL of the optic primordia of a 48-hour-old surface fish host showing lens apoptosis on (F) the transplant but not on (G) the control side. Scale bar, 100 μm; same magnification in (D) to (G). LE, lens epithelium. Other abbreviations are as in Fig. 1.

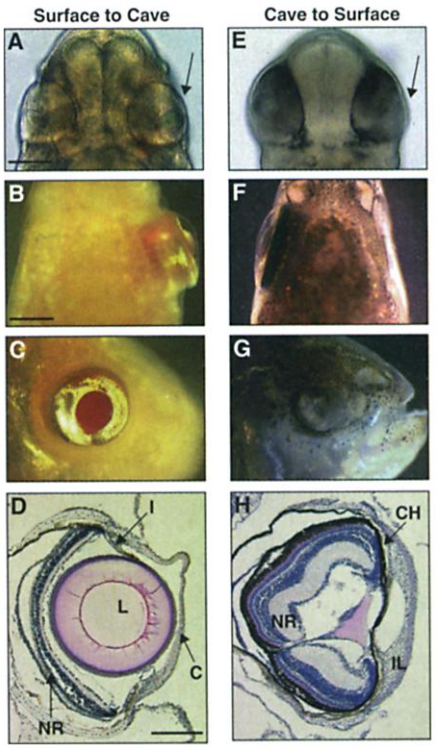

Fig. 3. Eye development following lens transplantation. Head regions of (**A** to **D**) cave fish or (**E** to **H**) surface fish hosts shown after 2 days (A and E) and 2 months (B to D and F to H) of development. (A and E) Ventral views. (B and F) Dorsal views. (C and G) Lateral views. Transplant side is on the right in (A), (B), (E), and (F) [arrows in (A) and (E)]. Scale bar in (A) is 150 μm, and the scale bar in (B) is 1 mm; the magnification is the same in (A) and (E), (B) and (F), and (C) and (G). (D and H) Sections through restored (D) and degenerate (H) eyes in cave fish and surface fish hosts, respectively. Scale bar in (D) is 150 μm; the magnification is the same in (D) and (H). CH, pigmented choroid layer. Other abbreviations are as in Fig. 1.

Neural retina development was determined by examining the expression of Pax6, Prox 1, proliferating cell nuclear antigen (PCNA), and rhodopsin, which are retinal markers for ganglion and amacrine cells, horizontal cells, photoreceptor precursor cells, and rod cells, respectively (*9, 14, 15*). Pax6, Prox 1, and PCNA were expressed in the appropriate retinal cells in both surface fish and cave fish [Web fig. 1 (*16*)], although the retinal layers were disorganized and only a few rod cells were present in cave fish (Fig. 4, E and F). In contrast, the restored eye exhibited well-organized layers of neuronal and glial cells, as well as a layer of rhodopsin-expressing rod cells (Fig. 4G). The teleost retina grows throughout life by adding new cells from the ciliary marginal zone (CMZ), which exhibits intense Pax6, Prox 1, and PCNA expression (*9, 15, 17, 18*). These markers were detected in the CMZ of the restored eye [Web fig. 1 (*16*)], implying that the retina is actively growing. Thus, the surface fish lens can promote normal patterning, growth, and rod cell differentiation in the cave fish retina.

The effect of the cave fish lens on surface fish eye development was determined by reciprocal lens transplantations (Fig. 3, E to H) (*19*). After 2 months of development, adult hosts exhibited a normal eye on the control side and a small eye (degenerate eye) on the transplant side (Fig. 3, F and G), which failed to develop a pupil, cornea, anterior chamber, and iris (Fig. 3H). As determined by the retinal markers [Fig. 4H and Web fig. 1 (*16*)], retinal cell differentiation occurred in the degenerate eye; however, the retina was small and distorted (Fig. 3H) in relation to its counterpart on the control side. The same results were obtained following extirpation of the surface lens vesicle. Although lens modification cannot completely account for the cave fish retinal phenotype, the results show that the cave fish lens has lost the ability to promote eye development.

We have shown that the surface fish lens is sufficient to rescue eye development after transplantation into the cave fish eye primordium. We conclude that a change in an inductive signal emanating from the lens is a major cause of eye regression in cave fish (*20*). Our results illustrate how an evolutionary modification in embryonic induction can result in dramatic changes in adult morphology.

References and Notes

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10. Surface fish, originally collected at Balmorhea State Park, TX, and cave fish, collected at Cueva de El Pachón, Tamaulipas, Mexico, were maintained at 25°C on a photoperiod consisting of 14 hours of light and 10 hours of darkness. For lens transplantation or extirpation, 24-hour-old (34- to 38-somite) embryos were washed for 30 min in calcium-free zebrafish

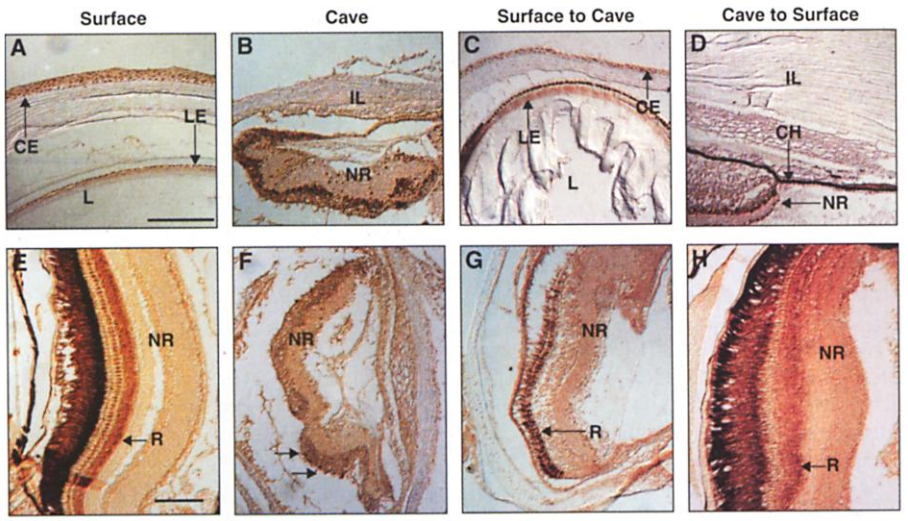

Fig. 4. Cornea and retina development following lens transplantation. Sections through the (**A** to **D**) anterior sector and (**E** to **H**) retina of a surface fish (A and E), a cave fish (B and F), a cave fish host with a transplanted surface fish lens (C and G), and a surface host with a transplanted cave fish lens (D and H). CE, corneal epithelium; R, rhodopsin-positive rod cells. Other abbreviations are as in Figs. 1 through 3. Arrows in (F) indicate a small number of cells expressing rhodopsin in the cave fish retina. Scale bar in (A) is 150 μm, and the scale bar in (E) is 100 μm; magnification is the same in (A) to (D) and in (E) to (H).

ringer (CFZFR) [116 mM NaCl, 2.9 mM KCl, and 10 mM Hepes (pH 7.2)] containing 0.2% EDTA, rinsed in CFZFR (40°C), and embedded in 1.2% agar in CFZFR (40°C). After cooling to room temperature, individual embryos were cut into agarose blocks. The operations were done with sharp tungsten needles in agarose blocks arranged side by side in CFZFR. The host embryos were grown to adults under the normal photoperiod.

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12. In 81 transplantations, 38 (49%) of the hosts survived. Nineteen (50%) of the survivors showed large external eyes on the transplant side, and the remainder showed eyes buried in the skin, although they were larger than those on the control side.
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14. Specimens were fixed in 4% paraformaldehyde, embedded in Paraplast, and sectioned at 8 μm. Sections were microwaved in 10 mM citric acid at pH 6.0 (three times) for 5 min to expose the antigens before immunostaining (9). Pax6 antibody was purchased from Babco (Richmond, CA), PCNA antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA), and rhodopsin antibody was purchased from Leinco Technologies (St. Louis, MO). S. I. Tomarev provided Prox 1 antibody. For primary incubations, the Pax6, Prox 1, and PCNA antibodies were diluted 100:1, and the rhodopsin antibody was diluted 50:1.
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19. Of 31 transplantations, 15 (44%) of the hosts survived. Thirteen (87%) of the survivors showed small degenerate eyes on the transplant side, and the remainder showed no effects on eye development.
20. Our results are relevant only to the Pachón cave fish studied here, one of at least 30 *Astyanax* cave fish populations that may have evolved the eyeless phenotype by different mechanisms (*4*, *6*).
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Synaptic Integration Mediated by Striatal Cholinergic Interneurons in Basal Ganglia Function

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The physiological role of striatal cholinergic interneurons was investigated with immunotoxin-mediated cell targeting (IMCT). Unilateral cholinergic cell ablation caused an acute abnormal turning behavior. These mice showed gradual recovery but displayed abnormal turning by both excess stimulation and inhibition of dopamine actions. In the acute phase, basal ganglia function was shifted to a hyperactive state by stimulation and suppression of striatonigral and striatopallidal neurons, respectively. D1 and D2 dopamine receptors were then down-regulated, relieving dopamine-predominant synaptic perturbation but leaving a defect in controlling dopamine responses. The acetylcholine-dopamine interaction is concertedly and adaptively regulated for basal ganglia synaptic integration.

The basal ganglia subserve motor and cognitive functions (*1–5*), and damage to this structure leads to abnormalities such as Parkinson's disease and Huntington's disease (*6–9*). In the basal ganglia circuit, cortical information reaches separate subpopulations of striatal γ-aminobutyric acid (GABA)–containing, medium-sized spiny neurons, which is then transmitted to substantia nigra pars reticulata (SNr)/entopeduncular nucleus (EPN) through two parallel routes named direct and indirect pathways (*6*, *10*, *11*). Striatonigral neurons in the direct pathway and striatopallidal neurons in the indirect pathway contain substance P (SP) and enkephalin (Enk), respectively. The two striatal principal neurons are thought to exert opposing effects upon the SNr/EPN neurons and the dynamic balance of basal ganglia–thalamocortical circuitry.

Activity of striatal principal neurons is modulated by dopaminergic and cholinergic inputs (*12–14*). Dopamine (DA) from substantia nigra pars compacta neurons excites and inhibits striatonigral and striatopallidal neurons, respectively. Acetylcholine (ACh) from striatal cholinergic neurons, opposing the DA action, inhibits and excites striatonigral and striatopallidal neurons, respectively (*13*, *15*, *16*). The modulatory role of DA transmission in the basal ganglia circuit has been well characterized (*12*, *17–20*), whereas the precise physiological and behavioral function of cholinergic neurons accounting for only 1 to 2% of the striatal neuronal

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