Why cavefish are blind

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Summary

Some fish exist as eyed, surface-dwelling and eyeless, cave-dwelling forms. The developmental processes that cause eye degeneration in different populations of *Astyanax* cavefish are similar. Although small optic primordia start to form, apoptosis of lens cells triggers developmental arrest and degeneration of the eyes. Degeneration has been linked to reduced expression of the transcription factor Pax6 in the anterior embryonic midline and optic primordia. Recently, Yamamoto and colleagues[1] reported that increased expression of the diffusible morphogen Sonic hedgehog (Shh) at the embryonic midline of cavefish reduces *pax6* expression and increases expression of Shh-regulated genes, which might confer selective advantages for life in caves. *BioEssays* 27:235–238, 2005. © 2005 Wiley Periodicals, Inc.

Introduction

Some animals that live in caves never venture out and are adapted to life in perpetual darkness. Many are not pigmented and blind.[2] For example, the teleost species *Astyanax mexicanus* (= *fasciatus*) comprises both eyed surface-dwelling (epigean) and numerous blind, eyeless cave-dwelling (troglomorphic) forms. A question that intrigued Darwin and many scientists since is why cave-dwelling forms do not develop eyes. The vertebrate eye has evolved to detect electromagnetic radiation with wavelengths visible to surface-dwellers and would, therefore, become redundant to members of a species that move into perpetual darkness. Redundant eyes might gradually degenerate because mutations preventing their development would no longer be selected against. Alternatively, losing the eyes might confer selective advantages on a cave-dwelling form. A major question puzzling researchers is how the molecular mechanisms controlling eye formation have evolved in epigean and troglomorphic forms of *A. mexicanus*.

The eyes of cavefish do start to form during embryonic development but, instead of developing normally, their primordia undergo developmental arrest and degeneration before finally sinking into the orbits where they are covered by skin. Degeneration is precipitated by apoptosis in the embryonic lens.[3,4] Genetic analyses indicate that eye degeneration involves multiple regulatory genes, including the well-known master regulator of eye development, *pax6*.[5–7] Changes in *pax6* expression precede the formation of small optic primordia and lens cell apoptosis. Until recently, it was unclear what induces apoptosis of the lens cells and whether mutations in regulatory genes upstream of *pax6* are the cause. Now, Yamamoto and colleagues[1] show that increased hedgehog (Hh) signaling at the anterior embryonic midline reduces *pax6* expression, promotes lens apoptosis, arrests eye growth and induces eye degeneration.

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Evidence for the importance of the lens in eye development

Normal lens development is crucial for the formation of the vertebrate cornea, pupil and iris, which are lacking in cavefish. Several years ago, Yamamoto & Jeffery[4] conducted elegant transplantation studies to decide where lens apoptosis and subsequent eye degeneration is controlled from; is it from the optic cup or from the lens itself? Transplantation of a lens vesicle from a cavefish embryo into the optic cup of a surface fish host embryo resulted in apoptosis. In contrast, transplantation of a surface fish lens into a cavefish optic cup prevented apoptosis, produced a differentiated lens and restored an eye made from cavefish tissue. This work demonstrated for the first time that the cavefish lens vesicle controls apoptosis autonomously.

These experiments also addressed the extent of eye restoration in cavefish that had been given surface fish lens vesicles. The development of the neural retina was compared between cavefish recipients of surface fish lenses and surface fish recipients of cavefish lenses using markers for ganglion and amacrine cells (*pax6*), horizontal cells (*prox1*), photoreceptor precursor cells (PCNA) and rod cells (rhodopsin). In adult cavefish recipients of surface fish lenses, the restored eye possessed an actively growing retina with normal laminar organization that differed from the disorganized and undersized retina of surface fish recipients of cavefish lenses. Nevertheless, restoration of an eye in cavefish does not restore their ability to respond to light.[8] The cavefish optic nerve is only partially developed and the optic lobes are reduced, suggesting that not all defects in the visual system of cavefish have their origin in defects of the lens.

In summary, the presence of a surface fish lens is sufficient to induce eye development in cavefish. Throughout evolution,

the ability of the cavefish lens vesicle to promote eye development has been lost, presumably through loss of an inductive signal, and changes outside the lens must also have occurred.

Evidence that changes in *pax6* expression precede eye degeneration in cavefish

The results of the lens transplantation experiments led researchers to focus on the expression of regulatory genes that are involved in lens development in the hope that a clear difference would be found between cavefish and surface fish. Initial expression analyses of *Prox1*, a homeobox gene involved in the differentiation of lens fiber cells, failed to reveal any differences.[9] *Prox1* expression appears after differences in optic vesicle size become apparent between cavefish and surface fish. This suggested that genetic changes might be occurring prior to optic primordia formation. Pax6 was an ideal candidate as it is expressed before the optic vesicles form. The *pax6* gene encodes an evolutionarily highly conserved transcription factor expressed in and essential for the development of the lens and retina of vertebrate embryos.[10–12] Loss-of-function mutations in *pax6* produce the *Small eye* phenotype in mice.[13] Heterozygotes show reduced Pax6 expression and microphthalmia, while homozygotes have no eyes at all. Recent experiments in mice showed that Pax6 is required autonomously by the surface ectoderm from which the lens and cornea develop.[14] Loss-of-function mutations in both copies of *pax6* specifically in this surface ectoderm allow lens development to begin but arrest it soon after and prevent normal retinal formation.

Previous experiments showed that *pax6* expression is reduced in the lens and optic vesicles of cavefish compared to surface fish.[1,3,7] In young surface fish embryos, bilateral *pax6* expression domains in the anterior neural plate join across the midline to demarcate the forebrain and optic primordia whereas the *pax6* expression domains of cavefish embryos are smaller and remain unfused leaving a gap in expression at the anterior midline (Fig. 1). At subsequent developmental stages, the *pax6* expression domain remains smaller in cavefish embryos than in surface fish. The reduction in *pax6* expression in the anterior neural plate is common to several independently derived cavefish populations, suggesting its importance in the evolution of eye degeneration. These findings still leave unanswered the question of whether lens apoptosis in cavefish is a direct or indirect consequence of changes in *pax6* expression.

Increased hedgehog signaling from the prechordal plate suppresses *pax6* expression in cavefish

The findings of Strickler and colleagues[7] also raised the question of what causes the reduction of *pax6* expression in cavefish. Experiments in *Xenopus* and chick demonstrate that signals from the prechordal plate (mesendoderm underlying the anterior neural plate and tube) suppress *pax6* expression in the overlying anterior neural plate and are crucial for resolving the retinal field into two separate optic primordia.[15] Work on several species of vertebrate has shown that hedgehog (Hh) proteins diffuse from the prechordal plate and repress *pax6* expression in nearby regions of the neural plate.[15–17] A likely cause of repressed *Pax6* expression was, therefore, enhanced Hh activity.

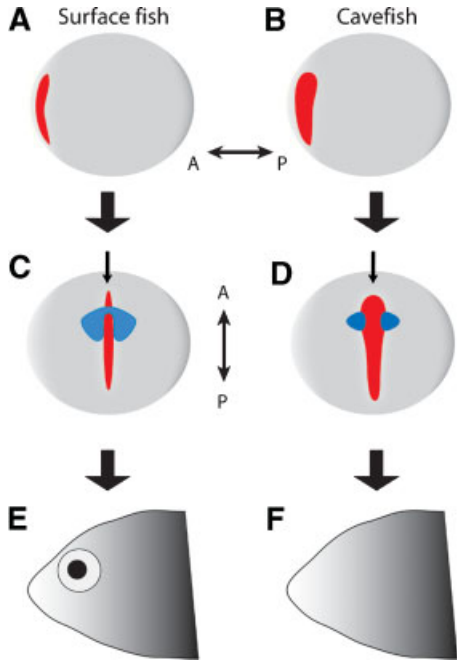

Figure 1. Schematic diagram showing the major differences in gene expression between surface and cavefish embryos. **A,B:** Lateral views of early tailbud embryos showing expansion of *Shh* (red) in cavefish. **C,D:** Dorsal views of late tailbud stage embryos showing *Shh* and *pax6* (blue) expression. **E,F:** The heads of adult surface fish and cavefish. Arrows in C, D indicate the embryonic midline. A, anterior; P, posterior.

In their recent paper, Yamamoto et al.[1] describe how early changes in eye morphogenesis in cavefish coincide with changes in the expression not only of *pax6* but also of the genes encoding transcription factors Pax2a and Vax1, whose domains in cavefish are larger than those in surface fish. The expression of *pax2* and *vax1* are also controlled by Hh signals emanating from the anterior embryonic midline.[18] Sonic hedgehog (Shh) specifies the optic stalk (future optic nerve) proximally by inducing its expression of *pax2*, which then represses *pax6* expression, limiting it to distal areas that will form the neural retina and retinal pigment epithelium.[17] To test directly the hypothesis that changes in Hh signaling are upstream of changes in *pax6, pax2a* and *vax1* expression in cavefish, *hh* expression was compared in surface fish and cavefish embryos. The expression domains of *shh* and

tiggy-winkle hedgehog (*twhh*) were expanded in the cavefish prechordal plate (Fig. 1A–D). The *shh*-expressing domain was wider in cavefish at the neural plate stage and extended further anteriorly and dorsally during optic vesicle formation. The expression of Hh downstream targets, such as the gene encoding the Shh receptor *ptc2* and the Hh-regulated transcription factor *nkx2.1a*, were expanded along the anterior embryonic midline, supporting the evidence for increased Hh signaling. The geographically separated populations of *Astyanax mexicanus* cavefish, which are thought to have evolved the eyeless phenotype separately,[5] all showed expansion of the *shh* expression domain, indicating that this phenomenon is not unique to one cavefish population.

To test whether *hh* expansion causes eye degeneration, *shh* and/or *twhh* mRNAs were injected into one side of a surface fish embryo to overexpress Hh protein. The resulting morphological and genetic changes mirrored those observed during cavefish eye degeneration. Importantly, apoptosis was detected in one or both lens vesicles in approximately 50% of *shh* mRNA-injected embryos, demonstrating that increased Hh signaling may account for lens apoptosis in cavefish. Treatment of cavefish embryos with the Hh inhibitor cyclopamine resulted in partial rescue, although eye development was never fully restored, possibly because the actions of Hh proteins required later in retinal development were blocked by cyclopamine.[19,20] These experiments indicate that an increase in *hh* expression lies at the root of eye degeneration in cavefish.

The evolution of eye loss

Cavefish have undergone constructive morphological changes that might compensate for blindness, such as an enhanced mechanosensory lateral line system, an enlarged jaw and the development of extra taste buds.[6,9,21–23] Multiple genes are responsible for cavefish taste bud expansion.[21,24,25] Shh, its receptor patched (Ptc) and the Shh-activated transcription factor Gli1 constitute a number of signaling molecules that are expressed in the developing taste papillae in mice.[26–29] However, it remains to be seen if the same is true in cavefish. Interestingly, an increase in taste bud number occurred in *shh*-injected surface fish embryos,[1] suggesting that Hh overexpression may account for the extra taste buds observed in cavefish relative to surface fish. This finding stresses the important point that Hh expression at the anterior embryonic midline controls more than just eye development[30] and that expansion of Hh expression in this region in cavefish will have had other developmental effects. Notably, Shh is involved in the morphogenesis and cell proliferation of vertebrate epithelial appendages, including the hair, teeth, taste buds and gut. If the eyes of cavefish degenerated because, under relaxed natural selection in perpetual darkness, loss-of-function mutations accumulated in one or more gene(s) that regulate Hh expression, then the effects of expanded Hh signalling on structures outside the eye presumably either conferred no selective disadvantages or conferred selective advantages. The Shh-mediated increase in taste buds might confer a selective advantage by enhancing a cavefish's ability to find food (assuming that the extra taste buds are functional, which needs to be tested). This suggests that there may have been positive selection for increased Hh expression since, by altering the balance between the development of different senses, it produced cavefish better adapted to their dark cave environment through the enhancement of non-visual senses. The alternative explanation, that the loss of eyes in cavefish is simply a consequence of a lack of selection against mutations that are deleterious to vision or eye development (but not to overall fitness), seems a less satisfactory explanation of recent findings.

It is still debatable whether all existing cavefish populations evolved from a common ancestor that lost its eyes as a result of hyperactive midline signaling by Shh or whether the same genetic changes took place independently in different populations. Genetic analyses favour the theory that at least four geographically separated *Astyanax mexicanus* cavefish populations evolved varying degrees of eye degeneration independently and that several different genes are involved.[5,6,31–33] At least for *Astyanax mexicanus*, however, the evolution of the developmental mechanisms controlling sensory development may have occurred because the changes led to advantages for cave-dwelling fish.

References

1. Yamamoto Y, Stock DW, Jeffery WR. 2004. Hedgehog signalling controls eye degeneration in blind cavefish. Nature 431:844–847.
2. Culver DC. 1982. Cave life. Evolution and ecology. Cambridge: Harvard University Press.
3. Jeffery WR, Martasian DP. 1998. Evolution of eye regression in the cavefish Astyanax: apoptosis and the Pax-6 gene. Amer Zool 38:658–696.
4. Yamamoto Y, Jeffery WR. 2000. Central role for the lens in cave fish eye degeneration. Science 289:631–633.
5. Wilkens H. 1971. Genetic interpretation of regressive evolutionary process: studies of hybrid eyes of two Astyanax cave populations (Characidae, Pisces). Evolution 25:530–544.
6. Wilkens H. 1988. Evolution and genetics of epigean and cave Astyanax fasciatus (Characidae, Pisces). Evol Biol 23:271–367.
7. Strickler AG, Yamamoto Y, Jeffery WR. 2001. Early and late changes in Pax6 expression accompany eye degeneration during cavefish development. Dev Genes Evol 211:138–144.
8. Romero A, Green SM, Lelonek MM, Stropnicky KC. 2003. One eye but no vision: cave fish with induced eyes do not respond to light. J Exp Zool Part B Mol Dev Evol 300:72–79.
9. Jeffery W, Strickler A, Guiney S, Heyser D, Tomarev S. 2000. Prox 1 in eye degeneration and sensory organ compensation during development and evolution of the cavefish Astyanax. Dev Genes Evol 210:223–230.
10. Walther C, Gruss P. 1991. Pax-6, a murine paired box gene, is expressed in the developing CNS. Development 113:1435–1449.
11. Puschel AW, Gruss P, Westerfield M. 1992. Sequence and expression pattern of pax-6 are highly conserved between zebrafish and mice. Development 114:643–651.
12. Nornes S, Clarkson M, Mikkola I, Pedersen M, Bardsley A, et al. 1998. Zebrafish contains two pax6 genes involved in eye development. Mech Dev 77:185–196.

13. Hill RE, Favor J, Hogan BL, Ton CC, Saunders G, et al. 1991. Mouse small eye results from mutations in a paired-like homeobox-containing gene. Nature 354:522–525.
14. Ashery-Padan R, Marquardt T, Zhou X, Gruss P. 2000. Pax6 activity in the lens primordium is required for lens formation and for correct placement of a single retina in the eye. Genes Dev 14:2701–2711.
15. Li H, Tierney C, Wen L, Wu JY, Rao Y. 1997. A single morphogenetic field gives rise to two retina primordia under the influence of the prechordal plate. Development 124:603–615.
16. Ekker SC, Ungar AR, Greenstein P, von Kessler DP, Porter JA, et al. 1995. Patterning activities of vertebrate hedgehog proteins in the developing eye and brain. Curr Biol 5:944–955.
17. Macdonald R, Barth KA, Xu Q, Holder N, Mikkola I, et al. 1995. Midline signalling is required for Pax gene regulation and patterning of the eyes. Development 121:3267–3278.
18. Amato MA, Boy S, Perron M. 2004. Hedgehog signaling in vertebrate eye development: a growing puzzle. Cell Mol Life Sci 61:899–910.
19. Neumann CJ, Nuesslein-Volhard C. 2000. Patterning of the zebrafish retina by a wave of sonic hedgehog activity. Science 289:2137–2139.
20. Stenkamp DL, Frey RA, Prabhudesai SN, Raymond PA. 2000. Function for Hedgehog genes in zebrafish retinal development. Dev Biol 220:238–252.
21. Schemmel C. 1974. Genetische Untersuchungen zur Evolution des Geschmacksapparates bei cavernicolen Fischen. Z Zool Syst Evolutionforsch 12:196–215.
22. Teyke T. 1990. Morphological differences in neuromasts of the blind cave fish Astyanax hubbsi and the sighted river fish Astyanax mexicanus. Brain Behav Evol 35:23–30.
23. Jeffery WR. 2001. Cavefish as a model system in evolutionary developmental biology. Dev Biol 231:1–12.
24. Schemmel C. 1967. Vergleichende Untersuchungen an den Hautsinnesorganene ober- und unterirdisch lebender Astyanax-Formen. Z Morphol Tiere 61:255–316.
25. Schemmel C. 1980. Studies on the genetics of feeding behaviour in the cave fish Astyanax mexicanus f. anoptichthys. An example of apparent monofactorial inheritance by polygenes. Z Tierpsychol 53:9–22.
26. Miura H, Kusakabe Y, Kato H, Miura-Ohnuma J, Tagami M, et al. 2003. Co-expression pattern of Shh with Prox1 and that of Nkx2.2 with Mash1 in mouse taste bud. Gene Exp Patterns 3:427–430.
27. Hall JM, Hooper JE, Finger TE. 1999. Expression of sonic hedgehog, patched, and Gli1 in developing taste papillae of the mouse. J Comp Neurol 406:143–155.
28. Hall JM, Bell ML, Finger TE. 2003. Disruption of sonic hedgehog signaling alters growth and patterning of lingual taste papillae. Dev Biol 255:263–277.
29. Jung HS, Oropeza V, Thesleff I. 1999. Shh, Bmp-2, Bmp-4 and Fgf-8 are associated with initiation and patterning of mouse tongue papillae. Mech Dev 81:179–182.
30. Chuong CM, Patel N, Lin J, Jung HS, Widelitz RB. 2000. Sonic hedgehog signaling pathway in vertebrate epithelial appendage morphogenesis: perspectives in development and evolution. Cell Mol Life Sci 57:1672–1681.
31. Mitchell RW, Russell WH, Elliot WR. 1977. Mexican eyeless characin fishes, genus Astyanax: Environment, distribution and evolution. Spec Publ Mus Texas Tech Univ 12:1–89.
32. Dowling TE, Martasian DP, Jeffery WR. 2002. Evidence for multiple genetic forms with similar eyeless phenotypes in the blind cavefish, Astyanax mexicanus. Mol Biol Evol 19:446–455.
33. Borowsky R, Wilkens H. 2002. Mapping a cave fish genome: polygenic systems and regressive evolution. J Hered 93:19–21.