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Commentary

Pax-6: Where to be conserved is not conservative

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Conserved sequences in the opsins of vertebrate and invertebrate photoreceptors (1), and homologous genes such as *Pax-6* involved in eye development across phyla (2), challenge the hypothesis that the eyes of vertebrates and invertebrates had distinct evolutionary origins (3, 4). This hypothesis was rooted in the dramatic differences in embryogenesis, phototransduction, and optical imaging mechanisms in the eyes of different species (5). Sequencing data suggest, however, that all metazoan photopigments probably had a common origin, so while the photopigment from the eye spots of flatworms has not been cloned, it would be surprising if it is not an opsin.

A photopigment, however, is not an eye. The evolution of eyes, as complex organs, could still be polyphyletic. Consider one of the most compelling cases of convergent evolution: the image-forming eyes of the cephalopod mollusks and those of the vertebrates (6). Though these eyes look extraordinarily similar in design, these similarities are not homologies. Neither primitive mollusks nor primitive vertebrates have much more than an eye spot, suggesting independent evolution of the camera eye in these two phyla starting from simple ancestral photoreceptive structures. The embryonic origins corroborate this dual ancestry (see Fig. 1). In developing vertebrates, the neural retina bulges out of the ventrolateral forebrain as an optic vesicle, presses against the inner layer of the overlying epidermis, and induces it to thicken and become a lens. The lens then induces the covering epidermis to clear into corneal tissue. The optic vesicle then involutes into an optic cup, the outer edges of which form the ciliary body and iris (7). In cephalopods, the embryonic origin of the neural retina is a peripheral placode and the lens, iris, and cornea form from successive folds of the ectoderm that encircle the developing eye. The lens is acellular; it is made of long fingerlike processes that coalesce into a central droplet (8). Thus, phylogenetic and embryological considerations strongly suggest that the two eyes must have evolved independently. Moreover, it seems highly unlikely that the structural similarities in the adult are due to a conserved developmental program. However, the expression of *Pax-6* in the development of the squid eye challenges this conclusion, as reported in this issue (9) from a collaboration between the laboratories of Gehring and Piatigorsky.

In 1994, Walter Gehring and his colleagues showed that the *Drosophila eyeless* gene is a homolog of the vertebrate *Pax-6* gene (10). Hypomorphic mutants in the *eyeless* (*ey*) gene in *Drosophila* compromise the development of the *Drosophila* eye disc, while heterozygotes for a mutant in the mammalian *Pax-6* gene, called *Small eye* (*Sey*) have eye abnormalities such as aniridia, and a reduction in eye size (11). The idea that a single developmental gene can govern eye formation in these two species, when every previous indication was that the compound eyes of insects and the camera eyes of vertebrates, if they shared anything, shared only ancestral photochemistry, was startling. The same laboratory then published an even more stunning result: the *ey* gene of *Drosophila*, if misexpressed in other imaginal discs such as the leg, wing, or antennal disc, transformed parts of these structures into compound eyes, resulting in flies with eyes growing all over their bodies (12). Hence, the *Pax-6*/*ey* gene was shown to be both necessary and sufficient (at least in some contexts) for eye formation. The mouse *Pax-6* gene under the same promoters also induces ectopic compound eyes in flies—not mouse eyes (12)! That the mouse protein is able to activate *Drosophila* genes, which carry out this organogenic program, strongly argues for common developmental mechanisms in eye formation that have been previously obscured by morphological considerations.

The paper in this issue of the *Proceedings* (9) extends the hypothesis that *Pax-6* has an evolutionarily conserved and critical role in eye development. It shows that there is a homolog of *Pax-6* in squid that is expressed in the eye-forming region and that squid *Pax-6* misexpression can lead to ectopic eye formation in *Drosophila*. If the morphological similarities between the eyes of cephalopods and vertebrates evolved twice, why then do these eyes share a conserved transcription factor that might regulate these processes? Is it a coincidence? The fly story makes this unlikely. The hypothesis that there has been an evolutionary reason to conserve the role of *Pax-6* in eye development must therefore be taken seriously. There are the conservative and bold forms of this evolutionary hypothesis. In the bold version, *Pax-6* is an organogenesis gene, a master regulator of eye development. In a more conservative version, *Pax-6* is patterning gene, expressed in the head, that has often been coopted to regulate particular aspects of eye development. But what exactly is it that *Pax-6* does?

Pax-6 is one of nine members of a family of vertebrate transcription factors that share a conserved paired box domain and a homeodomain (13). *Pax* genes are expressed in distinct patterns during development, and where mutants are available, obvious phenotypes consistent with the expression patterns often arise, indicating the essential nature of these genes (13). For example, *Pax-1* is involved with thymus and intervebral disc development; *Pax-2* is involved in optic stalk and kidney development; *Pax-3* is involved in neural crest development, and so on (13). In vertebrates, *Pax-6* is expressed not only in the eye but also in the nasal placodes, diencephalon, and the lateroventral hindbrain and spinal cord (14, 15). Homozygous *Sey* mutant mice die as embryos with defects in the formation of the nose, forebrain, and spinal cord (16). In flies, the *ey* gene is expressed in the brain and ventral nerve cord, as well as the eye disc, and null mutants do not survive. This indicates that in flies, *ey* is also involved in more than eye development. In squid, *Pax-6* is expressed in the brain and the arms as well as the eye tissue (9). So, perhaps categorizing *Pax-6* as a regulator of eye formation is not doing it full justice.

Pax-6 has important roles in several aspects of eye development (17). For instance, it is a clear that there is an autonomous defect in the lens-forming tissues of *Sey* homozygous mice and rats (16, 18). *Pax-6* mutant optic vesicles can induce normal epidermis to form a lens, but wild-type optic vesicles cannot induce a lens in mutant epidermis. Work in vertebrates suggests that *Pax-6* regulates crystallin genes in the lens (19). Squid *Pax-6* is expressed in the lens and is postulated to regulate squid crystallins (9). Interestingly, squid crystallins

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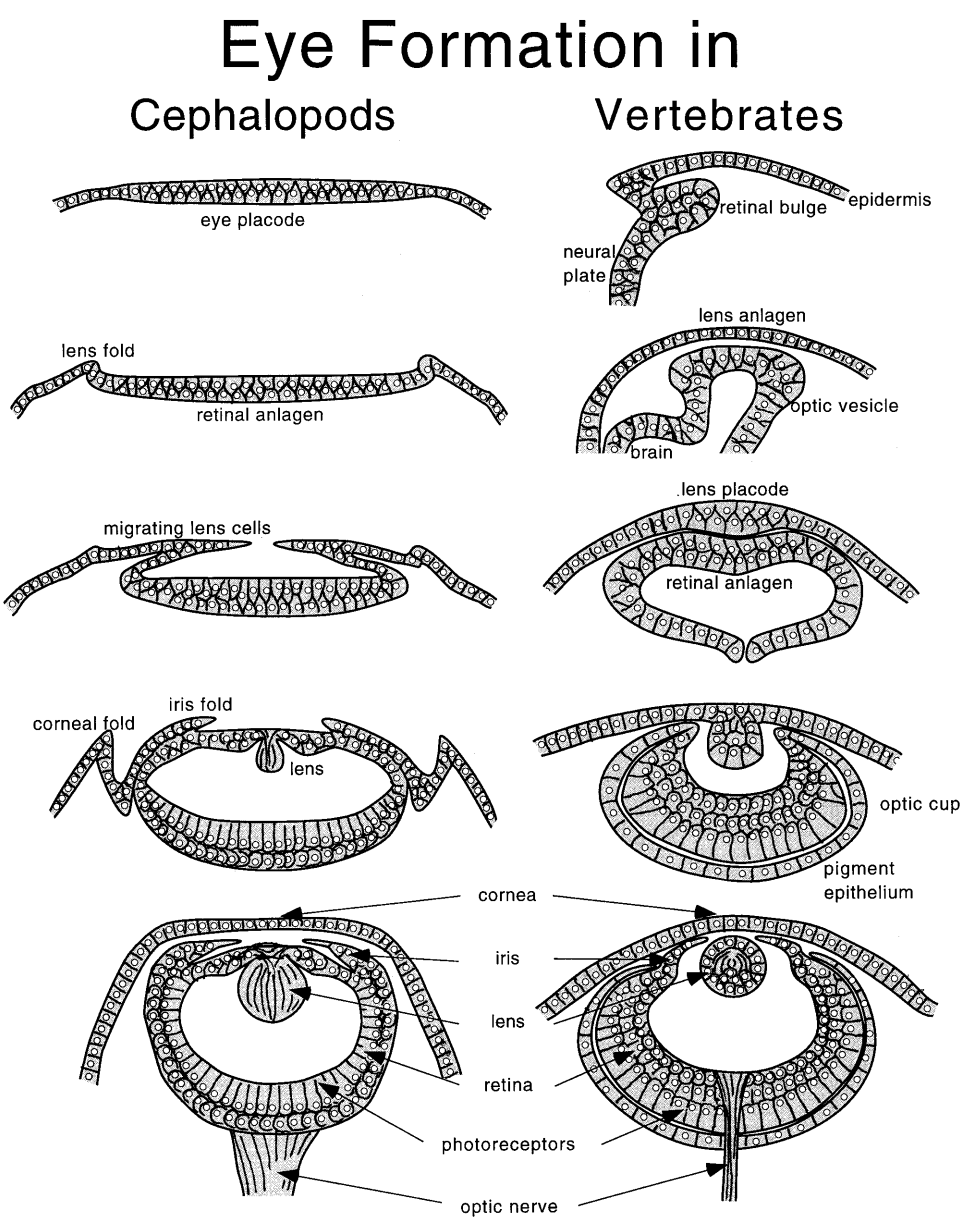

FIG. 1. Schematic diagram of cephalopod eye development (*Left*) and vertebrate eye development (*Right*) as explained in more detail in refs. 7 and 8. Development proceeds from top to bottom. Even though the adult structures are fairly similar, excepting certain obvious features such as the placement of the photoreceptors and lentigenic cells, the development is very different. The cephalopod eye forms from an epidermal placode through a series of successive infoldings, while the vertebrate eye emerges from the neural plate and induces the overlying epidermis to form the lens.

and vertebrate crystallins are totally unrelated proteins that originally had nothing to do with lens function (20). This suggests that *Pax-6* binding sites must have appeared secondarily in the promoters of these different genes. Thus, *Pax-6* would not be expected to regulate a common ancestral lens protein. If it does have an ancestral regulatory role in eye formation, *Pax-6* might be predicted to control some conserved genes in eyes. An obvious possibility is an opsin homolog because, as suggested above, it is likely that opsins are truly conserved elements of the photoreceptive organs. However, *Pax-6* is expressed in neither vertebrate nor cephalopod photoreceptors (9, 21).

The fact that both the mouse and squid *Pax-6*-encoded proteins can lead to ectopic eye formation in flies could be taken to imply the retention of the ability of *Pax-6* to fit into a conserved hierarchy of eye gene regulation across the metazoan kingdom. Yet, when all three species have such distinct structural and functional components, this interpretation seems almost too amazing. A more modest proposition is that *Pax-6* is autoregulatory, as many such transcription factors are. Thus, as the authors point out, it is possible that the squid or mouse *Pax-6* gene activates the native *ey* gene in flies, which then regulates the downstream fly genes (9). The obvious experiment, which should be done, is to try and rescue eye development in *ey* nulls by misexpression of the squid or mouse *Pax-6* genes. Another possibility is that DNA binding specificity of the different *Pax-6* homologs is similar, even though they regulate completely different genes in their native species. Therefore, more work on the molecular mechanisms and target specificity of these foreign gene inductions could reveal the presence, or absence, of conserved pathways in the molecular hierarchy of eye development.

Although zebrafish mutations that alter anterior neural patterning affect both *Pax-6* expression and eye formation (22), there has been no demonstration that misexpression of *Pax-6* in vertebrates can lead to ectopic eyes (21, 23). Perhaps

Pax-6 is higher in the regulatory pathway of eye development in flies than it is in vertebrates, where optic vesicles clearly form in the homozygous *Sey* mice (24). Another factor to consider in this regard is the special developmental potential of the imaginal discs of *Drosophila*. Studies have shown that wing discs may, if serially cultured in the abdomens of adult females, switch their fate spontaneously and make compound eyes when forced to differentiate by being removed from the host and inserted back into a larva about to pupate (25). Such spontaneous transdetermination from one fate to another is a peculiarity of imaginal discs. As structures set aside for later development, imaginal discs may repress inappropriate fates, but not shut them down completely. In this way, discs may be likened to pluripotential blast cells. Interestingly, much as ectopic expression of *ey* causes the transdetermination of wing discs into eyes, the ectopic expression of the *wingless* signaling molecule leads to the transdetermination of leg discs into wings (26). Thus, misexpression of these genes, and presumably others, in *Drosophila* imaginal discs may shift their fate potential in a way that is not possible in the vertebrate embryo, or any animal where development of adult structures is direct rather than delayed. The molecular hierarchy of early eye development is clearly an intriguing problem. In *Drosophila* several genes are involved, including *Pax-6*, *sine-oculis*, and *eyes absent*. Strikingly, a vertebrate homolog of *sine-oculis*, called *Six-3*, is expressed in the developing eye regions and may act upstream of *Pax-6*, since *Sey* mutants have normal *Six-3* expression (27). The discovery of other eye regulation factors shared between flies and vertebrates certainly strengthens the link between *Pax-6* and eye organogenesis, but at the same time threatens the primacy of *Pax-6* in this process.

To understand the special relationship between *Pax-6* and eyes it will be important to discover the ancestral pattern of *Pax-6* expression. Nematodes have *Pax-6* homologs and have often been positioned in evolutionary trees near the vertebrate/invertebrate branch point. The function of *Pax-6* homologs in these animals may shed light on the ancestral function of *Pax-6*. Mutants in these genes have defects in peripheral sense organs of the tail (*mab-18*), or abnormalities in the head (*vab-3*) (28, 29). But—nematodes do not have eyes or any known photoreceptive cells, and neither do adult sea urchins, which express a respectable *Pax-6* gene in their tube feet (30)! Echinoderms, as deuterostomes, are usually placed in evolutionary trees at the base of chordate evolution. The ribbon worm, however, which has eyes associated with *Pax-6* expression, seems to be on the line that preceded the vertebrate/invertebrate split (31). If nematodes and echinoderms lost their photosensory organs, *Pax-6* may have taken on new roles in these species. On the other hand, the existing evolutionary data on *Pax-6*, taken together, may also support the idea that *Pax-6* is not really an organogenesis gene, but rather a patterning gene for the head, the place where most, but not all, animals have evolved eyes.

There may be too many unresolved questions to allow skeptics to buy into the bold hypothesis at present, but neither can they rule it out. It will therefore be interesting to continue the evolutionary survey of *Pax-6* expression and function, especially in organisms that have either no eyes or very primitive eyes. For instance, there was initial speculation that the primitive eye spots of flatworms expressed *Pax-6* (32). This would be useful to know, since flatworms clearly represent a more ancestral state than the other animals so far studied. What if these animals had eyes but no *Pax-6*, or what if they had *Pax-6* but it wasn't expressed in their eyes?

1. Goldsmith, T. H. (1990) *Q. Rev. Biol.* **65,** 281–322.
2. Halder, G., Callaerts, P. & Gehring, W. J. (1995) *Curr. Opin. Genet. Dev.* **5,** 602–609.
3. Eakin, R. M. (1963) in *General Physiology of Cell Specialization*, eds. Mazia, D. & Tyler, A. (McGraw-Hill, New York), pp. 393–425.
4. Salvini-Plawen, L. V. & Mayr, E. (1977) *Evol. Biol.* **10,** 207–263.
5. Land, M. F. & Fernald, R. D. (1992) *Annu. Rev. Neurosci.* **15,** 1–29.
6. Packard, A. (1972) *Biol. Rev.* **47,** 241–307.
7. Balinsky, B. I. (1975) *An Introduction to Embryology* (Saunders, Toronto).
8. Arnold, J. M. (1971) in *Experimental Embryology of Marine and Fresh-Water Invertebrates*, ed. Reverberi, G. (North-Holland, Amsterdam), pp. 271–311.
9. Tomarev, S. I., Callaerts, P., Kos, L., Zinovieva, R., Halder, G., Gehring, W. & Piatigorsky, J. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 2421–2426.
10. Quiring, R., Walldorf, U., Kloter, U. & Gehring, W. J. (1994) *Science* **265,** 785–789.
11. Hanson, I. & Van, H. V. (1995) *Trends Genet.* **11,** 268–272.
12. Halder, G., Callaerts, P. & Gehring, W. J. (1995) *Science* **267,** 1788–1792.
13. Mansouri, A., Hallonet, M. & Gruss, P. (1996) *Curr. Opin. Cell Biol.* **8,** 851–857.
14. Li, H. S., Yang, J. M., Jacobson, R. D., Pasko, D. & Sundin, O. (1994) *Dev. Biol.* **162,** 181–194.
15. Amirthalingam, K., Lorens, J. B., Saetre, B. O., Salaneck, E. & Fjose, A. (1995) *Biochem. Biophys. Res. Commun.* **215,** 122–128.
16. Quinn, J. C., West, J. D. & Hill, R. E. (1996) *Genes Dev.* **10,** 435–446.
17. Macdonald, R. & Wilson, S. W. (1996) *Curr. Opin. Neurobiol.* **6,** 49–56.
18. Fujiwara, M., Uchida, T., Osumi-Yamashita, N. & Eto, N. (1994) *Differentiation* **57,** 31–38.
19. Cvekl, A. & Piatigorsky, J. (1996) *BioEssays* **18,** 621–630.
20. Tomarev, S. I. & Piatigorsky, J. (1996) *Eur. J. Biochem.* **235,** 449–465.
21. Hirsch, N. & Harris, W. A. (1997) *J. Neurobiol.* **32,** 45–61.
22. Macdonald, R., Barth, K. A., Xu, Q., Holder, N., Mikkola, I. & Wilson, S. W. (1995) *Development (Cambridge, U.K.)* **121,** 3267–3278.
23. Schedl, A., Ross, A., Lee, M., Engelkamp, D., Rashbass, P., van Heyningen, V. & Hastie, N. D. (1996) *Cell* **86,** 71–82.
24. Grindley, J. C., Davidson, D. R. & Hill, R. E. (1995) *Development (Cambridge, U.K.)* **121,** 1433–1442.
25. Hadorn, E. (1965) *Brookhaven Symp. Biol.* **18,** 148–161.
26. Maves, L. & Schubiger, G. (1995) *Development (Cambridge, U.K.)* **121,** 1263–1272.
27. Oliver, G., Mailhos, A., Wehr, R., Copeland, N. G., Jenkins, N. A. & Gruss, P. (1995) *Development (Cambridge, U.K.)* **121,** 4045–4055.
28. Chisholm, A. D. & Horvitz, H. R. (1995) *Nature (London)* **376,** 52–55.
29. Zhang, Y. & Emmons, S. W. (1995) *Nature (London)* **376,** 55–59.
30. Czerny, T. & Busslinger, M. (1995) *Mol. Cell. Biol.* **15,** 2858–2871.
31. Loosli, F., Kmita, C. M. & Gehring, W. J. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 2658–2663.
32. Zuker, C. S. (1994) *Science* **265,** 742–743.