

The genetic control of eye development and its implications for the evolution of the various eye-types[#]

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ABSTRACT Mutations in the Pax 6 homologs of mammals and insects prevent eye development and targeted expression of both mammal and insect Pax 6 homologs is capable of inducing functional ectopic eyes. Supported by RNA interference experiments in planarians and nemerteans, these findings indicate that Pax 6 is a universal master control gene for eye morphogenesis. Since all metazoan eyes use rhodopsin as a photoreceptor molecule and the same master control gene for eye development, we postulate a monophyletic origin of the various eye types. The finding of well developed eyes in jellyfish which essentially lack a brain, leads us to propose that the eye as a sensory organ evolved before the brain which is an information processing organ. The finding of highly developed eyes with a lens, vitreous body, stacked membranes like a retina and shielding pigment in unicellular dinoflagellates, raises the possibility that the prototypic eyes might have been acquired from symbionts.

KEY WORDS: Pax 6, eyeless, DNA microarrays, sine oculis, Notch, RNA interference

Introduction

The eye is a most fascinating organ since it allows us not only to visualize the outside world, but it is also a window to the soul, the world inside.

Evolution has generated at least three basically different types of eyes (Fig. 1): the camera-type eye with a single lens projecting onto a retina that is found in vertebrates and cephalopods; the compound eye with multiple ommatidia each consisting of a set of photoreceptor cells and a lens of its own, which is characteristic for insects and other arthropods; and the mirror eye which in the case of the scallop (*Pecten*) uses both a lens focussing the light onto a distal retina and a reflecting parabolic mirror projecting the light onto a proximal retina. Most of these eyes are positioned on the head of the animal and send their signals to the brain, which processes the information and transmits the appropriate signals to the effector organs, e.g. the muscles. However, in bivalves which do not form a head, the eyes are placed at the edge of the mantle allowing the animal to see when the two shells are slightly opened. In certain annelids which live head down in a tube, the eyes are placed at the hind end, where they are useful. Classical morphological studies have been used to argue for a polyphyletic origin of the various eye types. On the basis of morphological considerations Salvini-Plawen and Mayr (1961) claim "that the earliest invertebrates, or at least those that gave rise to the more advanced phyletic lines, had no photoreceptors" and that "photoreceptors have originated in at least 40 but possibly up to 65

or more phyletic lines". They strongly adhere to the dogma "that the lens eye of a vertebrate and the compound eye of an insect are independent evolutionary developments", a notion that you can find in nearly all zoology textbooks. I would like to challenge these ideas on the basis of our recent genetic work and argue for a monophyletic origin of the eyes from precambrian ancestors which already possessed primitive prototypic eyes. From these precambrian ancestors the different phyla with the various eye-types evolved by adaptive radiation. This hypothesis is much more compatible with Darwin's ideas (1882) than the assumption of a polyphyletic origin of the eyes. Before considering these issues, I would like to present the molecular genetic evidence which argues in favor of this radically different point of view.

Identification of the Master Control Genes for Eye Development

The term master control genes was proposed by Weintraub (1989) for the muscle determining gene *Myo D* and Lewis (1992) to designate homeotic genes which in a combinatorial fashion determine segmental identity along the anterior posterior axis in *Drosophila*. Deletion of one of these genes leads to dramatic

Abbreviations used in this paper: dac, dachshund; ERG, electroretinogram; ey, eyeless; eya, eyes absent; RNAi, RNA interference; so, sine oculis; toy, twin of eyeless.

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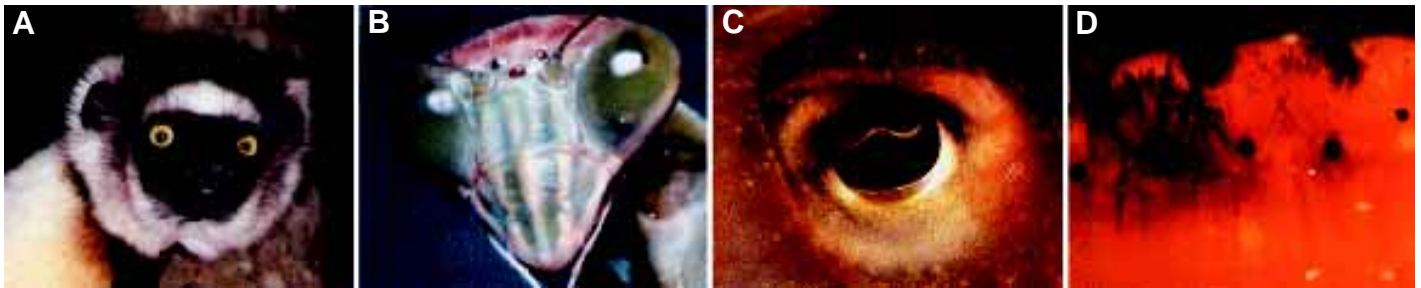


Fig. 1. Different types of eyes. (A) Camera-type eye from the Lemur *Propithecus verreauxi*. (B) Compound eye of the praying Mantis. (C) Camera-type eye from the Cephalopod *Sepia erostrata*. (D) Mirror eye from the clam *Chlamys nobilis*. (Courtesy of Dr. Kazuto Kato. Photographs kindly provided by Masahiro Iijima, Susumu Yamaguchi and Isamu Soyama).

homeotic transformations that allowed Lewis to construct the four-winged and the eight-legged fly. For the eye, a number of *Drosophila* mutants have been isolated which block eye development by inducing cell death rather than leading to a homeotic transformation. As early as 1915 Hoge isolated the first *eyeless* (*ey*) mutation which shows a rather variable phenotype from a total loss of eye facets on one or both sides of the head to more or less reduced eyes. For the mouse, a mutation *Small eye* was isolated and a similar mutant syndrome *Aniridia* was described in humans. Homozygous *Small eye* embryos are eyeless, noseless and suffer serious brain damage, whereas heterozygotes develop to adult mice with reduced eyes, suggesting that the eye is critically affected. Heterozygous *Aniridia* patients have a similar phenotype with a reduced or no iris at all; and two homozygous mutant human fetuses have been described which were eyeless and noseless, suffered brain damage and died prior to birth. Using the *Drosophila* paired box as a probe Walther and Gruss (1991) cloned the *Pax 6* gene of the mouse which was shown to correspond to the gene affected by the *Small eye* mutation (Hill *et al.*, 1991). Similarly, the human *Aniridia* gene was cloned and shown to encode Pax 6 (Ton *et al.*, 1991). Interestingly, the human and the murine Pax 6 proteins are identical in amino acid sequence indicating that there is a strong selective pressure on this protein. The protein contains both a paired and a homeodomain indicating that it is a transcription factor with two different DNA binding domains. It came as a total surprise, when my graduate student Rebecca Quiring accidentally cloned the *Pax 6* homolog of *Drosophila* and it turned out to be the *eyeless* gene. Again the degree of sequence conservation is very high, 95% in the paired domain and 90% in the homeodomain as compared to the mammalian proteins.

These observations suggested to me that *Pax 6* might be the universal master control gene for eye development, implying that the dogma of independent evolution of the vertebrate eye and the insect compound eye might be wrong. Encouraged by the fact that occasionally a transdetermination from wing to eye structures can be observed (Gehring *et al.*, 1968) and that the ectopic expression of the *Antennapedia* gene can induce antenna-to-leg transformations (Schneuwly *et al.*, 1987), I proposed to my collaborators Georg Halder and Patrick Callaerts to try to induce ectopic eyes in other regions of the body of the fly by targeted expression of the *Drosophila eyeless* gene and its mouse homolog (*Pax 6*). Using the gal 4 system and suitable genomic enhancers, we succeeded in inducing ectopic eyes on the antennae, wings and legs of the fly (Fig. 2) (Halder *et al.*, 1995). Thus, *eyeless* alias *Pax 6* became the paradigm for a master control gene. It represents a master switch initiating a cascade of at least 2000 genes which are required for eye morphogenesis.

Histologically, the ectopic eyes on the antenna are quite normal and 32 antennal eyes tested electrophysiologically produced an electroretinogram (ERG) suggesting that their photoreceptor cells are functional. From 8 of the antennal eyes a completely normal ERG was recorded indicating that these eyes are functional, whereas the other 24 lacked the on- and off-transients which are characteristic of photoreceptors which are functional but not postsynaptically connected to the brain. By following the axons from the antennal eye to the brain, we found that the axons project to the antennal rather than the optic centers of the brain (Callaerts *et al.*, unpubl.), suggesting that these flies might "smell the light". An interesting thought that we are going to pursue.

In collaboration with Meinrad Busslinger and Thomas Czerny (Czerny *et al.*, 1999) we discovered a second *Pax 6* gene in

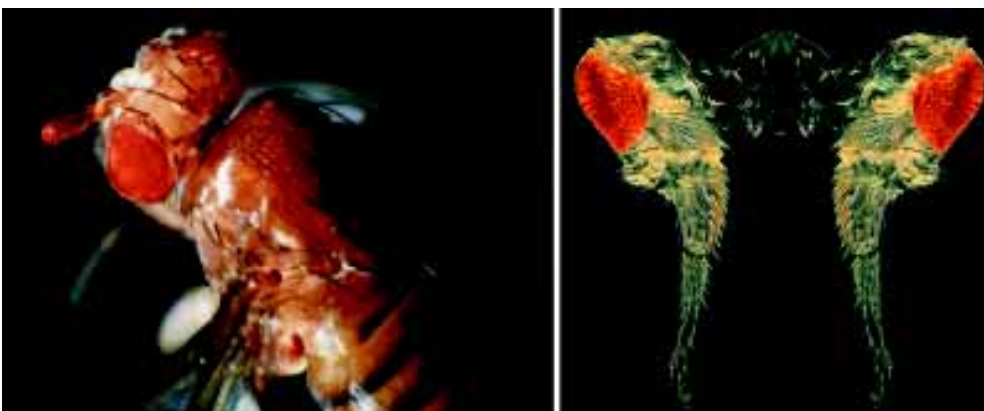


Fig. 2. (Left) Induction of ectopic eyes by targeted expression of the *eyeless* gene on the antennae and wings of *Drosophila*.

Fig. 3. (Right) Induction of ectopic eyes on the legs of *Drosophila* by targeted expression of the twin of *eyeless* gene.

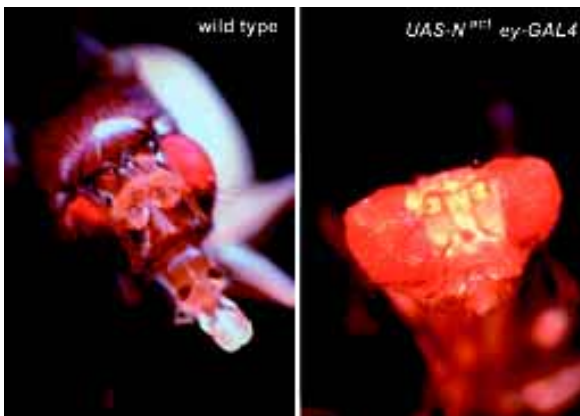


Fig. 4. "Hammerhead" flies induced by expression of a constitutively activated form of the Notch Receptor. Wild-type (left) and Hammerhead (right) flies with enlarged compound eyes (*UAS-N^{act} ey-GAL4*).

Drosophila called *twin of eyeless* which arose as a duplication during insect evolution. It is present in holometabolous insects (*Drosophila* and silk moth), whereas hemimetabolous (*Schistocerca*) and apterygote insects (springtail) have only a single gene. *Twin of eyeless* is more similar to vertebrate Pax 6 proteins than *eyeless* with regard to overall sequence conservation and DNA-binding function, and it is expressed earlier in embryogenesis, but the two genes share a similar expression pattern in the developing visual system and targeted expression of *twin of eyeless*, like *eyeless*, induces the formation of ectopic eyes (Fig. 3). Genetic and biochemical evidence indicates, however, that *twin of eyeless* functions upstream of *eyeless* by directly regulating the eye-specific enhancer of *eyeless* (Hauck *et al.*, 1999). *Twin of eyeless* is therefore required for the initiation of *eyeless* expression in the embryo and acts through *eyeless* to activate the eye developmental pathway. The isolation of loss-of-function mutants for both *eyeless* and *twin of eyeless* mutants indicates that the two genes can partially substitute one another and that *twin of eyeless* is required for the formation of ocelli whereas *eyeless* is predominantly involved in compound eye morphogenesis.

The Notch signalling pathway defines an evolutionarily conserved cell-cell interaction mechanism that throughout development controls the ability of precursor cells to respond to developmental signals. By targeting the expression of a dominant negative Notch receptor into the early eye primordia, we have been able to induce an *eyeless* phenotype, whereas the expression of a constitutively activated *Notch* receptor leads to the opposite effect and generates considerably enlarged eyes resembling so-called "hammerhead" flies (Fig. 4), as they occur in certain *Drosophila* species in nature (Kurata *et al.*, 2000). The dominant activated form of *Notch* can also induce ectopic eyes at the base of the proboscis which is derived from the antennal disc. The induction of ectopic eyes correlates with the induction of both *eyeless* and *twin of eyeless* expression by *Notch*, but we do not know whether this effect is directly mediated by the *Suppressor of hairless* transcription factor in the *Notch* signalling pathway, or whether the effect is indirect. *Notch* signalling regulates the master control genes *eyeless*, *vestigial* and *Distal-less* which in combination with different homeotic genes can induce the formation of ectopic eyes, wings, antennae and legs respectively (Kurata *et al.*, 2000). Thus, *Notch* is involved in a common regulatory pathway for the determination of the various *Drosophila* appendages.

To determine the extent of evolutionary conservation of the eye morphogenetic pathway, we have begun to identify subordinate target genes of *eyeless* further downstream in the gene regulatory cascade. An important gene in this respect is *sine oculis* whose expression is induced by *eyeless*. *Sine oculis* is a homeobox gene and also functions as a transcription factor. We have obtained evidence from ectopic expression studies in transgenic flies, from transcription activation studies in yeast, and from gel shift assays *in vitro*, that the Eyeless protein activates transcription of *sine oculis* by direct interaction with an eye-specific enhancer in the long intron of the *sine oculis* gene (Niimi *et al.*, 1999). Detailed analysis of this eye-specific enhancer has revealed functional binding sites for both Eyeless and Twin of eyeless proteins, which explains why *twin of eyeless* can partially substitute for *eyeless* (Punzo *et al.*, unpubl.). Even though *twin of eyeless* is acting upstream of *eyeless* and activates its expression, it also directly controls *sine oculis* which is downstream of *eyeless*. Since there is also a positive feedback from *sine oculis* to *eyeless*, the eye morphogenetic pathway is by no means linear but rather a complex genetic network (Fig. 5). Furthermore, *sine oculis* contains an ocelli-specific enhancer which is regulated by *twin of eyeless* and some other transcription factor(s), but not by *eyeless* (Punzo *et al.*, unpubl.). This explains the observation that *eyeless* null mutants which possess a functional *twin of eyeless* gene are still capable of forming normal ocelli.

As indicated in Fig. 5, the genetic cascade specifying eye morphogenesis involves many players that have to be analyzed one by one. However, modern genomics provides for the first time the means to get a general overview over a morphogenetic pathway. Using DNA microarrays and DNA chips we have begun to analyze the eye morphogenetic pathway. By comparing RNA from leg imaginal discs with leg discs in which an eye morphogenetic field has been induced by ectopic expression of *eyeless*, RNA from eye versus leg discs, and RNA from leg discs in which *eyeless* has been induced by a heat shock promoter, we are trying to identify the relevant genes that are activated (directly or indirectly) by *eyeless* when an eye is induced, and the "leg genes" that are repressed. An example of a microarray resulting from a collaboration with Kevin White is shown in Fig. 6. By hybridizing six candidate genes directly or indirectly induced by

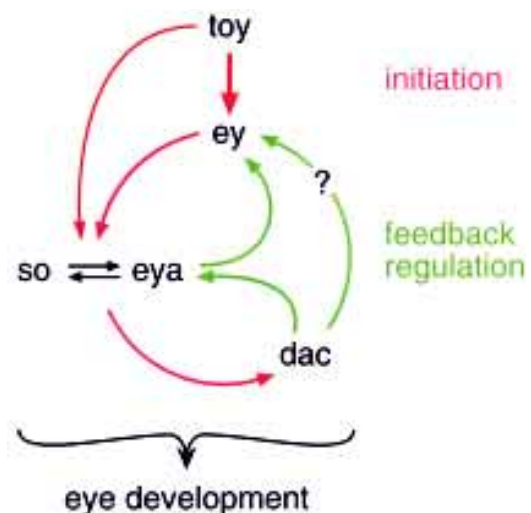


Fig. 5. Gene regulatory network controlling eye determination in *Drosophila*.

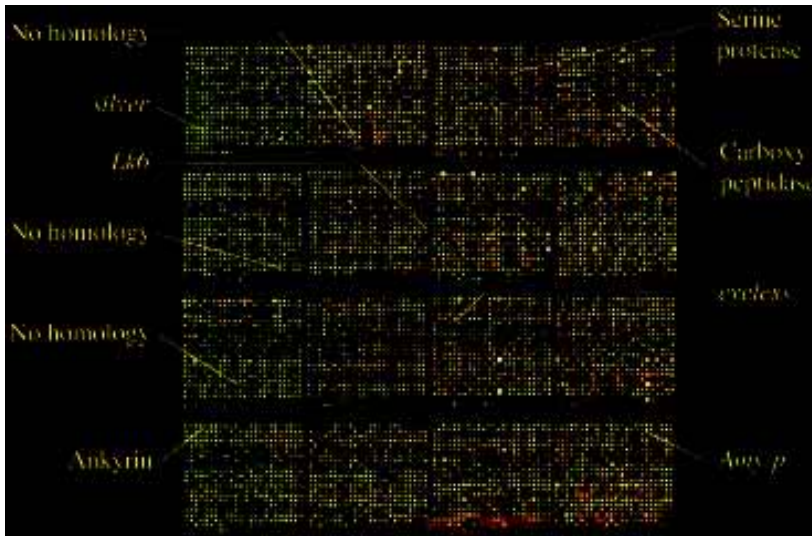


Fig. 6. DNA microarray probed with RNA from leg discs (green spots) versus eye discs in which an eye morphogenetic field is induced by ectopic expression of eyeless (red spots). A number of eyeless induced genes (spots) are labelled. Genes expressed in both kinds of discs are labelled yellow. No homology means that the respective gene is unknown. Courtesy of Lydia Michaut and Kevin White.

eyeless my collaborator Lydia Michaut was able to show that all six genes are expressed early in eye morphogenesis, i.e. in the eye disc anterior to the morphogenetic furrow, where *eyeless* is expressed, and / or in the morphogenetic furrow when and where differentiation sets in. It is our aim to compare the eye morphogenetic pathways between *Drosophila* and mouse, being analyzed by Peter Gruss and collaborators. Such a comparison will provide important insights into the parallel evolution of the compound and camera-type eye.

New Perspectives in Eye Evolution

Charles Darwin in "*The Origin of Species*" had great difficulties with eye evolution and devoted an entire chapter to "*Difficulties of the Theory*" in which he discusses "*Organs of extreme Perfection and Complication*":

"To suppose that the eye with all its inimitable contrivances for adjusting the focus to different distances, for admitting different amounts of light and for the correction of spherical and chromatic aberration, could have been formed by natural selection, seems, I freely confess, absurd in the highest degree".

But then he continues:

"Reason tells me, that if numerous gradations from a simple and imperfect eye to one complex and perfect can be shown to exist, each grade being useful to its possessor, as is certainly the case; if further, the eye ever varies and the variations be inherited, as is likewise certainly the case; and if such variations should be useful to any animal under changing conditions of life, then the difficulty of believing that a perfect and complex eye could be formed by natural selection, though insuperable by our imagination, should **not** be considered as subversive of the theory".

This pushes the question of eye evolution back to the problem of how the first primitive eye, the prototype has evolved. The evolution of an eye prototype would seem to be a highly improbable stochastic event, since selection can only work after the various components are assembled into a prototype that is at least partially functional as a photoreceptor organ.

"The simplest organ which can be called an eye consists of an optic nerve, surrounded by pigment-cells and covered by translucent skin, but without any lens or other refractive body".

Such primitive eyes are found, for example, in certain flatworms. Hesse (1897) has described the eyes of *Planaria torva* which consists of three photoreceptor cells and a single pigment cell only (Fig. 7), and there is a planarian species in Japan which has eyes with one photoreceptor cell and one pigment cell only, which corresponds exactly to the Darwinian prototype (Kiyokazu Agata, pers. comm.).

For the present discussion we adopt Darwin's definition of an eye as an organ consisting of at least two different cell-types, photoreceptor cells and pigment cells. The "eyes" of protists are organelles (and not organs) formed within a single cell and arise by the assembly of molecules within a cell rather than by the assembly of different cells, which is a fundamental difference with respect to the genetic control of morphogenesis.

Since the evolution of a prototypic eye is a highly improbable stochastic event that is not driven by selection, the hypothesis of a polyphyletic origin of the eyes, arising 40 to 65 times independently, is extremely unlikely

and incompatible with Darwin's ideas. Furthermore, all three major eye types (the camera-type, the compound eye and the mirror-eye) are found within the same class of molluscs, in the bivalvia, making an independent evolutionary origin even more unlikely.

Our finding of the same master control gene in insects and vertebrates suggested that *Pax 6* might be the universal master control gene for eye development in all metazoa. In order to test this hypothesis we expressed the mouse *Pax 6* gene ectopically in *Drosophila* and showed that the mouse homolog is capable of inducing ectopic compound eyes (Fig. 8). Of course, the induced eyes are *Drosophila* compound eyes, since we have only exchanged the master switch and the remaining 2000 genes required for eye morphogenesis come from *Drosophila*. Recently, we have also succeeded in a reciprocal kind of experiment in which *eyeless* or *twin of eyeless* mRNA is injected into *Xenopus* embryos at the two-cell stage and we were able to show that

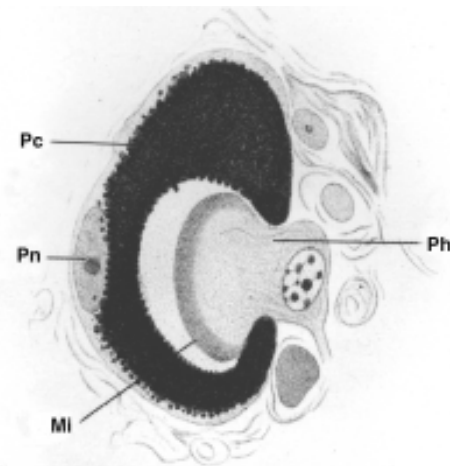


Fig. 7. Histological section across the eye of *Planaria torva* (after Hesse, 1897). Mi, microvilli; Pc, pigment cell; Ph, photoreceptor cell; Pn, pigment cell nucleus.

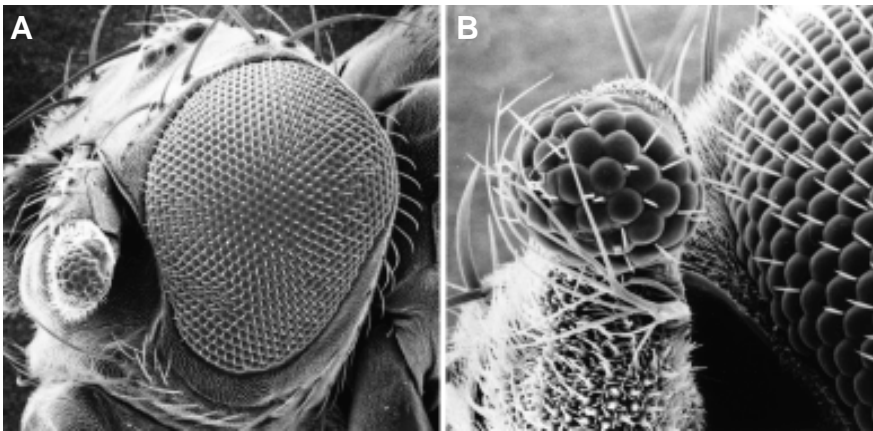


Fig. 8. Scanning electron-micrograph of an ectopic eye on the antennae induced by ectopic expression of the mouse *Small eye* gene (= *Pax 6*). (A) Overview. (B) Higher magnification. Courtesy of A. Hefti (REM Laboratory, University of Basel).

Xenopus eye structures can be induced by the ectopic expression of the *Drosophila* homologs (Kurata *et al.*, submitted). In addition, the activated form of the *Notch* receptor induces a considerable enlargement of the eye as in *Drosophila* and a duplication of the lens. This indicates that the top of the genetic cascade is highly conserved. At this point it has to be pointed out that there is no functional necessity to use a particular transcription factor like *Pax 6* for particular function e.g. eye morphogenesis, since a transcription factor can regulate any gene, if this gene is endowed with the appropriate regulatory elements in its enhancer or promoter. Therefore, if various eye types are controlled by the same *Pax 6* gene this is merely for evolutionary (historical) reasons and argues for descent from a common ancestor.

Until now *Pax 6* homologs (true orthologs) have been identified in vertebrates, Amphioxus, ascidians, sea urchins, cephalopods, nemerteans, nematodes and platyhelminths, and for Amphioxus, Phallusia, *Loligo* and *Caenorhabditis* (Plaza, Dozier & Seimiya unpubl.) we have shown that their *Pax 6* genes are capable of inducing ectopic eyes in *Drosophila*. The only exception is the *Dugesia tigrina* gene that has diverged to the extent that it is no longer capable of inducing ectopic eyes in *Drosophila* (the second *Dugesia* gene has not been tested yet). In all cases the *Pax 6* genes are expressed in the eyes, in the brain and in several cases in chemosensory organs.

The case of *Caenorhabditis* requires some special explanation, since this worm has no eyes. There are freeliving marine nematodes which possess eyes, but *C. elegans* lives underground and like many cave animals has lost its eyes. Why then should it have an intact *Pax 6* gene? It has been shown for blind cave animals that their *rhodopsin* genes rapidly degenerate and become pseudogenes (Iwabe *et al.*, 1996), which is due to the fact that rhodopsin has only one function, i.e. light perception, and in the absence of light in the

environment there is no selective pressure to maintain this gene intact. In contrast, *Pax 6* in addition to eye morphogenesis is also involved in morphogenesis of the brain and the nose (or other olfactory organs) and is therefore, indispensable even in the absence of light in the environment.

The observation that *Pax 6* is expressed during eye development and regeneration in planarians and nemerteans is of course only a correlation and does not provide any evidence for a causal relationship. However, recently the method of RNA interference (RNAi) by injection of double stranded RNA leading to the degradation of the targeted mRNA has been adapted to these worms and we have obtained genetic evidence for the involvement of both *Pax 6* and *sine oculis* homologs in eye morphogenesis of these worms. By injecting double stranded *Pax*

6 RNA into the regeneration blastema of regenerating nemerteans (*Lineus*) we have been able to knock out *Pax 6* function and to prevent eye regeneration (M. Tarpin, J. Bierne & W.J. Gehring, unpubl.). In the planarian *Dugesia tigrina*, the situation is more complicated because it possesses two *Pax 6* genes. However, we have been able to knock out the *sine oculis* homolog of *Dugesia* thereby preventing eye regeneration (Pineda *et al.*, 2000). These findings provide strong evidence that both *Pax 6* and *sine oculis* are structurally and functionally highly conserved in eye evolution.

A Retrograde Evolution of Biosynthetic Pathways

Histidine Biosynthesis



Evolution

- (0) Histidine has to be taken up from the environment
- (1) Step $\text{Y} \rightarrow \text{Z}$ (E9)
- (2) Step $\text{X} \rightarrow \text{Y} \rightarrow \text{Z}$ (E8)
- (3) Step $\text{W} \rightarrow \text{X} \rightarrow \text{Y} \rightarrow \text{Z}$ (E7)

B Intercalary Evolution of Morphogenetic Pathways

Eye Morphogenesis

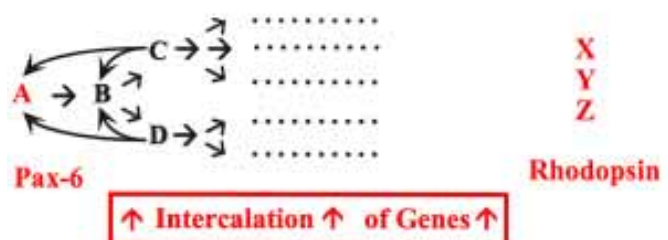


Fig. 9. Models for the evolution of biosynthetic and morphogenetic pathways. (A) Retrograde Evolution of biosynthetic pathways (after N. Horowitz, 1945), (B) Intercalary Evolution of morphogenetic pathways (after Gehring & Ikeo, 1999).

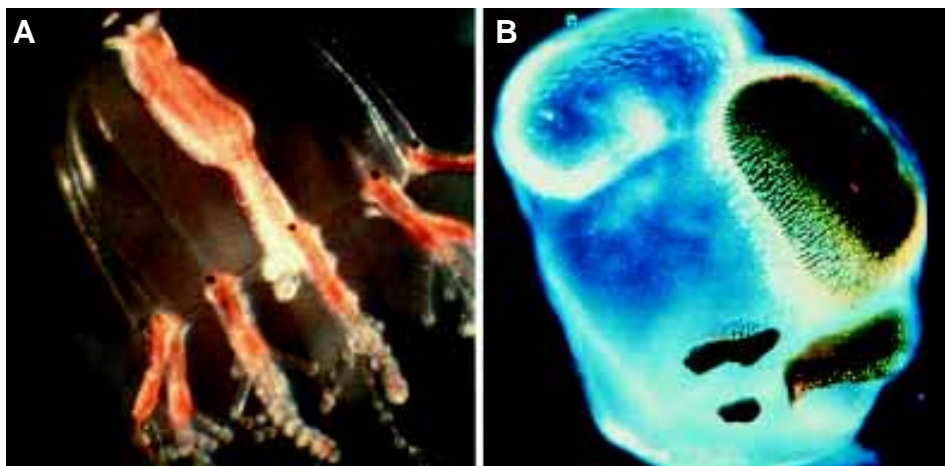


Fig. 10. Eyes of jellyfish medusae. (A) *Cladonema* eyes at the base of the tentacles. (B) *Chironex* eye (Courtesy of Volker Schmid).

How could the Eye Morphogenetic Pathways have Evolved?

The evolutionary events leading to a complex biological structure like the eye are difficult to reconstruct, since the evolutionary intermediates are frequently missing and often we do not know in which direction evolution has proceeded; from simple to more complex structures, or by reduction of highly complex to rudimentary structures. For biosynthetic pathways leading from one or more substrates via numerous enzymatic steps to more complex products, Horowitz (1945) has proposed the mechanism of retrograde evolution. For example, nine enzymes are involved in histidine biosynthesis in bacteria like *Salmonella*, starting from ATP and phosphoribosylpyrophosphate, (two substrates that are used in many other biochemical reactions) to synthesize histidine in a linear biochemical pathway. Horowitz hypothesized that the most primitive bacteria had to take up histidine from the environment (as many higher organisms do) and that the first enzyme to evolve was the enzyme (E9) catalyzing the last step in histidine biosynthesis (Fig. 7). The organism possessing E9 had a strong selective advantage when histidine became scarce in the envi-

ronment, since it could use the immediate precursor of histidine as a substrate. The next enzyme to evolve was presumably E8 which could have arisen by gene duplication of E9 and subsequent divergence, and so on until a completely linear and streamlined pathway had been established in a retrograde manner. Recent amino acid sequence and structural comparisons of these enzymes indicate that the Horowitz model is not entirely correct, but there is evidence that e.g. His A and His F, two enzymes with a β/α barrel structure have evolved by two-fold gene duplication and gene fusion from a common half-barrel ancestor (Lang *et al.*, 2000).

For the evolution of the eye morphogenetic pathway we have proposed a mechanism of intercalary evolution (Fig. 9) (Gehring and Ikeo, 1999). Since both the master control genes at the top of the gene cascade, *Pax 6* and *sine oculis*, as well as the essential photoreceptor genes of the rhodopsin family on the bottom of the hierarchy, are present already in the primitive eyes of flatworms, we propose that the more elaborate eyes have evolved from these primitive eyes by intercalation or recruitment of additional genes into the eye developmental pathway. The two best understood genetic mechanisms for intercalation are on the one hand gene duplication and subsequent divergence, and on the other hand enhancer fusions. We have found evidence for both of these mechanisms in *Drosophila* eye development. *Eyeless* and *twin of eyeless* are a good example of gene duplication, subsequent divergence and intercalation. In organisms with a single *Pax 6* gene that have been analyzed in detail, *Pax 6* is autoregulated and forms a positive autocatalytic feedback loop, which upon gene duplication in *Drosophila* has evolved into a heterocatalytic positive control loop, since *twin of eyeless* directly activates *eyeless* by interaction with the eye specific enhancer of *eyeless*. Therefore, *eyeless* has been intercalated into the eye pathway between *twin of eyeless* and *sine oculis*. The fact that both *twin of eyeless* and *eyeless* interact with the same enhancer in *sine oculis* (Punzo *et al.*,

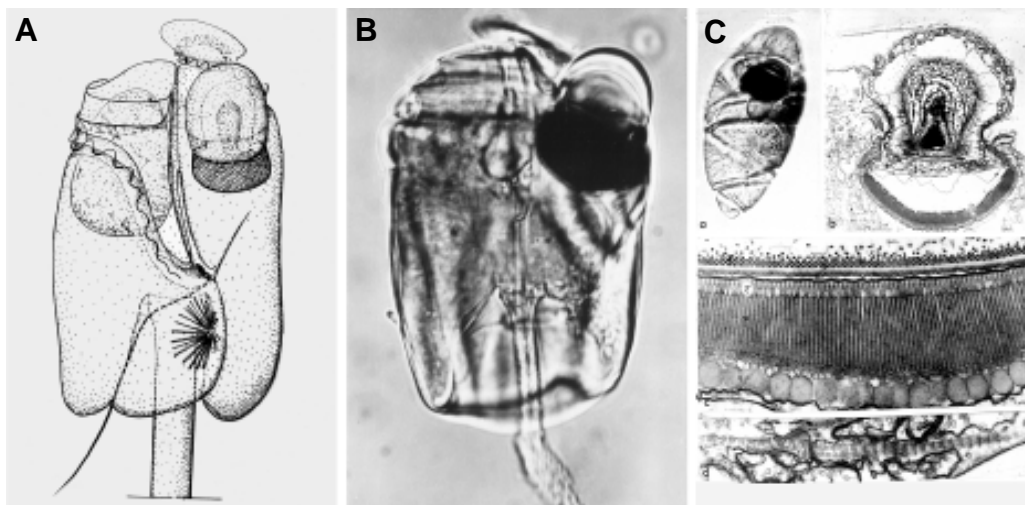


Fig. 11. Eye organelle of the unicellular dinoflagellate *Erythroopsis pavillardii*. (A) Schematic drawing. (B) Light micrograph showing the lens and the shielding pigment. (C) Stacked membranes resembling the arrangement in photoreceptor cells in the retina of multicellular organisms. After Greuet, 1965. (Courtesy of Marie-Odile Soyer).

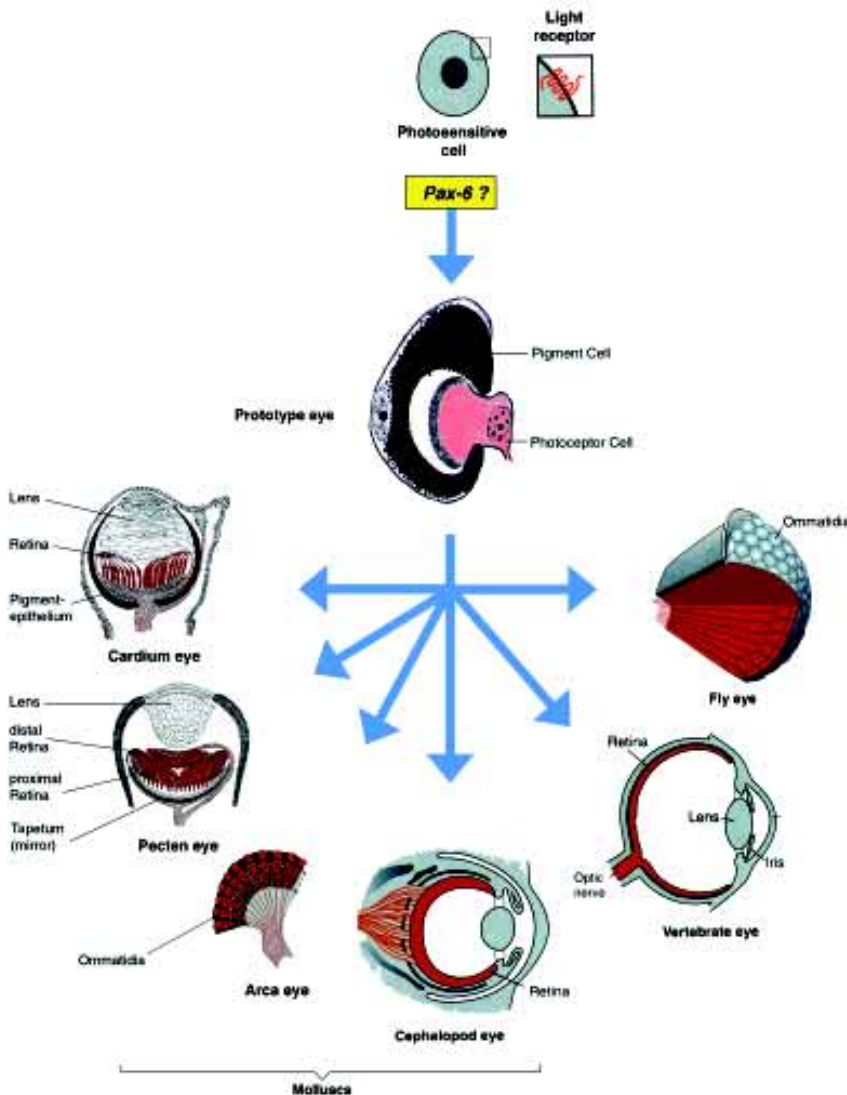


Fig. 12. Hypothetical evolution of photosensitive cells containing rhodopsin as a light receptor and monophyletic evolution of the various eye-types starting from a Darwinian prototype eye consisting of a single photoreceptor cell and a pigment cell assembled under the control of Pax 6 (after Gehring and Ikeo, 1999).

submitted) suggests that *eyeless* and *twin of eyeless* are derived from a common ancestral gene which is in line with the extensive similarity of their amino acid sequences. These two genes are partially redundant in their function, since they can partially substitute each other. However, they also have diverged to the extent that *eyeless* plays a major role in compound eye development, whereas *twin of eyeless* is required for the formation of ocelli.

A second case found in *Drosophila* is *Drosocrystallin* which like some crystallins in vertebrates has been recruited into the lens developmental pathway from another source (Piatigorsky & Wistow, 1989). In this case, there is strong evidence from sequence data that *Drosocrystallin* belongs to the family of cuticle proteins, suggesting it has been recruited into the eye developmental pathway by fusion to a lens-specific enhancer (Jansens & Gehring, 1999). *Drosocrystallin* represents a case for a gene that is not conserved in the course of evolution and contributes to lens development in *Drosophila* but not in vertebrates. However, even

highly conserved genes can be recruited for different functions. For example, the homeobox gene *Rx* plays a major role in vertebrate eye development, but despite the fact that *Drosophila* has a highly conserved *Rx* homolog, this gene is not expressed in *Drosophila* eye morphogenesis (Eggert *et al.*, 1998) and seems to serve a function in brain rather than eye development. Differential expression of conserved genes and recruitment of “new” genes into the eye morphogenetic pathway provide a likely mechanism for the evolution of different eye-types originating from the same eye prototype.

The Origin of Eyes and Brain

Gregory (1967) has considered the question of whether the eyes or the brain came first in evolution, assuming that the evolution of the mechanism of visual perception must entail the separate elaboration of eyes and brain. He regards the information given by the eyes as of indirect use to living creatures, since considerable “computing” is required to make any use of visual information. By contrast touch and chemical senses directly monitor biologically vital features of the environment, and their information requires but a minimal “computer”. Gregory therefore considers it reasonable to suppose that vision is a lately acquired sense. Furthermore, he supposes that the simple eye took over existing touch neural mechanism. Since probe touch can provide information in three dimensions, the underlying “computer” in the brain, was ideally suited to process visual information. Rüdiger Wehner (pers. comm.) has proposed the idea that rhodopsin may be considered as a chemoreceptor with a covalently bound ligand, retinal. In this case the ligand does not have to be bound by the receptor in order to activate it as in chemoreception, but it can be activated by photons. There is in fact some evidence supporting the hypothesis that rhodopsin is derived from chemoreceptors, since Klein *et al.* (1988) have found significant sequence homology between rhodopsin

and the cyclic AMP receptor of *Dictyostelium*, which serves as a chemoattractant receptor. This lends support to the idea that the olfactory and visual systems are closely related and that the visual system has evolved from chemoreception. In this context it is important to point out that Pax 6 is not only involved in eye morphogenesis but also in the development of the nose and other chemosensory organs, and in brain morphogenesis as well.

Concerning the question of who came first the eyes or the brain we have proposed that the sensory organs preceded the evolution of the brain (Gehring and Ikeo, 1999). The sensory organs are gathering information, whereas the brain is an information processing organ, similar to a computer. If no information is acquired, there is no need for an elaborate information processing organ. As more and more information is acquired by the sensory organs, the brain evolves in parallel to process this information and transmit it to the effector organs like the muscles. Evidence for this hypothesis comes from the analysis of jellyfish like *Cladonema* or *Chironex*, the sea

wasp (Fig. 10), which have highly evolved eyes, but no brain. The eyes are located at the base of the tentacles and transmit their information directly to the muscles without processing by a brain. There are very few interneurons between the eyes and the muscles, and there is only a ring nerve around the bell margin which may coordinate the movements of the tentacles, but there is no real brain. Of course, one may argue that jellyfish originally had a brain, but have reduced it in the course of evolution. However, there is no selective advantage to lose the brain in a freelifing pelagic animal. On the contrary, there is selective pressure to maintain it. Animals living underground or in caves may lose their eyes because of lack of selective pressure, however, they retain their brain. Parasites may even reduce their brains, by adapting to their hosts and become extremely reduced to reproduction "machines", but freelifing organisms can neither dispense with their sensory organs nor their brains. Therefore, I consider it likely that the eyes came before the brain.

This point of view is supported by the evolution of eye organelles in protists. *Chlamydomonas*, for example, has an eye organelle with a rhodopsin-related photosensitive pigment and a shielding pigment spot which allows it to determine the direction of the incoming light. Analogous to jellyfish, the eye organelle is located directly at the base of the effector organ, the flagellum, and the information is transmitted directly from the eye organelle to the flagellum without an intervening information processing organelle. It is worth pointing out that some dinoflagellates like *Erythrospira*, have evolved the most elaborate eye organelles consisting of a lens, shielding pigment and retina-like structures with membrane stacks, closely resembling the eyes of multicellular organisms (Fig. 11). We have no idea how such complicated organelles can evolve in single celled organisms. However, it raises the interesting possibility that the metazoan eye may have originated from symbionts, much like mitochondria and chloroplasts.

Concluding Remarks

The discovery of the universal master control genes for eye development has led us to a radically different concept of eye evolution than previously assumed. Our data argue strongly for a monophyletic origin of the various eye types starting from a Darwinian eye prototype present before the Cambrian explosion and leading to adaptive radiation in most or all phyla by parallel evolution, which can be divergent or convergent (Fig. 12). To base evolutionary studies exclusively on morphological criteria without considering the genetic basis can be misleading. Genetic criteria have also to be applied to the notion of evolutionary homology and this term has to be redefined in a quantitative rather than qualitative way as proposed by Zuckerkandl (1994). Developmental genetics and functional genomics will provide deeper insights into evolution, since DNA not only contains a detailed developmental program, but also vast information about the evolutionary history of organisms.

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