Pax 6 mastering eye morphogenesis and eye evolution

***Pax 6* genes from various animal phyla are capable of inducing ectopic eye development, indicating that *Pax 6* is a master control gene for eye morphogenesis. It is proposed that the various eye-types found in metazoa are derived from a common prototype, monophyletically, by a mechanism called intercalary evolution.**

Explaining the evolution of an organ as perfect as the eye is a great challenge for all evolutionary biologists. In his theory 'The Origin of Species' Charles Darwin devoted an entire chapter to the problem. Darwin freely admitted that the idea of an eye that is capable of adjusting the focus to different distances, of admitting different amounts of light and of correcting spherical and chromatic aberration, could have been formed by natural selection seems intuitively absurd. However, he subsequently found a way out of this dilemma by postulating a simple and imperfect eye, a prototype, from which the more perfect visual organs might have arisen gradually, by variation (mutation) and by natural selection. Darwin assumed the prototype to consist of at least two cells: an 'optic nerve' (photoreceptor cell) and a pigment cell shielding the photoreceptor cell from one side, covered by translucent skin, but without any lens or other refractive body. Such primitive eyes are found, for example, in some planarians (Fig. 1). Comparative anatomists have discovered numerous intermediates between this most primitive type of eye and the vertebrate eye, such as: eye cups; pinhole eyes; camera-type eyes with a single lens; reflecting mirror eyes; and compound eyes with numerous ommatidia, all of which lends support to Darwin's theory.

On the basis of comparative anatomical and ultrastructural studies of the various types of eye and photoreceptor cells, it has been postulated by Salvini-Plawen and Mayr[1], two strong proponents of darwinism, that photoreceptor organs have originated independently in at least 40, but possibly up to 65 or more different phyletic lines. However, there are some critical facts that are not consistent with this conclusion, and we would like to challenge this idea and argue for a monophyletic rather than a polyphyletic origin of the metazoan eye. Salvini-Plawen and Mayr argue purely on morphological grounds. Their section on 'the multiple origin of eyes' begins with the comment that 'it requires little persuasion to become convinced that the lens eye of a vertebrate and the compound eye of an insect are independent evolutionary developments'. This point has been taught to biology students for over a hundred years. However, in a later section (p. 237) these authors describe the observation that, in clams, all three major eye-types [the camera eye with a single lens (in the heart shell, *Cardium*), the mirror eye with a lens and a reflecting mirror (in the scallop, *Pecten*), and compound eyes that consist of 10–80 ommatidia each (in Noah's arc, *Arca noae*)], are found in the same phylogenetic class, the Bivalvia (Fig. 1). All of these types of eye are located at the same anatomical position – the edge of the mantle. The compound eyes of *Arca* are similar to those of arthropods, but they have only a single photoreceptor cell per ommatidium, whereas insects and crustaceans generally have eight or nine visual cells per unit. Salvini-Plawen and Mayr interpret the compound eyes of *Arca* as new formations, but an equally valid interpretation of these data is to assume that the camera-, mirror- and compound eyes of clams have evolved monophyletically from a common ancestral precursor. A monophyletic origin for the eye is also supported by the observation that all metazoans share the same visual pigment, rhodopsin.

Darwin was highly self-critical in his discussion of the eye prototype and admits that the origin of the prototype cannot be explained by natural selection, because selection can only drive the evolution of an eye once it is partly functional and capable of light detection. Therefore, selection cannot explain the origin of the eye prototype, which for Darwin represents the same problem as the origin of life. Therefore, both the origin of life and the origin of the eye prototype must have been very rare events, and a polyphyletic origin in over 40 different phyla is not compatible with Darwin's theory. In this review, we discuss more recent evidence in favor of a monophyletic origin of the eye and propose a new hypothesis explaining how morphogenetic pathways might have evolved.

Pax 6 is a master control gene for eye morphogenesis and evolution

Homeotic mutations in *Drosophila* have resulted in the identification of several master control genes that specify the body plan by controlling anterior–posterior polarity, segmental identity, organogenesis and identity of individual cells in great detail. The term 'master control genes' was introduced by Lewis[2] for the homeotic genes of the *Bithorax Complex*, and, perhaps the most impressive demonstration of their role in development has been the genetic construction of four-winged and eight-legged flies[3]. Targeted expression of the homeotic *Antennapedia* gene results in complete middle legs being induced in the antennal

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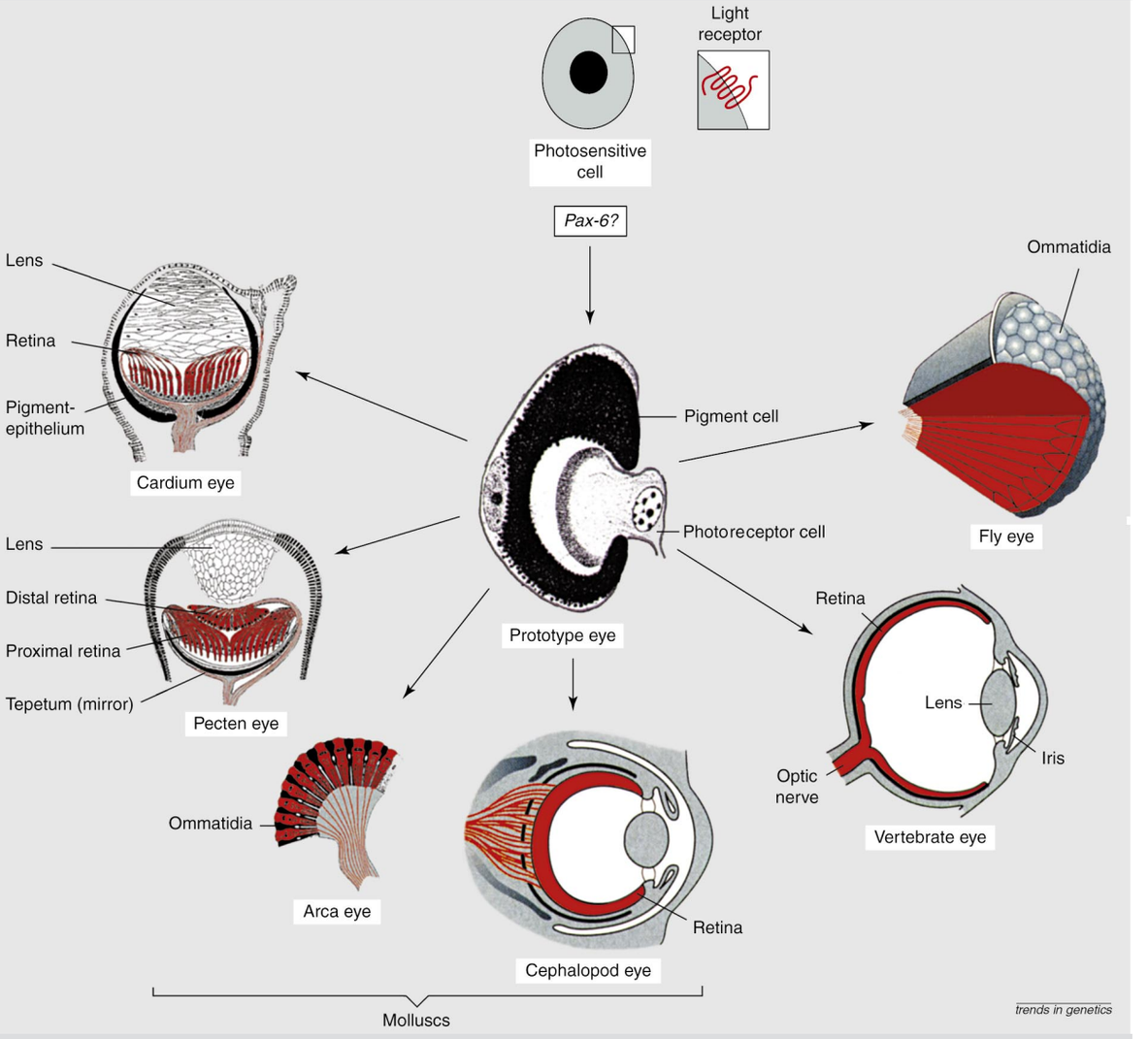
0168-9525/99/$ – see front matter PII: S0168-9525(99)01776-X

FIGURE 1. Eye evolution



A hypothetical scheme of the evolution of various eye-types from a common ancestral prototype. As a first step, photosensitive cells with a light receptor (opsin) have evolved. Under the control of the *Pax 6* gene, the photosensitive cell assembles with a pigment cell to form an organ, the prototype eye. By divergent, parallel and convergent evolution, the various eye-types are generated from the prototype: the compound eye of insects; the camera-type eye of vertebrates; and the large spectrum of eye-types in molluscs ranging from the primitive camera-type eye in *Cardium*, the mirror-plus-lens eye of *Pecten*, the compound eye of *Arca* to the highly evolved cephalopod eye, that greatly resembles the vertebrate camera-type eye.

discs of *Drosophila*[3]. Another striking example of a master control gene is *Pax 6*. This gene was first cloned in the mouse[4,5] and in humans[6] and subsequently shown to be affected in the mouse mutant, *Small eye*, and in human *Aniridia* patients. In humans and mice, eye defects are associated with *Pax 6* mutations in heterozygotes. The homozygous *Pax 6* mutation is lethal to mouse embryos: they lack eyes and a nose, and also have brain damage. *Pax 6* is expressed from the earliest stages of eye morphogenesis in the optic vesicle, giving rise to the retina and pigment retina, as well as in the overlying ectoderm that later forms the lens and the cornea. However, *Pax 6* is also expressed in the nasal epithelium, in specific regions of the brain and the spinal cord, and not exclusively in eye primordia.

Pax 6 encodes a transcription factor that contains a paired domain and a homeodomain. The *Pax* gene family clearly illustrates that novel genes are generated in the course of evolution by recombining parts of pre-existing genes in a process that Jacob called evolutionary tinkering[7]. The different *Pax* genes contain various combinations of paired domains, with homeodomains, a sequence called octapeptide, or parts of the homeodomain and paired domain, respectively. The murine and human PAX 6 proteins are identical in amino acid sequence. A *Pax 6* homolog in *Drosophila*[8] was subsequently discovered and this also shows extensive sequence similarity, both in the paired domain (94% identity), and in the homeodomain (90% identity). More surprising is the finding that the *Drosophila Pax 6* homolog is the *eyeless* (*ey*) gene known by a mutation affecting the eyes since 1915 (Ref. 9). This was unexpected because of the long-standing dogma, mentioned above, that the insect compound eye was non-homologous to the vertebrate camera eye, and that the two types of eye had evolved independently. The observation that *Pax 6* homologs of

both mammals and insects are essential for eye morphogenesis led to the idea that *Pax 6* might be the universal master control gene for eye morphogenesis and evolution[8].

We tested the master control gene hypothesis by constructing a gain-of-function mutation. In wild-type larvae, *ey* is expressed exclusively in the eye-antennal disc from the earliest stages when the disc primordia are formed in the embryo. Therefore, we used the Gal-4-system to target gene expression into imaginal discs other than eye discs[10]. By the use of different genomic enhancer lines, we were able to induce ectopic eyes on the legs, wings, halteres and the antennae of the fly, and recent electrophysiological experiments show that the ectopic eyes on the antenna can generate a normal electroretinogramme, which indicates that they are functional (P. Callaerts and W. Gehring, unpublished). This illustrates the role of *ey* as a master control gene that is capable of switching on a cascade of some 2500 genes required for eye morphogenesis[10]. Of course, eye morphogenesis cannot be induced in any tissue of the fly at any stage of development, but at least it does occur in all imaginal discs up to a certain stage of differentiation. The master control gene first has to interact with subordinate control genes to repress the resident genetic programme and to install the eye programme. If the cells have proceeded too far along their pathways and are firmly locked into a different pathway, the ectopic expression of *ey* has no effect.

Our next query was whether the mammalian *Pax 6* gene can functionally substitute for the *Drosophila* homolog. The ectopic expression of mouse *Pax 6* in *Drosophila* induces ectopic compound eyes[10], suggesting that *Pax 6* has a universal function of gene regulation in eye morphogenesis. The reciprocal experiment has not been completed yet, but it has been reported that *Xenopus Pax 6* is capable of inducing ectopic eye lenses[11]. However, by changing the timing and site of *Pax 6* RNA injection into the *Xenopus* embryo, it is possible to induce complete ectopic eyes (R. Chow, C. Altmann, R. Lang and A. Hemmati-Brivanlou, pers. commun.). These findings clearly indicate that *Pax 6* is a master control gene for eye morphogenesis in both insects and vertebrates.

The protein-coding regions of *Pax 6* are highly conserved in evolution, as are some of the regulatory sequences in the promoters and enhancers. Consequently, the regulatory mechanisms that direct ocular expression are also conserved between flies and mice. The eye-specific enhancer region of the *Drosophila ey* gene[8,12], when inserted upstream of either of the two mouse *Pax 6* promoters (P1 or P0), directs eye- and CNS-specific expression in transgenic mice that accurately reproduces features of endogenous *Pax 6* expression[13]. In a reciprocal experiment, the mouse P1 element is able to direct *lacZ* reporter gene expression into the eye imaginal discs of *Drosophila*. Here, the expression is restricted to the photoreceptor cells, although *lacZ* expression is delayed and occurs only posterior to the morphogenetic furrow, whereas endogenous *ey* expression is confined to the undifferentiated cells anterior to the morphogenetic furrow. However, the *Drosophila ey* enhancer itself shows the same spatio–temporal expression pattern as the mouse promotor, that could reflect perdurance of β-galactosidase or lack of regulatory sequences that confer repression posterior to the morphogenetic furrow[12]. Overall, there is evidence for conservation of *Pax 6* gene regulation, but there is uncertainty about the extent of the conservation.

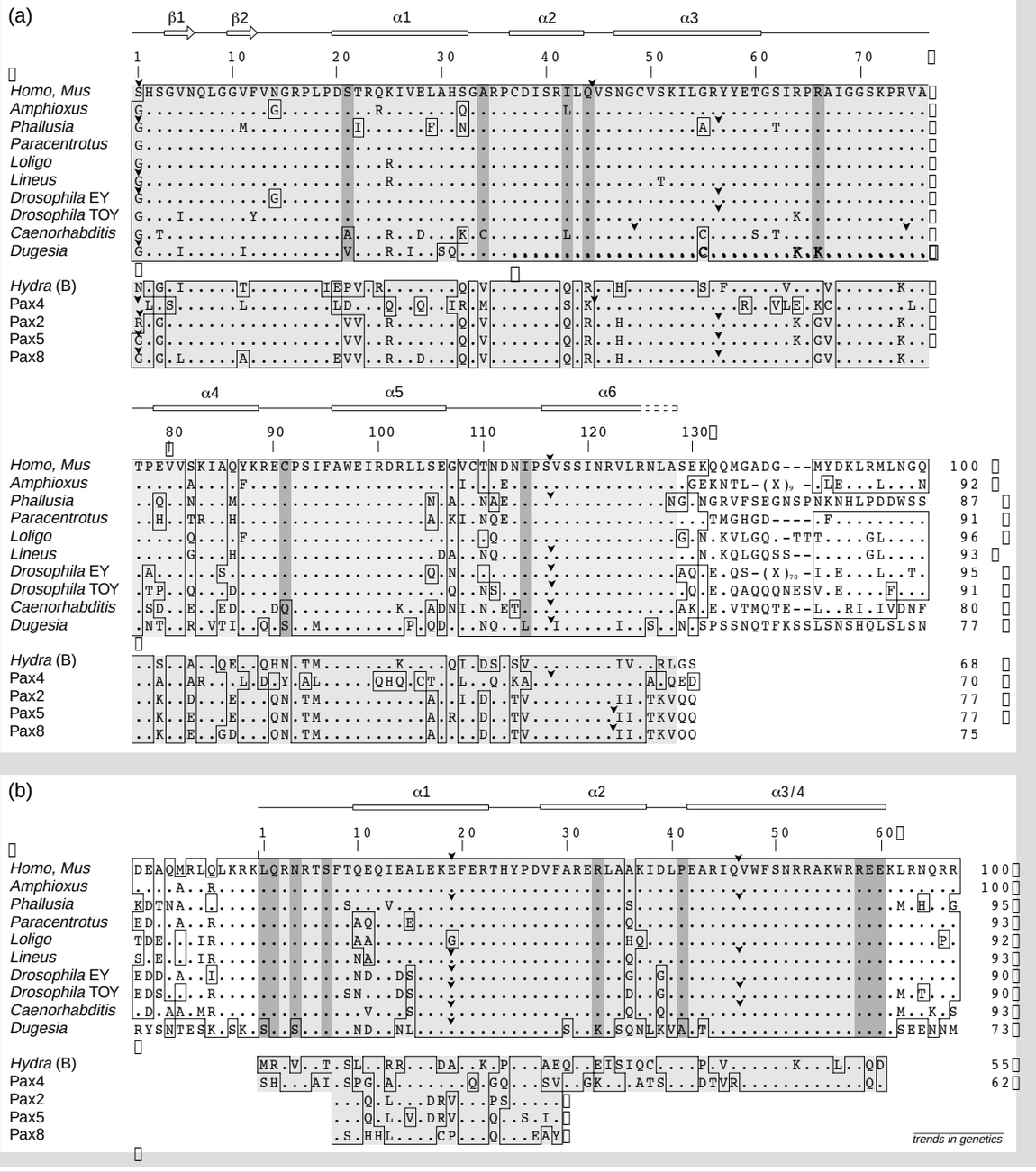
Genuine *Pax 6* genes have now been isolated from: mammals; amphibians; fish; amphioxus; sea squirts; sea urchins; squid; nematodes; ribbonworms; and planarians (Fig. 2). In Cnidarians the situation is less clear, because the genes found so far are either precursors of *Pax 6* or have diverged too far to be clearly identified as *Pax 6* homologs. In any case, this survey shows that *Pax 6* was present in the last common ancestor of all these triploblastic phyla, much like the rhodopsin gene. In addition to the mammalian *Pax 6* gene, its homologs from the sea squirt *Phallusia* and the squid *Loligo* are also capable of inducing ectopic eyes in *Drosophila*. With the exception of sea urchins and *Caenorhabditis elegans* (which presumably have lost their eyes during evolution because eyes are found in other echinoderms and nematodes), all *Pax 6* genes examined so far are expressed prominently in the developing eyes, including those of planarians, which come close to the darwinian prototype. Furthermore, *Pax 6* is specifically expressed in the differentiated eyes of the ribbonworm *Lineus*[14] and particularly during eye regeneration[15], strengthening the correlation between eye morphogenesis and *Pax 6* expression.

The evolution of *Pax 6*: *twin of eyeless*

More recently, a second *Pax 6* gene homolog in *Drosophila* called *twin of eyeless* (*toy*) was identified[16]. It shares 91% sequence identity in the paired domain and 90% in the homeodomain with the human and murine PAX 6 proteins (Fig. 2), compared with 95% and 90% for EY. Outside of these highly conserved domains, TOY is more similar to the mammalian proteins than EY, particularly in its overall length and at the C-terminus, where it shares a transcriptional activation domain with other PAX 6 proteins that is absent in EY. A survey by polymerase chain reaction (PCR) shows that two *Pax 6* genes are only found in holometabolous insects (*Drosophila* and *Bombyx*) and not in hemimetabolous (grasshopper) or apterygote insects (springtail), nor in all other phyla tested[16]. This indicates that the gene-duplication event leading to the two paralogs occurred during insect evolution, a conclusion that is also supported by the molecular phylogenetic analysis (Fig. 3). Besides the sequence similarity, the localization of the intron splice sites clearly indicates that both paralogs are bona fide *Pax 6* genes (Fig. 2). The first splice site at the N-terminus of the paired domain is missing in *toy*, but present in *ey*, whereas the second splice site in the homeodomain is present in *toy* and absent in *ey*, indicating that the ancestral gene had all four splice sites in the two boxes. The same four splice sites are also found in the nematode *Caenorhabditis elegans* and three out of four can be traced back to platyhelminths (*Dugesia*). This indicates that these introns are very old (precambrian) and that a bona fide *Pax 6* gene must have been present in the last common ancestor of triploblastic animals. Vertebrates share a splice site at codon 44/45 that is vertebrate-specific and is used for differential splicing in the paired box. It is absent in amphioxus and ascidians, indicating that this intron arose later in evolution, after vertebrates had separated from invertebrates.

Following gene duplication during insect evolution, the two paralogs *ey* and *toy* began to diverge in function. In particular, *toy* is expressed much earlier, at the blastoderm stage, when the *Drosophila* body plan is laid down, whereas *ey* is expressed only later, during germband extension. The spatial patterns at later stages are very similar although not identical. This earlier divergence with

FIGURE 2. Metazoan Pax 6 proteins



Comparison of the amino acid sequences for PAX 6 proteins from various metazoa. The paired domains are indicated in (a) and the homeodomains in (b). PAX 6 protein-specific amino acids are shaded more darkly. The positions of the intron splice sites are indicated by arrowheads. These have not yet been determined for Amphioxus, *Paracentrotus* and *Loligo*. The numbers indicate the percentage amino acid sequence identity as compared with the mouse and human proteins. For comparison the closely related Pax sequences from the mouse (m) are shown. Pax 2, 5 and 8 have only partial homeodomains. α, α-helices; β, β-sheets.

respect to temporal rather than spatial patterns of gene expression has been found in other duplicated developmental control genes, like *sloppy-paired 1* and *2* (Ref. 17), and might be a more general feature of evolution. Like *ey*, *toy* is also capable of inducing ectopic eyes in *Drosophila*, but *toy* requires a functional *ey* gene to induce eyes, suggesting that *toy* is upstream of *ey* in the genetic cascade controlling eye morphogenesis. Epistasis experiments, as well as biochemical and transgenic analyses, support the notion that *toy* acts upstream of *ey* in the eye developmental pathway[18] by directly regulating the eye-specific enhancer of the *ey* gene[12,16]. This observation reveals an interesting facet of the evolution of morphogenetic pathways: the single *Pax 6* in vertebrates is autoregulated by a positive feedback loop in which the PAX 6 protein binds to the enhancer in its own gene and activates its transcription[19]. In *Drosophila*, after gene duplication this positive autocatalytic feedback loop appears to have evolved into a heterocatalytic loop in which one of the paralogs regulates the other, leading to the

integration of *ey* into the eye developmental pathway underneath *toy*.

The genetic cascade specifying the eye developmental pathway

Following the discovery of *ey* as a master control gene, several groups have embarked upon the analysis of the genetic cascade leading to eye morphogenesis by identifying target genes and genetic interactions. However, the direct nature of a given genetic interaction and the molecular basis of the interaction has been demonstrated in only a few cases. In *Drosophila*, evidence for a direct activation of *ey* transcription by binding of TOY protein to the eye-specific enhancer of the *ey* gene has been described above. This puts the *toy* gene on top of the hierarchy and *ey* underneath[16]. The *toy* gene requires *ey* to induce eye formation; in turn, *ey* induces and requires *sine oculis* (*so*) and *eyes absent* (*eya*) for the induction of ectopic eyes[18]. There is strong evidence that *so* is a direct target for EY protein[20]. However, as more and more pieces are filled into the puzzle, the simple linear pathways turn into a complex network and several other genes have been found to be capable of ectopic eye induction. The *so* gene encodes a homeodomain protein that is required for the development of the entire visual system in *Drosophila*[21,22]. The *eya* gene encodes a novel type of nuclear protein involved in the development of the visual system as well as in the somatic gonadal precursors[23,24].

A gene called *dachshund* (*dac*) encodes a novel nuclear protein that is required for differentiation of the ommatidia, but is also essential for leg development[25,26]. The ectopic expression of *eya* or *dac* alone or in combinations of *eya* with *so* or *dac* induces ectopic eye formation, but also activates *ey* expression. The *ey*, *eya* and *dac* genes are all activated during eye induction[18] and there is evidence that the EYA protein forms a complex with SO (Ref. 27) and DAC (Ref. 28) proteins. Taken together, these findings can be explained by a model in which *ey* induces the initial expression of *so* and *eya* that regulates the activity of all four genes by positive feedback loops required for eye induction[16].

Targeted expression of the gene *teashirt* (*tsh*), which was shown to be required for the specification of the trunk segments in the *Drosophila* embryo, can also induce ectopic eyes[29]. This gene encodes a transcription factor with zinc-finger motifs and induces the expression of *ey*, *so* and *dac*. In turn, *ey* induces the expression of *tsh*, indicating that *tsh* is also a member of the regulatory network of genes that are connected to each other by positive feedback loops. However, it should be emphasized that *ey* is a much more potent inducer of ectopic eyes than any single gene in the later group, suggesting that no single gene can recapitulate the entire spectrum of *ey* activity, reinforcing the master control gene status of *Pax 6*.

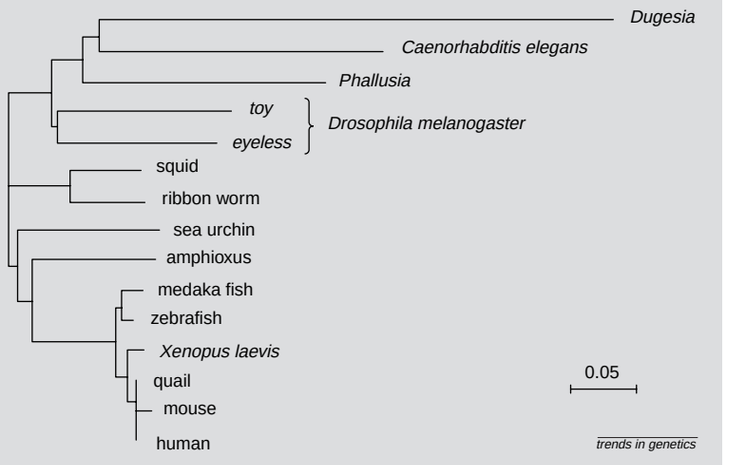
A second *Pax* gene, *eyegone* (*eyg*) apparently acts in parallel with *ey* in determining *Drosophila* eye development[30]. This gene contains only a partial paired domain, but a complete homeodomain. Loss-of-function mutations lead to a reduction of the eyes similar to *ey*, and ectopic expression leads to the induction of ectopic eyes. The two genes *eyg* and *ey* seem to have complementary functions because their coexpression leads to a synergistic enhancement of ectopic eye formation. The expression of *eyg* is not regulated by *ey* at the transcriptional level, nor does it regulate *ey* expression. However, homozygous *ey:eyg* double mutants are lethal, which indicates that the two genes interact. It has been proposed that the two protein products can form a heterodimer, which is compatible with the findings mentioned above[30].

One of our aims is to compare the genetic cascade from *Drosophila* with that of the mouse or other vertebrates to find out how many other genes besides *Pax 6* and the rhodopsin gene have been conserved during evolution. Several homologs for *so* and *eya* have been identified in vertebrates, and a second *so*-like gene has also been isolated from *Drosophila*[31]. However, sequence conservation of the protein-coding region does not necessarily imply that the function in eye morphogenesis is also conserved in evolution. For example, the mouse *Rx* gene that belongs to the paired-like class of homeobox genes was shown to be expressed both in the developing retina and forebrain. Loss-of-function mutants in mice do not form optic cups and, as a consequence, lack eyes[32]. Furthermore, misexpression of *Rx* induces ectopic retinal tissue in frogs[32]. However, a *Drosophila* homolog of *Rx* that has 100% sequence identity in the homeodomain is expressed only in the developing brain, but not in the embryonic or the larval eye primordia[33]. Eventually, it will be interesting to find how many new genes must be recruited into the eye-developmental pathway to generate either a mouse or a *Drosophila* eye, and how many of these genes are common. However, the major changes occurring during evolution are likely to occur at the level of gene regulation, and very different types of eye might be generated by the same set of regulatory genes.

The evolution of the different types of eye

The evolution of light-sensitive cells is intimately connected to the evolution of the visual pigment rhodopsin. Rhodopsin is the molecule of ultimate sensitivity because it is capable of sensing a single light quantum. Absorption of a single quantum of light converts all-*trans* retinal, that is covalently bound to the opsin protein molecule, into 11-*cis* retinal. This conversion causes a conformational change of the protein that is amplified by transducin, a G-protein and results in an electrical nerve impulse[34].

FIGURE 3. Phylogenetic tree of the *Pax 6* genes



The neighbor-joining method was used to generate a phylogenic tree of the *Pax 6* genes from various metazoa. Note that *Drosophila melanogaster eyeless* and *twin of eyeless* are closely related. The scale shows the number of amino acid substitutions per site. The monophyly of the *eyeless/Pax 6* group of genes is strongly supported by the phylogenetic analysis of Jacobs *et al.*[42]

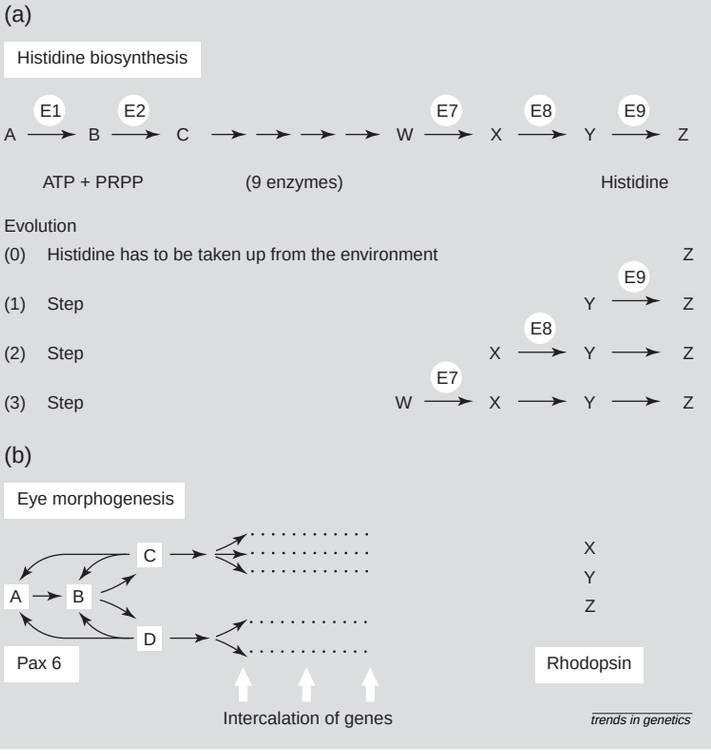
Rhodopsins are present in some bacteria, and some of these proteins also serve a sensory function. However, there is very little sequence conservation between bacteriorhodopsins and rhodopsins of higher organisms, even though both are structurally similar membrane proteins with seven transmembrane domains.

Protists have also developed visual systems that are based on rhodopsin. The unicellular green alga *Chlamydomonas* has developed a visual system that allows it to measure light intensity, as well as to determine the direction of the incident light. These abilities confer a strong selective advantage for an organism that depends on photosynthesis[35]. The direction of the incident light is determined with the help of the eyespot, a carotenoid-containing vesicle that presumably operates as an interference reflector. The action spectra for phototaxis and flash-induced phobic responses have a maximum close to 550 nm like rhodopsin, and in blind retinal-deficient cells, positive phototaxis can be restored by supplying the cells with all-*trans* retinal. Chlamydorhodopsin has recently been cloned[36], and it shows some sequence homology to invertebrate rhodopsins. However, it is not a typical seven-transmembrane receptor, and looks instead rather like an ion channel. Therefore, this primitive plant rhodopsin probably diverged from animal opsin early in evolution. In all vertebrates and invertebrates analyzed so far, typical rhodopsins belonging to one and the same gene family have been found.

The visual system of unicellular organisms is an organelle, rather than an organ, and it is formed by intracellular assembly processes, whereas the eyes of metazoa are organs made up of cells of at least two different types or of different tissues, as already pointed out by Darwin. There is accumulating evidence that *Pax 6* is the universal master control gene for eye morphogenesis in metazoa ranging from platyhelminths to humans. The universality of rhodopsin and *Pax 6* suggests that the different types of eye found in metazoa are derived from a single prototypic eye and are, therefore, of monophyletic origin. *Pax 6* serves as a regulatory gene to assemble the different cell-types, such as photoreceptor cells and pigment cells, into a light-sensing organ. This new concept of eye evolution is illustrated in Fig. 1. Originating from a precambrian prototype, the various types of eye are thought to have evolved by divergent, parallel and convergent evolution by recruiting numerous additional genes into the eye-developmental pathways, as discussed in the following section.

In higher metazoa, the eyes are connected to the brain, where visual information is processed and transmitted to the effector organs, such as muscles. In the more primitive (ancestral) cnidarians, such as cubomedusae (which do not have a brain, but only a nerve ring around the umbrella), the eyes are directly connected to the muscles in the tentacles. This suggests that the eye evolved as an information-gathering organ before the brain, the information-processing organ.

FIGURE 4. Retrograde and intercalary evolution



(a) Hypothetical retrograde evolution of histidine biosynthesis as proposed by Horowitz[37]. The last enzyme (E9) of the biosynthetic pathway evolves first, followed by E8 in a second step. This proceeds until all nine enzymes are lined up in a linear pathway. (b) Proposed intercalary evolution of morphogenetic pathways. First a rhodopsin-containing photosensitive cell has to evolve, that under the control of *Pax 6* is assembled with a pigment cell to form a functional eye prototype. The top of the cascade is formed by a master control gene (*Pax 6*), the bottom by essential structural genes, such as rhodopsin. In the course of evolution, new genes are intercalated between the top and bottom of the cascade: regulatory genes, such as *eyeless* downstream of *twin of eyeless*; and structural genes, such as the lens *crystallin* genes. The morphogenetic pathway is not linear but, rather, a complex network.

Evolution of biosynthetic and morphogenetic pathways

Horowitz[37] has proposed a mechanism for the evolution of biosynthetic (or biochemical) pathways that is based on the idea of retrograde evolution (Fig. 4a). This hypothesis assumes that, for example, the nine enzymes in histidine biosynthesis evolved in a retrograde fashion. Presumably, primitive organisms had to take up histidine from the environment. The organisms that evolved the last enzyme in the pathway presumably had a strong selective advantage when the supply of histidine (Z) in the environment was exhausted, because it could use compound Y and convert it to Z. The next step was the evolution of enzyme and so on, until all nine enzymes had evolved that made it possible to achieve histidine biosynthesis from PRPP and ATP. A similar mechanism of retrograde evolution has been proposed for the evolution of the sex-determination pathway[38].

Based on a similar kind of logic, we propose that morphogenetic (or developmental) pathways evolve by intercalary evolution (Fig. 4b). Prerequisite is the prior evolution of rhodopsin and of *Pax 6* to generate the prototypic eye. The prototype, as pointed out by Darwin, cannot be explained by selection, because selection can drive evolution only when the eye can function at least to a small extent. Once the prototype has evolved, presumably by stochastic events, selection can optimize it by a mechanism that can be called intercalary evolution to distinguish it from retrograde evolution mentioned above. The prototype has acquired two key genes, *Pax 6* on the top, and rhodopsin at the bottom of the genetic cascade. Increasingly complex and more-sensitive eyes can be generated by the intercalation of genes into the cascade (Fig. 4b). At least three genetic mechanisms for intercalation are

known. First, gene duplication and divergence, as described for *ey* and *toy*. The original autocatalytic feedback loop is converted to a heterocatalytic loop, where *toy* regulates *ey*, the latter becoming intercalated into the eye morphogenetic path-way downstream of *toy*[16]. Second, recruitment of novel genes into the morphogenetic pathway by fusion of the coding region of a gene to an eye-specific enhancer or promoter. Piatigorsky[39] has described several examples of this kind. Genes encoding enzymes like enolase or lactate dehydrogenase, or small heat shock proteins are recruited into the eye morphogenetic pathway as lens proteins called crystallins. This evolutionary process has been termed gene sharing or recruitment. Third, the recombination of various coding and regulatory regions of different genes by'evolutionary tinkering' might also lead to recruitment and intercalation into a new morphogenetic pathway.

These considerations clearly have a bearing on our concepts of homology. Homology is not an all-or-nothing phenomenon, because two different types of eye might only be partially homologous and they can also have acquired analogous features as proposed by Zuckerkandl[40]. This will resolve discrepancies in the interpretation[41] of these new findings in eye evolution.

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The Internet Section

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