Pax genes and eye organogenesis

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Pax6 is a highly conserved gene that controls eye development in all species where it has been tested. In spite of this common 'master control regulator', the eyes of different animals are morphologically very different and it is believed that they have evolved independently multiple times through evolution. Recent works looking at eye development in 'primitive' species offer some explanation as to the surprising amount of conservation in genetic and morphogenetic pathways involved in eye development. These studies not only implicate the Pax genes but also the So/Six gene family in playing a crucial ancestral role in visual system development.

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Abbreviations

Eyg	Eyegone
HD	homeodomain
PD	paired-domain
So*/*Six	*Sine oculis*

Introduction

The various visual systems are considered an example of convergent evolution, as it is widely accepted that such differing eyes have appeared independently many times through evolution. This view has been recently challenged because of the discovery of many intriguing molecular and genetic resemblances among widely divergent eyes [1]. The major argument comes from the conserved function of *Pax6*, a gene that is essential for eye development in all species in which it has been studied [1]. Do the vertebrate or invertebrate eyes have more in common than photoreceptors and share a common ancestor? The answer might lie in the understanding of Pax6 function and evolution and we here describe how various Pax genes, *Pax6*, *Pax2* and *Eye gone* (*Eyg*), control eye development.

Pax6 encodes a nuclear transcription factor from the Pax family. Pax genes are defined by the presence of a paired-box, which encodes a paired-domain (PD), a highly conserved 128 amino acid DNA binding domain (for review, see [2]). The PD is organized as two independent subdomains, the amino-terminal PAI and the carboxy-terminal RED that can both bind DNA, either independently or synergistically [3–5]. These two motifs are always found together except in a single *Drosophila* protein, Eyg, which lacks the PAI domain [6]. Beside their PD, Pax proteins often contain other conserved domains such as a complete or partial paired-type homeodomain (HD), or an octapeptide found between the PD and the HD. The HD is another DNA-binding domain the specificity of which depends on a crucial residue found at its position 50. Most homeoproteins, including all Hox proteins, bear a Gln at this position (Q_{50}) [7]. The HD found in Pax genes is always characterized by a S_{50}, and all Prd-class HDs bearing a S_{50} are found in Pax proteins. They can bind as homo- or as heterodimers with any paired-class HD to a palindromic DNA sequence [8]. The nine human Pax genes can be placed into five phylogenetic groups [9]: (1) Pax1 and Pax9; (2) Pax3 and Pax7; (3) Pax4 and Pax6; (4) Pax2, Pax5 and Pax8; (5) cnidaria PaxA and *Drosophila* Pox-*neuro*. Pax3/7, as well as Pax4/6, contain both a HD and a PD. The Pax proteins are, therefore, multifunctional transcription factors able to bind to a wide variety of sites through individual domains, or through cooperative interaction among these domains.

Origin of Pax genes: a role of *Pax6* in neurogenesis?

It has been proposed that, at the origin of modern Pax genes, a PD-containing protein (likely originating from a transposase) captured a HD through gene fusion, leading to a protein family able to bind complex cognate DNA sites [10••,11]. Consistent with this view, a Pax homologue whose PD is related to *Pax2/5/8* was identified in the freshwater sponge *Ephydatia fluviatilis* [12], one of the most primitive representatives of the animal kingdom (sponges probably diverged some 900 million years ago [Figure1]). This gene also encodes a degenerate but well-recognizable HD, suggesting that the Pax genes are of monophyletic origin with the capture of the HD occurring very early (Figure 1). Thus, the current HD-free Pax proteins (Pax1/2/9) might have evolved by losing their HD. In the basal class of Cnidaria — a phylum that diverged from bilaterians ~700 million years ago — where simple neurons first appeared, the coral Acrospora already presents four independent Pax genes [13••]: PaxA is related to Pox-*neuro*, PaxB is a Pax2/5/8 primitive homologue and PaxD is related to Pax3/7. PaxC, which was proposed to be related to Pax6, seems to be expressed in neurons, probably prefiguring its future role in cephalization and eye organogenesis. Interestingly, the jellyfish Podocoryne *PaxB* gene is also expressed during neurogenesis [14].

We focus our attention on three Pax proteins that are involved in eye development in a wide variety of species: Pax6, Pax2 and the divergent Eyegone (Eyg).

Pax6, *Pax2* and eye organogenesis

The camera-like eye of vertebrates is strikingly different from the compound eye of insects. These differences lie not only in the basic morphology of the two eyes, but also in their very different embryonic origins, their type of photoreceptors, and their phototransduction pathways. Nevertheless, Pax2

and Pax6 are both expressed in the vertebrate and invertebrate presumptive eye epithelium. Homozygous Pax6 mutants, from flies to humans, completely lack eyes (and nose). Pax6 gene dosage is also important: heterozygous mutations in Pax6 lead to eye defects, *Aniridia* or some of Peter's anomalies in human, *Small eye* in mice or rats [15,16]. Furthermore, extra copies of Pax6 in mice lead to similar eye defects to the loss of one copy of the locus in mice. Pax6 has thus been proposed to be *the* 'master control gene' for eye development. This implies that it sits on top of the hierarchy of eye determination genes and controls all aspects of eye development.The presumptive mammalian eye tissue is regionalized and specified through autonomous as well as inductive processes between apposed neural (optic vesicle) and surface ectoderm (lens placode), both of which require Pax6 (Figure 2). In flies, a single ectodermal epithelium forms both the retina and lens components of the eye. Although Pax6 mutants have no eyes, the vertebrate eye cup (and the eye disc in flies) does form in null Pax6 mutant mice, but then degenerates, suggesting that other genes are involved in parallel or upstream of Pax6 [17]. The precise early role of Pax6 in vertebrate eye development is thus not clear and it is also difficult to assign a common early role to Pax6 in vertebrates and in flies. Elegant conditional mouse knock-out experiments have suggested that Pax6 is required later in uncommitted retinal cells to retain their pluripotency, thus allowing them to generate the different cell types that compose the retina (e.g. retinal ganglion cells, cone and rods) [18••]. Pax6 is also required for all aspects of lens development, including its final differentiation and control of crystallin expression, although the crystallin originates from non-neural tissue.

A critical role of mouse Pax6 appears to be in its interaction with Pax2. Pax6 is first expressed in the optic primordium, whereas later it is found in all cells of the prospective retina, pigmented epithelium and lens epithelium [19,20]. Pax2 is primarily expressed in the ventral half of the optic vesicle, and becomes restricted to glial cells of the optic stalk soon after the invagination of the optic cup [21,22]. Pax2 is crucial for the generation of the optic stalk whereas Pax6 is required for the development of the optic cup. In Pax2 null mutant mice, no glial cells develop in the optic nerve and the optic chiasma fails to form. Thus, Pax2 and Pax6 are clearly involved in the regionalization of different presumptive eye tissues. Importantly their expression is mutually exclusive. Pax2 restricts Pax6 expression to the more distal portion of the optic vesicle. In Pax2-deficient mouse, the Pax6 expression domain expands and invades the optic cup/optic stalk boundary. Pax6 also restricts Pax2 expression to mid-regions [23].

Drosophila Pax2 (Sparkling) and Pax6 are also expressed in different cell types in the imaginal discs [24]. Pax6 is expressed in early mitotic uncommitted cells, before the morphogenetic wave of differentiation [25] (mouse Pax6 is also required in uncommitted retinal cells to retain their pluripotency). At the furrow, Pax6 is turned off while recruitment of all retinal cells occurs sequentially: photoreceptors, cone cells and pigment cells. *Sparkling* is expressed in cone cells (Figure 2), pigment and bristle cell precursors, but it is only required in cone cells, allowing them to recruit the pigment cells [24]. Although the functions of *Sparkling* and vertebrate Pax2 appear totally distinct, it has been noted that both genes are expressed in eye neuronal accessory cells and *Sparkling* is also expressed in glial cells in the fly peripheral nervous system.

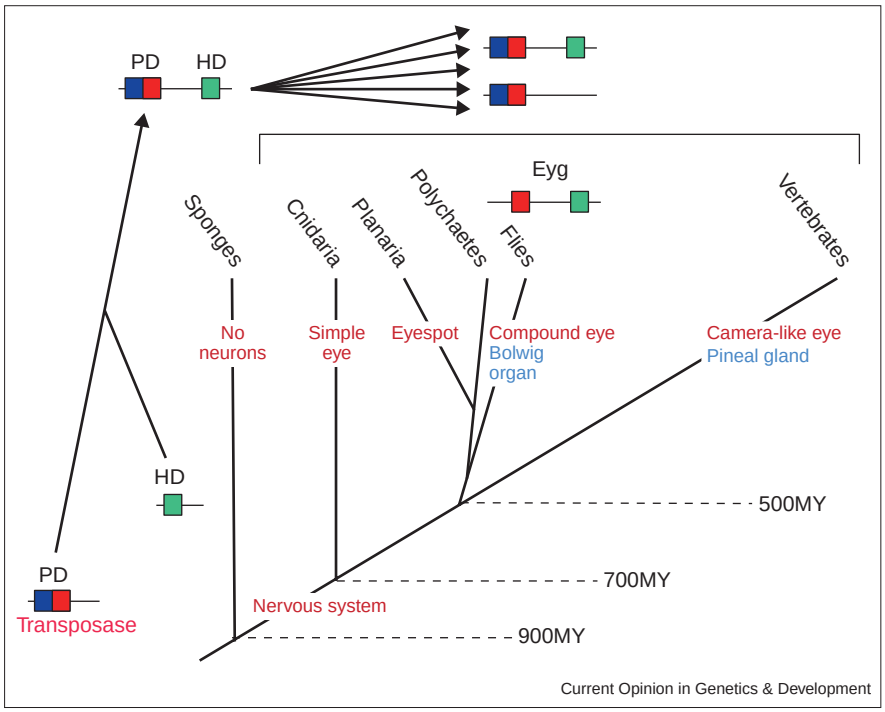
There is a third Pax gene that is required for *Drosophila* eye development. The *eyg* locus contains a duplicated gene (H Sun, personal communication). Each gene encodes a Pax protein with a partial PD lacking their amino-terminal PAI subdomain, but with a complete HD [6], thus dramatically affecting its DNA-binding specificity. Although the remaining part of the Eyg PD sequence is most related to Pax6, it does lack signature residues of Pax6, suggesting that Eyg diverged significantly from Pax6 before being duplicated. As its name indicates, *eyg* is required for eye development and it has been shown to repress *wingless* [26], a potent negative effector of eye development. Eyg can also induce ectopic eyes in flies and it has been proposed to act in a parallel pathway to Pax6 (H Sun, personal communication). However, the absence of mutants removing the function of both copies of either *Pax6* or *eyg* (*eyeless* and *twin of eyeless*, or *eyg* and *twin of eyg*) has hampered an understanding of their respective functions and epistasis. No *eyg* gene has been found in other species, further adding to the mystery of this gene.

Pax6 and the evolution of the eye

Until the realization that *Pax6* was involved in the development of very divergent types of eyes, it was widely accepted that various eyes represented examples of convergent evolution. Such convergence reflects the critical advantage of being able to perceive the environment as soon as the sophistication of the nervous system allowed the interpretation of images (i.e. after the split between major branches). Therefore, eyes are thought to have arisen independently ~40–60 times through evolution [27]. This view is also supported by the wildly divergent design of the contemporaneous eyes as well as by the fact that two types of photoreceptors, ciliary (e.g in vertebrates) and rhabdomeric (invertebrates) exist, sometimes in the same species. In addition, phototransduction uses significantly different pathways in the two types of photoreceptors (phosphodiesterase *versus* phospholipase C).

However, this view has recently been challenged by several intriguing resemblances between the genetic and patterning processes that control the development of eyes from very distant species, suggesting a common origin to these eyes [1]. One of these resemblances is the utilization of the Pax6 protein, which is essential for eye development in all species where it has been studied and was thus termed the 'master regulatory gene' for eye organogenesis. Strikingly, ectopic expression of many Pax6 genes is also

Figure 1



Evolution of Pax genes and of photodetection systems. Dates are only indicative and their exact value is subject to wide divergences. MY, million years. The paired domain is composed of two subdomains, PAI in blue and RED in red. The homeodomain is in green. The PAI, RED and HD are DNA binding domains. The acquisition of the HD by a proto-Pax gene occurred before the appearance of a nervous system. Duplication and loss of the HD led to four classes of Pax gene before the cnidarians diverged. Eyg has so far been found only in flies.

sufficient to redirect the developmental program of *Drosophila* (and in some cases of *Xenopus*) tissues toward an eye fate.

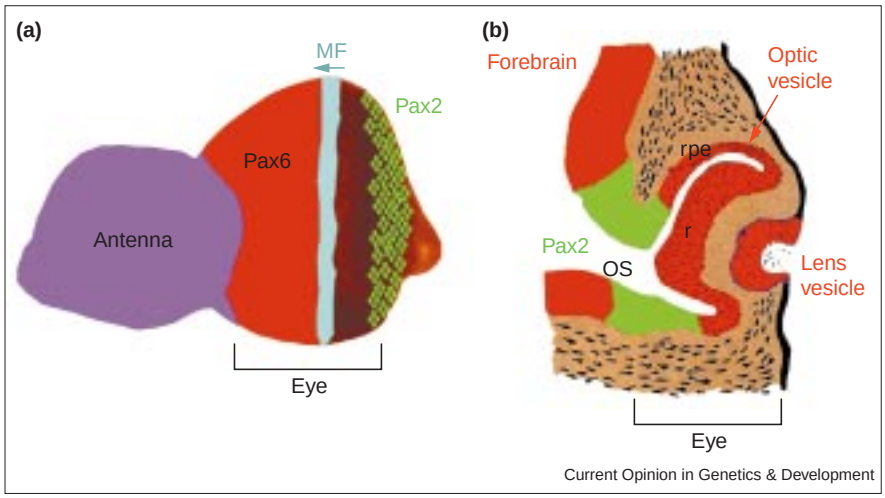
Other protagonists are shared for the development of many eyes. For instance, So/Six is a gene that encodes a transcription factor that has the capacity to induce ectopic eye structures in flies and is expressed in the eye in many species (see below [28]). Further similarities between the morphological routes that lead to retinal patterning have also been noted (e.g. waves of hedgehog in the morphogenetic furrow in the fly imaginal disc and of SHH in the developing fish retina, conservation of the *atonal* gene as the determinant of the first retinal cells; for review, see [29•]). Therefore, in spite of obviously distinct evolutionary origins, the fly and vertebrate eyes do share many molecular and morphogenetic components.

These 'functional homologies' are very surprising and suggest that, despite ~700 million years of independent evolution, and structures that are based on widely different designs, all contemporary eyes might share a common ancestral 'eye' whose development was controlled by Pax6.

How is Pax6 involved in eye development in various species? Was Pax6 already present very early on in a primitive species where it was involved in the development of a photosensitive organ ('proto-eye'), before functional image-forming eyes first appeared (a form of pre-adaptation)? Or instead, are we missing a critical early link when Pax6 first appeared, a real ancestor 'eye' from which all eyes evolved — that is, an organ with photoreceptors, pigment cells and the ability to focus light to form images or to detect motion? An important task to answer this question is to define the level of sophistication of such a 'primitive eye', or to find an alternative explanation to the re-utilization of similar processes. If PaxC in the very primitive coral Acrospora, which does not appear to have an 'eye', were a *bona fide* Pax6 gene [13••], this would suggest that Pax6 did precede the formation of eyes. It should be noted, however, that some cnidarians do have organized eyes, made up of two cell types, nerve cells and pigment cells with *Tripedalia cystophora* having genuine anatomical eyes. In addition, crystallin genes have been characterized in jellyfish [30]. Planaria, another group of less 'primitive' organisms, might also hold a key to this mystery [31••]. These animals present various light-detection systems that range from simple eyespots to more complex eyes. These complex eyes are composed of photoreceptors and pigment cells that form a cup capped by a lens, and are thus good candidates for an ancestral visual system. Strikingly, these eyes are characterized by the expression of a Pax6 homologue in both the pigment cells and in the photoreceptors and their organogenesis seems to rely on a conserved genetic module with Pax6 and So [31••]. This suggests that the different types of eyes — the camera-like eyes of vertebrates and compound eyes of invertebrates — might share a common ancestor 'proto-eye' (eyespot) and have diverged mostly due to modification of an ancient module, for instance by modification of the regulatory regions of these genes or of their targets. The apparition of a more elaborated 'image-forming eye' would then be considered as a convergent evolutionary process that occurred independently in many species.

Figure 2

Expression pattern of Pax6 (red) and Pax2 (green) in (a) the developing fly eye disc and (b) in vertebrates. In both systems, Pax6 and Pax2 are expressed in non-overlapping patterns. (Adapted from [29•].)
MF, morphogenetic furrow; os, optic stalk; r, retina; rpe, retinal pigment epithelium.



Another interesting example is the vertebrate pineal gland, an organ buried in the brain and involved in circadian rhythm entrainment. The presence of Pax6 in the pineal gland [19] in mice suggests that it shares a common ancestor with the lateral eyes. This organ might represent a primitive stage during eye evolution that has been retained to perform a highly specialized function in circadian rhythm entrainment. Alternatively the pineal gland might have evolved independently and relatively late to acquire its function in circadian rhythm. The fly larval visual system (Bolwig organ) is also responsible for entraining the clock and for other behavior of the larva (E Mazzoni, F Pichaud, C Desplan, unpublished data), and it does not form an image. Surprisingly, it does not appear to express *eyeless*, although it might express *twin of eyeless*, the other fly *Pax6* gene. Instead, the Bolwig organ requires the *So* gene for its morphogenesis. Does this mean that eye structures can form without Pax6? If this is the case, could So/Six be the real ancestral gene controlling eye development? In support of this model, the larval eye of the polychaete *Platynereis dumerilii* expresses both Pax6 and So/Six while the distinct adult eye expresses only So/Six [32••]. Furthermore, another Six gene, *optix*, is able to induce ectopic eyes in flies independently of *eyeless* (although it has not been shown that it does not require *toy*) [33]. Also consistent with this view, although planarian Girardia So is expressed together with Pax6 and is required in mature photoreceptors where it might be involved in rhodopsin expression [32••], recent results indicate that planarians can regenerate eyes in the absence of Pax6 [34•]. Are So/Six and Pax6 both recruited as a module for all eyes, with some eyes eventually losing Pax6, or is *So/Six* the ancestral regulator for a primitive light detection system that later acquired Pax6? Alternatively, there might be two types of 'proto-eyes' that have appeared independently with either Pax6 or So/Six controlling their organogenesis and opsin expression. In this perspective, it is interesting to note that Pax6 is not expressed in differentiated vertebrate rods and cones, suggesting that its ancestral role in controlling opsin expression [1] might have been lost, or that another early factor (So/Six?) plays this role. It will therefore be important to compare the expression and role of So/Six in ancient lineages such as sponges or cnidarians.

Conclusions

It is clear that Pax genes play an important role in eye development and that, at least in vertebrates, different Pax genes play antagonistic roles. The 'functional homologies' found in contemporaneous eyes might reflect the early recruitment of a module in a primitive light-gathering organ or 'proto-eye', presumably a transcriptional complex controlling photoreceptor differentiation. Gehring and Ikeo [1] have proposed an attractive model of 'intercalary evolution' whereby genes used for downstream functions are recruited for more upstream functions when the structures become more sophisticated.

In flies, many factors are able to induce ectopic eyes (Eyeless; Twin of Eyeless; Dachshund; Eye absent; sine oculis; Teashirt; Optix; Eyegone). To which extent are these genes part of a single ancestral biochemical module that has been recruited for eye development? It is likely that gene duplication or recruitment of additional genes to a core module (Six/Pax6) as well as modification in the gene-regulatory network might have led to the apparition of retinal accessory tissue such as pigment cells, lens or other retinal cells. The role of upstream factors, or the inductive cues, remains poorly defined although they are likely to have a major contribution for the adaptive design and optimization of various eyes.

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