PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B

Phil. Trans. R. Soc. B (2009) **364**, 2819–2832
doi:10.1098/rstb.2009.0079

Review

Eye evolution: common use and independent recruitment of genetic components

Pavel Vopalensky and Zbynek Kozmik*

Department of Transcriptional Regulation, Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Videnska 1083, Prague 4 CZ 14220, Czech Republic

Animal eyes can vary in complexity ranging from a single photoreceptor cell shaded by a pigment cell to elaborate arrays of these basic units, which allow image formation in compound eyes of insects or camera-type eyes of vertebrates. The evolution of the eye requires involvement of several distinct components—photoreceptors, screening pigment and genes orchestrating their proper temporal and spatial organization. Analysis of particular genetic and biochemical components shows that many evolutionary processes have participated in eye evolution. Multiple examples of co-option of crystallins, Gα protein subunits and screening pigments contrast with the conserved role of opsins and a set of transcription factors governing eye development in distantly related animal phyla. The direct regulation of essential photoreceptor genes by these factors suggests that this regulatory relationship might have been already established in the ancestral photoreceptor cell.

Keywords: eye; evolution; crystallin; opsin; pigment; gene

1. INTRODUCTION

Eyes of some sort occur in many animal phyla, but their anatomy, ontogenetic origin and degree of sophistication vary enormously (Land & Nilsson 2002). For the purpose of this text, we use the minimal definition of an eye as a photoreceptor cell in the close vicinity of a screening pigment (Arendt & Wittbrodt 2001; Land & Nilsson 2002)—a situation found, for example, in Hesse eye cups of amphioxus (Lacalli 2004). As these two components are formed by different genes and genetic pathways, which have different evolutionary histories, the evolution of an eye then becomes a question of evolutionary history of these separate components and their continuous or repeated integration to an organ called 'eye'. The aim of this review is to gather available genetic and biochemical data regarding evolution of particular eye components, i.e. phototransduction (which is extensively reviewed elsewhere in this issue), pigmentation and regulatory genes involved, and unite these processes.

2. GENETIC COMPONENTS OF PHOTORECEPTORS

Despite a remarkable variation in size and complexity, the common indispensable basis of all animal eyes is the photoreceptor cell containing photosensitive molecules, which are connected to a downstream phototransduction cascade. The photoreceptor cells are classified according to the morphology of membrane protrusions bearing visual pigments as 'rhabdomeric', which form microvilli, and 'ciliary', where the membrane surface is increased by folding the membrane of the cilium (Eakin 1979; Arendt 2003). Primary observations revealed the rhabdomeric photoreceptors as being predominantly present in the eyes of invertebrates, whereas the vertebrate eyes employ the ciliary type; however, several exceptions from this rule do exist (Arendt & Wittbrodt 2001; Land & Nilsson 2002). Both photoreceptor cell types have always co-existed in bilaterians, as suggested by both types found in amphioxus (Lacalli 2004) and confirmed by recent morphological and molecular studies (Arendt *et al.* 2004; Velarde *et al.* 2005). Still, it is not clear why the two types of photoreceptors were employed in the eyes of invertebrates versus vertebrates in a mutually exclusive way.

(a) *Opsins: variation on ancestral theme*

The first step of photoreception is mediated by light-sensitive transmembrane proteins containing retinal chromophore, generally termed rhodopsins. They have been found in most groups of organisms including archeal prokaryotes (Blanck & Oesterhelt 1987), unicellular eukaryotes (Nagel *et al.* 2002), fungi (Bieszke *et al.* 1999) and metazoa. The functions of rhodopsins in these organisms vary from photon-driven ionic pumps in prokaryotes (Blanck & Oesterhelt 1987), sensory molecules in fungi, to a light-gated ion channel in the eyespot of green algae (Spudich *et al.* 2000; Nagel *et al.* 2002). Metazoan

* Author for correspondence (kozmik@img.cas.cz).

Electronic supplementary material is available at http://dx.doi.org/10.1098/rstb.2009.0079 or via http://rstb.royalsocietypublishing.org.

One contribution of 13 to a Theme Issue 'The evolution of phototransduction and eyes'.

opsins are seven transmembrane proteins belonging to the superfamily of G-protein coupled receptors (GPCRs) and often are coupled to a Gα protein to mediate a phototransduction cascade. Despite these distinct functions of rhodopsins, several common features, such as a transmembrane structure, conserved retinal group covalently bound to a lysine residue and similarities in exon–intron structure, suggested the possibility of common evolutionary origin of all opsins. However, a recent bioinformatic study (Larusso *et al*. 2008) provided strong evidence that at least prokaryotic and metazoan opsins are not homologous, thus revealing their common features as a remarkable example of convergent evolution.

With increasing number of sequenced genomes available, the opsin gene repertoire has been described in several animal species (Raible *et al*. 2006; Plachetzki *et al*. 2007; Holland *et al*. 2008). This information then enables the understanding of metazoan opsin evolution and origin (figure 1), which is inseparably connected with the origin of eyes itself. The metazoan opsins seem to originate early in metazoan evolution from a single GPCR gene by acquisition of light sensitivity. As no opsin genes have been found in available genomic data for the choanoflagellate *Monosiga* and the poriferan *Amphimedon* (Plachetzki *et al*. 2007; Suga *et al*. 2008), it is probable that this event predated the cnidarian–bilaterian ancestor. Before the split of cnidarians and bilaterians, the newly formed ur-opsin gene underwent duplication producing two ancestral classes of opsins: ciliary opsin class (c-opsin) conserved in cnidarians and bilaterians and a second opsin gene eventually giving rise to the rest of bilaterian opsins (see below) and persisting in cnidarians as 'cnidopsin' class (figure 1) (Plachetzki *et al*. 2007).

In cnidarians, the c-opsins are expressed in the ciliary photoreceptors of adult eyes of hydrozoan *Cladonema*, cubozoans *Tripedalia* and *Carybdea* (Koyanagi *et al*. 2008; Kozmik *et al*. 2008*a*; Suga *et al*. 2008).

The bilaterian opsin repertoire comprises the c-opsin class and the second ancestral class which has diversified into several subclasses termed as rhabdomeric opsins (r-opsins), Go-coupled opsins, neuropsins and RGR (Terakita 2005). From these, only three groups (namely c-, r- and Go-coupled opsins) seem to have been recruited for visual purposes, whereas the function of other subclasses is probably supportive, as, for example, photoisomerases (RGR-opsin) involved in the retinal visual cycle (Radu *et al*. 2008). With one exception of Go-coupled opsin mediating the phototransduction in the ciliary part of the retina in scallop *Patinopecten yessoensis* (Kojima *et al*. 1997), the rhabdomeric and ciliary photoreceptors of bilaterians consistently employ r- and c-opsins, respectively.

(b) *Phototransduction in ciliary photoreceptors: promiscuity in Gα coupling*

The different morphology of rhabdomeric and ciliary photoreceptors is further reflected in the level of phototransduction cascades operating in these cells, although some common elements, such as opsins or arrestins, participate in both. The rhabdomeric phototransduction cascade, which is mediated by Gα_q and phospholipase C (Suzuki *et al*. 1995), seems to be evolutionarily conserved from protostomes to vertebrate retinal ganglion cells (Koyanagi *et al*. 2005; Panda *et al*. 2005; Contin *et al*. 2006; Graham *et al*. 2008). In contrast, the ciliary photoreceptors may employ both G_o-opsin (Kojima *et al*. 1997) and c-opsins, whose Gα protein partners may be rather variable. In vertebrate rods and cones, the c-opsin couples via transducin Gα_t to downstream hyperpolarizing phototransduction cascade. Because the Gα_t subfamily originated together with other rod and cone specific phototransduction genes during vertebrate-specific whole-genome duplications (Nordstrom *et al*. 2004; Milligan & Kostenis 2006), the Gα_t subunits are not present in invertebrates. A question arises—what then are the Gα proteins involved in ciliary phototransduction cascades in invertebrates and cnidarians? Apparently, the members of the G$\alpha_{i/o}$ protein subfamily, from which Gα_t proteins evolved, could be plausible candidates for this function. Consistent with this assumption, a Gα_{i1} protein subunit is expressed in the ciliary photoreceptor cells of *Ciona intestinalis* (Yoshida *et al*. 2002). In a reptile *Uta stansburiana*, a vertebrate c-opsin (parietopsin), expressed in the parietal eye retina, signals via a Gα_o protein (Su *et al*. 2006), and in the same cell, another vertebrate visual/non-visual opsin, pinopsin, couples with gustducin—a third vertebrate paralogue of transducin not used in rods and cones. In contrast to its reptile counterpart, chicken pinopsin has been shown to interact with Gα_{11} subunit (Kasahara *et al*. 2002), which is closely related to Gα_q protein. An exciting surprise came from a recent study (Koyanagi *et al*. 2008), which revealed that phototransduction in cubomedusan *Carybdea rastonii* is mediated by Gα_s cascade.

Taken together, these findings indicate that the coupling specificity of ciliary opsins could be rather promiscuous in comparison to the rhabdomeric opsins retaining more strictly the Gα_q specificity. Since the origin of G proteins pre-dated the origin of opsins, the opsin–Gα protein interaction evolved by co-option and subsequent coevolution (Plachetzki & Oakley 2007). Multiple co-option events during c-opsin evolution might explain their 'promiscuity' and reconcile an apparent discrepancy between the data pointing to $G_{i/o}$ phototransduction cascade in *Tripedalia* (Kozmik *et al*. 2008*a*) and G_s-mediated transduction in *Carybdea* (Koyanagi *et al*. 2008). Although *Carybdea* and *Tripedalia* are closely related, the opsin sequences identified in the studies are rather diversified and suggest that two different phototransduction cascades in structurally the same eyes might be possible. Moreover, all the opsins found to be expressed in the eye of hydrozoan *Cladonema radiatum* and assigned to the ciliary class (Suga *et al*. 2008) show even more sequence diversification (figure S1, electronic supplementary material). Together with low bootstrap support in phylogenetic trees and discrepancies in total number of *Hydra* and *Nematostella* opsins identified in two independent studies (Plachetzki *et al*. 2007; Suga

et al. 2008), further analyses are required to fully resolve the relationships among cnidarian opsins and address their Gα coupling specificity and role in phototransduction.

3. DARK PIGMENTS: REDEPLOYMENT WITHOUT LOGIC

The second essential component of a postulated minimal eye is a dark shielding pigment, which brings the organism additional information of light direction. Generally, three types of compounds—melanins, ommochromes and pterins—serve as shielding pigments in most animal eyes (see electronic supplementary material for detailed information). The distribution of screening pigments in the animal kingdom (figure 2) does not follow any rule and is not correlated with a certain eye or photoreceptor cell. This leads to an assumption that the union of a dark pigment and photoreceptor cannot be traced to any ancestral condition, but is rather an outcome of independent assembly of these two components in different animal phyla. Another possibility is that the pigments coexisted in ancient pigmented photoreceptor cells and were lost in several extant pigment cells.

4. LENS-CONTAINING EYES: A STORY OF ENDLESS CRYSTALLIN DIVERSITY

Lens-containing eyes are a feature of a surprisingly broad spectrum of organisms across the animal kingdom, which represent a significant improvement of the simple eye composed of just photoreceptor cells and pigment cells. Highly abundant, water-soluble proteins that contribute to the main optical properties of lens, transparency and refractive power, are collectively called crystallins (reviewed in Piatigorsky 2007). The molecular mechanism of their optical function is based on their exceptional solubility and stability at high concentration. In some cases, crystallins may account for up to 90 per cent of the dry weight of the lens. Remarkably and in striking contrast to the universal conservation of opsins as the visual pigments in the photoreceptors, the lens crystallins are enormously diverse proteins that are often taxon-specific, i.e. different proteins function as crystallins in different species (Wistow & Piatigorsky 1988; de Jong *et al*. 1989; Piatigorsky & Wistow 1989). Crystallin diversity might be a consequence, at least in some cases, of multiple independent origins of lenses during evolution. A surprising number of proteins has biophysical properties that enable them to fulfil an optical role as lens crystallins in different taxonomic groups. Nevertheless, the selection of a particular polypeptide as a crystallin appears not to be entirely random since certain proteins are preferentially recruited as lens crystallins. Proteins used as lens crystallins are often related or identical to ubiquitously expressed metabolic enzymes or physiological stress proteins. For example, all vertebrate lenses contain the α-crystallins that belong to the family of small heat shock proteins (Ingolia & Craig 1982; de Jong *et al*. 1993) and the β/γ-crystallins that are related to microbial stress proteins (Wistow 1990; D'Alessio 2002). Vertebrate α-crystallins are indeed effective chaperones that protect partially denatured proteins from aggregating in the lens (Horwitz 1992). The co-option of a protein with chaperone-like activity as a lens crystallin makes sense, in light of the fact that proteins in vertebrate lenses must remain functional and soluble for the entire lifespan of an organism. The selective advantage of recruiting enzyme-crystallins is much less clear. First, they do not fall within a common metabolic category. The catalytic activities include, but are not limited to, lactate dehydrogenase (ε-crystallin in crocodiles and birds), argininosuccinate lyase (δ-crystallin in reptiles and birds), α-enolase (τ-crystallin in turtles), glutathione *S*-transferase (S-crystallin in cephalopods), ADP-ribosylglycohydrolase (J1-crystallin in cubozoan jellyfish) or glyceraldehyde 3-phosphate dehydrogenase (π-crystallin in diurnal geckos) (Piatigorsky 2007). Furthermore, some enzyme-crystallins have no or diminished catalytic activity, e.g. aldehyde dehydrogenase/Ω-crystallin in scallops (Piatigorsky *et al*. 2000) or argininosuccinate lyase/δ1-crystallin in chickens (Piatigorsky *et al*. 1988), suggesting that their function in the lens is limited to a refractive role.

It turned out that most crystallins are not lens-restricted proteins being expressed at a low level in other tissues as well. This means that apart from their optical role in the lens, crystallins serve an apparently different function elsewhere. A concept of 'gene sharing' has emerged from crystallin studies (Piatigorsky *et al*. 1988; Piatigorsky & Wistow 1989; Piatigorsky 2007). Gene sharing proposes that the protein encoded by a single gene may perform two entirely different functions: a structural (refractive) function in the lens as a lens crystallin and a catalytic or stress function elsewhere in the organism. An important implication of gene sharing is that a protein can evolve a new role, without losing its original function, simply by a change in gene expression (Piatigorsky & Wistow 1991). It has become apparent over time that gene sharing and repeated use of proteins for new tasks are not limited to crystallins and lenses, but they rather represent a common evolutionary strategy (Piatigorsky 2007).

The overall diversity of crystallins throughout the animal kingdom is indicative of convergent evolutionary solution: lens cells simply choose to synthesize a suitable protein to high levels to make their lenses optically useful. Such opportunistic molecular strategy emphasizes the key role of transcriptional regulation in crystallin gene recruitment. The high level lens-preferred expression of crystallin genes is often regulated by transcription factors implicated in eye development as well (Duncan *et al*. 2004). Perhaps the best example of such a dual role of transcription factors as regulators of both eye development and crystallin gene expression is the case of Pax proteins (reviewed in Cvekl & Piatigorsky 1996; Gehring & Ikeo 1999; Cvekl *et al*. 2004; Kondoh *et al*. 2004; Kozmik 2005). The presence of Pax regulatory elements in non-homologous crystallin genes in phylogenetically distant animal species is due to convergent evolution (Carosa *et al*. 2002; Cvekl *et al*. 2004; Kozmik *et al*. 2008*b*). It reflects the situation that the diverse crystallin genes have been recruited independently during

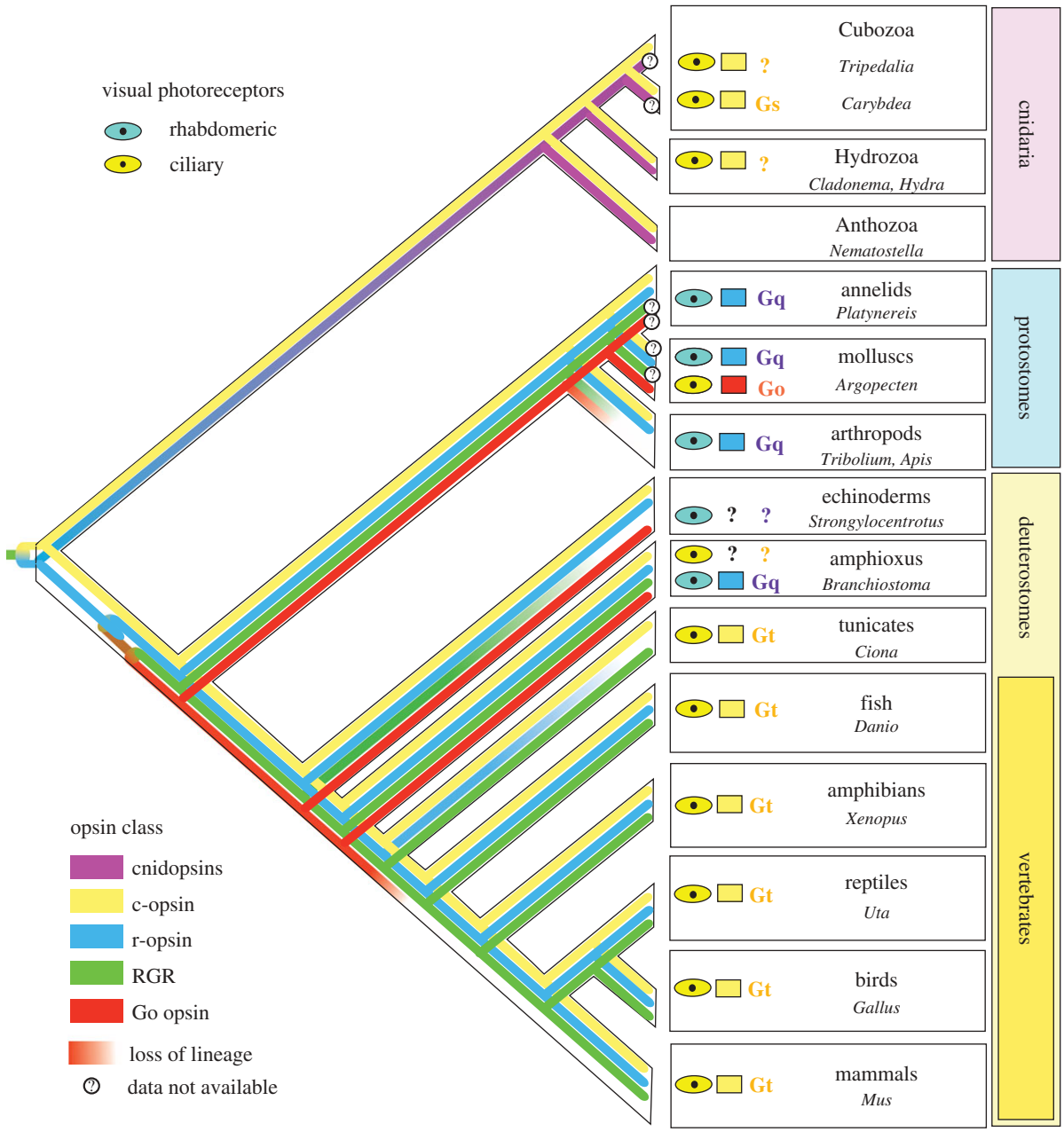

Figure 1. A schematic diagram of opsin distribution among eyes in different animal phyla. Particular opsins subfamilies are distinguished by different colouring. The lines leading to different phyla depict the hypothetical evolutionary fate of given opsin lineage based on available genomic and other data. The question mark denotes such animals where the presence of a given opsin lineage has not been confirmed yet. The colour of the eye-like pictogram corresponds to the type of photoreceptor cell employed in the eye. The class of opsin employed in the eye is represented by the colour of the rectangle next to the eye-like pictogram. If known, the Gα subunit interacting with the opsin is shown. Note that a small subset of vertebrate retinal ganglion cells expresses melanopsin coupled to a Gq signalling cascade (Panda *et al.* 2005). Although these cells fulfil the definition of a minimal eye, they are not the major photoreceptors of the eye and are not considered in this figure.

evolution and hence their regulatory elements to achieve lens-preferred expression must have been acquired by independent events as well (figure 3).

5. REDEPLOYMENT OF A SELECTED SET OF TRANSCRIPTION FACTORS FOR ANIMAL EYE DEVELOPMENT

Structural components of simple or complex (lens-containing) eyes described above are encoded by genes expressed during terminal cell differentiation at the end of developmental processes. Any developmental process to be completed properly requires tight regulation by a dedicated set of transcription factors. Given the enormous diversity of animal eyes, it came as a surprise that certain transcription factors are redeployed for visual system development far more often than others. In addition to governing eye morphogenesis, some of these transcription factors are directly involved in the regulation of differentiation genes encoding structural eye components.

(a) *Pax family of transcription factors*

Pax transcription factors are defined by the presence of a highly conserved DNA binding domain, the paired

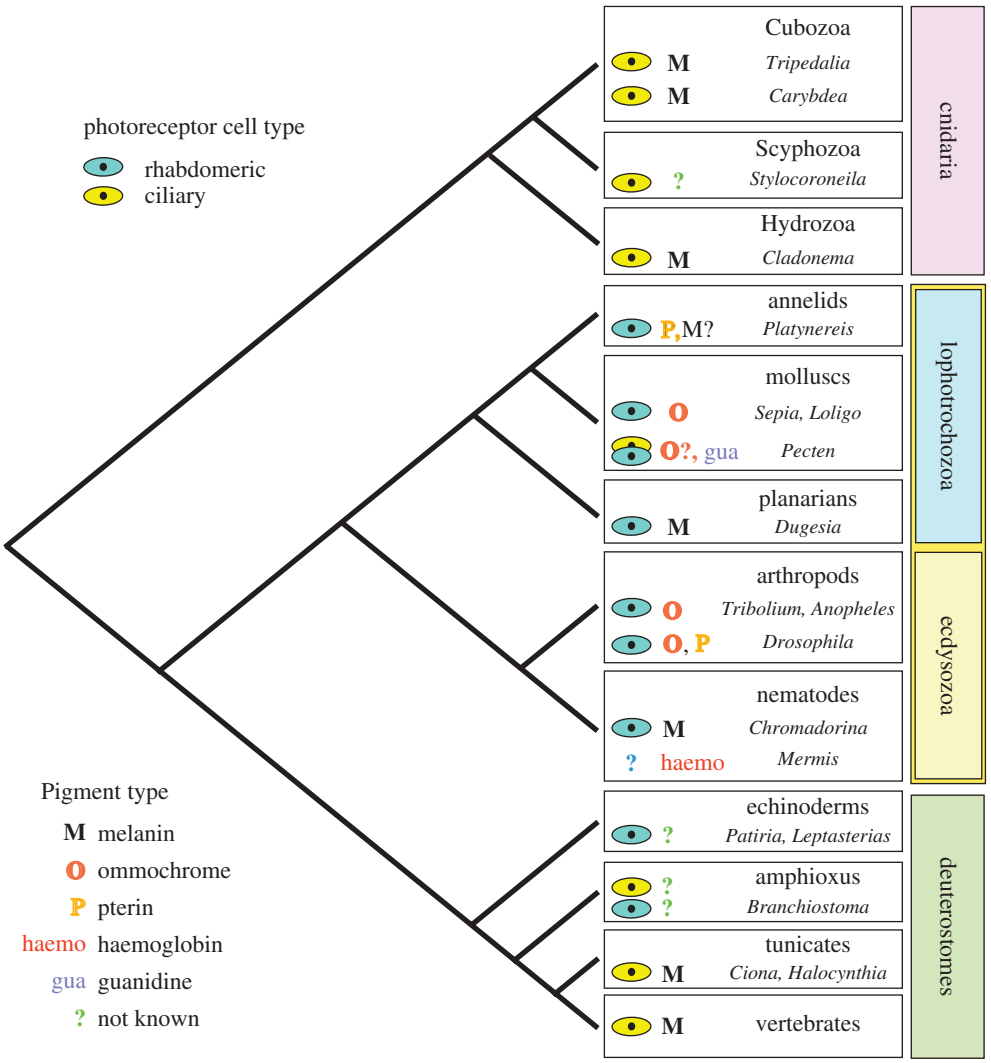

Figure 2. A schematic diagram of distribution of screening pigments in different animal phyla. The photoreceptor cell type screened by particular pigments is depicted on the left side of the box of each phylum. There is no apparent correlation between the photoreceptor cell type and shielding pigment used. Note the unknown type of photoreceptor and unique deployment of haemoglobin as a shielding pigment of nematode *Mermis*, caused probably by independent origin of the eye in this organism. See electronic supplementary material for further details and references.

domain (Burri *et al.* 1989; Treisman *et al.* 1991). In addition to a paired domain, some Pax proteins (such as Pax6) contain a second DNA binding domain, a homeodomain. Because a *PaxB*-like gene was identified in porifera (Hoshiyama *et al.* 1998) and placozoa (Hadrys *et al.* 2005), the origin of *Pax* genes predates the origin of eyes and the nervous system. The widespread use of *Pax* genes in the genetic programme underlying eye formation throughout the animal kingdom is remarkable. Mutations in the *Pax6* gene disrupt eye development in both mammals (Hill *et al.* 1991) and insects (Quiring *et al.* 1994). The ability to induce ectopic eyes through *Pax6* misexpression has furthermore been demonstrated in *Drosophila* and vertebrates (Halder *et al.* 1995; Chow *et al.* 1999). The key role of the *Pax6* gene for eye morphogenesis in such diverse species led to the proposal of *Pax6* being a 'master control gene' in animal visual system development (Gehring & Ikeo 1999). Such a simplified scenario, however, has to be dismissed. The term 'master control gene' implies that the Pax6 transcription factor is located at the top of a gene cascade and initiates eye development in almost any tissue where it is ectopically expressed. However, neither seems to be the case. For instance, in the absence of *Pax6*, presumptive retina can develop up to the optic cup stage, albeit abnormally (Grindley *et al.* 1995). Moreover, within the lens placode, *Pax6* expression is under the control of other transcription factors, Meis1 and Meis2 (Zhang *et al.* 2002). In *Drosophila*, the ability of the two *Pax6* paralogues, *ey* (Halder *et al.* 1995) and *toy* (Czerny *et al.* 1999), to induce ectopic eyes is restricted both spatially and temporally. These limitations suggest that *ey*/*toy* modify an existing programme of sensory organ development rather than initiate the entire eye morphogenesis. Furthermore, *toy* controls more than just eye morphogenesis because a loss-of-function mutation produces flies missing an entire head (Kronhamn *et al.* 2002). Likewise, in mice, *Pax6* is a pleiotropic regulator of development (Simpson & Price 2002).

Despite these limitations, there is no doubt that *Pax6* and other *Pax* genes have been frequently redeployed for visual system development. Apart from *Pax6*/*ey*/*toy*, three other *Pax* genes (*Pax2*, *Eyg*, *toe*) might have a role in the genetic programme underlying

Drosophila eye morphogenesis (Fu & Noll 1997; Jang *et al*. 2003; Dominguez *et al*. 2004). Likewise, in mice, *Pax2* cooperates with *Pax6* in the development of the retinal pigment epithelium (Baumer *et al*. 2003) and the mutual repression of *Pax6* and *Pax2* is responsible for morphogenesis of the entire mouse optic primordium (Schwarz *et al*. 2000). Even in species where genetic studies have not been done, there has been a generally good correlation between the presence of eyes and *Pax* gene expression (Loosli *et al*. 1996; Glardon *et al*. 1997, 1998; Tomarev *et al*. 1997; Callaerts *et al*. 1999; Arendt *et al*. 2002; Hartmann *et al*. 2003; Kozmik *et al*. 2003; Quigley *et al*. 2007), although the expression is never eye-restricted. There are few notable examples, however, known so far among bilaterians of eyes developing in the absence of *Pax6*. *Pax6* is apparently not expressed in developing *Limulus* eyes (Blackburn *et al*. 2008), developing *Platynereis* adult eyes (Arendt *et al*. 2002), Hesse eye cups of amphioxus (Glardon *et al*. 1998) and its function is not required for the eye regeneration in planarians (Pineda *et al*. 2002) as well as the Bolwig organ in *Drosophila* (Suzuki & Satoh 2000). Nonetheless, *Pax* genes arguably have an ancient and fundamental role in visual system development. The bipartite model (Kozmik 2005) proposes that the two independent DNA binding domains within a single Pax transcription factor have been co-opted for two essential features of the prototypical eye, production of a dark shielding pigment and production of a photopigment. Frequent deployment of *Pax* genes in eye development in phylogenetically diverse species may reflect their ancestral role in the regulation of key differentiation genes (figure 3).

(b) *Sine oculis* (*Six*) *and eyes absent* (*Eya*)

Eye specification in *Drosophila* is governed by the members of the retinal determination gene network that includes, apart from *Pax6* paralogues (*ey*, *toy*), *eyes absent* (*eya*), *sine oculis* (*so*/*six*) and *dachshund* (*dac*) (Pappu & Mardon 2004; Silver & Rebay 2005; Friedrich 2006). This highly interactive network of genes is sometimes referred to as *Pax–Six–Eya–Dach* network (PSEDN; Kawakami *et al*. 2000) to reflect the situation that it has been co-opted for non-retinal roles in other species and developmental contexts (Heanue *et al*. 1999; Xu *et al*. 1999; Ozaki *et al*. 2004; Kozmik *et al*. 2007). *Six* and *Eya* are evolutionarily old gene families, whose origin like in the case of *Pax* predates the origin of eyes and that are characterized by the conserved biochemical roles of the encoded proteins (Bebenek *et al*. 2004; Silver & Rebay 2005). This is perhaps best documented by *Eya* gene that encodes a protein phosphatase (Li *et al*. 2003; Rayapureddi *et al*. 2003; Tootle *et al*. 2003). Eya phosphatase activity is required for eye development in *Drosophila*, yet the same phosphatase activity is already found in the plant orthologue (Silver & Rebay 2005). Eya functions in the transcription factor complex with members of *Six* gene family (Ohto *et al*. 1999), which might explain consistent co-expression in many different developmental settings (Silver & Rebay 2005).

Nonetheless, the current evidence strongly suggests that *Eya* and *Six* have an ancient role in eye development. Orthologues of both genes are involved in visual system development in both invertebrates and vertebrates. In *Drosophila*, deficiency mutations of *so* and *eya* are characterized by loss of all visual sense organs (Bonini *et al*. 1993; Cheyette *et al*. 1994; Zimmerman *et al*. 2000; Friedrich 2006). Likewise, *eya* and *so* are expressed in the embryonic visual system of directly developing insects (Dong & Friedrich 2005) and their knockdown induces a long-term arrest of eye development (Dong & Friedrich in press). In addition, a divergent *Six3*/*6*-like gene, *optix*, does not synergize with *eya* and contributes to compound eye morphogenesis in *Drosophila* by a mechanism that is apparently *Pax6*/*ey* independent (Seimiya & Gehring 2000). In the annelid *Platynereis*, the orthologue of *Six1*/*2* is expressed in all components of the larval and adult visual system (Arendt *et al*. 2002). Orthologues of *eya* and *so* are functionally required for planarian eye regeneration (Pineda *et al*. 2002; Mannini *et al*. 2004). In the hydrozoan jellyfish *Cladonema*, a species with well-developed eyes, orthologues of *Six1*/*2* and *Six3*/*6* are expressed, both during normal development and during the process of regeneration (Stierwald *et al*. 2004). *Eya* and *Six4*/*5* genes in the chordate amphioxus are transiently expressed in the two rhabdomeric photoreceptive neurons that flank a biconcave pigment cell to comprise the first Hesse eyecup (Kozmik *et al*. 2007). Among the *Six* genes that are expressed in vertebrate eyes only *Six3*/*6*-like genes appear to have a critical role during development (Zuber *et al*. 1999; Kobayashi *et al*. 2001; Carl *et al*. 2002; Li *et al*. 2002; Zhu *et al*. 2002; Lopez-Rios *et al*. 2003; Liu *et al*. 2006). In addition, *Six3* has been implicated in the regulation of mouse rhodopsin gene (Manavathi *et al*. 2007). The analysis of a functional role of *Eya* in vertebrate eye development is complicated by the fact that three mouse *Eya* genes (*Eya1–3*) are expressed in the developing eye (Xu & Saunders 1997) and so their combined loss-of-function phenotype has to be generated in order to unmask possible redundancy. Unlike the other trascriptional regulators discussed in this review, *Eya* has so far not been associated with the expression of key differentiation genes such as opsins or genes regulating dark pigment formation (figure 3).

(c) *Orthodenticle-related homeobox* (*Otx*)

The first member of the *Otx* gene family, *orthodenticle* (*Otd*), has been isolated from *Drosophila* and shown to be necessary for development of photoreceptors in the compound eye, Bolwig organ and the ocelli (Finkelstein *et al*. 1990; Royet & Finkelstein 1995; Vandendries *et al*. 1996). *Otd* also participates in terminal photoreceptor differentiation. It has been shown to directly regulate opsins (Tahayato *et al*. 2003) and influence the expression of genes involved in rhabdomeric phototransduction cascade (Ranade *et al*. 2008). In other invertebrates, the expression of *Otx* genes in photoreceptors has been reported in planarians (Umesono *et al*. 1999), putative eye-field precursor of

the annelid *Hydroides elegans* (Arenas-Mena & Wong 2007), ciliary and rhabdomeric photoreceptor cells of *Platynereis dumerilii* (D. Arendt 2008, personal communication), sensory pigment cells of ascidians (Wada *et al*. 1996) and the frontal eye region of amphioxus (Williams & Holland 1996). Reciprocal rescue experiments with *Drosophila* and mammalian *Otx* orthologues demonstrated that at least part of ancestral genetic and biochemical interactions is still conserved between vertebrates and invertebrates (Acampora *et al*. 1998; Nagao *et al*. 1998).

Multiple vertebrate orthologoues of *otd* termed *Otx*1, *Otx*2 and *Crx*/*Otx*5 (Germot *et al*. 2001; Plouhinec *et al*. 2003) probably arose during whole genome duplication, since a single *Otx* gene is present in the genome of *C. intestinalis* (Wada *et al*. 2003) and amphioxus (Williams & Holland 1998). Besides the role of *Otx* genes in early vertebrate development of anterior neural structures and the brain (Simeone *et al*. 2002; Acampora *et al*. 2005), these genes are necessary for the proper development of the pineal gland and the eye (Martinez-Morales *et al*. 2001; Nishida *et al*. 2003; Plouhinec *et al*. 2005). Later in development, *Otx* genes play a crucial role in the terminal differentiation of photoreceptors and their maintenance during postnatal development (Nishida *et al*. 2003; Koike *et al*. 2007). The expression of *Otx* genes has been also detected in immature retinal ganglion cells (Bovolenta *et al*. 1997; Martinez-Morales *et al*. 2001; Rath *et al*. 2007)—putative descendants of the rhabdomeric photoreceptor line in vertebrates (Arendt 2003).

The role of vertebrate *Otx* genes in the regulation of eye-specific genes has been extensively studied and led to the discovery of many direct target genes. *Crx*, strongly expressed in differentiated photoreceptor cells, directly regulates the phototransduction genes, rhodopsin, β-PDE, arrestin and guanylate cyclase, via binding the PCE element in the promoters (Chen *et al*. 1997; Furukawa *et al*. 1997; Qian *et al*. 2005) (for a review, see Hennig *et al*. 2008). The expression of ciliary-phototransduction cascade genes in the vertebrate pineal gland is mediated by the action of *Otx* genes as well (Appelbaum & Gothilf 2006; Takechi *et al*. 2008). Besides the direct regulation of photoreceptor-specific genes, *Otx* genes are involved in the regulation of pigmentation. In ascidians, the *Tyrp* gene is a direct target of *Otx* (Wada *et al*. 2002). The vertebrate homologue *Otx*2 has been shown to bind to the promoters of *Mitf*, *tyrosinase* and *Tyrp1* (Martinez-Morales *et al*. 2003) as well as *Tyrp2* in the retinal pigmented epithelium (Takeda *et al*. 2003).

The fact that *Otx* genes are expressed in both rhabdomeric and ciliary photoreceptors across animal phyla points to their ancient role in photoreceptor cell differentiation (Ranade *et al*. 2008). The differentiation processes and regulatory subcircuits composed of differentiation genes regulated by certain transcription factors seem to be well conserved during evolution (Arendt 2008). The direct regulation of opsins by *Otx* could be an example of such a differentiation subcircuit dating back before the split of r- and c-opsins. Based on the scenario of opsin evolution proposed in Plachetzki *et al*. (2007), the regulatory relationship must have been already established between an ancestral *Otx* and ur-opsin before the split of cnidarians and bilaterians. Then, after the diversification of c-opsins and the second opsin class, this regulatory unit has been preserved in both rhabdomeric and ciliary photoreceptors (figure 4).

(d) *Retinal homeobox (Rx)*

During vertebrate development, *Rx* genes are expressed in the anterior forebrain, retinal primordia and pineal gland (reviewed in Bailey *et al*. 2004). The over-expression of *Rx* in *Xenopus* leads to ectopic formation of retinal tissue (Mathers *et al*. 1997). In zebrafish *chokh* mutants, a non-sense mutation in *Rx3* paralogue leads to the loss of eyes (Loosli *et al*. 2003) and *Rx* knock-out mice lack the eye and the anterior brain structures (Mathers *et al*. 1997). Similar to vertebrates, the ascidian homologue of *Rx* is expressed in the anterior brain and the knock-down resulted in the loss of photoreceptor cells (D'Aniello *et al*. 2006). In contrast, *Rx* is not expressed in planarian eyes (Salo *et al*. 2002) and genetic studies in *Drosophila* have shown a clear dispensability of *Rx* for compound eye development (Davis *et al*. 2003). The explanation of this result came from the emerging concept of sister cell types (Arendt 2003) and the fundamental discovery of *Rx* expression in the ciliary photoreceptors of *Platynereis* brain (Arendt *et al*. 2004). These findings led to the identification of *Rx* as a ciliary photoreceptor-specific marker. One may speculate, what is the reason for keeping the expression of *Rx* in differentiated ciliary photoreceptors in distantly related species. Analogous to the *Otx* scenario, the direct regulation of vertebrate c-opsins by *Rx* (Kimura *et al*. 2000; Wang *et al*. 2004; Pan *et al*. 2006) may point to an ancestral condition of ciliary photoreceptor cell type. In these cells, the regulation of c-opsins by *Rx* might have been already established and, being a differentiation subcircuit, it was conserved throughout evolution. *Rx* might be later co-opted for new roles in developmental regulatory networks operating in the anterior body part. Since this hypothesis is based on an *Rx*/c-opsin regulatory relationship so far confirmed solely in vertebrates, one has to keep in mind a possible co-option of *Rx* for this function.

(e) *Microphthalmia-associated transcription factor (Mitf)*

Mitf is a member of the Mitf/TFE family of bHLH-lecine-zipper transcription factors (Hodgkinson *et al*. 1993). Homozygous-mutant *Mitf* mice show severe defects of body pigmentation, have small unpigmented eyes, lack melanocytes in the inner ear and are deaf (reviewed in Steingrimsson *et al*. 2004). Genetic experiments have shown that multiple isoforms of *Mitf* are responsible for driving the expression of *Tyr* and *Tyrp* in melanocytes and retinal pigmented epithelium in cooperation with *Otx* and *Pax* genes (Martinez-Morales *et al*. 2004; Murisier & Beermann 2006; Bharti *et al*. 2008). Besides its role in melanogenesis, *Mitf* has been suggested to be involved in the regulation of the pteridine synthesis pathway in zebrafish (Ziegler 2003). In ascidians, *Mitf* is

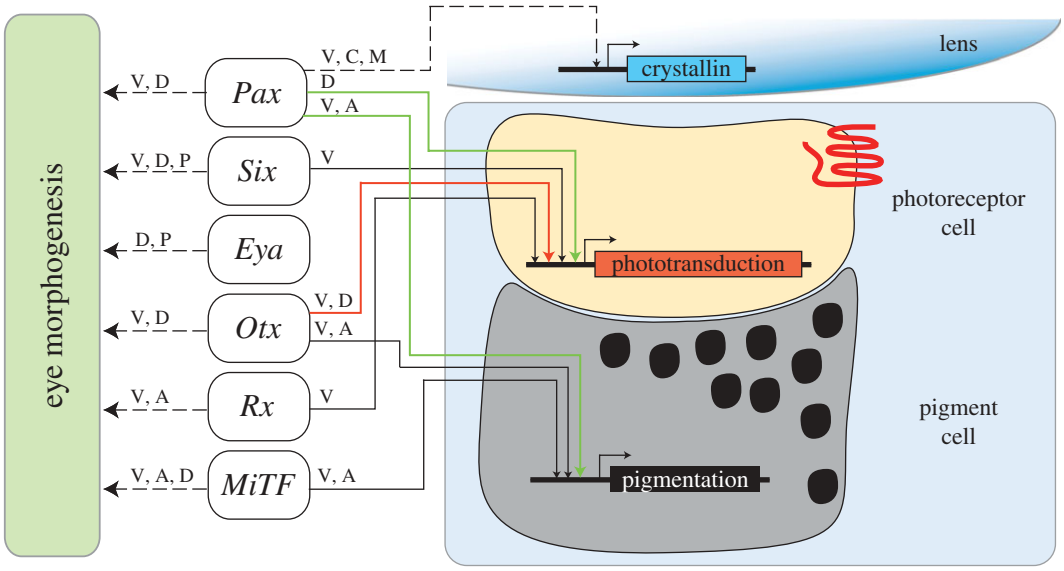

Figure 3. Dual role of transcription factors in regulation of both eye development and differentiation genes. The box on the left-hand side represents the sum of largely unknown developmental genes regulated by corresponding transcription factors based on functional data. The letters represent different animals (V, vertebrates; A, ascidians; D, *Drosophila*; C, cnidarians; M, molluscs; P, planarians). The arrows on the right-hand side represent a direct influence of a given factor on differentiation set of genes proved by biochemical methods (DNA-binding assay, ChIP, transgenesis, luciferase assays, etc.) The green arrows indicate the ancestral interaction proposed by the 'bipartite' model. The red arrow highlights the proposed role of *Otx* in the regulation of ancestral phototransduction genes. Co-option of a certain transcription factor to a new role is indicated by dashed line. We propose that the transcription factors were independently co-opted for regulation of genes governing eye development in different species and these downstream genes may vary among species. Please note that cross-regulatory interactions of transcription factors are not considered in this scheme for simplicity.

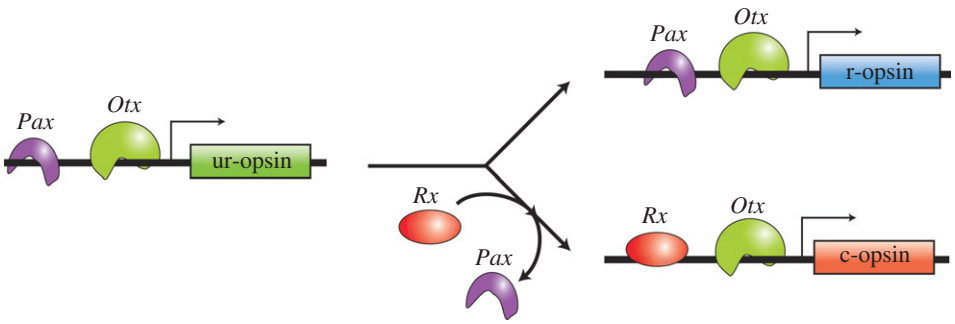

Figure 4. A hypothetical scenario suggesting the ancient regulatory relationship between Otx and ur-opsin predating the cnidarian–bilaterian split. The ur-opsin has been already regulated by the *Otx* gene and probably by *Pax* (not excluding other transcription factors involved). After the duplication and diversification of r- and c-opsin, the regulation by *Otx* has been preserved in both lineages, whereas *Pax*-dependent regulation has been lost in c-opsin lineage. Retinal homeobox *Rx* might have been recruited for regulation of c-opsin. Alternatively, *Rx* has regulated the ur-opsin and this role has been lost in r-opsin lineage. With increasing complexity of animal body plans, all the transcription factors have consequently acquired additional roles in eye development.

expressed in the precursors of pigmented cells in the brain vesicle (Yajima *et al.* 2003). Although the regulatory mechanism of ascidian *Tyr* and *Tyrp* genes is not yet fully understood (Toyoda *et al.* 2000), ascidian *Tyrp* gene has been shown to be directly regulated by *Otx* (Wada *et al.* 2002) and over-expression experiments suggest that *Mitf* and *Pax* are involved as well (Yajima *et al.* 2003; Toyoda *et al.* 2004).

The role of *Mitf* in eye development does not seem to be restricted to deuterostomes. *Drosophila* homologue of *Mitf* has been shown to be expressed in the eye-antennal imaginal disc and expression of dominant negative form of *Drosophila Mitf* resulted in enlarged photoreceptor field (Hallsson *et al.* 2004). Strikingly, a cnidarian homologue of *Mitf* has been isolated (Kozmik *et al.* 2008*a*) and shown to be expressed in the melanin-pigmented photoreceptor cells of the camera-type eye of *Tripedalia cystophora*. The expression of *Mitf* in cnidarians as well as bilaterian eyes and its interaction with *Otx* and *Pax* genes in deutorostomes raises speculations about *Mitf–Pax–Otx* cooperation within an ancestral pigmented photoreceptor cell.

6. CONCLUSIONS

The commonalities in the use of structurally similar seven-transmembrane receptors (opsins) as animal

eye photopigments stem from their shared evolutionary history. The handfull of dark (shielding) pigments that animals can make through various biosynthetic pathways are all apparently used for screening purposes without any evolutionary pattern or developmental logic. The recruitment of lens crystallins provides an extreme example of an opportunistic use of almost any soluble cytoplasmic protein for a refractive role. In contrast, there is a homology at the level of transcriptional regulators operating in developmental programmes in structurally diverse eyes. Although the expression of genes in all metazoa is generally regulated by a large number of diverse transcription factors often belonging to distinct families, certain transcription factors (i.e. Pax, Six, Eya, Otx, Mitf) have been deployed for the regulation of eye development far more often than others. The reasons for this phenomenon are not entirely clear at the moment, however, might be due to several underlying molecular mechanisms that are not necessarily mutually exclusive. Anteriorly expressed transcription factors are more likely to be co-opted for the regulation of an eye programme due to the fact that eyes are located more often at the front rather than at the back of the animal. Perhaps the most important aspect contributing to the observed homology of transcription factors is due to stochastically chosen ancestral regulatory connections. We can reasonably well argue that certain transcription factors were more or less randomly chosen for the regulation of essential genes in the ancestral (pigmented?) photoreceptor cell such as the gene encoding an ur-opsin. Gene duplication events generated opsin gene duplicates that began the process of divergence both in the coding sequence and in the regulatory regions. One might expect that the diverging genes encoding essential eye components have retained in their promoters binding sites of any transcription factor provided that such regulatory link was useful and the spatio-temporal expression pattern of the regulator was maintained. Such transcriptional regulators were later co-opted into new roles as 'higher structure' organizers of increasingly complex but diverse eyes. Frequent deployment in eye development in phylogenetically diverse species, thus, most likely reflects the ancestral role of a particular transcription factor in the regulation of a key differentiation gene.

We would like to thank three referees for valuable comments on our manuscript and Mr Jasper Manning for the correction of English. Work in Kozmik's laboratory is supported by grant nos. AV0Z50520514, IAA500520908 and IAA500520604 from the Academy of Sciences of the Czech Republic, by Center for Applied Genomics grant no. 1M6837805002 awarded by Ministry of Education, Youths and Sports of the Czech Republic and grant no. 91808 from the Grant Agency of the Charles University in Prague.

REFERENCES

Acampora, D., Avantaggiato, V., Tuorto, F., Barone, P., Reichert, H., Finkelstein, R. & Simeone, A. 1998 Murine Otx1 and Drosophila otd genes share conserved genetic functions required in invertebrate and vertebrate brain development. *Development* **125**, 1691–1702.

Acampora, D., Annino, A., Tuorto, F., Puelles, E., Lucchesi, W., Papalia, A. & Simeone, A. 2005 Otx genes in the evolution of the vertebrate brain. *Brain Res. Bull.* **66**, 410–420. (doi:10.1016/j.brainresbull.2005.02.005)

Appelbaum, L. & Gothilf, Y. 2006 Mechanism of pineal-specific gene expression: the role of E-box and photoreceptor conserved elements. *Mol. Cell. Endocrinol.* **252**, 27–33. (doi:10.1016/j.mce.2006.03.021)

Arenas-Mena, C. & Wong, K. S. 2007 HeOtx expression in an indirectly developing polychaete correlates with gastrulation by invagination. *Dev. Genes Evol.* **217**, 373–384. (doi:10.1007/s00427-007-0150-7)

Arendt, D. 2003 Evolution of eyes and photoreceptor cell types. *Int. J. Dev. Biol.* **47**, 563–571.

Arendt, D. 2008 The evolution of cell types in animals: emerging principles from molecular studies. *Nat. Rev. Genet.* **9**, 868–882. (doi:10.1038/nrg2416)

Arendt, D. & Wittbrodt, J. 2001 Reconstructing the eyes of Urbilateria. *Phil. Trans. R. Soc. B* **356**, 1545–1563.

Arendt, D., Tessmar, K., De Campos-Baptista, M. I., Dorresteijn, A. & Wittbrodt, J. 2002 Development of pigment-cup eyes in the polychaete *Platynereis dumerilii* and evolutionary conservation of larval eyes in Bilateria. *Development* **129**, 1143–1154.

Arendt, D., Tessmar-Raible, K., Snyman, H., Dorresteijn, A. W. & Wittbrodt, J. 2004 Ciliary photoreceptors with a vertebrate-type opsin in an invertebrate brain. *Science* **306**, 869–871. (doi:10.1126/science.1099955)

Bailey, T. J., El-Hodiri, H., Zhang, L., Shah, R., Mathers, P. H. & Jamrich, M. 2004 Regulation of vertebrate eye development by *Rx* genes. *Int. J. Dev. Biol.* **48**, 761–770. (doi:10.1387/ijdb.041878tb)

Baumer, N., Marquardt, T., Stoykova, A., Spieler, D., Treichel, D., Ashery-Padan, R. & Gruss, P. 2003 Retinal pigmented epithelium determination requires the redundant activities of Pax2 and Pax6. *Development* **130**, 2903–2915. (doi:10.1242/dev.00450)

Bebenek, I. G., Gates, R. D., Morris, J., Hartenstein, V. & Jacobs, D. K. 2004 sine oculis in basal Metazoa. *Dev. Genes Evol.* **214**, 342–351. (doi:10.1007/s00427-004-0407-3)

Bharti, K., Liu, W., Csermely, T., Bertuzzi, S. & Arnheiter, H. 2008 Alternative promoter use in eye development: the complex role and regulation of the transcription factor MITF. *Development* **135**, 1169–1178. (doi:10.1242/dev.014142)

Bieszke, J. A., Braun, E. L., Bean, L. E., Kang, S., Natvig, D. O. & Borkovich, K. A. 1999 The *nop-1* gene of *Neurospora crassa* encodes a seven transmembrane helix retinal-binding protein homologous to archaeal rhodopsins. *Proc. Natl Acad. Sci. USA* **96**, 8034–8039. (doi:10.1073/pnas.96.14.8034)

Blackburn, D. C., Conley, K. W., Plachetzki, D. C., Kempler, K., Battelle, B. A. & Brown, N. L. 2008 Isolation and expression of Pax6 and atonal homologues in the American horseshoe crab, *Limulus polyphemus*. *Dev. Dyn.* **237**, 2209–2219. (doi:10.1002/dvdy.21634)

Blanck, A. & Oesterhelt, D. 1987 The halo-opsin gene. II. Sequence, primary structure of halorhodopsin and comparison with bacteriorhodopsin. *EMBO J.* **6**, 265–273.

Bonini, N. M., Leiserson, W. M. & Benzer, S. 1993 The eyes absent gene: genetic control of cell survival and differentiation in the developing *Drosophila* eye. *Cell* **72**, 379–395. (doi:10.1016/0092-8674(93)90115-7)

Bovolenta, P., Mallamaci, A., Briata, P., Corte, G. & Boncinelli, E. 1997 Implication of OTX2 in pigment epithelium determination and neural retina differentiation. *J. Neurosci.* **17**, 4243–4252.

Burri, M., Tromvoukis, Y., Bopp, D., Frigerio, G. & Noll, M. 1989 Conservation of the paired domain in metazoans and

its structure in three isolated human genes. *EMBO J.* **8**, 1183–1190.

Callaerts, P., Munoz-Marmol, A. M., Glardon, S., Castillo, E., Sun, H., Li, W. H., Gehring, W. J. & Salo, E. 1999 Isolation and expression of a *Pax-6* gene in the regenerating and intact Planarian Dugesia(G)tigrina. *Proc. Natl Acad. Sci. USA* **96**, 558–563. (doi:10.1073/pnas.96.2.558)

Carl, M., Loosli, F. & Wittbrodt, J. 2002 Six3 inactivation reveals its essential role for the formation and patterning of the vertebrate eye. *Development* **129**, 4057–4063.

Carosa, E., Kozmik, Z., Rall, J. E. & Piatigorsky, J. 2002 Structure and expression of the scallop Omega-crystallin gene. Evidence for convergent evolution of promoter sequences. *J. Biol. Chem.* **277**, 656–664. (doi:10.1074/jbc.M107004200)

Chen, S., Wang, Q. L., Nie, Z., Sun, H., Lennon, G., Copeland, N. G., Gilbert, D. J., Jenkins, N. A. & Zack, D. J. 1997 Crx, a novel Otx-like paired-homeodomain protein, binds to and transactivates photoreceptor cell-specific genes. *Neuron* **19**, 1017–1030. (doi:10.1016/S0896-6273(00)80394-3)

Cheyette, B. N., Green, P. J., Martin, K., Garren, H., Hartenstein, V. & Zipursky, S. L. 1994 The *Drosophila* sine oculis locus encodes a homeodomain-containing protein required for the development of the entire visual system. *Neuron* **12**, 977–996. (doi:10.1016/0896-6273(94)90308-5)

Chow, R. L., Altmann, C. R., Lang, R. A. & Hemmati-Brivanlou, A. 1999 Pax6 induces ectopic eyes in a vertebrate. *Development* **126**, 4213–4222.

Contin, M. A., Verra, D. M. & Guido, M. E. 2006 An invertebrate-like phototransduction cascade mediates light detection in the chicken retinal ganglion cells. *FASEB J.* **20**, 2648–2650. (doi:10.1096/fj.06-6133fje)

Cvekl, A. & Piatigorsky, J. 1996 Lens development and crystallin gene expression: many roles for Pax-6. *Bioessays* **18**, 621–630. (doi:10.1002/bies.950180805)

Cvekl, A., Yang, Y., Chauhan, B. K. & Cveklova, K. 2004 Regulation of gene expression by Pax6 in ocular cells: a case of tissue-preferred expression of crystallins in lens. *Int. J. Dev. Biol.* **48**, 829–844. (doi:10.1387/ijdb.041866ac)

Czerny, T., Halder, G., Kloter, U., Souabni, A., Gehring, W. J. & Busslinger, M. 1999 Twin of eyeless, a second *Pax-6* gene of *Drosophila*, acts upstream of eyeless in the control of eye development. *Mol. Cell* **3**, 297–307. (doi:10.1016/S1097-2765(00)80457-8)

D'Alessio, G. 2002 The evolution of monomeric and oligomeric betagamma-type crystallins. Facts and hypotheses. *Eur. J. Biochem.* **269**, 3122–3130. (doi:10.1046/j.1432-1033.2002.03004.x)

D'Aniello, S. *et al.* 2006 The ascidian homolog of the vertebrate homeobox gene *Rx* is essential for ocellus development and function. *Differentiation* **74**, 222–234. (doi:10.1111/j.1432-0436.2006.00071.x)

Davis, R. J., Tavsanli, B. C., Dittrich, C., Walldorf, U. & Mardon, G. 2003 *Drosophila* retinal homeobox (drx) is not required for establishment of the visual system, but is required for brain and clypeus development. *Dev. Biol.* **259**, 272–287. (doi:10.1016/S0012-1606(03)00201-X)

de Jong, W. W., Hendriks, W., Mulders, J. W. & Bloemendal, H. 1989 Evolution of eye lens crystallins: the stress connection. *Trends Biochem. Sci.* **14**, 365–368.

de Jong, W. W., Leunissen, J. A. & Voorter, C. E. 1993 Evolution of the alpha-crystallin/small heat-shock protein family. *Mol. Biol. Evol.* **10**, 103–126.

Dominguez, M., Ferres-Marco, D., Gutierrez-Avino, F. J., Speicher, S. A. & Beneyto, M. 2004 Growth and specification of the eye are controlled independently by Eyegone and Eyeless in *Drosophila melanogaster*. *Nat. Genet.* **36**, 31–39. (doi:10.1038/ng1281)

Dong, Y. & Friedrich, M. 2005 Comparative analysis of Wingless patterning in the embryonic grasshopper eye. *Dev. Genes Evol.* **215**, 177–197. (doi:10.1007/s00427-004-0465-6)

Dong, Y. & Friedrich, M. In press. Enforcing biphasic visual system development in a directly developing insect by transient knockdown of single eye selector genes. *J. Exp. Zool. (Mol. Dev. Evol.)*

Duncan, M. K., Cvekl, A., Kantorow, M. & Piatigorsky, J. 2004 *Lens crystallins*. New York, NY: Cambridge University Press.

Eakin, R. M. 1979 Evolutionary significance of photoreceptors—retrospect. *Am. Zool.* **19**, 647–653.

Finkelstein, R., Smouse, D., Capaci, T. M., Spradling, A. C. & Perrimon, N. 1990 The orthodenticle gene encodes a novel homeo domain protein involved in the development of the *Drosophila* nervous system and ocellar visual structures. *Genes Dev.* **4**, 1516–1527. (doi:10.1101/gad.4.9.1516)

Friedrich, M. 2006 Ancient mechanisms of visual sense organ development based on comparison of the gene networks controlling larval eye, ocellus, and compound eye specification in *Drosophila*. *Arthropod Struct. Dev.* **35**, 357–378. (doi:10.1016/j.asd.2006.08.010)

Fu, W. & Noll, M. 1997 The Pax2 homolog sparkling is required for development of cone and pigment cells in the *Drosophila* eye. *Genes Dev.* **11**, 2066–2078. (doi:10.1101/gad.11.16.2066)

Furukawa, T., Morrow, E. M. & Cepko, C. L. 1997 *Crx*, a novel otx-like homeobox gene, shows photoreceptor-specific expression and regulates photoreceptor differentiation. *Cell* **91**, 531–541. (doi:10.1016/S0092-8674(00)80439-0)

Gehring, W. J. & Ikeo, K. 1999 Pax 6: mastering eye morphogenesis and eye evolution. *Trends Genet.* **15**, 371–377. (doi:10.1016/S0168-9525(99)01776-X)

Germot, A., Lecointre, G., Plouhinec, J. L., Le Mentec, C., Girardot, F. & Mazan, S. 2001 Structural evolution of *Otx* genes in craniates. *Mol. Biol. Evol.* **18**, 1668–1678.

Glardon, S., Callaerts, P., Halder, G. & Gehring, W. J. 1997 Conservation of Pax-6 in a lower chordate, the ascidian *Phallusia mammillata*. *Development* **124**, 817–825.

Glardon, S., Holland, L. Z., Gehring, W. J. & Holland, N. D. 1998 Isolation and developmental expression of the amphioxus Pax-6 gene (*AmphiPax-6*): insights into eye and photoreceptor evolution. *Development* **125**, 2701–2710.

Graham, D. M., Wong, K. Y., Shapiro, P., Frederick, C., Pattabiraman, K. & Berson, D. M. 2008 Melanopsin ganglion cells use a membrane-associated rhabdomeric phototransduction cascade. *J. Neurophysiol.* **99**, 2522–2532. (doi:10.1152/jn.01066.2007)

Grindley, J. C., Davidson, D. R. & Hill, R. E. 1995 The role of Pax-6 in eye and nasal development. *Development* **121**, 1433–1442.

Hadrys, T., Desalle, R., Sagasser, S., Fischer, N. & Schierwater, B. 2005 The Trichoplax PaxB gene: a putative Proto-PaxA/B/C gene predating the origin of nerve and sensory cells. *Mol. Biol. Evol.* **22**, 1569–1578. (doi:10.1093/molbev/msi150)

Halder, G., Callaerts, P. & Gehring, W. J. 1995 Induction of ectopic eyes by targeted expression of the eyeless gene in *Drosophila*. *Science* **267**, 1788–1792. (doi:10.1126/science.7892602)

Hallsson, J. H., Haflidadottir, B. S., Stivers, C., Odenwald, W., Arnheiter, H., Pignoni, F. & Steingrimsson, E. 2004

The basic helix–loop–helix leucine zipper transcription factor Mitf is conserved in *Drosophila* and functions in eye development. *Genetics* **167**, 233–241. (doi:10.1534/genetics.167.1.233)

Hartmann, B., Lee, P. N., Kang, Y. Y., Tomarev, S., De Couet, H. G. & Callaerts, P. 2003 Pax6 in the sepiolid squid *Euprymna scolopes*: evidence for a role in eye, sensory organ and brain development. *Mech. Dev.* **120**, 177–183. (doi:10.1016/S0925-4773(02)00456-2)

Heanue, T. A., Reshef, R., Davis, R. J., Mardon, G., Oliver, G., Tomarev, S., Lassar, A. B. & Tabin, C. J. 1999 Synergistic regulation of vertebrate muscle development by Dach2, Eya2, and Six1, homologs of genes required for *Drosophila* eye formation. *Genes Dev.* **13**, 3231–3243. (doi:10.1101/gad.13.24.3231)

Hennig, A. K., Peng, G. H. & Chen, S. 2008 Regulation of photoreceptor gene expression by Crx-associated transcription factor network. *Brain Res.* **1192**, 114–133. (doi:10.1016/j.brainres.2007.06.036)

Hill, R. E. *et al.* 1991 Mouse small eye results from mutations in a paired-like homeobox-containing gene. *Nature* **354**, 522–525. (doi:10.1038/354522a0)

Hodgkinson, C. A., Moore, K. J., Nakayama, A., Steingrimsson, E., Copeland, N. G., Jenkins, N. A. & Arnheiter, H. 1993 Mutations at the mouse microphthalmia locus are associated with defects in a gene encoding a novel basic-helix–loop–helix-zipper protein. *Cell* **74**, 395–404.

Holland, L. Z. *et al.* 2008 The amphioxus genome illuminates vertebrate origins and cephalochordate biology. *Genome Res.* **18**, 1100–1111. (doi:10.1101/gr.073676.107)

Horwitz, J. 1992 Alpha-crystallin can function as a molecular chaperone. *Proc. Natl Acad. Sci. USA* **89**, 10 449–10 453. (doi:10.1073/pnas.89.21.10449)

Hoshiyama, D., Suga, H., Iwabe, N., Koyanagi, M., Nikoh, N., Kuma, K., Matsuda, F., Honjo, T. & Miyata, T. 1998 Sponge Pax cDNA related to Pax-2/5/8 and ancient gene duplications in the Pax family. *J Mol Evol* **47**, 640–648. (doi:10.1007/PL00006421)

Ingolia, T. D. & Craig, E. A. 1982 Four small Drosophila heat shock proteins are related to each other and to mammalian alpha-crystallin. *Proc. Natl Acad. Sci. USA* **79**, 2360–2364. (doi:10.1073/pnas.79.7.2360)

Jang, C. C. *et al.* 2003 Two Pax genes, eye gone and eyeless, act cooperatively in promoting *Drosophila* eye development. *Development* **130**, 2939–2951. (doi:10.1242/dev.00522)

Kasahara, T., Okano, T., Haga, T. & Fukada, Y. 2002 Opsin-G11-mediated signaling pathway for photic entrainment of the chicken pineal circadian clock. *J. Neurosci.* **22**, 7321–7325.

Kawakami, K., Sato, S., Ozaki, H. & Ikeda, K. 2000 Six family genes—structure and function as transcription factors and their roles in development. *Bioessays* **22**, 616–626. (doi:10.1002/1521-1878(200007)22:7<616::AID-BIES4>3.0.CO;2-R)

Kimura, A., Singh, D., Wawrousek, E. F., KIkuchi, M., Nakamura, M. & Shinohara, T. 2000 Both PCE-1/RX and OTX/CRX interactions are necessary for photoreceptor-specific gene expression. *J. Biol. Chem.* **275**, 1152–1160. (doi:10.1074/jbc.275.2.1152)

Kobayashi, M., Nishikawa, K., Suzuki, T. & Yamamoto, M. 2001 The homeobox protein Six3 interacts with the Groucho corepressor and acts as a transcriptional repressor in eye and forebrain formation. *Dev. Biol.* **232**, 315–326. (doi:10.1006/dbio.2001.0185)

Koike, C. *et al.* 2007 Functional roles of Otx2 transcription factor in postnatal mouse retinal development. *Mol. Cell. Biol.* **27**, 8318–8329. (doi:10.1128/MCB.01209-07)

Kojima, D., Terakita, A., Ishikawa, T., Tsukahara, Y., Maeda, A. & Shichida, Y. 1997 A novel Go-mediated phototransduction cascade in scallop visual cells. *J. Biol. Chem.* **272**, 22 979–22 982. (doi:10.1074/jbc.272.37.22979)

Kondoh, H., Uchikawa, M. & Kamachi, Y. 2004 Interplay of Pax6 and SOX2 in lens development as a paradigm of genetic switch mechanisms for cell differentiation. *Int. J. Dev. Biol.* **48**, 819–827. (doi:10.1387/ijdb.041868hk)

Koyanagi, M., Kubokawa, K., Tsukamoto, H., Shichida, Y. & Terakita, A. 2005 Cephalochordate melanopsin: evolutionary linkage between invertebrate visual cells and vertebrate photosensitive retinal ganglion cells. *Curr. Biol.* **15**, 1065–1069. (doi:10.1016/j.cub.2005.04.063)

Koyanagi, M., Takano, K., Tsukamoto, H., Ohtsu, K., Tokunaga, F. & Terakita, A. 2008 Jellyfish vision starts with cAMP signaling mediated by opsin-Gs cascade. *Proc. Natl Acad. Sci. USA* **105**, 15 576–15 580. (doi:10.1073/pnas.0806215105)

Kozmik, Z. 2005 *Pax* genes in eye development and evolution. *Curr. Opin. Genet. Dev.* **15**, 430–438. (doi:10.1016/j.gde.2005.05.001)

Kozmik, Z., Daube, M., Frei, E., Norman, B., Kos, L., Dishaw, L. J., Noll, M. & Piatigorsky, J. 2003 Role of *Pax* genes in eye evolution: a cnidarian *PaxB* gene uniting Pax2 and Pax6 functions. *Dev. Cell* **5**, 773–785. (doi:10.1016/S1534-5807(03)00325-3)

Kozmik, Z. *et al.* 2007 Pax–Six–Eya–Dach network during amphioxus development: conservation *in vitro* but context specificity *in vivo*. *Dev. Biol.* **306**, 143–159. (doi:10.1016/j.ydbio.2007.03.009)

Kozmik, Z. *et al.* 2008*a* Assembly of the cnidarian camera-type eye from vertebrate-like components. *Proc. Natl Acad. Sci. USA* **105**, 8989–8993. (doi:10.1073/pnas.0800388105)

Kozmik, Z., Swamynathan, S. K., Ruzickova, J., Jonasova, K., Paces, V., Vlcek, C. & Piatigorsky, J. 2008*b* Cubozoan crystallins: evidence for convergent evolution of pax regulatory sequences. *Evol Dev* **10**, 52–61.

Kronhamn, J., Frei, E., Daube, M., Jiao, R., Shi, Y., Noll, M. & Rasmuson-Lestander, A. 2002 Headless flies produced by mutations in the paralogous *Pax6* genes eyeless and twin of eyeless. *Development* **129**, 1015–1026.

Lacalli, T. C. 2004 Sensory systems in amphioxus: a window on the ancestral chordate condition. *Brain Behav. Evol.* **64**, 148–162. (doi:10.1159/000079744)

Land, M. & Nilsson, E. 2002 *Animal eyes*. New York, NY: Oxford University Press.

Larusso, N. D., Rutterberg, B. E., Singh, A. K. & Oakley, T. H. 2008 Type II opsins: evolutionary origin by internal domain duplication? *J. Mol. Evol.* **66**, 417–423. (doi:10.1007/s00239-008-9076-6)

Li, X., Perissi, V., Liu, F., Rose, D. W. & Rosenfeld, M. G. 2002 Tissue-specific regulation of retinal and pituitary precursor cell proliferation. *Science* **297**, 1180–1183.

Li, X. *et al.* 2003 Eya protein phosphatase activity regulates Six1–Dach–Eya transcriptional effects in mammalian organogenesis. *Nature* **426**, 247–254. (doi:10.1038/nature02083)

Liu, W., Lagutin, O. V., Mende, M., Streit, A. & Oliver, G. 2006 Six3 activation of Pax6 expression is essential for mammalian lens induction and specification. *EMBO J.* **25**, 5383–5395. (doi:10.1038/sj.emboj.7601398)

Loosli, F., Kmita-Cunisse, M. & Gehring, W. J. 1996 Isolation of a Pax-6 homolog from the ribbonworm *Lineus sanguineus*. *Proc. Natl Acad. Sci. USA* **93**, 2658–2663. (doi:10.1073/pnas.93.7.2658)

Loosli, F., Staub, W., Finger-Baier, K. C., Ober, E. A., Verkade, H., Wittbrodt, J. & Baier, H. 2003 Loss of eyes in zebrafish caused by mutation of chokh/rx3.

EMBO Rep. **4**, 894–899. (doi:10.1038/sj.embor.embor919)

Lopez-Rios, J., Tessmar, K., Loosli, F., Wittbrodt, J. & Bovolenta, P. 2003 Six3 and Six6 activity is modulated by members of the groucho family. *Development* **130**, 185–195. (doi:10.1242/dev.00185)

Manavathi, B. *et al.* 2007 Repression of Six3 by a corepressor regulates rhodopsin expression. *Proc. Natl Acad. Sci. USA* **104**, 13 128–13 133. (doi:10.1073/pnas.0705878104)

Mannini, L., Rossi, L., Deri, P., Gremigni, V., Salvetti, A., Salo, E. & Batistoni, R. 2004 Djeyes absent (Djeya) controls prototypic planarian eye regeneration by cooperating with the transcription factor Djsix-1. *Dev. Biol.* **269**, 346–359. (doi:10.1016/j.ydbio.2004.01.042)

Martinez-Morales, J. R., Signore, M., Acampora, D., Simeone, A. & Bovolenta, P. 2001 Otx genes are required for tissue specification in the developing eye. *Development* **128**, 2019–2030.

Martinez-Morales, J. R., Dolez, V., Rodrigo, I., Zaccarini, R., Leconte, L., Bovolenta, P. & Saule, S. 2003 OTX2 activates the molecular network underlying retina pigment epithelium differentiation. *J. Biol. Chem.* **278**, 21 721–21 731. (doi:10.1074/jbc.M301708200)

Martinez-Morales, J. R., Rodrigo, I. & Bovolenta, P. 2004 Eye development: a view from the retina pigmented epithelium. *Bioessays* **26**, 766–777.

Mathers, P. H., Grinberg, A., Mahon, K. A. & Jamrich, M. 1997 The Rx homeobox gene is essential for vertebrate eye development. *Nature* **387**, 603–607. (doi:10.1038/42475)

Milligan, G. & Kostenis, E. 2006 Heterotrimeric G-proteins: a short history. *Br. J. Pharmacol.* **147**(Suppl. 1), S46–S55.

Murisier, F. & Beermann, F. 2006 Genetics of pigment cells: lessons from the tyrosinase gene family. *Histol. Histopathol.* **21**, 567–578.

Nagao, T., Leuzinger, S., Acampora, D., Simeone, A., Finkelstein, R., Reichert, H. & Furukubo-Tokunaga, K. 1998 Developmental rescue of *Drosophila* cephalic defects by the human *Otx* genes. *Proc. Natl Acad. Sci. USA* **95**, 3737–3742. (doi:10.1073/pnas.95.7.3737)

Nagel, G., Ollig, D., Fuhrmann, M., Kateriya, S., Musti, A. M., BAmberg, E. & Hegemann, P. 2002 Channelrhodopsin-1: a light-gated proton channel in green algae. *Science* **296**, 2395–2398. (doi:10.1126/science.1072068)

Nishida, A., Furukawa, A., Koike, C., Tano, Y., Aizawa, S., Matsuo, I. & Furukawa, T. 2003 Otx2 homeobox gene controls retinal photoreceptor cell fate and pineal gland development. *Nat. Neurosci.* **6**, 1255–1263. (doi:10.1038/nn1155)

Nordstrom, K., Larsson, T. A. & Larhammar, D. 2004 Extensive duplications of phototransduction genes in early vertebrate evolution correlate with block (chromosome) duplications. *Genomics* **83**, 852–872. (doi:10.1016/j.ygeno.2003.11.008)

Ohto, H., Kamada, S., Tago, K., Tominaga, S. I., Ozaki, H., Sato, S. & Kawakami, K. 1999 Cooperation of six and eya in activation of their target genes through nuclear translocation of Eya. *Mol. Cell. Biol.* **19**, 6815–6824.

Ozaki, H. *et al.* 2004 Six1 controls patterning of the mouse otic vesicle. *Development* **131**, 551–562. (doi:10.1242/dev.00943)

Pan, Y., Nekkalapudi, S., Kelly, L. E. & El-Hodiri, H. M. 2006 The Rx-like homeobox gene (*Rx-L*) is necessary for normal photoreceptor development. *Invest. Ophthalmol. Vis. Sci.* **47**, 4245–4253. (doi:10.1167/iovs.06-0167)

Panda, S., Nayak, S. K., Campo, B., Walker, J. R., Hogenesch, J. B. & Jegla, T. 2005 Illumination of the melanopsin signaling pathway. *Science* **307**, 600–604. (doi:10.1126/science.1105121)

Pappu, K. S. & Mardon, G. 2004 Genetic control of retinal specification and determination in *Drosophila*. *Int. J. Dev. Biol.* **48**, 913–924. (doi:10.1387/ijdb.041875kp)

Piatigorsky, J. 2007 *Gene sharing and evolution: the diversity of protein functions*. Cambridge, MA: Harvard University Press.

Piatigorsky, J. & Wistow, G. 1991 The recruitment of crystallins: new functions precede gene duplication. *Science* **252**, 1078–1079. (doi:10.1126/science.252.5009.1078)

Piatigorsky, J. & Wistow, G. J. 1989 Enzyme/crystallins: gene sharing as an evolutionary strategy. *Cell* **57**, 197–199. (doi:10.1016/0092-8674(89)90956-2)

Piatigorsky, J., O'Brien, W. E., Norman, B. L., Kalumuck, K., Wistow, G. J., Borras, T., Nickerson, J. M. & Wawrousek, E. F. 1988 Gene sharing by delta-crystallin and argininosuccinate lyase. *Proc. Natl Acad. Sci. USA* **85**, 3479–3483. (doi:10.1073/pnas.85.10.3479)

Piatigorsky, J., Kozmik, Z., Horwitz, J., Ding, L., Carosa, E., Robison JR, W. G., Steinbach, P. J. & Tamm, E. R. 2000 Omega-crystallin of the scallop lens. A dimeric aldehyde dehydrogenase class 1/2 enzyme-crystallin. *J. Biol. Chem.* **275**, 41 064–41 073. (doi:10.1074/jbc.M005625200)

Pineda, D. *et al.* 2002 The genetic network of prototypic planarian eye regeneration is Pax6 independent. *Development* **129**, 1423–1434.

Plachetzki, D. C. & Oakley, T. H. 2007 Key transitions during the evolution of animal phototransduction: novelty, 'tree-thinking,' co-option, and co-duplication. *Integr. Comp. Biol.* **47**, 759–769. (doi:10.1093/icb/icm050)

Plachetzki, D. C., Degnan, B. M. & Oakley, T. H. 2007 The origins of novel protein interactions during animal opsin evolution. *PLoS ONE* **2**, e1054. (doi:10.1371/journal.pone.0001054)

Plouhinec, J. L. *et al.* 2003 The mammalian Crx genes are highly divergent representatives of the Otx5 gene family, a gnathostome orthology class of orthodenticle-related homeogenes involved in the differentiation of retinal photoreceptors and circadian entrainment. *Mol. Biol. Evol.* **20**, 513–521. (doi:10.1093/molbev/msg085)

Plouhinec, J. L., Leconte, L., Sauka-Spengler, T., Bovolenta, P., Mazan, S. & Saule, S. 2005 Comparative analysis of gnathostome *Otx* gene expression patterns in the developing eye: implications for the functional evolution of the multigene family. *Dev. Biol.* **278**, 560–575. (doi:10.1016/j.ydbio.2004.11.019)

Qian, J., Esumi, N., Chen, Y., Wang, Q., Chowers, I. & Zack, D. J. 2005 Identification of regulatory targets of tissue-specific transcription factors: application to retina-specific gene regulation. *Nucleic Acids Res.* **33**, 3479–3491. (doi:10.1093/nar/gki658)

Quigley, I. K., Xie, X. & Shankland, M. 2007 Hau-Pax6A expression in the central nervous system of the leech embryo. *Dev. Genes Evol.* **217**, 459–468. (doi:10.1007/s00427-007-0156-1)

Quiring, R., Walldorf, U., Kloter, U. & Gehring, W. J. 1994 Homology of the eyeless gene of *Drosophila* to the Small eye gene in mice and Aniridia in humans. *Science* **265**, 785–789. (doi:10.1126/science.7914031)

Radu, R. A., Hu, J., Peng, J., Bok, D., Mata, N. L. & Travis, G. H. 2008 Retinal pigment epithelium-retinal G protein receptor-opsin mediates light-dependent translocation of all-trans-retinyl esters for synthesis of visual chromophore in retinal pigment epithelial cells. *J. Biol. Chem.* **283**, 19 730–19 738. (doi:10.1074/jbc.M801288200)

Raible, F., Tessmar-Raible, K., Arboleda, E., Kaller, T., Bork, P., Arendt, D. & Arnone, M. I. 2006 Opsins and clusters of sensory G-protein-coupled receptors in the sea urchin genome. *Dev. Biol.* **300**, 461–475. (doi:10.1016/j.ydbio.2006.08.070)

Ranade, S. S., Yang-Zhou, D., Kong, S. W., Mcdonald, E. C., Cook, T. A. & Pignoni, F. 2008 Analysis of the Otd-dependent transcriptome supports the evolutionary conservation of CRX/OTX/OTD functions in flies and vertebrates. *Dev. Biol.* **315**, 521–534. (doi:10.1016/j.ydbio.2007.12.017)

Rath, M. F., Morin, F., Shi, Q., Klein, D. C. & Moller, M. 2007 Ontogenetic expression of the *Otx2* and *Crx* homeobox genes in the retina of the rat. *Exp. Eye Res.* **85**, 65–73. (doi:10.1016/j.exer.2007.02.016)

Rayapureddi, J. P., Kattamuri, C., Steinmetz, B. D., Frankfort, B. J., Ostrin, E. J., Mardon, G. & Hegde, R. S. 2003 Eyes absent represents a class of protein tyrosine phosphatases. *Nature* **426**, 295–298. (doi:10.1038/nature02093)

Royet, J. & Finkelstein, R. 1995 Pattern formation in *Drosophila* head development: the role of the orthodenticle homeobox gene. *Development* **121**, 3561–3572.

Salo, E., Pineda, D., Marsal, M., Gonzalez, J., Gremigni, V. & Batistoni, R. 2002 Genetic network of the eye in Platyhelminthes: expression and functional analysis of some players during planarian regeneration. *Gene* **287**, 67–74. (doi:10.1016/S0378-1119(01)00863-0)

Schwarz, M., Cecconi, F., Bernier, G., Andrejewski, N., Kammandel, B., Wagner, M. & Gruss, P. 2000 Spatial specification of mammalian eye territories by reciprocal transcriptional repression of Pax2 and Pax6. *Development* **127**, 4325–4334.

Seimiya, M. & Gehring, W. J. 2000 The *Drosophila* homeobox gene optix is capable of inducing ectopic eyes by an eyeless-independent mechanism. *Development* **127**, 1879–1886.

Silver, S. J. & Rebay, I. 2005 Signaling circuitries in development: insights from the retinal determination gene network. *Development* **132**, 3–13. (doi:10.1242/dev.01539)

Simeone, A., Puelles, E. & Acampora, D. 2002 The Otx family. *Curr. Opin. Genet. Dev.* **12**, 409–415. (doi:10.1016/S0959-437X(02)00318-0)

Simpson, T. I. & Price, D. J. 2002 Pax6; a pleiotropic player in development. *Bioessays* **24**, 1041–1051. (doi:10.1002/bies.10174)

Spudich, J. L., Yang, C. S., Jung, K. H. & Spudich, E. N. 2000 Retinylidene proteins: structures and functions from archaea to humans. *Annu. Rev. Cell Dev. Biol.* **16**, 365–392. (doi:10.1146/annurev.cellbio.16.1.365)

Steingrimsson, E., Copeland, N. G. & Jenkins, N. A. 2004 Melanocytes and the microphthalmia transcription factor network. *Annu. Rev. Genet.* **38**, 365–411. (doi:10.1146/annurev.genet.38.072902.092717)

Stierwald, M., Yanze, N., Bamert, R. P., Kammermeier, L. & Schmid, V. 2004 The Sine oculis/Six class family of homeobox genes in jellyfish with and without eyes: development and eye regeneration. *Dev. Biol.* **274**, 70–81. (doi:10.1016/j.ydbio.2004.06.018)

Su, C. Y., Luo, D. G., Terakita, A., Shichida, Y., Liao, H. W., Kazmi, M. A., Sakmar, T. P. & Yau, K. W. 2006 Parietal-eye phototransduction components and their potential evolutionary implications. *Science* **311**, 1617–1621. (doi:10.1126/science.1123802)

Suga, H., Schmid, V. & Gehring, W. J. 2008 Evolution and functional diversity of jellyfish opsins. *Curr. Biol.* **18**, 51–55. (doi:10.1016/j.cub.2007.11.059)

Suzuki, M. M. & Satoh, N. 2000 Genes expressed in the amphioxus notochord revealed by EST analysis. *Dev. Biol.* **224**, 168–177. (doi:10.1006/dbio.2000.9796)

Suzuki, T., Terakita, A., Narita, K., Nagai, K., Tsukahara, Y. & Kito, Y. 1995 Squid photoreceptor phospholipase C is stimulated by membrane Gq alpha but not by soluble Gq alpha. *FEBS Lett.* **377**, 333–337. (doi:10.1016/0014-5793(95)01364-4)

Tahayato, A., Sonneville, R., Pichaud, F., Wernet, M. F., Papatsenko, D., Beaufils, P., Cook, T. & Desplan, C. 2003 Otd/Crx, a dual regulator for the specification of ommatidia subtypes in the *Drosophila* retina. *Dev. Cell* **5**, 391–402. (doi:10.1016/S1534-5807(03)00239-9)

Takeda, K., Yokoyama, S., Yasumoto, K., Saito, H., Udono, T., Takahashi, K. & Shibahara, S. 2003 OTX2 regulates expression of DOPAchrome tautomerase in human retinal pigment epithelium. *Biochem. Biophys. Res. Commun.* **300**, 908–914. (doi:10.1016/S0006-291X(02)02934-0)

Takechi, M., Seno, S. & Kawamura, S. 2008 Identification of *cis*-acting elements repressing blue opsin expression in zebrafish UV cones and pineal cells. *J. Biol. Chem* **283**, 31 625–31 632. (doi:10.1074/jbc.M806226200)

Terakita, A. 2005 The opsins. *Genome Biol.* **6**, 213. (doi:10.1186/gb-2005-6-3-213)

Tomarev, S. I., Callaerts, P., Kos, L., Zinovieva, R., Halder, G., Gehring, W. & Piatigorsky, J. 1997 Squid Pax-6 and eye development. *Proc. Natl Acad. Sci. USA* **94**, 2421–2426. (doi:10.1073/pnas.94.6.2421)

Tootle, T. L., Silver, S. J., Davies, E. L., Newman, V., Latek, R. R., Mills, I. A., Selengut, J. D., Parlikar, B. E. & Rebay, I. 2003 The transcription factor Eyes absent is a protein tyrosine phosphatase. *Nature* **426**, 299–302. (doi:10.1038/nature02097)

Toyoda, R., Sato, S., Ikeo, K., Gojobori, T., Numakunai, T., Goding, C. R. & Yamamoto, H. 2000 Pigment cell-specific expression of the tyrosinase gene in ascidians has a different regulatory mechanism from vertebrates. *Gene* **259**, 159–170. (doi:10.1016/S0378-1119(00)00433-9)

Toyoda, R., Kasai, A., Sato, S., Wada, S., Saiga, H., Ikeo, K., Gojobori, T., Numakunai, T. & Yamamoto, H. 2004 Pigment cell lineage-specific expression activity of the ascidian tyrosinase-related gene. *Gene* **332**, 61–69. (doi:10.1016/j.gene.2004.01.030)

Treisman, J., Harris, E. & Desplan, C. 1991 The paired box encodes a second DNA-binding domain in the paired homeo domain protein. *Genes Dev.* **5**, 594–604. (doi:10.1101/gad.5.4.594)

Umesono, Y., Watanabe, K. & Agata, K. 1999 Distinct structural domains in the planarian brain defined by the expression of evolutionarily conserved homeobox genes. *Dev. Genes Evol.* **209**, 31–39. (doi:10.1007/s004270050224)

Vandendries, E. R., Johnson, D. & Reinke, R. 1996 orthodenticle is required for photoreceptor cell development in the *Drosophila* eye. *Dev. Biol.* **173**, 243–255. (doi:10.1006/dbio.1996.0020)

Velarde, R. A., Sauer, C. D., Walden, K. K., Fahrbach, S. E. & Robertson, H. M. 2005 Pteropsin: a vertebrate-like non-visual opsin expressed in the honey bee brain. *Insect Biochem. Mol. Biol.* **35**, 1367–1377. (doi:10.1016/j.ibmb.2005.09.001)

Wada, S., Katsuyama, Y., Sato, Y., Itoh, C. & Saiga, H. 1996 Hroth an orthodenticle-related homeobox gene of the ascidian, *Halocynthia roretzi*: its expression and putative roles in the axis formation during embryogenesis. *Mech. Dev.* **60**, 59–71. (doi:10.1016/S0925-4773(96)00600-4)

Wada, S., Toyoda, R., Yamamoto, H. & Saiga, H. 2002 Ascidian otx gene Hroth activates transcription of the brain-specific gene HrTRP. *Dev. Dyn.* **225**, 46–53. (doi:10.1002/dvdy.10135)

Wada, S. *et al.* 2003 A genomewide survey of developmentally relevant genes in *Ciona intestinalis*. II. Genes for homeobox transcription factors. *Dev. Genes Evol.* **213**, 222–234. (doi:10.1007/s00427-003-0321-0)

Wang, Q. L. *et al.* 2004 *QRX*, a novel homeobox gene, modulates photoreceptor gene expression. *Hum. Mol. Genet.* **13**, 1025–1040. (doi:10.1093/hmg/ddh117)

Williams, N. A. & Holland, P. W. H. 1996 Old head on young shoulders. *Nature* **383**, 490–490. (doi:10.1038/383490a0)

Williams, N. A. & Holland, P. W. 1998 Gene and domain duplication in the chordate Otx gene family: insights from amphioxus Otx. *Mol. Biol. Evol.* **15**, 600–607.

Wistow, G. 1990 Evolution of a protein superfamily: relationships between vertebrate lens crystallins and microorganism dormancy proteins. *J. Mol. Evol.* **30**, 140–145. (doi:10.1007/BF02099940)

Wistow, G. J. & Piatigorsky, J. 1988 Lens crystallins: the evolution and expression of proteins for a highly specialized tissue. *Annu. Rev. Biochem.* **57**, 479–504. (doi:10.1146/annurev.bi.57.070188.002403)

Xu, Z. P. & Saunders, G. F. 1997 Transcriptional regulation of the human *PAX6* gene promoter. *J. Biol. Chem.* **272**, 3430–3436.

Xu, P. X., Adams, J., Peters, H., Brown, M. C., Heaney, S. & Maas, R. 1999 Eya1-deficient mice lack ears and kidneys and show abnormal apoptosis of organ primordia. *Nat. Genet.* **23**, 113–117.

Yajima, I. *et al.* 2003 Cloning and functional analysis of ascidian Mitf *in vivo*: insights into the origin of vertebrate pigment cells. *Mech. Dev.* **120**, 1489–1504. (doi:10.1016/j.mod.2003.08.009)

Yoshida, R., Kusakabe, T., Kamatani, M., Daitoh, M. & Tsuda, M. 2002 Central nervous system-specific expression of G protein alpha subunits in the ascidian *Ciona intestinalis*. *Zool. Sci.* **19**, 1079–1088. (doi:10.2108/zsj.19.1079)

Zhang, X., Friedman, A., Heaney, S., Purcell, P. & Maas, R. L. 2002 Meis homeoproteins directly regulate Pax6 during vertebrate lens morphogenesis. *Genes Dev.* **16**, 2097–2107. (doi:10.1101/gad.1007602)

Zhu, C. C., Dyer, M. A., Uchikawa, M., Kondoh, H., Lagutin, O. V. & Oliver, G. 2002 Six3-mediated auto repression and eye development requires its interaction with members of the Groucho-related family of co-repressors. *Development* **129**, 2835–2849.

Ziegler, I. 2003 The pteridine pathway in zebrafish: regulation and specification during the determination of neural crest cell-fate. *Pigment Cell Res.* **16**, 172–182. (doi:10.1034/j.1600-0749.2003.00044.x)

Zimmerman, J. E., Bui, Q. T., Liu, H. & Bonini, N. M. 2000 Molecular genetic analysis of *Drosophila* eyes absent mutants reveals an eye enhancer element. *Genetics* **154**, 237–246.

Zuber, M. E., Perron, M., Philpott, A., Bang, A. & Harris, W. A. 1999 Giant eyes in *Xenopus laevis* by overexpression of XOptx2. *Cell* **98**, 341–352. (doi:10.1016/S0092-8674(00)81963-7)