

Development and evolution of eyes and photoreceptors: From Cyanobacteria to humans¹

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Recent experiments on the genetic control of eye development have opened up a completely new perspective on eye evolution. The demonstration that targeted expression of a single master control gene, that is, Pax6 can induce the formation of ectopic eyes in both insects and vertebrates, necessitates a reconsideration of the dogma of a polyphyletic origin of the various eye types in various animal phyla. The involvement of Pax6, Six1 and Six3 genes, which encode highly conserved transcription factors, in the genetic control of eye development in organisms ranging from planarians to human argues strongly for a monophyletic origin of the eye. Because transcription factors can control the expression of any target gene, provided it contains the appropriate gene regulatory elements, the conservation of the genetic control of eye development by Pax6 among all bilaterian animals is not due to functional constraints, but a consequence of its evolutionary history. The prototypic eyes postulated by DARWIN to consist of two cells only, a photoreceptor and a pigment cell, were accidentally controlled by Pax6 and the subsequent evolution of the various eye types occurred by building onto this original genetic program. Indeed, Pax6 was found to be expressed in the prototypic eyes of the trochophora larva of annelids. A hypothesis of intercalary evolution is proposed that assumes that the eye morphogenetic pathway is progressively modified by intercalation of genes between the master control genes on the top of hierarchy and the structural genes like rhodopsin at the bottom. The recruitment of novel genes into the eye morphogenetic pathway can be due to at least two different well known genetic mechanisms, gene duplication and enhancer fusion.

In tracing back the evolution of eyes beyond bilaterians, we find highly developed eyes in some box-jellyfish as well as in some hydrozoans. In hydrozoans the same orthologous Six genes (Six1 and Six3) are required for eye regeneration as in planarians, and in the box jellyfish *Tripedalia* a pax B gene, which may be a precursor of Pax6, was found to be expressed in the eyes. In contrast to the adults, which have highly evolved eyes, the Planula larva of *Tripedalia* has single-celled photoreceptors similar to

some unicellular protists. We propose that the evolution of the prototypic eye started with a single step of cellular differentiation from a single-celled pigmented photoreceptor cell into two cell types, a photoreceptor cell specified by Pax6 and a pigment cell specified by the *microphthalmia transcription factor (MITF)*, which is also conserved from jellyfish to humans. From the prototypic eye more complex eyes can evolve by recruiting additional genes into the eye developmental pathway by intercalary evolution.

Using gene chips we have begun to decipher the eye developmental program in *Drosophila* at larval, pupal and adult stages of development. By comparing leg imaginal discs from wildtype larvae with leg discs in which a compound eye field was induced by Pax6, we found that approximately one hundred genes are induced, mostly transcription factors. At the pupal stage about 400 additional genes are expressed, mostly structural genes required for eye morphogenesis, and in the adult eye another 500 become active which are involved in the visual process, like opsins, transducin, arrestin etc. Therefore, we estimate that besides the genes involved in the imaginal disc formation, about 1000 genes are required to specify a compound eye.

Furthermore, we have obtained evidence that not only Pax6 is highly conserved in evolution, but also its DNA target sites in enhancers which are regulated by Pax6. As an example the Delta 1-crystallin enhancer of the chicken is interpreted correctly by the *Drosophila* lens – secreting cells indicating that the genetic circuits are conserved even though the lens protein genes are not conserved.

In tracing back the evolution of vision, we have to return to the earliest history of living organisms. Sunlight is a primary source of energy for life. Among the oldest known fossils are the stromatolites from Western Australia. They represent cyanobacterial deposits and are some 3500 million (3.5 billion) years old. Stromatolites can still be found in some lagoons of Western Australia. Cyanobacteria are capable of photosynthesis and light perception by proteorhodopsin, and they show circadian rhythms. Some cyanobacteria have become symbionts of eukaryotic cells, and

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they have been incorporated first into red algae as primary chloroplasts with two cyanobacterial membranes. Subsequently, the red alga with its symbiont has been taken up by other eukaryotes, where they form secondary chloroplasts with 3 or 4 membranes, two cyanobacterial membranes, a primary host plasma membrane from the red alga, and a secondary host phagosomal membrane. In dinoflagellates (and euglenids) the primary host plasma membrane has been lost, so that they have three membranes around their secondary chloroplasts. Some dinoflagellates have lost their chloroplast and are heterotrophic. Some of these heterotrophs have evolved highly complex photoreceptor structures, resembling a human eye with “cornea”, “lens”, “retina” and “pigment cup”, all in a single cell. During cell division, these “ocelloids” arise from thylakoid membranes, suggesting that they are derived from the chloroplast. The dinoflagellate *Pyrocystis* does indeed contain a proteorhodopsin which is very similar to that of cyanobacteria. Also, in flagellates like *Chlamydomonas* and *Volvox* the “eye spot” (ocelloid) is located in the chloroplast, sug-

gesting that the photoreceptors of flagellates and metazoan might originate from cyanobacteria. These considerations lead to the proposal of a “Russian Doll” hypothesis which assumes that photoreception originated in cyanobacteria and was transferred in at least two symbiotic steps to dinoflagellates. It is not known how the most primitive photoreceptors found in cnidarians have evolved. A first hypothesis assumes another symbiotic step from dinoflagellates to jellyfish. Since dinoflagellates are common symbionts in many cnidarians, their “eye morphogenetic genes” might have been incorporated into some jellyfish genomes. Alternatively, colonial flagellates might have evolved their photoreceptors by cellular differentiation.

Reference

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