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Reinventing a Common Strategy for Patterning the Eye

Minireview

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Master regulatory genes have been defined by their ability to trigger the complete development of an organ or appendage, such as an eye or a wing. One of the most striking examples is *Pax6*, a gene whose function is required to produce eyes in most species and whose expression is sufficient to induce ectopic eye development in flies and in frogs (Chow et al.,1999; Gehring and Ikeo, 1999). The extreme degree of conservation of function for this factor in species with clearly different types of eyes was surprising, given the widely accepted model that eyes have appeared *independently* many times throughout evolution (Salvini-Plawen and Mayr, 1977). Now, recent reports further challenge our views on how eyes have evolved by identifying common differentiation mechanisms and strategies for fly and vertebrate eyes, and by showing that Pax6 requires upstream signals to allow it to specify an eye. Do these apparent homologies result from the evolution of diverse eyes from a common primitive ancestral visual organ? Since the common ancestor of flies and vertebrates did not have a complex eye capable of forming an image, it is more likely that we are witnessing the reutilization of developmental principles designed for the elaboration of other specialized structures.

Restriction of Pax6 Expression

The camera eye of vertebrates is strikingly different from the compound eye of insects; these differences lie not only in the basic morphologies of the two types of eyes, but also in their very different embryonic origins. It is widely believed that they have evolved independently. Thus, the common role of Pax6 has been explained by its recruitment for organogenesis from an ancestral role in a common "primitive unit" for photoreceptor differentiation and light detection (Sheng et al., 1997; Gehring and Ikeo, 1999). Indeed, the light-sensitive pigment (opsin) is highly conserved, and most opsin genes contain essential binding sites that are recognized in *Drosophila* by a dimer of the Pax6 homeodomain, supporting the idea that opsin and Pax6 form a conserved ancestral module (Sheng et al., 1997). Some aspects of this function may have been lost in vertebrates, since Pax6 is not expressed in differentiated cones and rods.

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The vertebrate eye develops from apposed neural (optic vesicle) and surface (lens placode) ectoderm and its early development involves reciprocal inductive processes between these two tissues, both of which express and require Pax6. In flies, a single ectodermal epithelium forms both the retinal and lens components of the eye as well as the antenna. Pax6 expression is activated by the embryonic patterning system and is then restricted to the eye-antennal primordium as well as to subsets of cells in the nervous system. Although fly and vertebrate eyes appear to develop very differently, it is possible that the greater emphasis on induction in vertebrate eye development results from the use of transplantation methods rather than the genetic techniques used in flies.

In a recent issue of this journal, Kumar and Moses (2001) reported that early determination of the *Drosophila* eye may also be the result of an inductive process, as the choice between eye vs. antennal fates is controlled by antagonistic interactions between the Notch (N) and epidermal growth factor receptor (EGFR) pathways (Figure 1). EGFR activity suppresses Pax6 expression in the antennal disc through an as yet unknown mechanism. This restricts to the eye disc the activation of the conserved cascade of downstream "eye determination genes" (Desplan, 1997) at the second instar larval stage, i.e., relatively late in development. The N pathway antagonizes EGFR and allows eye development to proceed. This work is consistent with the previous observation that ectopic activation of N can turn on Pax6 expression in the antennal disc (Kurata et al., 2000). Thus, it seems that N and EGFR control the late subdivision of an earlier anterior embryonic domain, the eye-antennal field. The relevant inducers of EGFR and N signaling have not been located; they could be presented by the embryonic ventral midline and brain, respectively, or be activated autonomously in the eye-antennal disc at a later stage.

In vertebrate eye development, Pax6 is similarly first expressed in a broad anterior ectodermal domain that is resolved into bilateral fields under the influence of prechordal mesoderm. Within the surface ectoderm, Pax6 is then further restricted to the lens placode region. However, Pax6 is also expressed and required in the nasal placode, which could be considered analogous to the antennal disc, based on its origin from an ectodermal region close to the lens placode and its function in chemosensation (Grindley et al., 1995). Although vertebrate Pax6 does not seem to be excluded from the nasal placode by a mechanism like that described by Kumar and Moses (2001), other aspects of Pax6 regulation may be conserved, as some Pax6 enhancers have been shown to respond to signals present in differentiating eye tissues of both flies and vertebrates (Xu et al., 1999).

Patterning the Fly Eye

After determination of the eye field by Pax6 and the other eye determination genes, the photoreceptor clusters are specified during the third larval instar in a posterior to anterior wave of differentiation led by an indentation called the morphogenetic furrow. Propagation of this

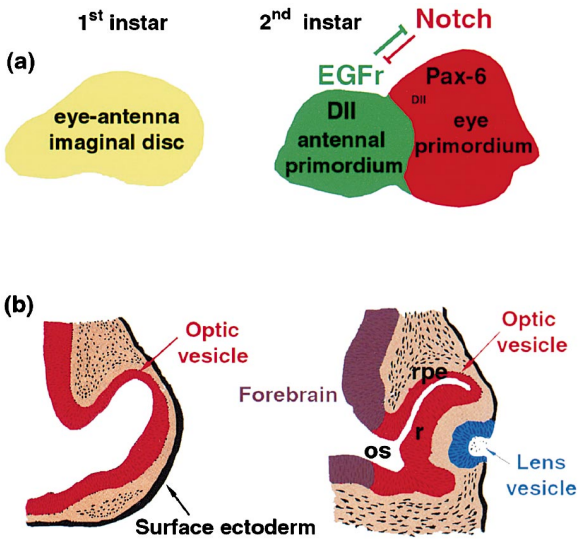

Figure 1. Comparison between *Drosophila* and Vertebrate Eye Development

(a) Fly eye-antennal primordia. In second instar larvae, territorial allocation takes place and leads to the definition of antenna vs. eye fields. At this stage the eye disc still expresses the antennal gene *Distalless* (*Dll*). (b) Early onset of vertebrate eye development. os, optic stalk; rpe, retinal pigmented epithelium; r, retina. (After Gilbert, Developmental Biology, Fourth Edition, p. 268.)

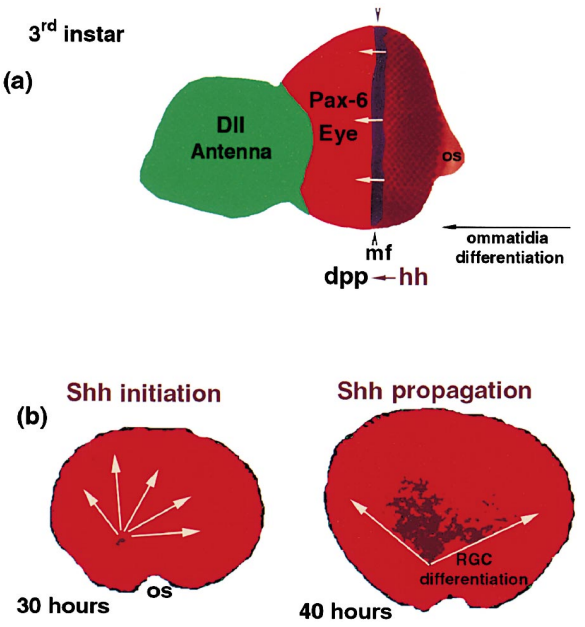

Figure 2. Wave of Differentiation in the Fly and Zebrafish Retina

(a) Third instar larval disc: the morphogenetic furrow crosses the disc from the posterior to the anterior. The differentiating photoreceptor clusters are represented in brown. (b) Initiation and subsequent radial progression (shown by the arrows) of the differentiating wave in the zebrafish retinal ganglion cells (RGC). Differentiated RGC are shown in brown.

wave, initiated at the posterior tip of the eye disc, requires the two diffusible molecules Hedgehog (hh) and Decapentaplegic (dpp), a *Drosophila* BMP homolog. *hh* is initially expressed at the posterior margin, and then turns on in the differentiating photoreceptors; hh activates *dpp* expression in a stripe just anterior to its own expression domain. This relationship between *hh* and *dpp* is highly reminiscent of the induction of *dpp* by hh expressed in the posterior compartment of the *Drosophila* wing imaginal disc. The static stripe of *dpp* at the anteroposterior compartment boundary produces a gradient of dpp that patterns both compartments of the wing (Lawrence and Struhl, 1996). However, in the eye disc, cells that receive the hh signal themselves turn on *hh* expression, allowing the domains of *hh* and *dpp* to progress dynamically across the eye disc. Thus, it is interesting to note that, although the same relationship between Hh and Dpp is used to pattern the wing and eye, this relationship is employed differently in the two tissues. This differential utilization of common modules is a perspective that might be brought to bear on comparisons between species as well.

Common Mechanism or Common Origin?

Besides the functional conservation of Pax6, what other similarities are revealed by the comparison of fly and vertebrate eye development? Although differentiation of the diverse cell types in the vertebrate and fly eyes does not rely on a strict lineage system in either case, the mechanisms of cell specification are quite different. In *Drosophila*, photoreceptors and ommatidial accessory cells are recruited after their final division by sequential inductive processes after the morphogenetic furrow. In vertebrates, cell fate determination occurs differently: multipotent progenitor cells exit the cell cycle at different times during embryonic and postnatal development, giving rise successively to the seven cell types present in the retina, including cone and rod photoreceptors (Cepko et al., 1996). However, two recent reports have shown that differentiation of the zebrafish retina uses the same strategy as the fly retina. A wave of differentiation marked by Sonic hedgehog (Shh) sweeps through the fish retina, leaving behind differentiated retinal cells (Neumann and Nuesslein-Volhard, 2000; Stenkamp et al., 2000; Figures 2 and 3). Shh expression is first detected in a single patch of newly formed retinal ganglion cells (RGCs) close to the optic stalk, and then progresses circumferentially within the RGC layer as a wave that follows the ontogenesis of RGCs. The RGCs, the projection neurons, are the first retinal cells to differentiate and, like the early differentiating R8 photoreceptors in *Drosophila*, they require a homolog of the proneural gene *atonal* for their differentiation (Wang et al., 2001). It is interesting to note that Pax6 also plays a similar role in flies and vertebrates at this stage of retinal development. In flies, Pax6 is restricted to the undifferentiated dividing precursor cells ahead of the furrow, and is turned off in the differentiating photoreceptors posterior to the morphogenetic furrow. Marquardt et al. (2001) show in this issue that mouse Pax6 is required specifically in undifferentiated retinal precursor cells to maintain their ability to give rise to the full set of retinal cells.

In analogy with the requirement for hh in photoreceptor differentiation and furrow progression in flies, Shh and the related protein Tiggywinkle hh are required both for RGC differentiation and to activate Shh expression in fish. These observations are very surprising and excit-

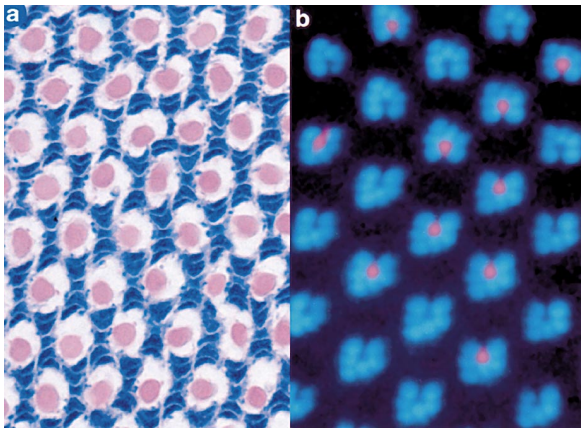

Figure 3. Crystalline Organization of the Fish and Fly Eyes

(a) Tangential section through the layer of cone inner segments in an adult zebrafish retina. The large pink profiles are the UV cones while the blue bow ties are the other three cones. The centers of the bow ties are the blue cones, and the "wings" are the red-green double cones (From P. Raymond, Univ. Michigan). (b) Imaging of photoreceptors in a live adult fly eye using optical neutralization of the cornea (Pichaud and Desplan, 2001). The outer photoreceptors are shown in blue from their autofluorescence in UV light, while a subset of R7 inner photoreceptors marked by a *rhodopsin 3*-GFP transgene are shown in pink.

ing as they almost perfectly reproduce what happens in the fly retina. As the eyes of zebrafish and flies have evolved independently, how can one interpret this similarity? Is it a fabulous evolutionary coincidence, or are we witnessing the utilization of a reliable genetic module that developed before the divergence of these species? Similar "convergent" developmental strategies have been noted for the fly and chick wings, which, like the eyes, clearly share no common ancestor (Panganiban et al., 1997).

On the Patterning of a Periodic Structure

How general is the utilization of a reiterative morphogenetic wave? There are other precise patterning processes both in vertebrates and in invertebrates that do not rely on this mechanism, even if many do use the *hh* module to trigger cell differentiation. These patterning systems rely on lateral inhibition to achieve regular arrays of sensory structures, for example the bristles on the insect scutellum that appear stochastically but end up in a regular array. The fly eye also uses lateral inhibition for ommatidial self-assembly. However, the addition of a temporal component, the morphogenetic furrow, allows each ommatidial row to be used as a template for the next, thus giving the pattern a long-range order (Figure 3). Interestingly, Baker and Yu (2001) argued in a recent issue of *Cell* that the progressive recruitment of photoreceptors also permits the number of late-differentiating precursors to be adjusted to match the number of early-differentiating clusters.

The introduction of a time axis during *Drosophila* eye development might also be explained by the necessity of forming retinotopic photoreceptor projections to the neural cartridges of the lamina, the first optic ganglion. Each differentiating row of ommatidia sends a set of axons that reach the lamina after those of the previous row, thus contacting the next available region of the lamina and inducing its differentiation by secreting hh and the EGFR ligand Spitz. In this system, anteroposterior retinotopy is specified by the temporal component, and only the dorsoventral axis needs a separate patterning mechanism. The meaning of a morphogenetic wave of Shh in the fish retina remains to be understood. Pathfinding by RGC axons relies on a graded system of ephrin and netrin expression (Holmberg et al., 2000) and there is as yet no well-defined requirement for sequential innervation of optic areas within the brain. Nevertheless, patterning of the retina or, more generally, patterning of any precise periodical structure, might opt for such a progressive, iterative strategy simply because local cell–cell interactions (local diffusion of short-range molecules), although reliable and robust, are not sufficient for the most precise patterning events.

In this respect, it is very interesting to consider the patterning of the chicken feather buds. Feathers are arranged in specific tracts over the body and their development occurs sequentially from the midline toward the lateral region, following a propagated morphogenetic stripe. In analogy with the retina, feathers are not specified by a cell lineage mechanism, since all the cells seem to be capable of differentiating into feathers (Chuong et al., 2000). The morphogenetic stripe is characterized by homogeneous expression of Shh that is then resolved to the individual feather buds. Shh is secreted over a very short range, and induces feather bud specification, while the same differentiating bud secretes BMPs that are able to diffuse further and inhibit a similar cell fate. Cells at a distance from the source of BMP can again undergo morphological reorganization accompanied by changes in adhesion that locally affect cell density. This in turn could trigger the expression of Shh, reiterating the process (Chuong et al., 2000). In addition, lateral inhibition through both the N and FGFR pathways seems to be required to resolve the individual feather buds (Crowe et al., 1998). The analogy between feather and retinal patterning seems striking; both use iterative expression of an hh family protein to promote differentiation over time, coupled with lateral inhibition through N and a receptor tyrosine kinase to allow spacing of differentiated structures. In addition, a BMP family member is a target of hh in both cases and may play a similar role. Although dpp promotes the formation of *Drosophila* photoreceptors, while BMPs inhibit the formation of chick feather buds, dpp also induces the expression of an inhibitor of differentiation, *hairy*, at a distance from the front of differentiation in the fly eye.

Concluding Remarks

All together, these findings must be placed in the context of the recent realization, based on complete genome sequences, that evolution toward a higher level of complexity does not rely on the dramatic acquisition of new regulatory functions, or even on a large increase in gene number. It has become clear that Nature uses over and over again a limited number of biochemical modules, such as hh/Shh or dpp/BMP, to achieve various simple patterning tasks. It appears now that patterning of complex tissues also reuses networks of these modules simply by changing the rules of their interaction. For instance, changes in the promoters of *hh* and *dpp* as well as changes in the targets of these signaling molecules

through the influence of the genes that determine the nature of the structure (master regulators) might allow the development of new structures during evolution.

It is very likely that the primitive ancestral unit from which eyes evolved was simply a photoreceptive cell that used Pax6 to activate expression of an opsin. As different eyes became more complex, Pax6 became restricted to progenitor cells within the retina. Then, a convergent strategy that used the interactions between several preexisting biochemical modules such as hh, dpp, and N was superimposed onto the Pax6 module to pattern the crystalline arrays of photoreceptors (Figure 3). Differences between phyla in the details of the regulation of such conserved genes and networks support the idea that they have been recruited *independently* through evolution to elaborate new eye structures. It is striking that very complex and distinct developmental processes are achieved with a relatively restricted and simple number of interlinked genetic modules such as the hh, dpp, N, and EGFR pathways. This leads to the notion that similar strategies do not necessarily imply common ancestry of the organ, but instead reflect the reuse of an efficient mechanism invented for a similar task.

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