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Mechanisms of Drosophila Retinal Morphogenesis: The Virtues of Being Progressive

Minireview

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The adult Drosophila eye is such an exquisitely precise neurosensory structure that it is often compared to a crystal. Approximately 800 individual eyes (or ommatidia) are arranged in a two-dimensional lattice, the accuracy of which is crucial for proper vision. This precision is accompanied by relative structural simplicity, as each ommatidium includes only eight photoreceptor neurons and 12 accessory cells that are arranged in a stereotypical pattern. Perhaps more striking than the structure itself is the fact that its development does not depend on cell lineage but rather is driven purely by positional information (Ready et al., 1976; Lawrence and Green, 1979).

The critical developmental stages (when pattern formation and cell-type allocation begin) occur in a monolayer epithelium, the eye imaginal disc. Provided with an eye disc and all required tools, an assignment to build a fly eye could be approached by two fundamentally different strategies. In the first scenario, the 800 ommatidia (or their precursors) would be dispersed simultaneously onto the epithelium and then arranged, one by one, until they occupy exactly the right position. Alternatively, one might start from one end of the epithelium and lay down one ommatidial row at a time in a progressive and organized manner, such that the leading row can be used as a template for positioning the next. Nature has dealt with this assignment by using the latter strategy. Retinal differentiation is a repetitive process that begins at the posterior margin and progresses across the eye disc, one row at a time, reaching the anterior border about 2 days later (Figure 1A). The forward edge of neural differentiation coincides with an indentation in the apical surface of the epithelium, the morphogenetic furrow (MF). Thus, the MF is, by definition, a transient change in cell shape, which crosses the eye disc much as "the wave" sweeps along the stands of a sporting arena. Cells located posterior to the MF assemble gradually into ommatidial clusters, while cells located anteriorly are unpatterned and divide actively (Ready et al., 1976; Tomlinson and Ready, 1987). Owing to the asynchronous nature of retinal development, the various developmental stages are laid out spatially and can thus be observed simultaneously in a single eye disc. We will review recent advances in our knowledge of the mechanisms that drive and coordinate retinal differentiation.

How Does the Furrow Move?

The mechanisms underlying MF progression in Drosophila were addressed experimentally by a series of eye disc surgery and transplantation experiments (Lebovitz and Ready, 1986). Eye disc fragments from which the differentiating (posterior) portions, including the MF, had been removed were shown to develop fairly normally when cultured in larval hosts. This suggested that the information necessary to generate a new MF and to ensure its propagation was mapped onto the disc ahead of the MF. Insights into the nature of this map and how it is generated and interpreted have recently come from a convergence of genetic and molecular approaches. A crucial insight came from the observation that certain rare mutations in the segment polarity gene *hedgehog* (*hh*) lead to a failure in MF progression (Heberlein et al., 1993; Ma et al., 1993). In a viable, partial loss-of-function allele, the MF stops after having moved approximately half way across the disc; the resulting adult fly lacks the anterior eye. Similarly, in a temperature-sensitive *hh* mutation, the MF stops upon shifting larvae to the restrictive temperature. Hh is expressed (and presumably secreted) by differentiating photoreceptors located posterior to the MF, and its function is required for the expression of the transforming growth factor β (TGFβ) family member *decapentaplegic* (*dpp*) in the MF. Loss of *dpp* function in the eye also results in a failure of morphogenesis, leading to the proposal that hh acts by inducing the expression of *dpp*. This proposal has recently been strengthened by the observation that ectopic expression of *hh* in groups of cells anterior to the MF leads to ectopic induction of *dpp* and the generation of new MFs (Heberlein et al., 1995). Although it is unclear whether dpp mediates all functions of hh, it is clear that the MF is driven from posterior to anterior across the eye disc by a signal, hh, that emanates from differentiating photoreceptor cells. Neither *hh* nor *dpp* is required autono-

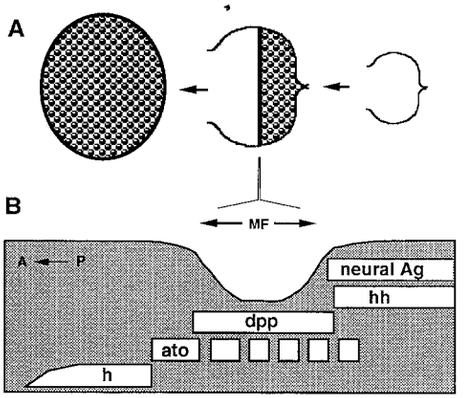

Figure 1. Expression Domains of Genes Involved in MF Progression

(A) Diagram of the retina at different developmental stages. From right to left are shown eye disc prior to MF initiation, eye disc during MF progression, and adult retina. The MF progresses from right to left. View is from the top.

(B) Relative expression domains (along the anterior [A]–posterior [P] axis) of genes involved in MF progression. A lateral view of a portion of an eye disc is shown. Posterior is to the right. Ag, antigen.

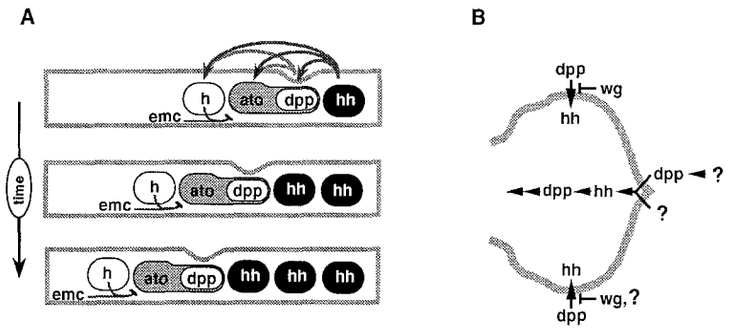

Figure 2. Models for MF Progression and Initiation

(A) Model for MF progression. A lateral view of an eye disc region is shown in which the MF progresses from right to left as time passes from top to bottom.

(B) Model for furrow initiation. Posterior is to the right; dorsal is up.

mously for photoreceptor differentiation, as ommatidia within homozygous mutant clones (analyzed in mosaic animals) develop relatively normally. Rather, these genes are likely to coordinate events by which more anteriorly located cells are instructed to enter the MF and to begin their journey toward differentiation. Therefore, a disruption in the signaling event will lead, indirectly, to an inhibition of neural differentiation.

A signaling cascade that involves hh and dpp also plays a crucial role in signaling across the anterior–posterior compartment border in developing appendages (reviewed by Perrimon, 1995). Hh is expressed and secreted by cells in the posterior compartment and diffuses across the anterior–posterior boundary, where it induces the expression of *dpp*. Dpp, in turn, appears to convey long-range organizing and patterning information in both compartments (Capdevila and Guerrero, 1994). In developing wings and in dorsal legs, *dpp* expression is regulated negatively by the products of the segment polarity gene *patched* (*ptc*) and the gene encoding the major catalytic subunit of protein kinase A (*Pka*), a repression that is alleviated by hh (recently reviewed by Kalderon, 1995; Perrimon, 1995). The same regulatory circuits appear to operate in the eye disc, based on the observation that the consequences of loss of *ptc* or *Pka* function in the eye (analyzed in clones of mutant cells) are indistinguishable from those caused by ectopic activation of *hh* (Pan and Rubin, 1995; Strutt et al., 1995; Wehrli and Tomlinson, 1995; Ma and Moses, 1995). These phenotypes include ectopic expression of *dpp*, the formation of ectopic MFs, and precocious neural differentiation in the anterior eye disc. Most strikingly, the mutant tissue can act as an organizing center: it can alter both the normal growth and the polarity of the surrounding wild type tissue. Thus, the MF coincides with a boundary that has long-range patterning properties similar to those attributed to the anterior–posterior border. Although the exact genetic hierarchy in which *hh*, *ptc*, *Pka*, and *dpp* act has not been conclusively established, it appears that these genes carry out similar functions in the MF and along the anterior–posterior border. A striking difference among these systems is, however, that the boundary in the eye disc is transient, it moves, and it does not involve stably inherited cell fates. In more molecular terms, a requisite for MF progression is that cells that receive the hh signal become in time cells that send the hh signal (Figure 2A).

Role of Helix-Loop-Helix Proteins

This transition from recipient to sender of the hh signal requires that the receiving cells differentiate as photoreceptor neurons; hh is expressed in differentiating photoreceptors (Ma et al., 1993), and this expression requires that cells differentiate as neurons (Heberlein et al., 1993). In the fly's peripheral nervous system (PNS), which includes the photoreceptors, the formation of neural precursors and their subsequent differentiation require the function of the so-called proneural genes (reviewed by Ghysen et al., 1993). Recently, it has been shown that the allocation of neural precursors in the eye disc requires the proneural gene *atonal* (*ato*) (Jarman et al., 1994, 1995). *ato* encodes a basic–helix-loop-helix (bHLH) protein that heterodimerizes with another ubiquitous bHLH protein encoded by *daughterless*. The heterodimer acts as a sequence-specific DNA-binding complex in vitro (Jarman et al., 1993). Loss of *ato* function during eye development leads to a failure in neural differentiation. This failure is due to a defect in the specification of the R8 photoreceptor cell. R8, the so-called ommatidial founder cell, is the first cell to begin neural differentiation posterior to the MF and is believed to recruit the remaining seven photoreceptors by local cell–cell interactions.

ato is expressed in a stripe of cells immediately anterior to the MF. As the furrow passes, expression becomes refined to evenly spaced groups of cells and, shortly thereafter, to single R8 precursors (Jarman et al., 1994). The process of selection (and spatial arrangement) of single cells from a zone of neural competence is believed to occur by lateral inhibition and involves the functions of *scabrous*, *Notch*, *Delta*, and *ato* (Baker and Zitron, 1995; Jarman et al., 1995). The exact underlying mechanisms are not fully understood and are beyond the scope of this minireview. Ectopic expression of *hh* in patches of cells anterior to the MF induces an ectopic proneural zone evidenced by ectopic induction of *ato* (Heberlein et al., 1995). Neural competence is then restricted to single cells, which later differentiate as neurons and begin to express their endogenous *hh* gene. This, in turn, induces neighboring cells to express *dpp*, resulting in a self-propagating morphogenetic wave through reiteration of the same inductive sequence, a process that clearly recapitulates the events of normal MF progression (Figure 2A).

Two other members of the bHLH family appear to regulate neural differentiation negatively. The product of the *hairy* (*h*) gene is expressed anterior to the MF in a band of cells with a sharp posterior border that coincides closely with the anterior border of ato expression (Brown et al.,

1991). Unexpectedly, and despite its interesting and conserved expression pattern, loss of *h* function has little effect on normal eye development. The *extra macrochaetae* (*emc*) gene encodes an HLH protein lacking the basic DNA-binding domain. Loss of *emc* function also leads to only minor defects. However, loss of function of both *h* and *emc* (analyzed in mosaic animals owing to their requirement for viability), causes unexpected disruptions in developing eye tissue (Brown et al., 1995). Most commonly, the MF accelerates by as much as eight ommatidial rows while traversing the doubly mutant tissue. This advanced MF appears relatively normal by morphological and molecular criteria. More rarely, neural differentiation occurs in domains that are completely separated from the normal differentiating field; in these cases, however, the doubly mutant tissue is always located primarily anterior to the MF. It will be important to determine whether such islands of neural cells express *hh*, induce *dpp*, and, eventually, form ectopic furrows. These data clearly show that *h* and *emc* act in concert to regulate negatively the rate of furrow progression and neural differentiation. However, the tight linkage between these two processes in the developing retina makes the establishment of a causal relationship between them difficult.

The regulatory network in which the genes encoding bHLH and HLH proteins function during eye development is unknown. However, some clues may come from potential parallels between the events that lead to R8 selection and the better understood process of sensory organ precursor formation in other regions of the PNS (reviewed by Ghysen et al., 1993). For example, while *h* and *emc* both negatively regulate the transcription of the proneural gene *achaete* during sensory organ precursor selection, they do so by utilizing different molecular strategies (see Brown et al., 1995, and references therein). It is possible that during normal eye development, h functions together with emc (directly or indirectly) by repressing *ato* expression anterior to the MF. Curiously, ectopic expression of *hh* (or loss of PKA) anterior to the MF leads to ectopic induction of h in more anterior regions of the eye disc (commonly surrounding precociously differentiating neural clusters) (Heberlein et al., 1995; Pan and Rubin, 1995). This suggests that the normal stripe of *h* expression may be induced by the advancing MF via the hh signaling cascade. We are therefore presented with the apparently paradoxical situation in which hh induces not only an activator of neural competence, ato, but also an inhibitor, h (Figure 2A). This dual regulation may define precisely the rate at which the proneural zone, and consequently the MF, traverses the eye disc.

How Does the Furrow Start?

While it is clear that hh function is necessary for continued MF progression, it is equally clear that it is not required for its formation or its initial movement away from the posterior border. Not only is *hh* expression absent before the furrow begins to move (Ma et al., 1993), but, in a viable *ato* mutant, where neural precursors (and thus hh-expressing cells) never form, the furrow is present and appears to move a short distance from the posterior margin (Jarman et al., 1995). Thus, the process of MF initiation is distinct and genetically separable from MF progression.

In early developing eye discs (before the MF initiates), *dpp* is expressed all around the disc margin. Why is it then that the MF does not initiate and progress away from the dorsal and ventral margins? Recent developments have started to provide some clues (Ma and Moses, 1995). It turns out that another familiar player, the product of the *wingless* (*wg*) gene, helps in restricting MF initiation to the posterior margin. Prior to MF initiation, *wg* is expressed around the periphery of the eye field, excluding the posterior margin. When *wg* function is removed (with a temperature-sensitive mutation), the MF initiates (in addition to its normal posterior site) at the dorsal margin and propagates ventrally. This ectopic MF appears to posses all of the attributes of a normal furrow; i.e., it coincides with *dpp* expression, and it leaves in its wake assembling clusters of hh-expressing differentiating photoreceptor cells. A similar, but much weaker, effect is observed along the ventral margin, suggesting the presence of another yet unidentified molecule inhibiting furrow initiation there. Because wg does not appear to interfere with the expression of *dpp*, it is likely to act somewhere downstream by antagonizing dpp function, perhaps by inhibiting the generation of hh-expressing cells at the lateral margins (Figure 2B). Although we are far from understanding the mechanisms underlying MF initiation, the experiments just described suggest that the position along the margin where the MF starts is at least partly defined by a restrictive process that involves wg. What determines the timing of MF initiation still remains a complete mystery.

While much remains to be elucidated about the mechanisms by which dpp, hh, and wg act in MF initiation and progression, they and their homologs work in concert to coordinate many developmental events, not only in the fly but also in vertebrates. Therefore, it appears that although these signaling molecules have been co-opted for many developmental purposes, their relationship to each other has remained flexible.

Concluding Remarks

Although our knowledge of retinal morphogenesis has increased substantially in the past few years, there are still many remaining questions. For example, how are growth and cell cycle synchronization, two events that closely parallel furrow progression, coordinately regulated? And how is it that the complete process of eye development can be set in motion by a single master regulatory gene, the *PAX-6* homolog *eyeless* (Hadler et al., 1995)? Regardless of what the answers to these questions will be, it is clear that differentiation in the fly's retina occurs by repetitive cycles of induction. We believe that such a progressive mechanism enables a structure to achieve an unusual degree of precision. In addition, it allows for connections to an associated structure to be formed with the same precision. For example, asynchronous neural development in the fly's retina coordinates the temporal sequence of proliferation and synaptogenesis in the first optic ganglion, the lamina (reviewed by Kunes and Steller, 1993). Interestingly, a wave of asynchronous differentiation, oc-

curring in a central-to-peripheral direction, has recently been described in the developing primate retina (Wikler and Rakic, 1994). Moreover, developing chick feather buds, which have long been known to develop asynchronously, have recently been found to coexpress *Sonic hedgehog* and the *dpp* homolog *BMP-2* (Nohno et al., 1995). Although it is unclear whether these systems utilize progressive mechanisms for pattern formation, the parallels are striking and warrant further investigation.

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