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Transcription factors in eye development: a gorgeous mosaic?

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The dramatic physical differences between compound eyes of insects (such as flies) and camera eyes of vertebrates (like mice and men) support the orthodox view that these two eye types have evolved independently. This view is based on the obvious anatomical differences between these two eyes but also on striking physiological and cell biological differences between the photoreceptor neurons they contain. Vertebrate eyes are comprised of a single optical unit, with one lens projecting an image onto a continuous neural retina. The vertebrate retina not only includes photoreceptor neurons but also four types of interneurons (bipolar, horizontal, amacrine, and ganglion cells), all of which are considered to be part of the central nervous system (for review, see Saha et al. 1992). In contrast, insect compound eyes consist of many repeated unit eyes or ommatidia, each of which (in Drosophila) contain 12 accessory cells and eight photoreceptor neurons. The fly’s photoreceptors project directly to the optic lobes of the brain without any retinal interneurons, and the insect neural retina is considered part of the peripheral nervous system (Ready et al. 1976). Vertebrate photoreceptors carry their visual pigment (opsin) on intracellular membrane sacs in the rod or cone cell outer segments that are supported by a ciliary body, whereas insect photoreceptor cells carry their opsin on extracellular microvilli (forming an aggregate known as the rhabdomere) and have no ciliary bodies. Both types of photoreceptors use a G-protein-based system of phototransduction but differ in their response to light. Vertebrate photoreceptor cells use a cyclic GMP-linked phosphodiesterase and hyperpolarize upon light reception, whereas insect photoreceptor cells use phospholipase C and depolarize upon stimulation by light (for review, see Zuker 1994). The expression of Pax6 protein in Drosophila imaginal discs is necessary and sufficient to induce ectopic eyes, and Pax6 has been proposed as a candidate “master gene” for the visual system. It is likely, however, that things are more complex than that. Recently, another gene has been shown to produce ectopic eyes in flies (Shen and Mardon 1997). Moreover, the expression of Pax6 is not restricted to the developing visual system in mice, nor is Pax6 ectopic expression sufficient to induce ectopic eyes in transgenic mice (for review, see Hanson and Van Heyningen 1995). Thus, although the homology of vertebrate and arthropod eyes is not yet proven, there is certainly an active debate taking place and further data supporting that hypothesis are now available.

In this issue, Fu and Noll present a paper on the isolation of the Drosophila gene sparkling, which has a role in the development of nonphotoreceptor accessory cells in the compound eye. Furthermore they show that the Sparkling protein is a homolog of the murine Pax2, which plays a role in the development of optic nerve glial (nonphotoreceptor) cells in the mouse eye (MacDonald and Wilson 1996). In the mouse, Pax2 is required for the development of non-neuronal optic stalk glial cells and also for the development of the inner ear (Torres et al. 1996). Fu and Noll (this issue) find that Sparkling is expressed in several nonphotoreceptor accessory cell types in the developing Drosophila eye and is required for their development. Despite the dissimilarity of these cells to the mouse glia, which require Pax2 function, and the lack of definitive data that sparkling is a functional ho-
...molog of Pax2, its role in fly eye development is interesting nonetheless.

Drosophila retinal development occurs in two distinct processes: the initiation and propagation of the morphogenetic furrow, and ommatidial assembly. sparkling plays a role only in ommatidial assembly, and this process is our focus here. In the early fly embryo, small groups of cells are set aside to form most of the adult body, and these remain inside the larva until pupal metamorphosis. Among these are the eye-antennal imaginal discs that include the presumptive eye epithelium. This remains an unpatterned columnar epithelium until the final larval stage, when a coordinated wave termed the morphogenetic furrow (Ready et al. 1976) begins to move across it. The furrow is driven by the forward diffusion of Hedgehog protein from the posterior side and is associated with changes in cell shape, synchronization of the cell cycle, and the establishment of the initial cell clusters that will form the future ommatidium (for review, see Heberlein and Moses 1995). The furrow lays down successive columns of ommatidial “preclusters,” at ~2-hr intervals, and sweeps across the eye field in ~2 days. These preclusters initially contain five cells and will later form the first five photoreceptors (R8, R2, R5, R3, and R4, Fig. 1A; Ready et al. 1976). It is likely that furrow progression requires the formation of these preclusters and the specification and initial differentiation of the R8 “founder” cell.

Once the ommatidial founder cell (R8) is specified, the subsequent steps may be considered as ommatidial assembly. Among the five cells of the precluster, the R8 cell is the first to differentiate as a neuron. It is followed by three successive pairs of photoreceptors, R2/5, R3/4, and R1/6 and, after this, the last photoreceptor, R7. Following this, the accessory cells are recruited into the cluster, beginning with the four lens-secreting cone cells, and then (in several steps) the screening pigment cells. Finally, one cell is determined as the mother cell for a mechanosensory bristle. The first model to account for ommatidial assembly proposed that a “combinatorial code” of cell type-specific ligands and generally expressed receptors could convey local positional information and produce a patterned, sequential induction of cell types (Fig. 1B; for review, see Tomlinson 1988). The R8 cell would express some specific ligand that would be sensed by the adjacent cells. These would thus be specified as R2 and R5 and respond by expressing a second specific ligand. The next two cells in the precluster lie in contact with both R8 and either R2 and R5. They would thus receive input from the first and second ligands, and this combination would specify them as R3 and R4. This process would continue through a series of ligand-receptor interactions until all of the 20 cells of the ommatidium are specified and in place.

The first mutation found to affect ommatidial assembly gave strong support to this combinatorial code model. In the sevenless mutation the putative R7 cell is not specified as a neuron at all but instead becomes a cone cell. sevenless was later found to encode a transmembrane, receptor tyrosine kinase (RTK) and to be expressed on all of the developing cells except for the first three (R8, R2, and R5). The ligand for Sevenless was identified (Bride of Sevenless) and was found to be required in and expressed on only the developing R8 cell, which contacts R7. This ligand-receptor pair is exactly what the combinatorial code model predicts (for review, see Simon 1994). One potential problem is that in addition to the putative R7 cell, R3, R4, R1, and R6 all express Sevenless and all contact the R8 cell and thus are exposed to Bride of Sevenless. Why is it that these four cells also do not develop as R7 cells? The answer to this was found in the function of the seven-up gene product, which is a zinc finger transcription factor expressed in only these four cells. In seven-up mutant retinal clones, these four cells do become R7s, and thus the normal function of Seven-up protein in the retina is to cause these cells to ignore the Bride of Sevenless→Sevenless signal (Mlodzik et al. 1990). Once a target cell has received the...
inductive signal, it must respond by altered gene expression to begin differentiation and to express its own specific ligand to form the next link in the chain. A great deal of attention has been focused on how this cell surface signal is transduced to the nucleus, not least because the cascade involves the products of a number of oncogene homologs and cell-cycle regulators: the Ras signal transduction cascade. A series of genetic screens and biochemical experiments in several laboratories has uncovered the major elements of this cascade from the receptor to the nuclear transcription factors (for review, see Simon 1994).

A problem with the simplest version of the combinatorial code model is that the action of the Ras pathway does not appear to be very cell type specific. The Ras pathway acts downstream of other RTKs in oogenesis and in embryonic development, yet these Ras-mediated signals do not produce R7 photoreceptors in the embryo. Even in the eye, ectopic activation of the Ras cascade in other cells does not always lead to the specification of extra R7 cells. The expression of activated Sevenless or Ras constructs in unspecified retinal cells produces several different ectopic cell types (for review, see Simon 1994). Thus, a Ras pathway signal can be interpreted by a receiving cell to specify more than one cell fate. It is even possible to produce R7 cells without any Sevenless at all: When both sevenless and seven-up are removed, some R7 cells remain (Modzik et al. 1990).

It would thus appear that the Bride of Sevenless → Sevenless → Ras signal does not specifically induce the R7 cell fate and does not act alone, as the combinatorial model would predict. Moreover, no other cell type-specific ligands or receptors have been recovered, despite genetic screens specifically designed to find them (Baker et al. 1992). This problem has become ever more perplexing recently with new data on the function of the epidermal growth factor (EGF) receptor’s function in the developing fly eye. Like Sevenless, the Drosophila EGF receptor homolog (Egfr) is a transmembrane RTK that acts through the Ras cascade but, unlike Sevenless, has many functions outside the retina (for review, see Schweitzer and Shilo 1997). A gain-of-function dominant mutation of the Drosophila Egfr causes a rough eye phenotype and, when homozygous, results in a deficit of ommatidia (Baker and Rubin 1989). This led to the suggestion that the Egfr acts in spacing ommatidia, but this does not now seem to be the case. The simple loss-of-function retinal phenotype for the Egfr was more difficult to determine than that of sevenless because it has earlier functions in development (in oogenesis and embryonic development), and retinal mosaics are not interpreted easily because the receptor is required early for cell proliferation (Xu and Rubin 1993). A ligand for the Egfr (Spitz) was found to act in the eye and is required for ommatidial development (Tio and Moses 1997). Analyses of spitz phenotypes, Spitz expression, and dominant-negative Egfr constructs have shown that early precluster cells express Spitz, which then acts through the Egfr on later cells to induce these cells to differentiate (Freeman 1996; Tio and Moses 1997). However, it appears that this process is common to most or all of the cells of the developing ommatidium. Furthermore, a negative ligand, Argos, is produced by the undifferentiated cells and is proposed to dampen this process (Freeman 1996). This has suggested a new model for ommatidial assembly that resembles the combinatorial model, in that there are a series of inductive events, but differs in proposing that each event uses the same ligand and receptor. The simple version of this new EGF receptor model does account for the numerous steps at which the receptor acts but cannot account for the different cell types in the ommatidium nor for their positions (it could only produce successive rings of similar cells; Fig 1C). Another problem is that both known receptors (Sevenless and the Egfr) act through the same signal transduction cascade—via Ras. It is thus hard to see how similar signals can produce different cell types.

Thus, neither the combinatorial code nor the Egfr models account for the different cell type of the ommatidium. There is some hope to be found in the function of Seven-up (see above), a transcription factor that can control the context in which the Sevenless signal is interpreted: a cell that already expresses Seven-up and then receives a Bride of Sevenless → Sevenless signal ignores the signal, and a cell without Seven-up, which receives the same signal, is specified as an R7 cell. Thus, a series of transcription factors, expressed in defined subsets of cells, could act to modulate Ras-inductive signals to produce specific cell types. These factors may have overlapping expression patterns or, in some cases, may interact negatively to prevent overlap. A similar “transcription factor mosaic” has been proposed to underlie floral organ identity in higher plants (for review, see Coen 1996).

In Drosophila, 13 transcription factors have been found that may have roles in this mosaic, and in this issue, Fu and Noll provide evidence that Sparkling may be the fourteenth (Table 1). The first “tile” in this transcription factor mosaic may be Atonal, a basic helix-loop-helix (bHLH) protein that is the proneural gene for the ommatidial founder cell R8. Atonal is expressed initially in a broad band of cells in the furrow, but this pattern resolves to single, evenly spaced proto-R8 cells (Jarman et al. 1995). This focusing of the Atonal pattern is likely to involve extracellular signals that include Scabrous and elements of the Notch pathway (Cagan and Ready 1989; Baker and Yu 1997). The next tile may be Rough, which is initially expressed in a reciprocal pattern to Atonal and may be antagonistic to it (Dokucu et al. 1996). Later, Rough acts to specify the R2/5 cell-type (Tomlinson et al. 1988). Following Rough expression, Bar, Spalt, and Seven-up have roles in R3, R4, R1, and R6 (Modzik et al. 1990; Higashijima et al. 1992; Reuter et al. 1996), and Phyllodactyls act in R1, R6, and R7 (Chang et al. 1995; Dickson et al. 1995). Prospero is expressed in R7 and the cone cells (Kaufmann et al. 1996), and Cut is specific to cone and bristle cells (Blochlinger et al. 1993). Seven in Absentia and Tramtrack appear to have opposite roles in the R7 cell (Carthew and Rubin 1990; Kaufmann et al. 1996).

Lozenge is expressed in all of the undifferentiated cells
posterior to the morphogenetic furrow and is retained in all of the postprecluster cells (R1, R6, and R7, cone, and pigment cells; G. Shirley and U. Banerjee, pers. comm.). Lozenge interacts with other factors in this system, positively regulating Bar and negatively regulating Seven-up (Daga et al. 1996). Lozenge is homologous to the human AML1 transcription factor, and consensus AML1 sites are seen in the fourth intron of sparkling. Furthermore, in lozenge null mutations, Sparkling expression is eliminated (G. Shirley and U. Banerjee, pers. comm.). Some sparkling alleles were isolated originally as enhancers of lozenge (Lindsay and Zimm 1992). As Fu and Noll (this issue) now show that Sparkling regulates Cut in cone cells and Bar in some pigment cells, it may be that Lozenge acting through Sparkling may set the pattern of pigment and cone cells. Finally in this gorgeous mosaic we come back to the Ras pathway: Two transcription factors, Pointed and Yan, are phosphorylation targets of the mitogen-associated protein (MAP) kinase and may thus be the factors that ultimately drive each successive cell into the next available developmental slot as delimited by the mosaic in response to signals from Sevenless and Egfr (O'Neill et al. 1994).

It would be pleasant to be able to stop here and propose that nonspecific Ras pathway signals, acting through a complex mosaic of transcription factors, can account for all of the cell types of the Drosophila ommatidium. Unfortunately this will not work either: Although we may have achieved a deeper level of understanding, we still cannot account for this prepattern of transcription factors. More signals must be at work and scientists in this field cannot retire yet. We can perhaps console ourselves with the observation that the ommatidium is a simple model for the more complex Drosophila nervous system. A real understanding of the development of the human brain may be some way off.

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**Table 1.** Genes encoding transcription factors that may act in ommatidial assembly

<table>
<thead>
<tr>
<th>Name</th>
<th>Type of factor</th>
<th>Function in eye</th>
<th>Function elsewhere</th>
<th>Vertebrate homologs</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>atonal (ato)</td>
<td>HLH</td>
<td>founder cell (R8) specification</td>
<td>chordotonal organs</td>
<td>Math-1</td>
<td>Ben-Arie et al. (1996); Jarman et al. (1995)</td>
</tr>
<tr>
<td>Bar (B)</td>
<td>homeodomain</td>
<td>R1 and R6 and 1° pigment cell-type specification</td>
<td>PNS</td>
<td>Barx1 and Barx2</td>
<td>Higashijima et al. (1992); Jones et al. (1997)</td>
</tr>
<tr>
<td>cut (ct)</td>
<td>homeodomain</td>
<td>expressed in cone and bristle cells</td>
<td>sensory organ identity antenna, tarsal claws, ovaries</td>
<td>Clox, Cux-1, and Cux-2 AML1</td>
<td>Blochlinger et al. (1993); Quaggin et al. (1996); Daga et al. (1996)</td>
</tr>
<tr>
<td>lozenge (lz)</td>
<td>Runt/AML1</td>
<td>R1, 6, 7, cone, and pigment cells</td>
<td>embryonic terminal development</td>
<td></td>
<td></td>
</tr>
<tr>
<td>phyllopod (phyl)</td>
<td>novel nuclear protein</td>
<td>R1, 6, and 7 cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pointed (pnt)</td>
<td>ETS domain</td>
<td>R7 and other cells</td>
<td>CNS</td>
<td>Ets1</td>
<td>O'Neill et al. (1994); Treier et al. (1995)</td>
</tr>
<tr>
<td>prospero (pros)</td>
<td>homeodomain</td>
<td>expressed in R7 and cone cells</td>
<td>CNS</td>
<td>Prox-1</td>
<td>Kauffmann et al. (1996)</td>
</tr>
<tr>
<td>rough (ro)</td>
<td>homeodomain</td>
<td>R2 and R5 cell-type specification</td>
<td>none</td>
<td></td>
<td>Tomlinson et al. (1988); Kimmel et al. (1990)</td>
</tr>
<tr>
<td>seven in absentia (sina)</td>
<td>ring finger</td>
<td>R7 cell specification</td>
<td>fertility, PNS</td>
<td>Siah-1 and Siah-2</td>
<td>Carthew and Rubin (1990); Kauffman et al. (1996)</td>
</tr>
<tr>
<td>seven-up (svp)</td>
<td>C2C2-type zinc finger</td>
<td>R1, 6, 3, and 4 cell-type specification</td>
<td>CNS</td>
<td>COUP and Svp[40] Hsal-1, Hsal-2, Msal, Xs Sal-1</td>
<td>Mlodzik et al. (1990)</td>
</tr>
<tr>
<td>spalt (sal)</td>
<td>zinc finger</td>
<td>expressed in R3 and 4</td>
<td>embryonic and other development</td>
<td></td>
<td>Reuter et al. (1996)</td>
</tr>
<tr>
<td>sparkling (spa)</td>
<td>paired/ homeodomain</td>
<td>cone and pigment cell type specification</td>
<td>CNS</td>
<td>Pax-2</td>
<td>Fu and Noll (this issue)</td>
</tr>
<tr>
<td>tramtrack (ttt)</td>
<td>C2H2-type zinc finger</td>
<td>represses R7 cell</td>
<td>regulates segmentation genes, sensory organs embryonic development</td>
<td>Ets1</td>
<td>Xiong and Montell (1993); Lai et al. (1996)</td>
</tr>
<tr>
<td>yan/ anterior open (aop)</td>
<td>ETS domain</td>
<td>R7 and cone cells</td>
<td></td>
<td></td>
<td>Lai and Rubin (1992); O'Neill et al. (1994)</td>
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References


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