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REVIEW ARTICLE

R8 development in the *Drosophila* eye: a paradigm for neural selection and differentiation

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SUMMARY

The *Drosophila* eye is an outstanding model with which to decipher mechanisms of neural differentiation. Paramount to normal eye development is the organized selection and differentiation of a patterned array of R8 photoreceptors – the founding photoreceptor of each ommatidium that coordinates the incorporation of all other photoreceptors. R8 development is a complex process that requires the integration of transcription factors and signaling pathways, many of which are highly conserved and perform similar functions in other species. This article discusses the developmental control of the four key elements of R8 development: selection, spacing, differentiation and orchestration of later events. New questions that have surfaced because of recent advances in the field are addressed, and the unique characteristics of R8 development are highlighted through comparisons with neural specification in other *Drosophila* tissues and with ganglion cell development in the mammalian retina.

Key words: Drosophila, eye, R8 photoreceptors

INTRODUCTION

The compound eye of *Drosophila melanogaster* has long served as a model for many biological processes and its study has led to major advances in our understanding of cell-fate specification, cell-cell communication and tissue patterning. A component of the *Drosophila* peripheral nervous system, each eye consists of 750-800 light-sensing units (ommatidia) containing eight photosensitive neurons (photoreceptors) (Ready et al., 1976). The presence of such a large number of neurons (>6000), the highly reproducible and consistent nature of ommatidial development, the potential for easily observable phenotypes and the power of *Drosophila* genetics makes the *Drosophila* eye an extremely useful tool with which to study many aspects of neural development. Moreover, virtually all of the genes required for proper *Drosophila* eye development are highly conserved, and many perform similar functions in a wide range of species.

The chronology of ommatidial development is well characterized. First, differentiation of developmentally equipotent cells of the eye imaginal disc begins when movement of the morphogenetic furrow (MF) initiates at the posterior margin of the disc and progresses anteriorly. With its passage, the MF leaves in its wake clusters of differentiating neurons that will ultimately become the photoreceptors of the adult eye (Fig. 1A) (Ready et al., 1976; Tomlinson and Ready, 1987). The order in which photoreceptors differentiate within an ommatidium is invariant and begins with the R8 photoreceptor (Jarman et al., 1994; Tomlinson and Ready, 1987). The R2/R5, R3/R4 and R1/R6 photoreceptors are then sequentially recruited in a pairwise fashion. Last, the R7 photoreceptor is recruited. In *Drosophila*, each ommatidium is not clonally derived (Lawrence and Green, 1979). Instead, the R8 photoreceptor orchestrates ommatidial construction via induction of surrounding uncommitted cells to become photoreceptors in a process that is dependent on Epidermal Growth Factor Receptor (EGFR) signaling (Freeman, 1996; Freeman, 1994; Jarman et al., 1994; Tio et al., 1994; Tio and Moses, 1997; Wasserman et al., 2000).

The central role for R8 in ommatidial development and its selection prior to all other photoreceptors within each ommatidium begs the following question: what factors control the development of R8? The initial aspect of R8 development – selection of single R8 neurons from among a field of uncommitted cells – is a difficult developmental challenge that is carried out with remarkable precision in the maturing *Drosophila* eye. Indeed, selection of single R8 cells requires the integration of cell-autonomously acting transcription factors with secreted growth factor signaling pathways, and failure of R8 selection or selection of too many R8 cells per

ommatidium has profound adverse effects on later recruitment events. Once selected, the presumptive R8 cell must then complete differentiation, as well as coordinate the recruitment of subsequent photoreceptor cells. These later aspects of R8 development, differentiation and orchestration of later recruitment events, occur simultaneously but independently. Further complicating the process of R8 development is the fact that R8 cells are not selected randomly or simultaneously, but rather in a precise progressive pattern to ensure that R8 cells are evenly spaced throughout the eye disc. Specifically, each column of R8 cells is staggered with respect to subsequent and previous columns such that successive columns are exactly out of phase, which ultimately gives rise to the characteristic hexagonal pattern of the adult eye (Wolff and Ready, 1993). Proper R8 spacing also requires the interaction of many factors, particularly members of the Notch and EGFR signaling pathways, and disruptions in spacing can affect both the number and composition of ommatidia. Thus, in the *Drosophila* eye, as for most neural systems, the timing and placement of neural differentiation is crucial.

Despite the temporal overlap of the many processes that underlie R8 maturation, the roles of individual transcription factors and signaling pathways are specific and genetically separable. Thus, it is both feasible and instructive to subdivide R8 maturation into distinct processes that are defined by the genetic pathways that govern them. This article is therefore subdivided into four sections: first, positively and negatively acting factors required for proper R8 selection are reviewed; second, the roles of the Notch and EGFR signaling pathways, which coordinate the spacing of the selected R8 cells, are discussed; third, the genes involved in differentiation of the selected R8 cell are analyzed; and fourth, the organizing properties of R8 are reviewed. As several recent discoveries have called into question some of the basic assumptions of R8 development and function, these data, their impact on the field of R8 development, and possible controversies that surround them are highlighted throughout the review. Next, some of the unique and important developmental characteristics of R8 specification are discussed by comparing it with another paradigm for neural differentiation: *Drosophila* sensory organ precursor (SOP) development. Last, perhaps the most direct relevance of R8 development to neural specification is addressed by discussing the striking parallels between R8 differentiation and ganglion cell development during mammalian retinal morphogenesis.

Positive selection of R8: proneural genes

Neural development in *Drosophila* involves a complex interplay among positively and negatively acting factors. One family of positively acting factors are the proneural genes, which encode basic helix-loop-helix proteins. These proneural genes are required for development of the *Drosophila* peripheral nervous system (reviewed by Bray, 2000). Two such proneural genes, *atonal* (*ato*), which is required for chordotonal organ development, and *daughterless* (*da*), which is required for many processes, are required for R8 selection (Jarman et al., 1993) (Table 1).

A semi-lethal *ato* allele that behaves as a genetic null (ato^{1}) results in complete failure of both R8 selection and all subsequent recruitment events. Mosaic analysis reveals an absolute cell-autonomous requirement for *ato* in R8, but no requirement in any other photoreceptor (Jarman et al., 1994). The expression pattern of both *ato* transcript and protein are consistent with this central role in R8 development. Prior to MF initiation, Ato is expressed at the posterior edge of the eye imaginal disc in a broad dorsoventral stripe of cells. As the MF progresses anteriorly, Ato expression continues in a broad stripe within and just anterior to the MF, and is resolved posteriorly into evenly spaced clusters of cells of decreasing number until it is expressed exclusively in the single cell per ommatidium that will become R8 (Fig. 1B) (Jarman et al., 1994; Jarman et al., 1995). This progressive resolution of Ato-expressing cells can be divided into four defined stages (Fig. 1C). Immediately after the broad dorsoventral expression of Ato (stage 1), clusters of approximately 10 cells, termed intermediate groups (stage 2), can be identified (Jarman et al., 1995). From within the intermediate group, the nuclei of two or three cells migrate apically in the imaginal disc to form the R8 equivalence group, a group of cells that are likely to be equipotent to become R8 (stage 3) (Fig. 1D) (Dokucu et al., 1996). One of these two to three cells continues to express Ato

Table 1. Selected genes involved in R8 development

Gene	Encoded protein	Selected references
Selection		
atonal (*ato*)	bHLH, nuclear	Jarman et al., 1994; Jarman et al., 1995
daughterless (*da*)	bHLH, nuclear	Brown et al., 1996
hairy (*h*)	bHLH, nuclear	Brown et al., 1995
extramacrochaete (*emc*)	HLH, nuclear	Brown et al., 1995
hedgehog (*hh*)	Novel, secreted	Dominguez, 1999; Heberlein et al., 1995
Spacing		
scabrous (*sca*)	Fibrinogen-like, secreted	Baker et al., 1990; Mlodzik et al., 1990
Notch (*N*)	Single-pass transmembrane receptor	Baker and Zitron, 1995; Cagan and Ready, 1989
Delta (*Dl*)	Transmembrane ligand	Baker and Zitron, 1995
Enhancer of split [*E*(*spl*)]	bHLH, nuclear	Ligoxygakis et al., 1998
Epidermal growth factor receptor (*EGFR*)	Transmembrane receptor	Dominguez et al., 1998; Freeman, 1996
Differentiation		
senseless (*sens*)	Zinc finger, nuclear	Frankfort et al., 2001; Nolo et al., 2000
rough (*ro*)	Homeodomain, nuclear	Dokucu et al., 1996; Tomlinson et al., 1988
spalt major (*salm*)	Zinc finger, nuclear	Mollereau et al., 2001
Organization		
spitz (*spi*)	EGF-like transmembrane, similar to TGF-α	Freeman, 1994; Tio et al., 1994; Tio and Moses, 1997
boss	Seven-pass transmembrane ligand	Hart et al., 1990; Reinke and Zipursky, 1988

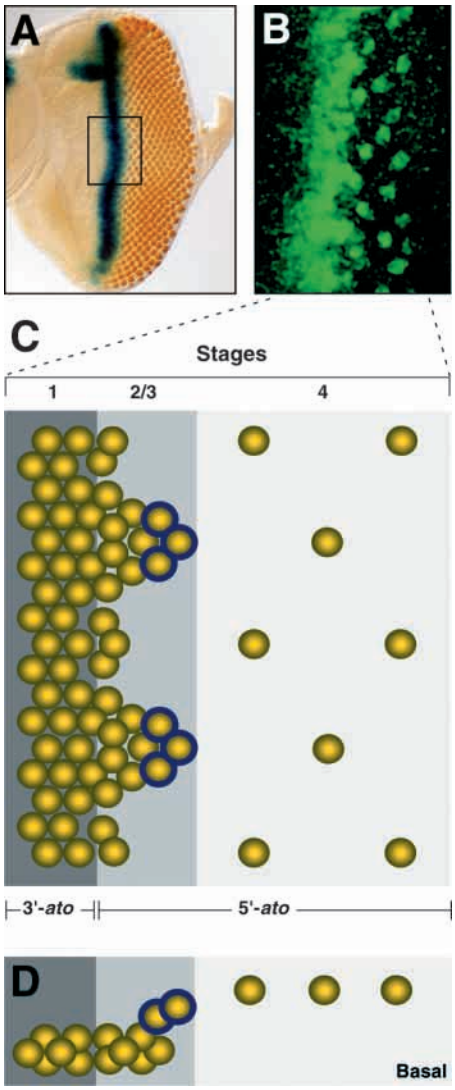

Fig. 1. Dynamic Atonal expression within the morphogenetic furrow. (A) The morphogenetic furrow (MF, blue vertical stripe) traverses the eye imaginal disc from posterior to anterior. Photoreceptors are recruited progressively – ommatidia close to the MF are immature and consist of fewer photoreceptors than do ommatidia located more posteriorly. The first photoreceptor to differentiate with passage of the MF is R8, which then coordinates recruitment of all subsequent photoreceptors. Anterior is towards the left in all panels. (B) Region of box in A. Atonal (Ato) is expressed in a dynamic pattern. Anterior Ato expression is ubiquitous, while posteriorly Ato is resolved to single cells that will become R8s. (C) Schematic of Ato expression. Ato expression occurs in four distinct stages. In this and subsequent figures, gray shading corresponds to the stage of Ato expression. Stage 1 (dark gray): Ato is expressed in a broad band of virtually all nuclei within and just anterior to the morphogenetic furrow. Stage 2 (medium gray): Ato is detected in alternating clusters (intermediate groups) of approximately 10 nuclei each. These intermediate groups are separated by a 'bridge' of three or four Ato-expressing cells. Stage 3 (medium gray): Two or three posterior nuclei of the intermediate group migrate apically to form the R8 equivalence group (blue outlines), a group of cells believed to be equipotent to differentiate as the R8 photoreceptor. Stage 4 (light gray): three columns of Ato-expressing nuclei are positioned exactly out of phase with one another to mark the future R8 cell and prefigure the adult hexagonal array. An enhancer located 3′ to the *ato*-coding region controls *ato* transcription during stage 1 and is not dependent on endogenous *ato* function, whereas a 5′ enhancer is autoregulatory and directs *ato* transcription during stages 2-4. (D) Lateral view of Ato expression. Ato-expressing nuclei are positioned basally during stages 1 and 2, but migrate toward the apical surface of the imaginal disc during stage 3 and remain apical throughout stage 4.

and ultimately becomes the R8 photoreceptor (stage 4). Ato expression in stage 1 is controlled by a downstream (3′) enhancer that does not require endogenous *ato* function, while all later Ato expression is governed by an upstream (5′) enhancer that does require endogenous *ato* activity (Sun et al., 1998). This implies a role for *ato* autoregulation in R8 selection. Indeed, the regulatory ato^2 allele probably partially disrupts the 5′ enhancer and causes the formation of R8 cells that are deficient in photoreceptor recruitment but nevertheless appear to be properly selected (White and Jarman, 2000). By contrast, in the more severe ato^1 allele, it is likely that the cells of the intermediate groups are never selected because Ato expression under the control of the 5′ enhancer requiring endogenous *ato* function does not occur. This results in failure of R8 selection and complete absence of photoreceptor differentiation. Taken together, these data suggest that early *ato* expression is required for the establishment of an initial field of cells that are capable of becoming R8 and that late, *ato*-dependent Ato expression is essential for R8 differentiation. Additional support for this idea comes from the observation that induction of *ato* under the control of a heat-shock promoter is sufficient to induce only one or two ectopic R8 cells per ommatidium (Dokucu et al., 1996). This implies that increased levels of *ato* expression may induce extra R8 cells only after the potential to do so has been established, which presumably occurs when Ato is restricted to the two to three cells of the R8 equivalence group. It is not known whether autoregulation of *ato* is direct or indirect, though there is some evidence to suggest an indirect mechanism of action mediated by another transcription factor, Senseless (Sens). There is also evidence that the Notch signaling pathway is required to establish high levels of Ato expression in stage 1 during a process known as 'proneural enhancement'.

The protein encoded by *da* is a requisite binding partner for Ato, as well as for other basic helix-loop-helix proteins (Cabrera and Alonso, 1991; Jarman et al., 1993; Murre et al., 1989; Van Doren et al., 1991). Clonal analysis of a null mutation in *da* reveals a phenotype that is similar to that of the ato^1 mutation – failure of all photoreceptor development. Moreover, there is an absolute cell-autonomous requirement for *da* in R8, as well as a partial requirement for *da* in R2 and R5 (Brown et al., 1996). Consistent with its predicted function, Da is expressed in a pattern that is very similar to that of Ato, beginning with a broad dorsoventral stripe within the MF. Posterior to the MF, Da is expressed in most cells at a low level, but in R8 at a high level. Da and Ato also appear to crossregulate each other. Only early Da expression is detected in ato^1 mutant eye discs, and *da* function is required for proper spatiotemporal expression of Ato (Brown et al., 1996). Moreover, analysis of loss-of-function clones of *da* and *ato* have similar effects on Ato expression – both lead to expansion of Ato to all cells of the clone in a manner similar to the initial

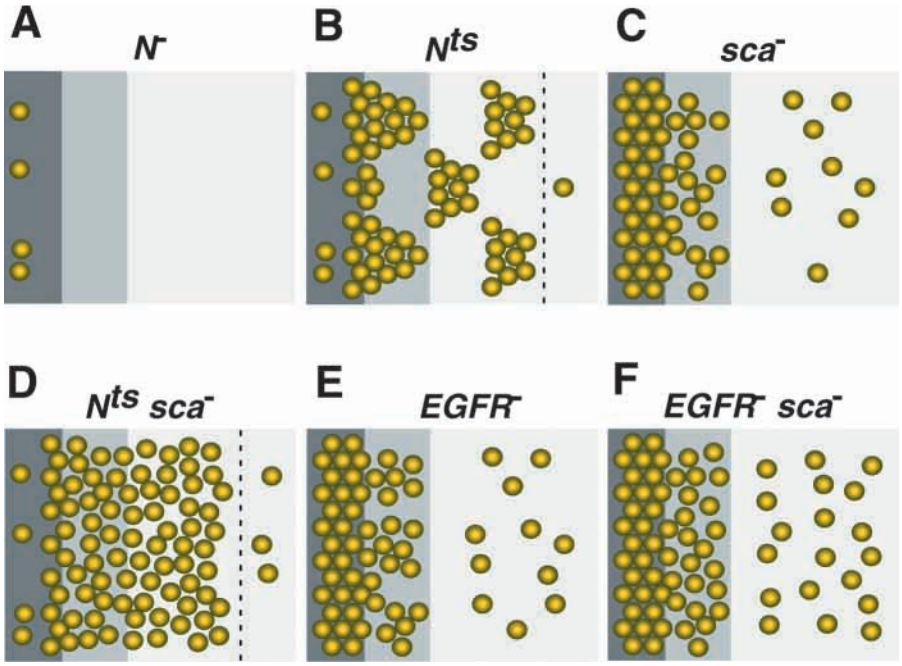

Fig. 2. Loss-of-function phenotypes affecting R8 spacing. (A) Loss-of-function *N* clones result in low levels of Ato expression in stage 1. This represents a failure of 'proneural enhancement.' No Ato expression is detected from stage 2-4. (B) Removal of *Notch* (*N*) function with a temperature-sensitive mutation results in failure of lateral inhibition. Stage 2 intermediate groups do not resolve and large clusters of well-spaced R8 precursors develop. Stage 1 Ato expression is also reduced at the restrictive temperature. Return to the permissive temperature restores lateral inhibition and single R8 precursors develop. (C) Loss of *scabrous* (*sca*) function prevents formation of stage 2 intermediate groups and nuclei at this stage are unpatterned. Notch-mediated inhibition of R8 occurs in stage 2, resulting in single R8 precursors in stage 4, but these cells are too closely spaced and phase relationships are lost. (D) Absence of both *N* and *sca* function results in massive overinduction of R8 precursors and nearly all nuclei express Ato during stage 2, owing to a failure of both intermediate group establishment and lateral inhibition. Stage 1 Ato expression is reduced at the restrictive temperature. Upon return to the permissive temperature, single R8 precursors develop, but they are unpatterned. (E) Loss-of-function *Epidermal Growth Factor Receptor* (*EGFR*) clones fail to form discrete intermediate groups and have additional Ato-expressing cells in stages 2 and 3. R8 precursors are too closely spaced and lack phase relationships. Note resemblance to *sca*$^{-}$ phenotype. (F) Removal of both *EGFR* and *sca* function leads to a more severe phenotype than either alone. Intermediate groups do not form in stage 2 and many unpatterned Ato-expressing R8 precursors develop in stage 4.

pattern of Ato expression (Chen and Chien, 1999). This finding suggests that while not required for initiation of Ato expression, both Ato and Da are required for proper autoregulation of late Ato expression. However, a detailed analysis of the mechanisms underlying Ato/Da crossregulation has not been reported.

It therefore appears that many components of the canonical proneural paradigm are present during R8 selection. First, a specific proneural gene (*ato*) controls the development of a specific organ or cell type (R8). Second, the expression of this proneural gene is highly regulated. Third, this essential proneural gene requires binding to Da for its proper function and regulation. However, many questions remain. For example, the details of *ato* autoregulation in the eye, including the factors that physically interact with the crucial *ato* enhancers are not known. Moreover, no obvious potential direct downstream targets of Ato and Da have been identified in the eye thus far. Finally, the mechanism by which putative eye-specific factors control the transcriptional environment in the developing eye to ensure that an R8 cell, and not a chordotonal organ, develops from an Ato-expressing cell are not known.

Negative selection of R8: *hairy*, *extramachrochaete*, *rough* and *hedgehog*

Two other genes that encode members of the helix-loop-helix class of proteins, *hairy* (*h*) and *extramacrochaete* (*emc*), are required for the timing of initiation of stage 1 Ato expression and act as repressors of proneural expression in other tissues and in vitro (Brown et al., 1995; Skeath and Carroll, 1991; Van Doren et al., 1991). Hairy and Emc are expressed in eye imaginal tissue anterior to both the MF and stage 1 Ato expression, and loss-of-function mutations in either gene have only subtle effects on R8 development. However, *emc*$^{-}$ *h*$^{-}$ double mutant clones show ectopic neural differentiation, accelerated MF progression and Ato expression, but no expanded Ato expression at any stage (Brown et al., 1995). Moreover, the spacing of ommatidial clusters in the double mutants is irregular, suggesting that later aspects of the regulation of Ato expression are also disrupted (Brown et al., 1995). Thus, *h* and *emc* appear to act in concert to negatively regulate both Ato expression and patterning. The details of this relationship in the developing eye are only vaguely understood, but it appears that Notch repression of H and Emc is required to induce high levels of Ato during stage 1 (Baonza and Freeman, 2001).

rough (*ro*) encodes a homeodomain-containing protein that helps to ensure that only one cell of each R8 equivalence group differentiates as R8, and loss-of-function mutations in *ro* often result in ommatidia with two or three R8 cells each (Dokucu et al., 1996; Heberlein et al., 1991). However, the mechanism by which this occurs is not known. Furthermore, loss of *ro* function results in an expansion of stage 1 Ato expression, and misexpression of *ro* is capable of both repressing Ato prior to final R8 selection and preventing photoreceptor differentiation (Dokucu et al., 1996; Kimmel et al., 1990). Thus, Ro acts as a repressor of both Ato expression and R8 selection. Consistent with this role, Ro is expressed in a pattern that is mutually exclusive with that of Ato. Moreover, Ro expression begins in the MF at about the same time Ato is expressed in the R8 equivalence group (stage 3), the precise time at which Ro is believed to exercise its function in R8 selection. Establishment of the mutually exclusive expression patterns of Ro and Ato

does not require Notch signaling (Dokucu et al., 1996). This observation suggests the existence of some unidentified intermediate that relays a signal from Ro-expressing cells to the R8 equivalence group in order to repress all but one of the Ato expressing cells from being selected as R8. There are currently no stellar candidates for this relay signal, but the results of a recent genetic screen for modifiers of a dominant *ro* mutant phenotype has already uncovered genes that regulate Ato and may yet unveil other missing links in this process (Chanut et al., 2000).

The Hedgehog signaling pathway also plays a role in the regulation of Ato expression (Borod and Heberlein, 1998; Dominguez, 1999; Dominguez and Hafen, 1997; Greenwood and Struhl, 1999; Heberlein et al., 1995). As Hedgehog signaling is required for early events involving the MF, it is not surprising that Hedgehog signaling is required for early expression of Ato and that ectopic Hedgehog signaling leads to increased Ato or precocious Ato expression in the eye disc (Borod and Heberlein, 1998; Dominguez, 1999; Greenwood and Struhl, 1999; Heberlein et al., 1995; Heberlein et al., 1993b; Ma et al., 1993). However, Hedgehog signaling also exerts a powerful repressive effect during later stages of Ato expression, and loss of Hedgehog signaling during stage 4 results in expansion of Ato expression to many cells, though at an expression level lower than endogenous Ato at this stage. These alternate effects are thought to result from a presumed Hh gradient – high levels of Hh at the posterior boundary of Ato expression inhibits Ato, whereas low levels further anteriorly induce Ato (Dominguez, 1999). It is possible that the inhibitory function of Hedgehog signaling is mediated by Ro; in addition to the increased Ato levels observed in loss-of-function clones of *smoothened* (*smo*), which prevent all Hedgehog signaling, levels of Ro in the MF are reduced. However, interior *smo* clones in adult eyes present relatively normal ommatidial organization (Dominguez, 1999; Dominguez and Hafen, 1997). As the Notch signaling target *Enhancer of split* [*E*(*spl*)] is expressed appropriately and Sca protein is detected in single cells within *smo* clones, it is possible that Notch-mediated selection of single R8 cells occurs despite the failure of Ato resolution to single cells (Dominguez, 1999). This potential uncoupling of Ato expression and R8 selection is striking and unexpected because Ato expression and R8 selection were thought to be inextricably linked. However, a possible explanation is suggested by other data involving the aforementioned ato^2 regulatory mutation. In these mutants with a compromised 5′ *ato* enhancer, single R8 cells are selected but have disrupted Ato expression, beginning with the stage 2 intermediate groups and lack all Ato expression by stage 4 (White and Jarman, 2000). Thus, Ato expression and R8 selection are also uncoupled in this mutant. Taken together, these data demonstrate that late Ato expression (stages 3 and 4) is necessary for normal R8 function but not sufficient for R8 selection.

R8 spacing: *Notch*, *scabrous* and *EGFR*

The factors controlling the perfect positioning and spacing of adult ommatidia, and in particular the developing R8 photoreceptors, have been of great interest for nearly two decades. Early research in the eye implicated members of the Notch signaling pathway and the *scabrous* (*sca*) locus in this

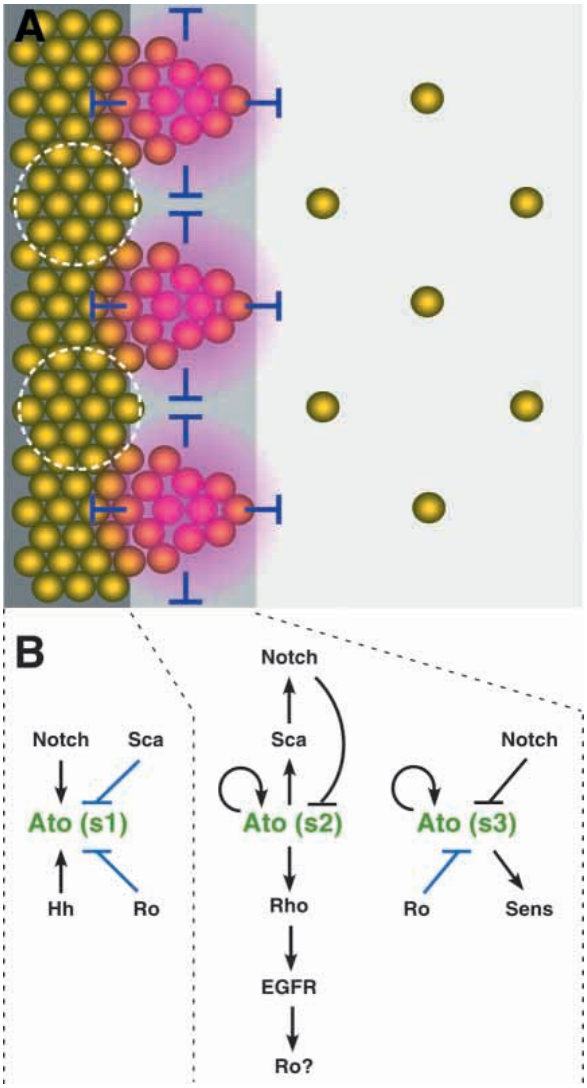

Fig. 3. R8 selection and spacing. (A) Model for spacing and phase of R8. At least two factors (Scabrous and an unknown EGFR-dependent factor) are secreted from the Atonal-expressing intermediate group to establish a repressive gradient that extends outward in all directions (purple shading). In this model, *ato* transcription immediately anterior (left) to the intermediate group is repressed (blue inhibitory arrows) even though pre-existing *ato* transcript and Ato protein are still detectable. The interdigitating areas of ubiquitous Ato (dotted circles) that lie outside the repressive gradient maintain *ato* transcription and will become the intermediate groups of the next column, thus establishing alternate phase and spacing.
(B) Regulation of Ato expression. Stage 1 Ato is controlled by the combined inputs of positively and negatively acting factors. Stage 2 Ato (intermediate groups) marks the onset of Ato autoregulation and induction of Scabrous (Sca), one of the secreted factors that establishes the repressive gradient required for R8 spacing. Sca also potentiates Notch pathway activity during lateral inhibition of stage 2 and 3 Ato. Stage 2 Ato simultaneously induces Rhomboid family members which lead to local EGFR signaling. EGFR signaling is then hypothesized to induce a second factor that contributes to the spacing gradient. This factor is thought to induce Rough (Ro) which represses *ato* transcription. In stage 3 (R8 equivalence group), Ato induces Sens and is repressed by the actions of both Ro and the Notch pathway. See text for further details. Modified from Baonza et al. (Baonza et al., 2001).

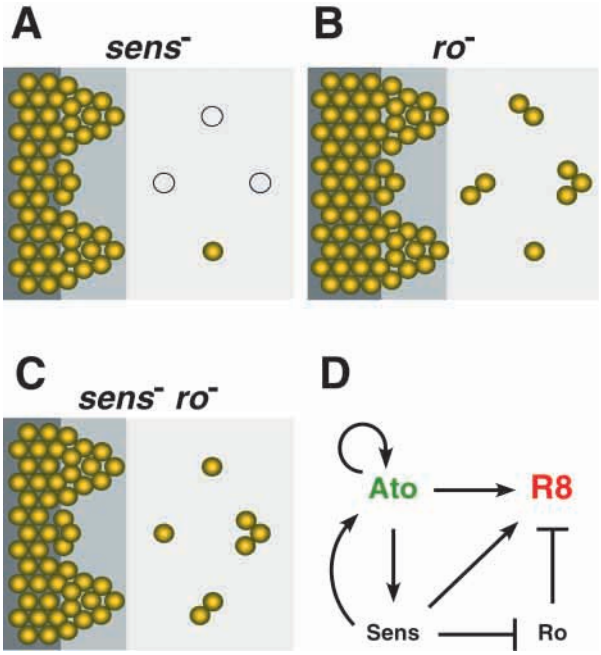

Fig. 4. Genetic control of R8 differentiation. (A) Loss-of-function *senseless* (*sens*) clones do not express Ato in stage 4 R8 precursors in most cases and these precursors display proper space and phase relationships. Expression of Ato in stage 1-3 is unaffected. (B) Loss of *rough* (*ro*) function prevents resolution of the stage 3 R8 equivalence group into single R8 precursors and two or three R8 precursors express Ato during stage 4 in many ommatidia. Phase relationships and spacing are not affected. Expression of Ato in stage 1 is expanded. (C) Removal of both *sens* and *ro* function results in a phenotype that is very similar to *ro*. (D) Relationships controlling R8 differentiation. *sens*-mediated repression of *ro*, a repressor of R8 differentiation, is crucial for R8 differentiation. See text for details.

process (Baker et al., 1990; Cagan and Ready, 1989; Dietrich and Campos-Ortega, 1984; Mlodzik et al., 1990). More recent studies reveal that the Notch (N) and Sca proteins physically interact, and that the relationships between the Notch pathway and Sca are highly specific, reflecting both temporal and spatial requirements (Baker et al., 1996; Baker and Yu, 1997; Baker and Yu, 1998; Baker and Zitron, 1995; Li and Baker, 2001; Ligoxygakis et al., 1998; Powell et al., 2001). Moreover, the timing of action of both the Notch pathway and Sca are linked with the progressive restriction of Ato expression within and posterior to the MF. Finally, the EGFR pathway, among its myriad roles in eye development, is also required for proper R8 spacing.

The Notch signaling pathway has been reviewed extensively (Baker, 2000; Greenwald, 1998). In brief, *Notch* (*N*) encodes a receptor that can be bound by two distinct membrane-bound ligands, Delta (Dl) and Serrate (Ser). Receptor/ligand interactions activate Notch signaling which leads to changes in activity or expression of downstream members of the pathway, including *Suppressor of Hairless* [*Su*(*H*)] and members of the *E*(*spl*) complex. Transduction of the Notch signal can lead to a number of outcomes. In some cases, cell fate determination and differentiation are delayed, often via the repression of proneural genes in during a process known as lateral inhibition. In other cases, inductive cues are generated (Baker and Yu, 1997; Fortini et al., 1993; Jennings et al., 1994). Examples of both types of effects are seen in the *Drosophila* eye.

The combined actions of the Notch pathway and Sca are required for establishing the dynamic Ato expression pattern and R8 spacing. Loss-of-function clones of either *N* or *Dl* display low initial levels of Ato expression during stage 1 that do not increase or resolve into later stages of expression, as well as a complete failure of R8 differentiation (Fig. 2A). Moreover, expression of either constitutively active *N* or *Dl* ligand induces high levels of Ato anterior to the MF (Baker and Yu, 1997; Baonza and Freeman, 2001). These findings suggest that Notch signaling is not required for initiation of Ato expression, but for achieving high levels of Ato expression or 'proneural enhancement.' Furthermore, as both proneural gene expression and differentiation are rescued non-autonomously near the borders of *Dl* clones, it seems that proneural enhancement is a specific function of cells in which Notch signaling is activated (Baker and Yu, 1997). Mechanistically, proneural enhancement requires alleviation of Su(H) repressor function by Notch signaling but does not rely on conversion of Su(H) to its active form or on E(spl) function (Li and Baker, 2001; Ligoxygakis et al., 1998). Moreover, recent data suggests that proneural enhancement requires Decapentaplegic (Dpp) signaling for full efficiency and that Notch signaling represses H and Emc proteins to derepress Ato expression (Baonza and Freeman, 2001; Greenwood and Struhl, 1999). Thus, both activation of Ato expression and alleviation of Ato repression appear to contribute to proneural enhancement.

As a lack of proneural enhancement results in the absence of all Ato expression beyond stage 1 and failure of R8 differentiation, and the timing of proneural enhancement coincides with the transfer of *ato* regulation from the *ato*-independent 3′ enhancer to the *ato*-dependent 5′ enhancer, it is possible that Notch signaling enables Ato levels to rise sufficiently for this transfer to occur. Beyond this point, however, the role of Notch signaling in R8 differentiation becomes very different, and the Notch pathway begins to cooperate with Sca to ensure that single R8 cells are selected and that they are evenly spaced in the emerging eye.

The expression of N and Dl are not mutually exclusive in the developing eye, and loss of either protein has similar effects on R8 selection and spacing (Baker and Yu, 1998). When *N* function is removed using a temperature sensitive allele (Fig. 2B), a neurogenic phenotype that leads to premature photoreceptor differentiation of most cells in the MF is observed (Cagan and Ready, 1989). Specifically, photoreceptors are present in evenly spaced clusters of approximately 10 cells expressing R8 markers, including Ato, while Dl^{ts} mutations result in slightly smaller clusters of cells (Baker et al., 1996; Baker and Zitron, 1995). Thus, when N signaling is removed, Ato stage 2 intermediate clusters fail to resolve into single R8 cells. This implies that N signaling represses Ato during stage 2 and is required for establishing ommatidia with only one R8 cell. This is an example of classic lateral inhibition. Consistent with this interpretation, the pattern of E(spl) expression is mutually exclusive with that of Ato beginning with stage 2 and misexpression of activated *N* is sufficient to repress Ato and induce E(spl) in intermediate groups (Baker et al., 1996; Dokucu et al., 1996). Furthermore, unlike the process of proneural enhancement, lateral inhibition

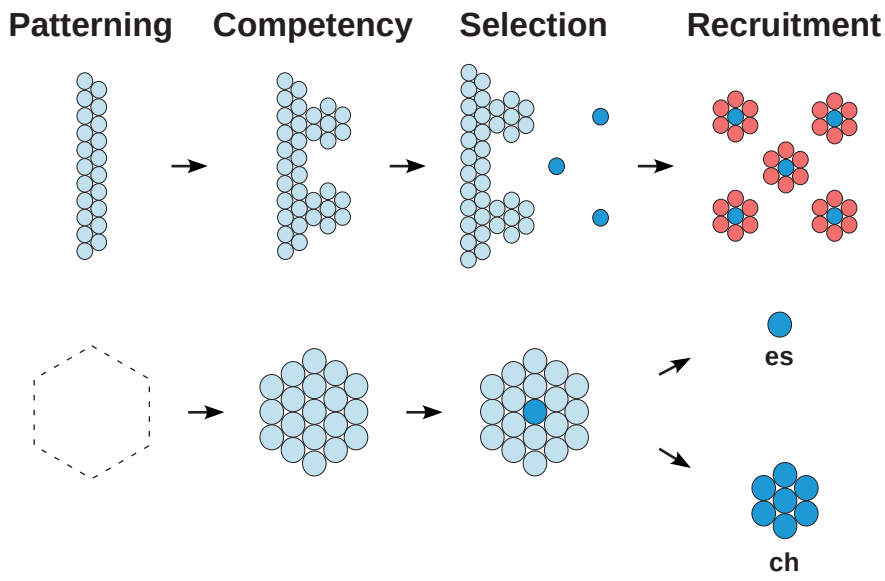

Fig. 5. Comparison between *Drosophila* R8 and SOP development. R8 and SOP development can be divided into four roughly analogous stages: patterning of neural fields, establishment of zones of neural competency (proneural clusters), selection of the presumptive R8/SOP and R8/SOP-mediated recruitment. R8 patterning begins with ubiquitous expression of Ato (light-blue circles) in all retinal cells. The R8 competency zone is determined when ubiquitous Ato is reshaped into clusters of about 10 cells (intermediate groups) via the action of endogenously activated factors. Lateral inhibition represses Ato in all members of the intermediate group except for the selected presumptive R8 (dark blue circles). The differentiating R8 cell recruits neighboring cells, which do not express Ato, to become non-R8 photoreceptors (red circles). These non-R8 photoreceptors have a different function, morphology and axonal projection from R8 cells. As eye development occurs progressively, all stages of R8 specification are visualized simultaneously. By contrast, the future region of an SOP is established by exogenous prepatterning genes (hexagon). All cells within this prepatterned region form a competency zone (proneural cluster) of 15-20 cells and express proneural genes (light-blue circles). Selection occurs when the future SOP expresses proneural genes at a high level and, by lateral inhibition, prevents this enhanced expression in the surrounding cells (dark-blue circles). SOP-mediated recruitment does not occur during external sensory organ (es) development but does occur during chordotonal organ (ch) development. However, the SOP of a chordotonal organ is only sufficient to recruit proneural-expressing cells from within the existing proneural cluster to become SOPs (dark-blue circles). These SOPs then produce equivalent chordotonal organs.

is mediated by 'traditional' N signaling molecules as loss-of-function clones of *Su(H)* and *E(spl)* both lead to neurogenic phenotypes (Li and Baker, 2001; Ligoxygakis et al., 1998).

The diametrically opposed effects of Notch signaling in R8 specification raise the following question: what mediates the abrupt transition in *Notch* function from promoting Ato elevation during proneural enhancement to repressing Ato during lateral inhibition? One explanation is that elevated levels of Ato following proneural enhancement cause a change in sensitivity to Notch signaling. Possible mechanisms for such a change include Ato autoregulation (see above) and induction of potential target genes such as *sens*, which interacts strongly with the Notch pathway in the developing wing (Nolo et al., 2000). Other explanations are certainly possible, and only future experiments can resolve this conundrum.

The *sca* locus encodes a fibrinogen-like secreted peptide that is expressed in a subset of Ato-expressing cells beginning in the stage 2 intermediate groups and is maintained in R8 at high levels until just after Ato expression in R8 ceases (Baker et al., 1990; Baker et al., 1996; Lee et al., 1996; Mlodzik et al., 1990). *sca* mutant eye discs contain more R8 cells than expected. Moreover, these R8 photoreceptors are located too close together and Ato intermediate groups do not form correctly (Fig. 2C) (Baker and Zitron, 1995; Lee et al., 1996). Strikingly, misexpression of high levels of Sca has a similar phenotype (Ellis et al., 1994). The similarity in gain- and loss-of-function phenotypes suggest that differential levels of Sca establish the R8 spacing pattern. In one model for *sca* function, secreted Sca protein diffuses away from its source in the intermediate groups to surrounding cells where it represses Ato anteriorly. This establishes both the phase and spacing of the developing eye field (Baker and Zitron, 1995; Baonza et al., 2001). The mechanism for this function of *sca* is not known, but it is likely to be independent of Notch signaling (as N^{ts} mutants show proper phase and spacing of R8) and require an unidentified receptor.

Analysis of developing eye tissue mutant for both *sca* and members of the Notch pathway firmly established their roles in R8 spacing. Tissue mutant for *sca* and either *N* or *Dl* have similar phenotypes: a worsening of either single mutant phenotype such that a nearly continuous field of R8 cells that lack any trace of clustering or organization is observed (Fig. 2D) (Baker and Zitron, 1995). These data, in conjunction with *sca* and N^{ts} phenotypes, suggest that Sca establishes the intermediate groups and that Notch signaling restricts the intermediate groups to single R8 cells. As *sca* and *N* genetically interact, and their encoded proteins bind one another, it is possible that *sca* provides a bias for Notch signaling in particular regions (Baker et al., 1990; Baker and Zitron, 1995; Ellis et al., 1994; Powell et al., 2001). Establishment of such a bias by Sca is distinct from its role in determining intermediate groups and would occur within the intermediate groups themselves to potentiate Notch activity and generate single R8 cells through lateral inhibition. Consistent with this model, removal of only the Notch pathway results in large, evenly spaced clusters of R8 cells in which the initial function of *sca* is preserved, whereas removal of Sca or increased levels of Sca causes a lack of intermediate groups and unbiased Notch activity which results in multiple, randomly spaced, single R8 cells (Baker and Zitron, 1995; Ellis et al., 1994).

The role of the EGFR in ommatidial spacing has long been debated, but despite earlier conclusions to the contrary, recent evidence suggests that EGFR signaling via the Ras pathway does participate in R8 spacing (Baonza et al., 2001; Kumar et al., 1998; Spencer et al., 1998; Yang and Baker, 2001). In fact,

clones of null mutations in *EGFR*, other members of the Ras signal transduction pathway, and members of the Rhomboid (Rho) family, which are required for EGFR ligand processing, all result in R8 cells that are spaced too close together (Fig. 2E) (Baonza et al., 2001; Wasserman et al., 2000; Yang and Baker, 2001). Indeed, in both *EGFR* and *rho1*, *rho3* double mutants, Ato expression is disrupted such that stage 1 expression is expanded and intermediate group formation is disrupted (Baonza et al., 2001; Wasserman et al., 2000).

Interestingly, *ato* function is absolutely required for activation of MAP kinase in the eye (Chen and Chien, 1999). This activation begins in a pattern that overlaps the stage 2 intermediate groups, and MAP kinase signaling then leads to repression of Ato non-autonomously (Chen and Chien, 1999). As loss- and gain-of-function data suggest that non-autonomous Ato repression via EGFR signaling is crucial in establishing intermediate groups (Lesokhin et al., 1999); it therefore appears that Ato induces a negative feedback loop to regulate both its own expression and R8 spacing via EGFR-mediated (and Sca-mediated) non-autonomous repression. The mechanism governing EGFR control over R8 spacing is not entirely clear, but does appear to rely on an EGFR ligand that is not Spitz (Spi). The identity of this ligand is unknown, but data suggest that it probably is not a known EGFR ligand, is almost certainly processed by Rho family members and may be encoded by the uncharacterized *spitz2* locus (Baonza et al., 2001; Kumar et al., 1998; Wasserman et al., 2000; Yang and Baker, 2001). As Rough expression is dependent on EGFR signaling and Rough plays a role in both R8 selection and the regulation of Ato, it has been hypothesized that MAP kinase activation in the intermediate groups leads to non-autonomous induction of Rough in the surrounding areas to repress Ato. In existing models, this non-autonomous induction of Rough would be accomplished by a proposed secreted factor that diffuses from the intermediate groups (Baonza et al., 2001; Dominguez et al., 1998).

Recently, it was reported that removal of both *EGFR* and *sca* function results in a more severe phenotype of multiple R8 cells than is seen in either mutant alone (Fig. 2F). Given the established roles of both EGFR signaling and Sca in intermediate group formation and R8 spacing, this result indicates that the EGFR pathway and Sca probably act independently, implying parallel pathways for establishing R8 spacing and phase. Thus, an inclusive model for R8 spacing that requires both Sca and EGFR signaling has been presented (Baonza et al., 2001; Dominguez et al., 1998). A key assumption of this model is that the self-organizing properties of the developing *Drosophila* eye have their roots in the intermediate groups, which produce an inhibitory gradient that represses *ato* transcription in all directions. New intermediate groups emerge outside of the repressive domain established by the preceding intermediate groups such that they are evenly spaced and out of phase (Fig. 3).

While a singular spacing model incorporating both *sca* and EGFR signaling is certainly enticing, it is possible that EGFR signaling works somewhat differently during R8 specification. For example, the disruption of intermediate groups when EGFR signaling is removed does not appear to be as profound as the effects of *sca* on this process. Moreover, 'twinning' of R8 cells is occasionally observed when EGFR signaling is removed, and is frequently observed when *ro*, a downstream target of EGFR signaling, is removed (Dominguez et al., 1998; Heberlein et al., 1991; Yang and Baker, 2001). Therefore, it is possible that EGFR signaling acts at multiple stages of R8 specification, and not only during intermediate group formation. Additional work is required to distinguish between these possibilities.

R8 differentiation: *senseless*, *rough* and *spalt*

It has long been assumed that the processes of R8 selection and differentiation were one, and that *ato* function controlled both processes. However, recent studies suggest that this is not the case, and that once the presumptive R8 is selected, the interactions of additional factors control the process of R8 differentiation. Foremost among these factors are two genes, *sens*, which encodes a zinc-finger transcription factor, and *ro* (Frankfort et al., 2001; Heberlein et al., 1991; Kimmel et al., 1990; Nolo et al., 2000).

sens is genetically downstream of *ato* and Sens expression specifically overlaps Ato beginning in the two to three cells of the R8 equivalence group (stage 3). Expression of Sens is then maintained solely in R8 throughout and beyond larval development. Mosaic analysis of null mutations in *sens* reveal a cell-autonomous requirement for *sens* in R8, and no requirement for *sens* function in any other photoreceptor. Indeed, loss of *sens* function leads to a complete failure of R8 differentiation and loss of Ato expression in stage 4 in most (75%) cases. Consistent with the position of *sens* downstream of *ato*, this failure of R8 differentiation occurs after proper selection and spacing of the presumptive R8 cell has occurred (Fig. 4A). Finally, s*ens* acts at or near the top of the cascade of R8 differentiation as misexpression of *sens* induces non-R8 photoreceptors to express R8-specific markers and to adopt specific adult morphologic characteristics of an R8 cell (Frankfort et al., 2001).

ro is expressed only in the eye imaginal disc and is a major negative regulator of R8 selection and spacing, the loss of which generates ommatidia containing two or three R8 cells (Fig. 4B) (Dokucu et al., 1996; Dominguez, 1999; Dominguez et al., 1998; Heberlein et al., 1991; Kimmel et al., 1990). After the initial broad expression of Ro in the MF, this pattern of expression is lost and Ro is later expressed in the emerging R2 and R5 photoreceptors and then in the R3 and R4 photoreceptors, remaining mutually exclusive with Ato and Sens at all stages (Dokucu et al., 1996; Frankfort et al., 2001; Kimmel et al., 1990). Furthermore, Ro is required in R2 and R5 for their proper function and cell-specific expression of *ro* can induce R7 to adopt the fate of an outer photoreceptor in a process that is dependent on *sevenless* activation. This implies that Ro functions after photoreceptor selection to induce a specific photoreceptor subtype identity (Tomlinson et al., 1988; Kimmel et al., 1990).

Thus, it appears that Sens and Ro have analogous functions during eye development: to control differentiation of a specific cell-type after selection has occurred. Moreover, a mutual antagonism between Sens and Ro is suggested by their non-overlapping patterns of expression, as well as by data demonstrating that the presumptive R8 adopts the R2/R5 fate and expresses Ro when *sens* function is removed. This is consistent with a model where Sens represses Ro in the developing R8 cell, and is supported by the observation that misexpression of *sens* is sufficient to repress Ro in non-R8 photoreceptors. Furthermore, simultaneous removal of both

sens and *ro* function results in the restoration of R8 differentiation in many ommatidia (Fig. 4C). Together, these results suggest that *sens*-mediated repression of Ro regulates the process of R8 differentiation (Fig. 4D) (Frankfort et al., 2001). This finding highlights that an important developmental mechanism, repression of a repressor of cell fate, is at work during a crucial stage of R8 development. A similar mechanism was recently identified during Notch-mediated repression of Hairy and Emc during proneural enhancement, and may be at the root of other aspects of R8 development.

It is important to note that EGFR signaling, though required for the differentiation of all non-R8 photoreceptors, is not required for R8 differentiation, but is required for both R8 cell survival and R8 spacing (Baonza et al., 2001; Kumar et al., 1998; Yang and Baker, 2001). However, as ascertained from both ectopic expression experiments and *EGFR* gain-of-function *Ellipse* mutations, hyperactive EGFR signaling can hinder or prevent R8 selection (Baker and Rubin, 1989; Baker and Rubin, 1992; Chen and Chien, 1999; Lesokhin et al., 1999). Furthermore, MAP kinase activation is highest in the intermediate group – the future source of R8. Thus, despite exposure to EGFR ligands, expression of EGFR protein, and probably activation of MAP kinase within its cytoplasm, the presumptive R8 somehow remains refractory to the effects of EGFR signaling. How is this accomplished? One published hypothesis is that autoregulatory Ato expression makes the future R8 cell refractory to EGFR signaling (Baonza et al., 2001). However, another plausible explanation is that Sens represses EGFR signaling in the developing R8 cell. This is suggested by the observations that in *sens* mutant tissue, MAP kinase activation in the intermediate group is not altered and that Ro is expressed in the presumptive R8 cell (Frankfort et al., 2001). As EGFR signaling is required for Ro expression, one interpretation is that EGFR signaling is effective in *sens* mutant presumptive R8 cells, which leads to Ro expression and assumption of the R2/R5 fate. Furthermore, *sens* mutant clones induced in animals heterozygous for the *Ellipse* mutation have a phenotype that is reminiscent of *Ellipse* homozygotes, possibly suggesting that Sens represses EGFR signaling (B. J. F. and G. M., unpublished).

Last, while R8 specification occurs during third instar larval development, terminal R8 photoreceptor differentiation occurs during pupal stages 3 to 4 days later. These late events, which include rhabdomere morphogenesis and opsin expression, are under the control of the *spalt* (*sal*) gene complex in both R8 and the other UV-sensitive photoreceptor, R7. Sal proteins are expressed specifically in the R8 and R7 photoreceptors beginning in late-pupal stages (Mollereau et al., 2001). How is the 3 day gap between R8 fate specification and highly specific Sal expression and terminal differentiation bridged? As Sens expression is maintained in R8 into adult life and misexpression of *sens* is sufficient to induce R8 rhabdomere morphology (B. J. F. and G. M., unpublished) (Frankfort et al., 2001), Sens is a good candidate for this role. Indeed, a genetic relationship between *sens* and the *sal* gene complex has been identified, but the details of this relationship are not understood at this time (B. Mollereau, personal communication).

Photoreceptor recruitment: role of R8 as a coordinator

The differentiation of all photoreceptors other than R8 is controlled in a manner that is strikingly different from that used for R8 differentiation. For non-R8 photoreceptors, activated EGFR signaling via the MAP kinase pathway is both necessary and sufficient for differentiation, and the timing of signaling is a crucial determinant of which cell fate is adopted (Freeman, 1996). However, the primary and initial source of secreted Spitz (Spi) protein, the activating ligand in the eye for the EGFR that is required for all non-R8 photoreceptor differentiation, is R8 (Freeman, 1994; Tio et al., 1994; Tio and Moses, 1997). Interestingly, this function of R8 is needed after R8 selection but before R8 differentiation, and does not require R8 differentiation to occur (Frankfort et al., 2001). In addition, R8 is the singular source of Boss protein in an ommatidium. Boss is the ligand for the Sevenless (Sev) tyrosine kinase receptor, and contact between Boss and Sev is required to induce R7 differentiation. Thus, the R8 photoreceptor serves as a kind of recruiting center for each ommatidium.

The function of R8 in the recruitment of R7 has been reviewed extensively and will not be presented in detail here (Cagan, 1993; Raabe, 2000; Zipursky and Rubin, 1994). In short, *boss* encodes a membrane-associated protein with a large extracellular domain that is produced exclusively in the R8 photoreceptor and is absolutely required for R7 development (Hart et al., 1990; Reinke and Zipursky, 1988). Boss is presented on the apical surface of R8 such that it is able to bind to the Sev receptor tyrosine kinase (RTK), which is also required for R7 development (Hafen et al., 1987; Kramer et al., 1991; Tomlinson and Ready, 1986). This interaction leads to internalization of the Boss protein in the presumptive R7, phosphorylation of the Sev RTK, induction of MAP kinase signaling and ultimately R7 differentiation (Fortini et al., 1992; Hart et al., 1993; Kramer et al., 1991; Simon et al., 1991). The position of R8 and precise localization of Boss to the apical cell surface are crucial in restricting the inductive cue to only the presumptive R7 cell and represent a key regulatory role in R8 organizer function (Van Vactor et al., 1991). Finally, MAP kinase signaling via Spi/EGFR interactions is concomitantly required in R7 for proper differentiation, and combinatorial mechanisms of these two signals have been proposed (Freeman, 1996; Tio and Moses, 1997).

The activating ligand for EGFR that is required for photoreceptor recruitment is Spi (Freeman, 1997; Freeman, 1996; Freeman, 1994; Lesokhin et al., 1999; Spencer et al., 1998; Tio et al., 1994; Tio and Moses, 1997; Yang and Baker, 2001). Moreover, the initial source of this ligand is R8, and there is a cell-autonomous requirement in R8 for *spi* function in normal ommatidial formation (Freeman, 1994; Tio et al., 1994). However, R8 is not the only source of Spi, and the next two sets of photoreceptors to be recruited, R2/R5 and R3/R4, have partial requirements for *spi* function as well (Freeman, 1994; Tio et al., 1994). The *spi* gene is expressed in photoreceptors other than R8, R2/R5 and R3/R4, but proper processing by both Star (S) and Rhomboid family members is thought to occur only in at most the first five photoreceptors (Heberlein et al., 1993a; Heberlein and Rubin, 1991; Hsiung et al., 2001; Kolodkin et al., 1994; Pickup and Banerjee, 1999; Wasserman et al., 2000). This process of expression and secretion of Spi from the R8 photoreceptor begins before overt differentiation. Specifically, *sens* mutations that allow R8 selection but not R8 differentiation show successful activation of the EGFR pathway (Frankfort et al., 2001). Moreover, the

same study revealed that R8 differentiation is not required for photoreceptor recruitment, and that differentiation of the presumptive R8 cell as an R2 or R5 photoreceptor results in a founder cell that is nonetheless sufficient to recruit outer photoreceptors of all subtypes (Frankfort et al., 2001). Thus, all that is required to begin photoreceptor recruitment and differentiation is an initial source of Spi from a founding photoreceptor that need not be R8.

This conclusion helps to explain a puzzling result – that ectopic expression of the proneural gene *scute* (*sc*) in an *ato* mutant background is capable of inducing ommatidia that typically lack a discernable R8 photoreceptor (Sun et al., 2000). One explanation for this result is that Sc replaces currently undiscovered basic helix-loop-helix proteins which direct photoreceptor differentiation and that are normally induced by Ato, thereby bypassing the requirement for Ato (Sun et al., 2000). Another plausible interpretation is that Sc is capable of 'priming' the eye disc so that it is possible for the selection to occur, much like the presumed earliest function of Ato. Then, selection of non-R8 founding photoreceptors occurs and some recruitment of other photoreceptors follows. Future experimentation is required to distinguish these two models.

A paradigm for neural selection and differentiation

A fantastic interplay among transcription factors and major signaling pathways used in many other genetic systems is required to coordinate the patterning of an undifferentiated eye into a field of evenly spaced, single R8 cells. The involvement of such developmentally conserved factors alone is sufficient to make R8 development a useful paradigm for neural selection and differentiation. Moreover, the facility of *Drosophila* R8 development as a model paradigm is highlighted by its unique nature and by its direct implications on mammalian retinal development.

Comparison of R8 development to another well-studied *Drosophila* neural paradigm, sensory organ precursor (SOP) specification, reveals both similarities and differences. As each presumptive R8 cell becomes a single photoreceptor, each SOP gives rise to a single sensory organ, either of the external or chordotonal class. Moreover, the overall mechanism of each is the selection of a single neural precursor from among a field of equipotent undifferentiated cells. Both rely on proneural genes as the key protagonists to establish a zone of neural competency (proneural clusters for SOPs, intermediate groups for R8) from which a single proneural-expressing cell is selected. Finally, both require proneural self-stimulation, repression by other HLH proteins (Hairy and Emc), and Notch-mediated lateral inhibition to achieve this selection (Cubas et al., 1991; Culi and Modolell, 1998; Jarman et al., 1993; Skeath and Carroll, 1991; Van Doren et al., 1991). However, there are many differences between R8 and SOP selection (Fig. 5).

One major difference is the mechanism of establishment of the zone of neural competency. SOP proneural clusters are positioned according to exogenous cues from prepattern genes that act along the major axes of the tissue. During R8 specification, ubiquitous proneural gene expression in the eye imaginal disc is reshaped into intermediate groups in a process that is achieved via the interaction of endogenously activated factors. Indeed, the self-organizing nature inherent to R8 spacing is perhaps the single greatest difference between R8 and SOP specification. Along the same lines, the progressive nature of R8 specification allows for visualization of multiple stages of development simultaneously. This is not possible during SOP development. A third distinction is in the later function of SOPs and R8. Each SOP give rise to a clonally derived sensory organ, and only in chordotonal organ generation is there any inductive signaling from an SOP to neighboring cells, and this occurs on a limited scale to recruit additional SOPs from an already established proneural cluster (zur Lage and Jarman, 1999). *Drosophila* ommatidia are not clonally derived and instead rely heavily on R8 to initiate and orchestrate signaling to neighboring cells to induce photoreceptor fate assumption. Interestingly, chordotonal organ SOPs express Ato, suggesting that the recruitment function of R8 and chordotonal organ SOPs may be specific to Ato-expressing cells. Moreover, induced photoreceptors are no longer part of the intermediate group at the time they differentiate. Thus, R8 has a distinct role as a coordinator. The breadth of R8 development is another distinguishing feature. Because approximately 750 R8 cells differentiate in a wild-type *Drosophila* eye, this vast neuronal field facilitates the identification of extremely subtle phenotypes. The smaller scale of SOP development is not conducive to such analysis. Finally, there are differences in gene function between the two systems. One example is the interaction between Notch and EGFR signaling in the two systems. In SOPs, multiple lines of evidence suggest antagonism between the two pathways (Culi et al., 2001; zur Lage and Jarman, 1999). In R8 specification, Notch signaling both enhances and represses proneural gene expression while EGFR signaling represses proneural gene expression during R8 spacing and is not required for R8 differentiation. Thus, no clear antagonism has been demonstrated, and the pathways probably work in parallel.

The process of R8 specification also has a direct parallel in the mammalian retina: retinal ganglion cell (RGC) development. Evidence for this parallel originally was correlative. For example, analogous to R8 in the *Drosophila* retina, RGCs are the first cells to be 'born' in the mammalian retina. Moreover, RGCs are selected from among a group of competent progenitor cells that comprise much of the early embryonic mammalian retina, and their differentiation is regulated by Notch (Austin et al., 1995). Furthermore, RGCs develop as a patterned, spaced array at the leading edge of a wave that spreads from the central to peripheral retina during a process reminiscent of MF progression in *Drosophila* (McCabe et al., 1999). In addition, despite the fact they are not photoreceptors, RGCs project axons directly to the brain, much as R8 cells do. Recently, a genetic basis for the relationship between R8 and RGCs was established. Two groups reported that *Math5*, a murine ortholog of *ato*, is expressed in RGCs and their progenitors coincident with the onset of RGC differentiation, and that *Math5* function is required for proper RGC differentiation (Brown et al., 2001; Wang et al., 2001). These results imply that *Math5* is a proneural gene required for RGC differentiation. Therefore, despite seemingly dissimilar function, R8s and RGCs appear to have much in common with regard to axonal projection, timing of differentiation, and genetic control of cell fate (Kumar, 2001).

These distinctions make R8 development in *Drosophila* a unique and relevant paradigm for the study of neural specification. In particular, its self-organizing nature provides an opportunity for the study of relationships that may not be

accessible in other systems. Furthermore, despite a common goal of neural differentiation, R8 development and SOP development are very distinct, and comparisons of gene function between the two has already identified a number of example where genetic relationships are changed between the two systems. Finally, as a system with a direct mammalian correlate in RGC differentiation, it follows that the study of R8 specification in *Drosophila* will probably yield important insights to the more complicated process of mammalian eye development.

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REFERENCES

Austin, C. P., Feldman, D. E., Ida, J. A., Jr and Cepko, C. L. (1995). Vertebrate retinal ganglion cells are selected from competent progenitors by the action of Notch. *Development* **121**, 3637-3650.

Baker, N. E. (2000). Notch signaling in the nervous system. Pieces still missing from the puzzle. *BioEssays* **22**, 264-273.

Baker, N. E. and Rubin, G. M. (1989). Effect on eye development of dominant mutations in *Drosophila* homologue of the EGF receptor. *Nature* **340**, 150-153.

Baker, N. E. and Rubin, G. M. (1992). *Ellipse* mutations in the *Drosophila* homologue of the EGF receptor affect pattern formation, cell division, and cell death in eye imaginal discs. *Dev. Biol.* **150**, 381-396.

Baker, N. E. and Yu, S. Y. (1997). Proneural function of neurogenic genes in the developing *Drosophila* eye. *Curr. Biol.* **7**, 122-132.

Baker, N. E. and Zitron, A. E. (1995). *Drosophila* eye development: *Notch* and *Delta* amplify a neurogenic pattern conferred on the morphogenetic furrow by *scabrous*. *Mech. Dev.* **49**, 173-189.

Baker, N. E. and Yu, S. Y. (1998). The R8-photoreceptor equivalence group in *Drosophila*: fate choice precedes regulated *Delta* transcription and is independent of *Notch* gene dose. *Mech. Dev.* **74**, 3-14.

Baker, N. E., Mlodzik, M. and Rubin, G. M. (1990). Spacing differentiation in the developing *Drosophila* eye: a fibrinogen-related lateral inhibitor encoded by *scabrous*. *Science* **250**, 1370-1377.

Baker, N. E., Yu, S. and Han, D. (1996). Evolution of proneural *atonal* expression during distinct regulatory phases in the developing *Drosophila* eye. *Curr. Biol.* **6**, 1290-1301.

Baonza, A. and Freeman, M. (2001). Notch signalling and the initiation of neural development in the Drosophila eye. *Development* **128**, 3889-3898.

Baonza, A., Casci, T. and Freeman, M. (2001). A primary role for the epidermal growth factor receptor in ommatidial spacing in the *Drosophila* eye. *Curr. Biol.* **11**, 396-404.

Borod, E. R. and Heberlein, U. (1998). Mutual regulation of *decapentaplegic* and *hedgehog* during the initiation of differentiation in the *Drosophila* retina. *Dev. Biol.* **197**, 187-197.

Bray, S. (2000). Specificity and promiscuity among proneural proteins. *Neuron* **25**, 1-2.

Brown, N. L., Paddock, S. W., Sattler, C. A., Cronmiller, C., Thomas, B. J. and Carroll, S. B. (1996). Daughterless is required for *Drosophila* photoreceptor cell determination, eye morphogenesis, and cell cycle progression. *Dev. Biol.* **179**, 65-78.

Brown, N. L., Sattler, C. A., Paddock, S. W. and Carroll, S. B. (1995). *Hairy* and *emc* negatively regulate morphogenetic furrow progression in the *Drosophila* eye. *Cell* **80**, 879-887.

Brown, N. L., Patel, S., Brzezinski, J. and Glaser, T. (2001). Math5 is required for retinal ganglion cell and optic nerve formation. *Development* **128**, 2497-2508.

Cabrera, C. V. and Alonso, M. C. (1991). Transcriptional activation by heterodimers of the *achaete-scute* and *daughterless* gene products of *Drosophila*. *EMBO J.* **10**, 2965-2973.

Cagan, R. (1993). Cell fate specification in the developing *Drosophila* retina. *Development Supplement* 19-28.

Cagan, R. L. and Ready, D. F. (1989). *Notch* is required for successive cell decisions in the developing *Drosophila* retina. *Genes Dev.* **3**, 1099-1112.

Chanut, F., Luk, A. and Heberlein, U. (2000). A screen for dominant modifiers of *ro(Dom)*, a mutation that disrupts morphogenetic furrow progression in *Drosophila*, identifies *groucho* and *hairless* as regulators of *atonal* expression. *Genetics* **156**, 1203-1217.

Chen, C. K. and Chien, C. T. (1999). Negative regulation of *atonal* in proneural cluster formation of *Drosophila* R8 photoreceptors. *Proc. Natl. Acad. Sci. USA* **96**, 5055-5060.

Cubas, P., de Celis, J. F., Campuzano, S. and Modolell, J. (1991). Proneural clusters of *achaete-scute* expression and the generation of sensory organs in the *Drosophila* imaginal wing disc. *Genes Dev.* **5**, 996-1008.

Culi, J. and Modolell, J. (1998). Proneural gene self-stimulation in neural precursors: an essential mechanism for sense organ development that is regulated by Notch signaling. *Genes Dev.* **12**, 2036-2047.

Culi, J., Martin-Blanco, E. and Modolell, J. (2001). The EGF receptor and N signalling pathways act antagonistically in *Drosophila* mesothorax bristle patterning. *Development* **128**, 299-308.

Dietrich, U. and Campos-Ortega, J. A. (1984). The expression of neurogenic loci in imaginal epidermal cells of *Drosophila melanogaster*. *J. Neurogenet.* **1**, 315-332.

Dokucu, M. E., Zipursky, S. L. and Cagan, R. L. (1996). *atonal*, *rough* and the resolution of proneural clusters in the developing *Drosophila* retina. *Development* **122**, 4139-4147.

Dominguez, M. (1999). Dual role for *hedgehog* in the regulation of the proneural gene *atonal* during ommatidia development. *Development* **126**, 2345-2353.

Dominguez, M. and Hafen, E. (1997). Hedgehog directly controls initiation and propagation of retinal differentiation in the *Drosophila* eye. *Genes Dev.* **11**, 3254-3264.

Dominguez, M., Wasserman, J. D. and Freeman, M. (1998). Multiple functions of the EGF receptor in *Drosophila* eye development. *Curr. Biol.* **8**, 1039-1048.

Ellis, M. C., Weber, U., Wiersdorff, V. and Mlodzik, M. (1994). Confrontation of *scabrous* expressing and non-expressing cells is essential for normal ommatidial spacing in the *Drosophila* eye. *Development* **120**, 1959-1969.

Fortini, M. E., Simon, M. A. and Rubin, G. M. (1992). Signalling by the Sevenless protein tyrosine kinase is mimicked by Ras1 activation. *Nature* **355**, 559-561.

Fortini, M. E., Rebay, I., Caron, L. A. and Artavanis-Tsakonas, S. (1993). An activated Notch receptor blocks cell-fate commitment in the developing *Drosophila* eye. *Nature* **365**, 555-557.

Frankfort, B. J., Nolo, R., Zhang, Z., Bellen, H. J. and Mardon, G. (2001). *senseless* repression of *rough* is required for normal R8 photoreceptor differentiation in the developing *Drosophila* eye. *Neuron* **32**, 403-414.

Freeman, M. (1994). The *spitz* gene is required for photoreceptor determination in the *Drosophila* eye where it interacts with the EGF receptor. *Mech. Dev.* **48**, 25-33.

Freeman, M. (1996). Reiterative use of the EGF receptor triggers differentiation of all cell types in the *Drosophila* eye. *Cell* **87**, 651-660.

Freeman, M. (1997). Cell determination strategies in the *Drosophila* eye. *Development* **124**, 261-270.

Greenwald, I. (1998). LIN-12/Notch signaling: lessons from worms and flies. *Genes Dev.* **12**, 1751-1762.

Greenwood, S. and Struhl, G. (1999). Progression of the morphogenetic furrow in the *Drosophila* eye: the roles of Hedgehog, Decapentaplegic and the Raf pathway. *Development* **126**, 5795-5808.

Hafen, E., Basler, K., Edstroem, J. E. and Rubin, G. M. (1987). *sevenless*, a cell-specific homeotic gene of *Drosophila*, encodes a putative transmembrane receptor with a tyrosine kinase domain. *Science* **236**, 55-63.

Hart, A. C., Kramer, H., Van Vactor, D. L., Jr., Paidhungat, M. and Zipursky, S. L. (1990). Induction of cell fate in the *Drosophila* retina: the Bride of sevenless protein is predicted to contain a large extracellular domain and seven transmembrane segments. *Genes Dev.* **4**, 1835-1847.

Hart, A. C., Kramer, H. and Zipursky, S. L. (1993). Extracellular domain of the Boss transmembrane ligand acts as an antagonist of the Sev receptor. *Nature* **361**, 732-736.

Heberlein, U. and Rubin, G. M. (1991). *Star* is required in a subset of photoreceptor cells in the developing *Drosophila* retina and displays dosage sensitive interactions with *rough*. *Dev. Biol.* **144**, 353-361.

Heberlein, U., Mlodzik, M. and Rubin, G. M. (1991). Cell-fate determination in the developing *Drosophila* eye: role of the *rough* gene. *Development* **112**, 703-712.

Heberlein, U., Hariharan, I. K. and Rubin, G. M. (1993a). *Star* is required for neuronal differentiation in the *Drosophila* retina and displays dosage-sensitive interactions with *Ras1*. *Dev. Biol.* **160**, 51-63.

Heberlein, U., Wolff, T. and Rubin, G. M. (1993b). The TGF beta homolog *dpp* and the segment polarity gene *hedgehog* are required for propagation of a morphogenetic wave in the *Drosophila* retina. *Cell* **75**, 913-926.

Heberlein, U., Singh, C. M., Luk, A. Y. and Donohoe, T. J. (1995). Growth and differentiation in the *Drosophila* eye coordinated by *hedgehog*. *Nature* **373**, 709-711.

Hsiung, F., Griffis, E. R., Pickup, A., Powers, M. A. and Moses, K. (2001). Function of the *Drosophila* TGF-alpha homolog Spitz is controlled by Star and interacts directly with Star. *Mech. Dev.* **107**, 13-23.

Jarman, A. P., Grau, Y., Jan, L. Y. and Jan, Y. N. (1993). *atonal* is a proneural gene that directs chordotonal organ formation in the *Drosophila* peripheral nervous system. *Cell* **73**, 1307-1321.

Jarman, A. P., Grell, E. H., Ackerman, L., Jan, L. Y. and Jan, Y. N. (1994). *atonal* is the proneural gene for *Drosophila* photoreceptors. *Nature* **369**, 398-400.

Jarman, A. P., Sun, Y., Jan, L. Y. and Jan, Y. N. (1995). Role of the proneural gene, *atonal*, in formation of *Drosophila* chordotonal organs and photoreceptors. *Development* **121**, 2019-2030.

Jennings, B., Preiss, A., Delidakis, C. and Bray, S. (1994). The Notch signalling pathway is required for Enhancer of split bHLH protein expression during neurogenesis in the *Drosophila* embryo. *Development* **120**, 3537-3548.

Kimmel, B. E., Heberlein, U. and Rubin, G. M. (1990). The homeo domain protein Rough is expressed in a subset of cells in the developing *Drosophila* eye where it can specify photoreceptor cell subtype. *Genes Dev.* **4**, 712-727.

Kolodkin, A. L., Pickup, A. T., Lin, D. M., Goodman, C. S. and Banerjee, U. (1994). Characterization of *Star* and its interactions with *sevenless* and EGF receptor during photoreceptor cell development in *Drosophila*. *Development* **120**, 1731-1745.

Kramer, H., Cagan, R. L. and Zipursky, S. L. (1991). Interaction of Bride of sevenless membrane-bound ligand and the Sevenless tyrosine-kinase receptor. *Nature* **352**, 207-212.

Kumar, J. P. (2001). Signalling pathways in Drosophila and vertebrate retinal development. *Nat. Rev. Genet.* **2**, 846-857.

Kumar, J. P., Tio, M., Hsiung, F., Akopyan, S., Gabay, L., Seger, R., Shilo, B. Z. and Moses, K. (1998). Dissecting the roles of the *Drosophila* EGF receptor in eye development and MAP kinase activation. *Development* **125**, 3875-3885.

Lawrence, P. A. and Green, S. M. (1979). Cell lineage in the developing retina of *Drosophila*. *Dev. Biol.* **71**, 142-152.

Lee, E. C., Hu, X. X., Yu, S. Y. and Baker, N. E. (1996). The *scabrous* gene encodes a secreted glycoprotein dimer and regulates proneural development in *Drosophila* eyes. *Mol. Cell. Biol.* **16**, 1179-1188.

Lesokhin, A. M., Yu, S. Y., Katz, J. and Baker, N. E. (1999). Several levels of EGF receptor signaling during photoreceptor specification in wild-type, *Ellipse*, and null mutant *Drosophila*. *Dev. Biol.* **205**, 129-144.

Li, Y. and Baker, N. E. (2001). Proneural enhancement by Notch overcomes Suppressor-of-Hairless repressor function in the developing *Drosophila* eye. *Curr. Biol.* **11**, 330-338.

Ligoxygakis, P., Yu, S. Y., Delidakis, C. and Baker, N. E. (1998). A subset of Notch functions during *Drosophila* eye development require Su(H) and the E(spl) gene complex. *Development* **125**, 2893-2900.

Ma, C., Zhou, Y., Beachy, P. A. and Moses, K. (1993). The segment polarity gene *hedgehog* is required for progression of the morphogenetic furrow in the developing *Drosophila* eye. *Cell* **75**, 927-938.

McCabe, K. L., Gunther, E. C. and Reh, T. A. (1999). The development of the pattern of retinal ganglion cells in the chick retina: mechanisms that control differentiation. *Development* **126**, 5713-5724.

Mlodzik, M., Baker, N. E. and Rubin, G. M. (1990). Isolation and expression of *scabrous*, a gene regulating neurogenesis in *Drosophila*. *Genes Dev.* **4**, 1848-1861.

Mollereau, B., Dominguez, M., Webel, R., Colley, N. J., Keung, B., de Celis, J. F. and Desplan, C. (2001). Two-step process for photoreceptor formation in *Drosophila*. *Nature* **412**, 911-913.

Murre, C., McCaw, P. S., Vaessin, H., Caudy, M., Jan, L. Y., Jan, Y. N., Cabrera, C. V., Buskin, J. N., Hauschka, S. D., Lassar, A. B. et al. (1989). Interactions between heterologous helix-loop-helix proteins generate complexes that bind specifically to a common DNA sequence. *Cell* **58**, 537-544.

Nolo, R., Abbott, L. A. and Bellen, H. J. (2000). Senseless, a Zn finger transcription factor, is necessary and sufficient for sensory organ development in *Drosophila*. *Cell* **102**, 349-362.

Pickup, A. T. and Banerjee, U. (1999). The role of *star* in the production of an activated ligand for the EGF receptor signaling pathway. *Dev. Biol.* **205**, 254-259.

Powell, P. A., Wesley, C., Spencer, S. and Cagan, R. L. (2001). Scabrous complexes with Notch to mediate boundary formation. *Nature* **409**, 626-630.

Raabe, T. (2000). The sevenless signaling pathway: variations of a common theme. *Biochim. Biophys. Acta* **1496**, 151-163.

Ready, D. F., Hanson, T. E. and Benzer, S. (1976). Development of the *Drosophila* retina, a neurocrystalline lattice. *Dev. Biol.* **53**, 217-240.

Reinke, R. and Zipursky, S. L. (1988). Cell-cell interaction in the *Drosophila* retina: the *bride of sevenless* gene is required in photoreceptor cell R8 for R7 cell development. *Cell* **55**, 321-330.

Simon, M. A., Bowtell, D. D. L., Dodson, G. S., Laverty, T. R. and Rubin, G. M. (1991). Ras1 and a putative guanine nucleotide exchange factor perform crucial steps in signaling by the Sevenless protein tyrosine kinase. *Cell* **67**, 701-716.

Skeath, J. B. and Carroll, S. B. (1991). Regulation of *achaete-scute* gene expression and sensory organ pattern formation in the *Drosophila* wing. *Genes Dev.* **5**, 984-995.

Spencer, S. A., Powell, P. A., Miller, D. T. and Cagan, R. L. (1998). Regulation of EGF receptor signaling establishes pattern across the developing *Drosophila* retina. *Development* **125**, 4777-4790.

Sun, Y., Jan, L. Y. and Jan, Y. N. (1998). Transcriptional regulation of *atonal* during development of the *Drosophila* peripheral nervous system. *Development* **125**, 3731-3740.

Sun, Y., Jan, L. Y. and Jan, Y. N. (2000). Ectopic Scute induces *Drosophila* ommatidia development without R8 founder photoreceptors. *Proc. Natl. Acad. Sci. USA* **97**, 6815-6819.

Tio, M. and Moses, K. (1997). The *Drosophila* TGF-alpha homolog *spitz* acts in photoreceptor recruitment in the developing retina. *Development* **124**, 343-351.

Tio, M., Ma, C. and Moses, K. (1994). *spitz*, a *Drosophila* homolog of transforming growth factor-alpha, is required in the founding photoreceptor cells of the compound eye facets. *Mech. Dev.* **48**, 13-23.

Tomlinson, A. and Ready, D. F. (1986). Sevenless: A cell specific homeotic mutation of the *Drosophila* eye. *Science* **231**, 400-402.

Tomlinson, A. and Ready, D. F. (1987). Neuronal differentiation in the *Drosophila* ommatidium. *Dev. Biol.* **120**, 366-376.

Tomlinson, A., Kimmel, B. E. and Rubin, G. M. (1988). *rough*, a Drosophila homeobox gene required in photoreceptors R2 and R5 for inductive interactions in the developing eye. *Cell* **55**, 771-784.

Van Doren, M., Ellis, H. M. and Posakony, J. W. (1991). The *Drosophila* Extramacrochaetae protein antagonizes sequence-specific DNA binding by Daughterless/Achaete-Scute protein complexes. *Development* **113**, 245-255.

Van Vactor, D. L., Jr., Cagan, R. L., Kramer, H. and Zipursky, S. L. (1991). Induction in the developing compound eye of *Drosophila*: multiple mechanisms restrict R7 induction to a single retinal precursor cell. *Cell* **67**, 1145-1155.

Wang, S. W., Kim, B. S., Ding, K., Wang, H., Sun, D., Johnson, R. L., Klein, W. H. and Gan, L. (2001). Requirement for math5 in the development of retinal ganglion cells. *Genes Dev.* **15**, 24-29.

Wasserman, J. D., Urban, S. and Freeman, M. (2000). A family of *rhomboid*-like genes: *Drosophila rhomboid-1* and *roughoid*/*rhomboid-3* cooperate to activate EGF receptor signaling. *Genes Dev.* **14**, 1651-1663.

White, N. M. and Jarman, A. P. (2000). *Drosophila atonal* controls photoreceptor R8-specific properties and modulates both receptor tyrosine kinase and Hedgehog signalling. *Development* **127**, 1681-1689.

Wolff, T. and Ready, D. F. (1993). Pattern formation in the *Drosophila* retina. In *The Development of* Drosophila melanogaster (ed. M. Bate and A. Martinez Arias), pp. 1277-1326. Plainview, NY: Cold Spring Harbor Laboratory Press.

Yang, L. and Baker, N. E. (2001). Role of the EGFR/Ras/Raf pathway in specification of photoreceptor cells in the *Drosophila* retina. *Development* **128**, 1183-1191.

Zipursky, S. L. and Rubin, G. M. (1994). Determination of neuronal cell fate: lessons from the R7 neuron of *Drosophila*. *Annu. Rev. Neurosci.* **17**, 373-397.

zur Lage, P. and Jarman, A. P. (1999). Antagonism of EGFR and Notch signalling in the reiterative recruitment of *Drosophila* adult chordotonal sense organ precursors. *Development* **126**, 3149-3157.