CMLS, Cell. Mol. Life Sci. 57 (2000) 195–214
1420-682X/00/020195-20 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Determination of *Drosophila* photoreceptors: timing is everything

C. A. Brennan and K. Moses*

Emory University School of Medicine, Department of Cell Biology, 1648 Pierce Drive, Atlanta (Georgia 30322-3030, USA), Fax + 1 404 727 4717, e-mail: kmoses@cellbio.emory.edu

Abstract. This review covers recent findings concerning the specification of the photoreceptor subtypes in the *Drosophila* eye. Particular attention is paid to aspects of retinal patterning and differentiation where relative timing of events seems to be tightly controlled and essential for proper assembly of the compound eye. For example, specification of the founding photoreceptors of each cluster requires sequential positive and negative signaling through the Notch pathway, and reiterated signaling through the epidermal growth factor receptor leads to the pairwise recruitment of the distinct types of photoreceptors in discrete zones across the eye. Results suggest that different signaling environments for these two receptors may exist across the disc, and that receiving cells may constantly shift their predisposition to respond to such signals by adopting given fates. In addition, considerable data exist that the rate of expansion of retinal patterning across the disc is restricted to allow the orderly patterning of retinal precursors, and that one mechanism for controlling this rate may be the co-ordinated expression anterior to the furrow of factors which both inhibit and promote the expansion of retinal patterning. Finally, this review considers the possibility that the morphogenetic furrow serves as a moving source of morphogens which supply spatial information to both anterior and posterior tissue, providing temporal cues that regulate the many events involved in orderly assembly of the precise array of retinal cell types in the compound eye.

Key words. Eye; *Drosophila*; Notch; EgfR/DER; Hedgehog.

Introduction

The convergence of the powerful genetic tools available in *Drosophila* with the repetitive and progressive nature of retinal specification has helped the study of fly eye development to become one of the most fruitful fields in cell and developmental biology. Study of the fly eye has yielded insight into many aspects of cell signaling, especially the definition of cellular transduction cascades [1–6]. Other productive areas have included regulation of the cell cycle and cell death [7–9], neural specification [10], axon path finding [11, 12], epithelial polarity [13, 14], specification of organ identity [15–18], and the evolution of the eye [19, 20]. In addition, for many biologists, the beauty of fly eye development has made it worth studying for its own sake.

The compound eye of the adult fly is composed of about 800 individual 'facet eyes,' or ommatidia, each capturing light from distinct, but overlapping, visual fields. The highly ordered appearance of the eye surface (fig. 1A) indicates an unparalleled degree of cellular organization in the underlying retina. Each ommatidium is composed of 20 cells: a stereotypic asymmetric array of 8 photoreceptor cells along with 12 accessory bristle, lens-secreting, and pigment cells (fig. 1B) [21]. The derivation of all these cells from a single monolayer epithelium is reflected in the columnar appearance of the adult ommatidium; almost all of the cells retain their apical and basal connections. Each ommatidium widens basally to apically, producing the characteristic curvature of the eye [21].

The photopigments of each photoreceptor are embedded in microvillar membrane stacks, called rhabdomeres, that extend from each photoreceptor cell into a central space in the ommatidium. The rhabdomeres of the outer six photoreceptors, R1–R6, can be individually identified by the position they occupy in an asym-

* Corresponding author.

metric trapezoid [21]. These rhabdomeres are large, reaching the full height of the ommatidium, and all have a blue-sensitive rhodopsin. The outer photoreceptors provide 'high-sensitivity' light capture properties to the fly retina, perhaps in analogy to the scotopic vision of vertebrate rod cells [22–24]. The inner photoreceptors R7 and R8 have smaller rhabdomeres, positioned in the center of the trapezoid. R7 cells are UV sensitive and R8s are generally blue-green sensitive; these two cells are thought to confer 'high acuity' photoreceptive capacity, similar to vertebrate cone cells [23, 24].

The eight photoreceptor axons from each ommatidium project through the basement membrane supporting the retina (fig. 1C), and extend directly to the brain through the optic stalk. The projections to the optic lobe are retinotopic, with an additional level of complexity: since

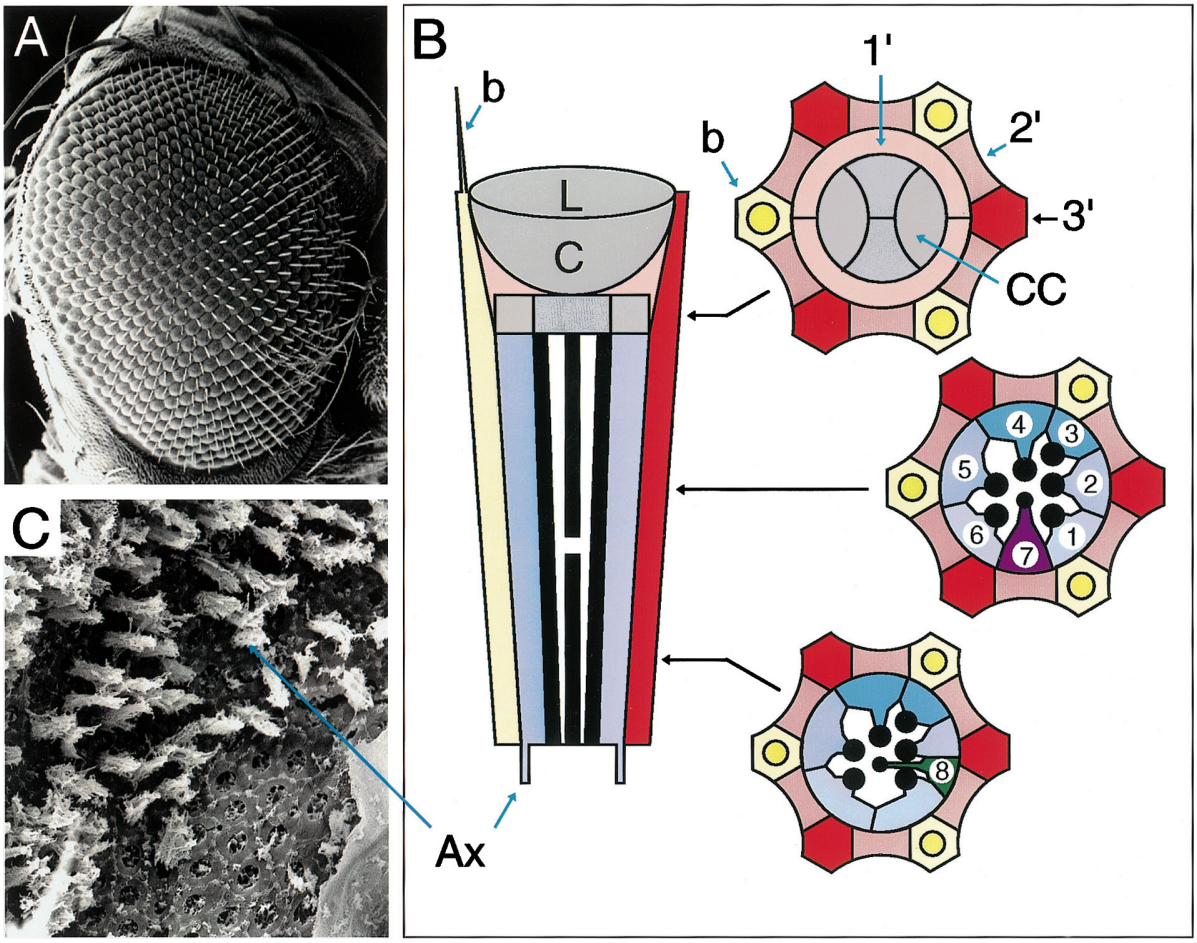

Figure 1. Structure of the compound eye. (*A*) A scanning electron micrograph of the compound eye. Anterior is to the right and dorsal is up. Note the regular array of lenses which overly each ommatidium and the mechanosensory bristles in each alternate vertex. The height of the eye is approximately 0.5 mm. (*B*) A diagram of the cells in one ommatidium. A longitudinal section is shown to the left, and three cross-sections to the right at the levels indicated. The cells of the ommatidium can be classified into four functional groups: (i) the light-sensitive photoreceptors (shown in blue, green, and violet); (ii) the screening pigment cells (red); (iii) the dioptic elements secreted by the cone cells (gray), and (iv) the mechanosensory bristle (yellow). (i) The photoreceptors: the six blue-sensitive outer photoreceptor cells are shown in shades of blue, and numbered in one of the two sections in which they can be seen. Their rhabdomeres are shown in black. Note the asymmetric trapezoidal arrangement of the rhabdomeres. The ultraviolet-sensitive apical central photoreceptor (R7) is shown in violet, and the blue/green-sensitive basal central cell (R8) is shown in green. Note the axons (labeled Ax) which project from the base of the ommatidium and innervate the optic lobes of the brain (not shown). (ii) The 1°, 2°, and 3° pigment cells are shown in shades of red, and one example of each class is labeled in the uppermost cross-section (1° pigment cells are in lightest red, and 3° in darkest). (iii) The cone cells (labeled CC in the uppermost section) secrete the lens (L) and the fluid-filled pseudocone (C). (iv) The two cells that make up the mechanosensory bristle (b) are shown in yellow. Note that the bristle and 2° and 3° cells are shared among ommatidia. The convention is to count 20 cells per ommatidium: 8 photoreceptors, 4 cone cells, 2 1° pigment cells, 3 2° pigment cells, 1 3° pigment cell, and 2 bristle cells. (*C*) A scanning electron micrograph showing ommatidial axons (Ax) emerging from the fenestrated membrane (formed by the feet of the 2° and 3° pigment cells) on the basal side of the retina.

each ommatidium is cone shaped, the rhabdomeres within an ommatidium are not parallel, and each captures a slightly different angle of light. Thus each individual photoreceptor axon separates from the axons of its own ommatidial cohort, and regroups with axons from those photoreceptors in adjacent ommatidia with parallel optical axes before projecting to the brain. This arrangement, known as neural superposition, allows for a high degree of sensitivity while maintaining visual acuity [25–27]. In addition, the axons of the outer and inner photoreceptors diverge: R1–R6 project to the first optic ganglion, the lamina, while the axons of R7 and R8 extend through the lamina and synapse at the medulla. Higher-order integration and image formation occurs at the lobula [24, 26, 28]. For such a complex visual system to work, its components must be arranged in an extremely precise array.

Like other ectodermal structures of the adult fly, the eye develops from an imaginal disc, an epithelial pouch set aside during embryogenesis which grows inside the animal during larval life. The onset of differentiation is postponed until the final larval stage; during metamorphosis, differentiation is completed, and the disc evaginates and fuses with other imaginal tissues [29–31].

In order for the highly tuned optics of the eye to function, the ommatidia must be assembled with near crystalline precision. Like a crystal, the repetitive structure of the eye is built by accretion, starting with a small number of units as a template, and then building on them and repeating the structure as the eye grows [21, 32]. The repeated units are the ommatidia, and development begins at the posterior edge and expands anteriorly. The morphogenetic furrow is an indentation in the eye disc that marks the anterior boundary of neural patterning as it expands anteriorly across the disc (fig. 2A). Ommatidial photoreceptor clusters are specified one column at a time, beginning at the posterior margin of the eye imaginal disc during the final larval instar and emerging from the furrow at the rate of about one column every 2 h [21, 33–35]. This sequential differentiation of ommatidial columns is mirrored by a stereotypic sequence of specification of the individual photoreceptors within each cluster. The founding photoreceptor in each cluster is the R8 cell, which is required for the pairwise recruitment of the other seven photoreceptors [32]. The assembly of the ommatidia is lineage independent, and relies entirely on cell-cell interactions [34, 36]. The simplicity and reiterative nature of the specification of the retinal cell types has facilitated the dissection of the multiple highly conserved signaling pathways which underlie the inductive interactions.

The effect of the periodic addition of new columns of R8 founder cells followed by sequential recruitment of the remaining seven photoreceptors by each R8 cell is to produce a gradient of retinal maturity from anterior to posterior across the disc, with single-spaced R8 cells at the anterior edge of differentiation followed by rows of progressively larger photoreceptor clusters: at any given time, all developmental stages are represented (fig. 2B, C). These steps in retinal determination are highly controlled, this being essential to build a compound eye with such high resolution. It appears that one strategy used in the eye to ensure orderly retinal assembly is tight control of the timing of events including the rate of expansion of differentiation, synchronization of cell cycles, and the order of recruitment of cell types to the ommatidial clusters. This review discusses examples of temporal regulation in the eye, and suggests some mechanisms by which it may be achieved.

Ommatidial determination

One hundred thirty-five years ago, August Weismann [31] reported an indentation in the eye imaginal disc which appeared to mark a boundary between anterior unpatterned epithelial tissue, and posterior tissue with a highly ordered appearance. This indentation, now known as the morphogenetic furrow, is caused by the co-ordinated apical-basal contraction of cells, and moves like a wave across the disc over a period of about 2 days [31–33]. Anterior to the furrow, cells divide randomly, but at the furrow, they synchronize their mitotic cycles by arresting in G1 phase. In and posterior to the furrow, a subset of cells are subsequently determined as photoreceptor neurons in the initial five-cell ommatidial precluster, and remaining uncommitted cells undergo another round of co-ordinated mitosis several hours later [37]. This final division provides a pool of cells for the remaining ommatidial assembly steps in which the final three photoreceptors, the four cone cells and then after pupariation, the bristle-producing and primary, secondary, and tertiary pigment cells are added [21, 34].

R8 specification

The R8 photoreceptor is the first to be specified, and it is indispensable for the recruitment of the other seven [10, 34]. Proper R8 specification requires both the correct spacing of these founding photoreceptors and their specific determination as R8 cells. The earliest molecular markers of photoreceptor determination in the eye are the expression of two basic helix-loop-helix (bHLH) transcription factors, Atonal (Ato) and Daughterless (Da) [38, 39], in a pattern that travels anteriorly with the furrow, with a characteristic expression profile. Anterior to the furrow is a band of moderate expression, followed by the up-regulation and progressive resolution in the furrow into intermediate groups of

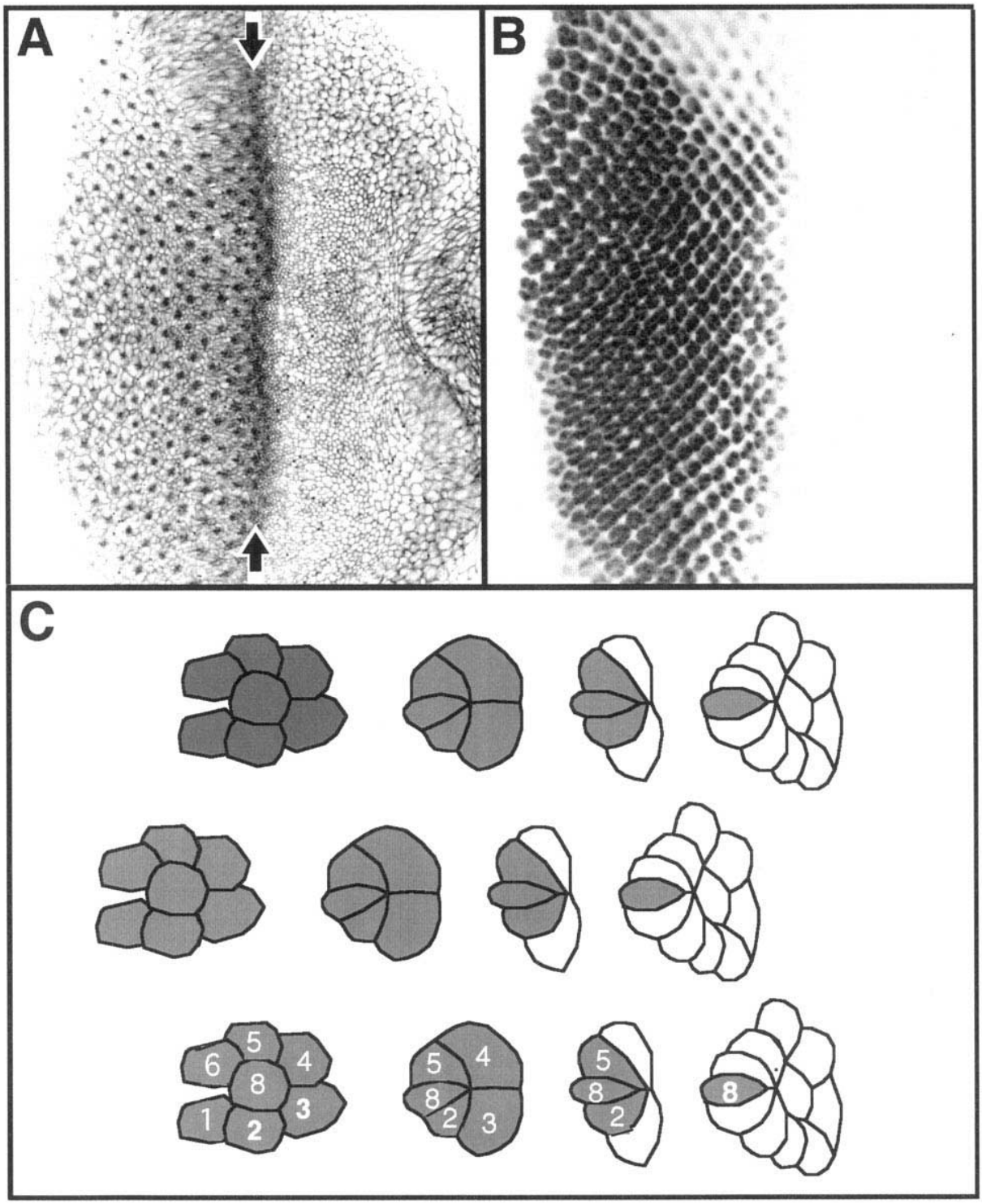

Figure 2. The eye disc during retinal specification. (*A*) A late third-instar eye disc stained (with phalloidin) to reveal filamentous cytoplasmic actin. Anterior is to the right. The entire disc is about 100 μm wide. Note the intense condensation of apical cell profiles in the morphogenetic furrow (marked with arrows). (*B*) A late third-instar eye disc stained with α-Elav antibody to show neural differentiation. Note the gradient of maturity with groups of two staining cells at the anterior edge of differentiation, followed by groups of three photoreceptors (R8, R2, R5), then the five-cell preclusters (R8, R2, R5, R3, R4), and eventually the clusters with all eight photoreceptors. (*C*) A diagram to illustrate the progressive and synchronized development posterior to the furrow. The first photoreceptor to differentiate is R8, which is specified in the furrow. After R8 specification, R2 and R5 join the precluster, followed by R3 and R4, then R1 and R6, and finally R7. The growth of the cluster is highly ordered, with photoreceptors recruited in pairs in distinct zones behind the furrow. Presumptive future photoreceptor cell fates are indicated by numbers.

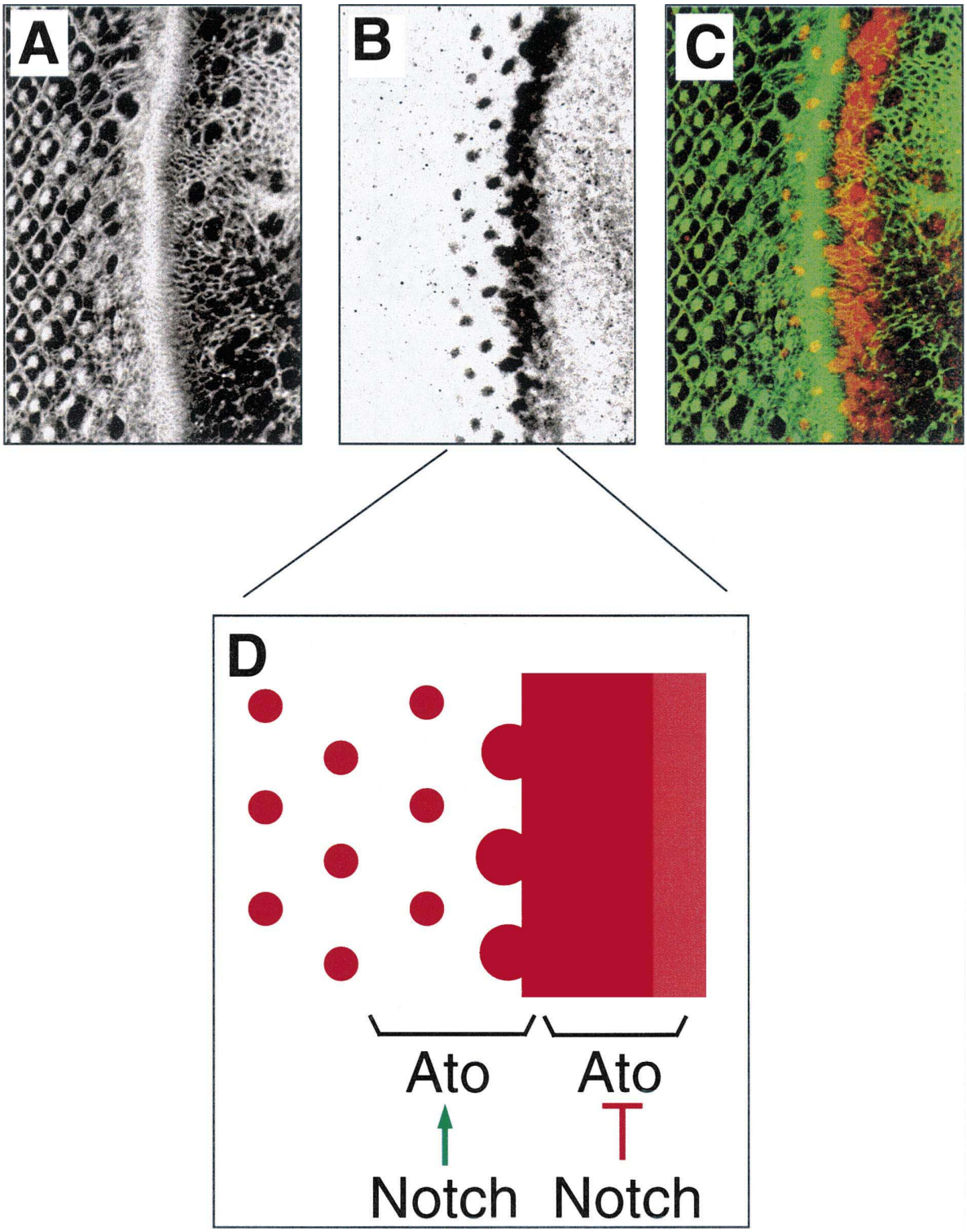

Figure 3. Atonal expression pattern and changing regulation by Notch. (*A*) Filamentous actin (phalloidin) stain showing the furrow. Anterior on the right. (*B*) The same field stained to show the Atonal protein. Note that this protein is nuclear, is expressed in a broad and increasing domain anterior to the furrow (to the right) and then becomes restricted first to the 'intermediate groups' and then to single cells over a space of about three columns (about 6 h). Atonal then persists in the single cells (the future R8 photoreceptors) for about three columns (about 6 h). (*C*) A merged image of panels (*A*) and (*B*) showing cytoplasmic actin in green and Atonal in red. Note that Atonal expression begins anterior to the furrow, and is resolved into single cells in the furrow. (*D*) A diagram to illustrate how regulation of Ato by Notch signaling has sequential, opposing functions. Notch is required for increasing Ato expression levels anterior to the furrow (although not for onset of Ato expression). In the furrow, Notch is then required for repression of Ato in all non-R8 cells [60, 64].

cells, the R8 equivalence groups, and finally into individual evenly spaced cells, the R8 precursors, where Ato and Da persist for three columns [40, 41]. Since these events are reiterative, all phases of expression can be seen at once (fig. 3B). *ato* is the proneural gene for photoreceptors in the eye, and certain other sensory organs [10, 41]. Proneural genes have been characterized as defining subpopulations of cells or equivalence groups within an epithelium that are competent to become sensory neurons [42, 43]. Daughterless acts as a more general bHLH protein, able to dimerize with many different proneural protein products to form functional heterodimers [44, 45].

Many of the genes involved in *Drosophila* eye development have earlier roles in other developmental processes, and so the loss-of-function phenotype in the eye can often only be examined in mosaic mutant patches in the eye [46, 47]. Mosaic clones lacking *ato* function in the eye both fail to specify founding R8 photoreceptors, and consequently lack all neural differentiation [48]. Ectopic expression of Ato in all cells in the eye disc directs all cells in the equivalence group into an R8 fate (although cells outside the equivalence group are unaffected) [48]. Therefore, understanding the regulation of *ato* has been seen as one key to understanding R8 specification.

Factors regulating the initiation of Ato expression in all cells anterior to the furrow are not fully characterized, but other bHLH genes are expressed farther anterior to Ato and antagonize its expression, and slowing its expansion into anterior cells. Hairy, known to repress proneural gene expression in other tissues [49, 50], is expressed in a tight band just anterior to but not overlapping Ato [51]. Extramacrochaetae (Emc), an HLH protein that lacks the basic DNA-binding domain, is able to sequester and inactivate positively acting bHLH proneural genes, and is expressed at its highest level in the eye just anterior to Hairy [39, 51, 52] (fig. 4B). These two genes act redundantly to repress neural development: mosaic clones lacking function of both genes permit the progression of Ato expression to accelerate and shift anteriorly, followed by precocious neural differentiation [51]. In such clones, the accelerated neural differentiation is disordered, with incorrect spacing between the clusters and abnormal cluster morphology [51]. Therefore, although factors which directly initiate Ato expression are unknown, Hairy and Emc prevent premature expression, and slow down the furrow and the anterior spread of neural patterning.

Several mutations have been described which disrupt the resolution of Ato expression into preclusters and R8 cells. Many of these represent genes in or interacting with the Notch pathway. Notch is a transmembrane glycoprotein with 36 extracellular epidermal growth factor repeats [53]. Signaling through the Notch receptor has been studied in many organisms and tissues, and appears to inhibit differentiation in some contexts, and promote it in others [reviewed in refs 54, 55]. Both activities are seen in the regulation of Ato expression in the eye disc, in distinct phases. Notch is expressed in all cells in the eye disc, and at higher levels in cells just ahead of and in the morphogenetic furrow. When the preclusters from which R8s will be selected emerge from the furrow they retain higher Notch protein levels than surrounding cells [56–59].

While the initiation of Ato expression is Notch independent, Notch signaling is autonomously required for up-regulating Ato anterior to the furrow. Removal of Notch with a temperature-sensitive allele ($Notch^{ts}$) or in a mosaic clone prevents this normal up-regulation, and ubiquitous expression of an activated form of the Notch receptor under heat shock control increases levels of Ato expression anterior to the furrow [60]. Unlike many Notch functions in *Drosophila*, this early requirement of Notch for proneural enhancement is not mediated through the downstream components E(spl) or Su(H) [61].

As the *Notch*-dependent up-regulation of Ato continues in the furrow in some cells, Ato is down-regulated in the remaining cells, allowing the intermediate groups to emerge. This repression of *ato* is also *Notch* dependent: under $Notch^{ts}$ conditions, too many neurons differentiate upon emerging from the furrow, reflecting a failure to appropriately space out the preclusters [62–64]. Thus, Notch wields opposite effects on Ato expression in adjacent groups of cells, promoting Ato expression in the intermediate groups while repressing Ato in all other cells. Moreover, Notch is next required within the non-R8 members of the intermediate groups for the further restriction of Ato; in addition to having too

Figure 4. Hh and Dpp regulate furrow progression. (*A*) A late third-instar eye imaginal disc showing Scabrous expression (immunostain). The normal furrow lies between the two red arrows. A circular ectopic furrow is propagating away from a clone in which the Hh pathway has been ectopically activated by loss of function of *patched*, the negatively acting receptor for Hh. (*B*) A diagram to show some of the genetic interactions near and in the furrow. Domains of protein expression shown are: Hh (orange), Dpp (blue), Hairy (green), Emc (pink), and Ato (red; note that Ato expression ramps up toward the furrow, and then resolves to intermediate groups and ultimately individual R8 cells). Inductive interactions are shown with green arrows: Hh induces Dpp and Dpp may induce Hairy over a long range, and cell cycle arrest in the furrow at a short range. Repressive interactions are shown in red: Hairy and Emc repress Ato, and Hh (directly or indirectly) represses Hairy close to the furrow.

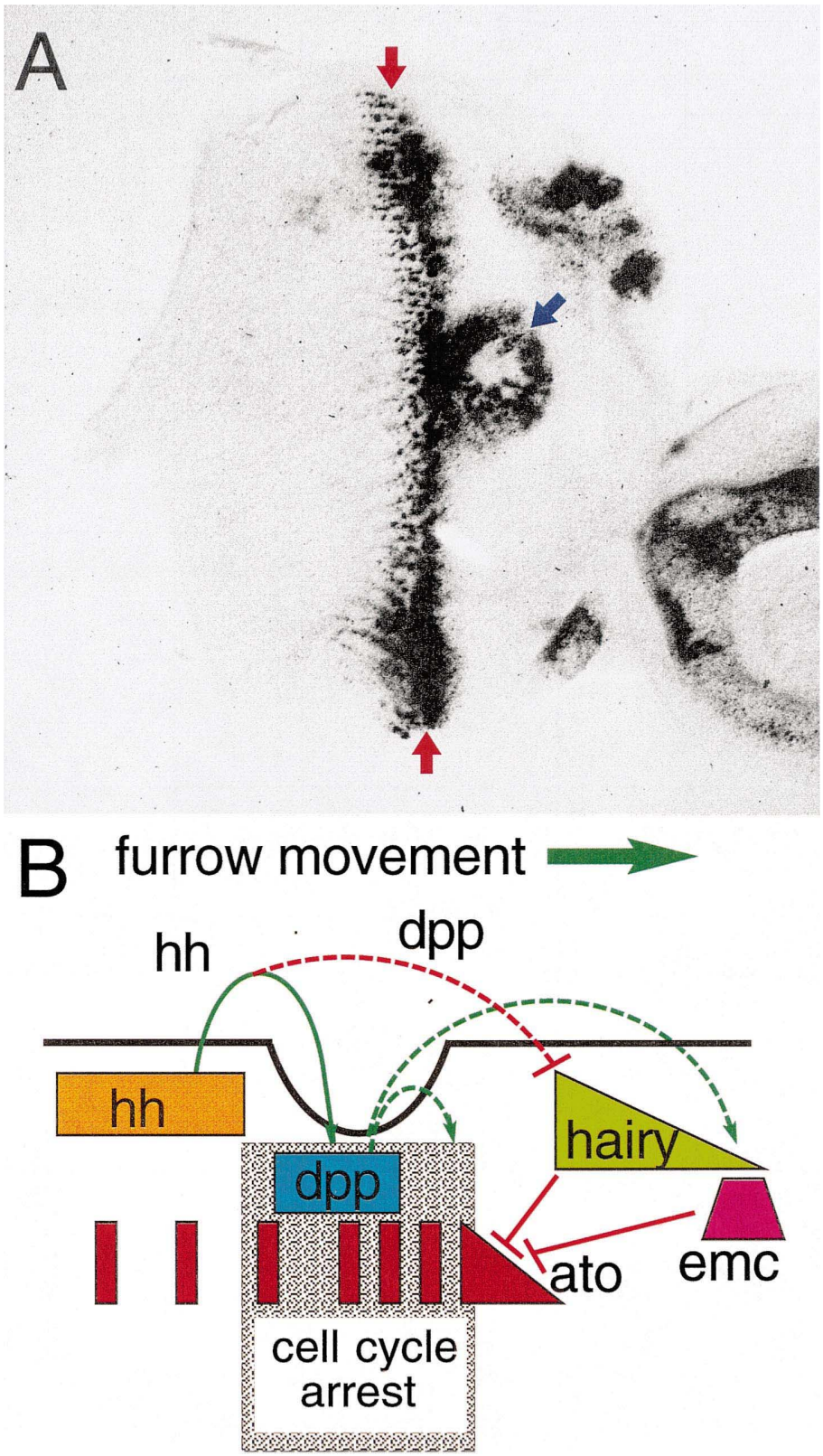
A
B
furrow movement
hh
dpp
hh
dpp
hairy
ato
emc
cell cycle
arrest

Fig. 4.

many ommatidial clusters, regions of the disc deprived of Notch function in the furrow can also show multiple R8 cells in individual clusters [64], This 'neurogenic' function of Notch has been well-documented in the development of other sensory organs in *Drosophila* [reviewed in ref. 65]. So, Notch signaling can also have sequentially opposite effects within some cells, first promoting and then repressing Ato expression (fig. 3D).

What regulates the switch in Notch activity from proneural enhancing to its classic proneural-repressing, or neurogenic, function? It is not likely that different Notch ligands are responsible for the different signaling activities. Delta appears to be the main ligand for Notch in the eye. Like Notch, Delta is a large transmembrane protein, and has 9 epidermal growth factor repeats [66, 67]. *Delta* is first expressed in the furrow, at uniform levels and in almost all cells, and is subsequently expressed in individual photoreceptors around the time of their specification, for which it is required [57, 68, 69]. *Delta* mutant clones show both *Notch* phenotypes: the centers of the clones lack photoreceptor differentiation, representing the proneural *Notch* function, whereas the edges of the clones have too many photoreceptors, indicative of the neurogenic phenotype [60]. Delta and Notch expression patterns are dynamic, but are not sufficient to explain the switch in Notch function from proneural enhancing to proneural repressing. Nor do subtle differences in protein or signaling levels appear to predict which cell will emerge as R8, unlike the competitive inhibition seen in other sensory organ proneural fields in the fly, in which initially small differences in Notch protein or signaling levels are magnified, leading to the selection of individual neuronal precursors [57, 65, 70, 71].

Two other genes interacting with the Notch pathway in the eye, *scabrous* (*sca*) and *kuzbanian* (*kuz*), may contribute to some aspects of Notch signaling in R8 specification. Scabrous (Sca) is a glycoprotein with homology to vertebrate tenascins found in the extracellular matrix of developing vertebrate neural tissues [72, 74]. In the eye, Sca is first expressed in R8 equivalence groups, and is then restricted to R8 cells where it persists for several columns [73]. Sca protein is diffusible and acts as an inhibitory morphogen, emanating from newly established preclusters to position the subsequent R8 cells at maximum distance, ensuring that each successive column is out of register with the last [63, 74, 75]. The profile of Sca protein levels in the furrow probably sets the prepattern for Notch and Delta signaling, and while direct biochemical interaction has not been shown, interaction with the Notch pathway at some level has been suggested [63, 76, 77].

The *kuz* gene encodes a disintegrin metalloproteinase that cleaves Delta, releasing an extracellular ligand [78, 79]. Mosaic *kuz* clones in the eye resemble *Delta* clones in that the centers lack photoreceptors while the periphery has an excess of photoreceptors [78]. This suggests that Kuz-cleaved diffusible Delta may have a different signaling activity than the membrane-bound form of the ligand. Perhaps the diffusible Delta mediates the proneural Notch signaling activity while the tethered form signals the anti-neural function. Experiments with secreted forms of Delta suggest that the diffusible form of the ligand may antagonize Notch function in *Drosophila* [80, 81]. The expression pattern of Kuz has not been reported, but it is possible that temporal regulation of Delta cleavage might explain sequential opposing Notch activities at the furrow.

Since R8 cells are the founding photoreceptors of each ommatidial cluster, their correct spacing and specification are essential for the subsequent integrity of the retinal array. Expression of the *ato* proneural gene appears to specify R8 fate, and two aspects of the regulation of Ato have been considered here. The rate of anterior progression of the Ato expression domain is controlled by the expression of the negatively acting HLH factors *hairy* and *emc* anterior to the furrow. Restriction of the rate at which columns of R8 are specificied may be essential for proper patterning, perhaps to allow time for Notch-dependent interactions to occur; in *hairy emc* mosaic clones, there are defects in the spacing and morphology of the preclusters. A great deal remains to be explained about the functions of Notch in patterning the array of founder R8 photoreceptors. Notch first promotes, then represses Ato expression and R8 fate in distinct zones relative to the furrow. The molecular mechanisms underlying this switch in activity are not known, but changes in ligand processing, presence or absence of interacting extracellular factors, or the underlying developmental state of the cells may influence the outcome of Notch signaling. Although the specific mechanisms controlling the temporal differences in response to Notch signaling are not known, some general strategies the eye may employ to regulate responsiveness across the disc will be addressed below.

Specification of remaining cells of the ommatidial cluster

Following R8 specification, the remaining complement of seven photoreceptors are recruited to the ommatidial cluster. The photoreceptors differentiate in pairs: R2 and R5, then R3 and R4 join, forming the five-cell precluster. After a final round of cell division among all remaining unpatterned cells, R1 and R6 are the first cells to be recruited, and finally R7 joins, several hours later [21] (fig. 2C). Following the photoreceptors, the four lens-secreting cone cells are added to the cluster,

and after pupariation, the 1°, 2° and 3° pigment cells and bristle cells are recruited, completing the ommatidium [82]. As each photoreceptor is specified, its nucleus rises, and it begins expression of neural antigens.

The sequential differentiation of all the remaining cells following R8 is triggered by repeated activation of the epidermal growth factor receptor (EgfR) tyrosine kinase in each cell. Signaling through a receptor tyrosine kinase (RTK) such as EgfR starts with the binding of a ligand to the membrane-bound RTK. Upon ligand binding, RTK dimers transphosphorylate each other and stimulate a cellular cascade of events including the activation of the nucleotide exchange factor Ras, and a series of phosphorylation reactions. Near the bottom of the cascade, MAP kinase (MAPK) is phosphorylated, and then activates a number of targets, including nuclear proteins. Exposure of eye discs carrying a temperature sensitive allele of *EgfR* (*Egfr*^{ts}) to the nonpermissive temperature causes a block in ommatidial development at the one-cell stage, and mosaic clones lacking EgfR function contain only isolated R8 cells [83–85]. Expression of a dominant negative EgfR protein (EgfR^{dn}) behind the furrow can block cluster formation at different stages depending on when it is expressed [86]. In addition, ectopic expression of an activated form of EgfR anterior to the furrow can result in ectopic, but disordered photoreceptor differentiation in an *ato*- and R8-independent manner [83]. These results suggest that signaling through EgfR is required for the differentiation of all photoreceptors except R8.

Spitz (Spi) is a secreted transforming growth factor (TGF)-α homolog with a single EGF repeat [87], and is a necessary recruiting ligand acting through EgfR in the eye; ubiquitous overexpression of active Spi ligand causes over-recruitment of some photoreceptors, and in mosaic *spi* clones, while R8 specification is normal, recruitment of the remaining photoreceptors fails [86, 88]. Mosaic analysis showed that *spi* is nonautonomously required in the photoreceptors of the five-cell precluster for successful ommatidial assembly [89]. The strength of the requirement in these photoreceptors corresponds to the order of their recruitment: R8 has the strongest requirement, followed by R2 and R5, then R3 and R4, suggesting that new photoreceptors are recruited to the ommatidia by existing photoreceptors by signaling through Spi ligand and EgfR RTK [89]. While *spi* mosaic clones are only defective in the recruitment of photoreceptors after R8, loss of EgfR function results in failure of R8 maintenance and in death of tissue behind the furrow [83, 84], suggesting that another ligand must be acting through EgfR to promote cell survival.

Spi is expressed steadily in differentiating photoreceptors, but *spi*-dependent recruitment of photoreceptors occurs discretely, in pairs [21, 88, 89]. One model to explain this discontinuous recruitment has invoked the simultaneous expression by differentiating photoreceptors of Spi and the inhibitory EgfR ligand, Argos (Aos) [90]. Aos is a secreted protein with a single EGF repeat that can antagonize EgfR signaling, and which is often expressed in response to positive EgfR signaling, likely acting as a negative feedback signal [91–94]. Since Aos is more highly diffusible than Spi, its inhibitory signal could initially dominate the environment surrounding the precluster. Eventually, Spi concentration would reach locally elevated levels, sufficient for its activating signal to prevail, and it would trigger the next round of Ras activation and differentiation in the closest cells [86, 90, 92, 93, 95]. This model is probably insufficient to fully explain the dynamics of the successive rounds of induction, which do not exactly occur in the concentric rings of cells envisioned by this model; however, it appears to be basically valid. Another factor functioning in surrounding cells to oppose recruitment to the ommatidial cluster is Fat facets (Faf), which protects specific ubiquitin-tagged proteins from degradation. Action of the *faf* gene is required in cells which surround, but do not join, the clusters and may prevent excessive photoreceptor recruitment [96–98]. While the cellular targets of this deubiquitinating enzyme are not known, it may negatively regulate signaling through the Ras pathway [99]. The expression of *faf* may further restrict the ability of cells to respond to Spi signaling.

It is likely that Spi signaling in the eye is also regulated post-transcriptionally in the sending cell: *spi* encodes a pro-protein with perinuclear localization that must be cleaved to generate an active ligand [86–100]. The *rhomboid* (*rho*) and *Star* genes both encode novel membrane proteins that are probably required for the processing or cleavage of the Spi protein, releasing the active ligand [100–105]. Star is also perinuclearly localized, and is briefly expressed in a narrow band at the furrow, and subsequently in R8, R2, and R5, and more cells as the cluster grows [102, 103]. *Star* mutant clones in the eye lack all photoreceptors, including R8, and mosaic analysis reveals that Star function is required in R8, R2, and R5 [103, 106, 107]. Rho protein localizes to the apical membranes of cells, and in the eye is expressed in R8 cells in the furrow and in R2 and R5 shortly thereafter [105, 108, 109]. Removal of *rho* function in mutant clones in the eye has only subtle phenotypes, but ectopic expression results in the addition of extra outer photoreceptors [105, 109]. While *Star* has more severe phenotypes and *rho* has weaker effects in the eye than *spi*, it is possible that the regulation of Spi ligand production by Star and Rho could contribute to the distinct rounds of recruitment of retinal cells to the ommatidium.

Notch and Delta are also involved in the recruitment of all the photoreceptors and accessory cells which follow

R8 [62, 69]. *Notch*^{ts} experiments revealed that Notch both promotes and represses the specification of later ommatidial cell types at different stages, similar to its opposing roles in R8 determination. A 4-h larval exposure to the nonpermissive temperature results in distinct phenotype zones in the adult eye: a region with excess photoreceptors followed by a more posterior region with too few photoreceptors [62]. In addition, it was noticed that cells needed Notch signaling at the onset of their differentiation, and if Notch function was temporarily removed then restored, a given cell would assume a different fate, one appropriate to the new time [62]. It seems that cells have restricted windows of opportunity in which to assume their normal fate. When differentiation is temporarily blocked by removal of Notch, the fate possibilities of a cell are not preserved, but change so that they keep pace with a normally advancing furrow, producing the distinct developmental zones in the disc which represent the successive stages of ommatidial recruitment.

R7 is the last photoreceptor to be recruited, and requires signaling through two RTKs, EgfR and Sevenless (Sev), to adopt its fate [reviewed in refs 86, 110]. The ligand for Sev is Bride-of-sevenless (Boss), which is expressed on the surface of R8 [111–113]. Although many cells in the eye disc express Sev, only R7 responds to Boss signaling; the fate of R1–R6 is already determined by the time of Boss presentation, and cone and pigment cell precursors do not contact R8 [114]. The requirement for *sev* was discovered before the role of EgfR in any recruitment step was known, and the finding that it was only required for R7 specification prompted the expectation that different RTKs would be required for the recruitment of each photoreceptor, and that the unique RTK or combination of RTKs would specify the individual identity of each [115]. However, Sev and EgfR both signal through the same Ras pathway, and it was even found that the intracellular signaling domain of Sev could be replaced by that of another RTK, and still function as Sev [116–118]. This led to a new model in which RTK signaling through the Ras pathway was merely a trigger, permitting or instructing a cell when to differentiate, but conferring no identity information [86, 88].

The recruitment and differentiation of all the cell types of the ommatidium except R8 are signaled through both Notch and RTK, either Sev or EgfR. Ligand-dependent RTK signaling through the Ras pathway is fairly well characterized biochemically, and can either promote or repress differentiation, depending on the type and quantity of ligand. The activating and inhibiting contributions of Notch signaling to differentiation are less well understood [119]. However, it is clear that the signaling pathways themselves do not specify the identity of newly differentiating retinal cells. The same pathways are used reiteratively for the generation of many different cell types. What, then, determines the fates of the sequentially recruited photoreceptor and accessory cell types?

Transcription factors providing ommatidial cell subtype specificity

A number of experiments have revealed a prepattern of cellular factors influencing the identity assumed by a cell in the eye disc upon Ras and Notch signaling. Most of these are nuclear proteins, and some are expressed nowhere outside the eye. These have been recently reviewed [118, 120–123], and so just three examples will be given here.

The *rough* (*ro*) gene encodes a homeobox transcription factor which is initially expressed in the furrow in all non-Ato-expressing cells, where it is thought to participate in the proper resolution of the Ato expression pattern and selection of single R8 precursors [48, 124–126]. In addition to its role in restricting Ato expression to single R8 cells, *ro* is required for the specification of subsequent photoreceptors. After expanding in the furrow to all non-R8 cells, its expression is shortly restricted to four cells, the precursors of R2, R5, R3, and R4 [126]. Initially, by repressing *ato* in R2 and R5, Ro prevents them from assuming an R8 fate [48, 127]. Subsequently, Ro is required in R2 and R5, and possibly also in R3 and R4, to prevent them from assuming an R1/R6 fate [127]. Ectopic expression of Ro in presumptive R7 cells transforms them into outer photoreceptors (R1–R6) [128]. Ro is therefore expressed in the four non-R8 members of the five-cell precluster, and is required in at least two, and perhaps all four of them for proper fate determination.

The *seven-up* (*svp*) gene encodes two orphan nuclear receptors differing in their putative ligand-binding domain [129]. An enhancer detector in *svp* is initially expressed in R3 and R4, and soon also in R1 and R6 [129]. Mosaic analysis reveals that *svp* must be expressed in R3, R4, R1, and R6 for their proper specification; these cells will all assume the inner photoreceptor fate of an R7 in its absence [129]. A brief pulse of ectopic expression of Svp under heat shock control causes multiple defects seen in stripes in the adult eye, indicating that it can affect photoreceptor determination at several distinct stages [130]. Targeted misexpression in the precursors of various ommatidial cell subtypes can cause the late loss of R8 identity, and autonomous transformations of R2 and R5 into the R3/4/1/6 fate, of R7 into an outer photoreceptor fate, and cone cells into an R7 fate [131]. Thus, the expression of *svp* in ommatidial precursor cells must be under tight temporal and spatial control.

The homeodomain transcription factor BarH2 is initially expressed in all cells in the furrow, and is then down-regulated before reappearing with the related homeoprotein BarH1 in R1 and R6, and also the primary pigment cells, where the two proteins are required for specifying the identity of all of these cells [132]. Misexpression of BarH1 in cone cells results in their transformation to either R1/6 photoreceptors or primary pigment cells [133].

In ommatidia doubly mutant for both *ro* and *svp*, all photoreceptors assume an inner photoreceptor fate, either R8 or R7 [127]. Indeed, the activities of Ro, Svp, and Bar seem sufficient to specify the fates of most of the cell types in an ommatidium. However, requirements for about a dozen other prepattern factors have been described [reviewed in refs 120, 121]. Many of these factors serve a similar function to Ro, Svp, and Bar: loss of expression or ectopic expression directs the transformation of a subgroup of ommatidial cell types to a new subgroup fate. The nuclear proteins seem to confer a potential identity to a precursor cell, which assumes this identity if signaling through the Ras pathway occurs. The transcriptional targets of these prepattern factors in the photoreceptor cells are largely unknown, with a few exceptions [48, 134]. The prepattern factors may direct appropriate rhodopsin expression or the expression of cell surface recognition molecules that might be required for the axon reshuffling that occurs prior to synapsing in the brain.

While the prepattern of such fate-specifying transcription factors may explain how non-specific extrinsic signals can trigger specific fates, the question still remains as to how these patterning factors are placed in the appropriate cells at the appropriate times. The *lozenge* (*lz*) gene encodes a putative transcription factor of the Runt/AML-1 family that plays a role in the establishment of the prepattern of transcription factors, especially those required in the specification of cells types that occur after precluster formation and the second mitotic wave [135, 136]. The Lz protein is found in all cells behind the furrow except the five precluster photoreceptors (R8, 2, 5, 3, 4); it is seen in all basal undifferentiated cells, and apically in R1, R6, R7, and the cone cells as they are specified. Additionally, Lz is expressed in the sequence of primary, secondary, and tertiary pigment cells as they are specified during pupation [136]. In the R7 equivalence group (R7 + cone cells), Lz represses *svp*, differentiating this group from R1 and R6, in which Lz positively regulates the transcription of *Bar* [135]. Additional evidence suggests that Lz may also positively regulate other prepattern factors in cone and primary pigment cells [136]. Studies of Lz homologs support the notion that it could both activate and repress transcription, and suggest that these activities are likely accomplished as a heterodimer with structurally unrelated proteins [135].

Perhaps the discovery of activating and repressing dimerization partners for Lz will help us understand how the prepattern of ommatidial subtype transcription factors is positioned in the appropriate precursor cells at the appropriate time. However, it is likely that there are multiple complex mechanisms that remain to be discovered. For example, no gene analogous to *lz* is yet known to regulate the prepattern transcription factors in the early five-cell precluster. And if the activities of Lz are regulated by dimerization partners, how are such partners positioned? Furthermore, the $Notch^{ts}$ experiments revealed that the developmental potential of a cell changes over time, demonstrating that the positioning of these prepattern factors is highly dynamic and constantly shifting. Possible mechanisms for directing this ever-changing prepattern will be addressed below.

Temporal co-ordination of steps in retinal patterning

Under some conditions, photoreceptor differentiation can be uncoupled from the furrow; for example, ectopically expressing activated EgfR in random patches anterior to the furrow induces precocious but disordered neural differentiation [83], and surgical explants of disc tissue from the anterior to the furrow will differentiate into photoreceptors [137]. The function of the furrow and associated events does not seem to be to induce neuronal differentiation per se, but rather to control such differentiation, so that it proceeds in an orderly manner.

How is the orderly expansion of retinal patterning across the eye disc achieved? Superimposed on the system of Notch and EgfR signaling among ommatidial precursors as they form is a network of longer-range signaling molecules which organize the presumptive retinal tissue and restrict ommatidial formation to one column at a time. As discussed, newly patterned preclusters inhibit the formation of new ommatidial founders, secreting inhibitory signals such as Sca to ensure that subsequent R8 cells are at sufficient distance and that each column is out of register with the last. This inhibition of R8 formation is balanced by a default tendency of anterior cells to all differentiate as R8 cells, as shown by the ectopic R8 cells that form when elements of the inhibitory pathway are lacking. This default R8 fate is explained, at least in part, by the expression of the proneural gene *ato* in a stripe of cells anterior to the furrow. However, the rate at which the domain of Ato expression progresses anteriorly is restricted by the expression of *hairy* and *emc* anterior to the Ato stripe: in mutant patches lacking both *hairy* and *emc* function, Ato expression shifts anteriorly, and the pace of furrow progression and ommatidial patterning accelerate. But something must control the rate of progression of the *hairy* and *emc* expression domains.

A signaling network of secreted proteins originating in newly specified photoreceptors controls the rate of furrow progression by both inhibiting and stimulating steps leading to ommatidial patterning in anterior tissue. The key molecules in this signaling network are Hedgehog (Hh), Wingless (Wg), and members of the BMP family, including Decapentaplegic (Dpp). All three classes of secreted proteins have roles in many aspects of *Drosophila* development, and are able to act as morphogens, inducing developmental events at a distance and specifying fates in a concentration-dependent manner [138–142].

Hh is expressed in photoreceptors behind the furrow, and is required for furrow progression and expansion of neural patterning; when Hh function is removed using a temperature-sensitive allele, the furrow stops [143]. When this happens, ommatidial clusters behind the furrow continue to mature, but no anterior cells enter into the differentiation pathway, causing in the disc the loss of the gradient of maturity (no young forms are found), and in the adult, a small, bar-shaped eye. Remarkably, ectopic activation of the Hh pathway in patches anterior to the furrow is sufficient to trigger ectopic neural differentiation, with a circular furrow that radiates out from the initial site of ectopic Hh signaling [2, 144, 145] (fig. 4A). Associated with these circular furrows are ectopic rings of Sca, Ato, and Hairy, with expression patterns in and ahead of the ectopic furrow, just as with a normal furrow [144, 145]. Thus Hh, directly or indirectly, drives the expression of genes which promote furrow progression, such as *ato*, as well as genes which oppose furrow progression, such as *hairy*.

Hh protein probably cannot diffuse as far anteriorly as would be needed to directly activate *hairy* expression. Such distant effects of Hh have been thought to be mediated by the more highly diffusible TGF-βs. *dpp* is expressed at the furrow, and is dependent upon Hh expression as shown in loss of Hh function and ectopic Hh pathway activation experiments [2, 144–146]. Localized, *hh*-dependent Dpp expression is known to mediate long-range patterning effects in other tissues, such as the *Drosophila* wing [147, 148], and an early model has been that Hh behind the furrow drives Dpp expression in the furrow which diffuses anteriorly mediating the effects of Hh [149]. Experiments have failed to fully support this model: mutant patches lacking TGF-β or Hh receptors or cellular transduction elements in mosaic eye discs fail to arrest the furrow in the dramatic way seen from global loss of Hh function, although the furrow is slowed in many cases [3, 150–153]. Furthermore, removal of temperature-sensitive *dpp* function only arrests the furrow in the latter stages of progression, and ectopic expression of *dpp* in random clones anterior to the furrow does not generate circular furrows radiating from the site of misexpression as does *hh* [154, 155].

The failure of overexpression of or loss-of-function of *dpp* to mimic the *hh* phenotypes could be due to redundancy with other TGF-βs expressed in the eye such as *glass bottom boat* [159]. However, removal of downstream signaling components in either the Hh or TGF-β pathways in mosaic clones has consistently failed to inhibit neuronal development in the ways predicted if reception of those signals were absolutely required for photoreceptor differentiation. The restricted expression domains of Hh and Dpp probably more likely serve as a moving source of somewhat redundant morphogens, imposing a temporal schedule of developmental stages both far ahead and just anterior to the furrow.

Hh signaling not only drives Hairy expression far ahead of the furrow (probably indirectly), but also represses Hairy as the furrow nears. The anterior and posterior boundaries of the broad Hairy expression domain are independently specified by *hh*-dependent positional information emanating from the furrow; when an ectopic furrow converges with the endogenous furrow, Hairy expression between the two furrows is lost, suggesting that the concentration of morphogens between the two furrows is too high to permit *hairy* expression [144]. Ato expression is initiated following loss of Hairy, and while Ato expression is Hh dependent, its anterior boundary

Figure 5. Models for specification of the sequence of ommatidial recruitment steps. Although secretion of Spitz from differentiating photoreceptors is believed to activate the Ras pathway in surrounding cells and recruit them to the ommatidial cluster, it is still unknown how unpatterned cells advance through the set of potential fates that they could acquire upon Ras signaling. Three ways in which undifferentiated cells behind the furrow might know where they are and what fate to assume upon Ras signaling can be imagined. (*A*) In the first model, sequential local interactions instruct cells to progress through a series of states of developmental potential. For example, once R2 and R5 are specified, they might instruct surrounding cells to abandon the R2/R5 potential fate and advance to the R3/R4 potential fate. Likewise, upon differentiation, R3 and R4 might instruct surrounding cells to divide. (*B*) In the second model, cells intrinsically know 'what time' it is by some internal clock mechanism. Such a clock could allow presumptive photoreceptors to progress through a series of potential fates, for example, by expressing different sets of transcription factors at different times. This could instruct them to respond to Ras signalling at '2 o'clock' by differentiating as R2/R5, or if not yet determined by '8 o'clock', to divide once more [167]. (*C*) In the third model, cells know 'what time' it is by reading an exogenous field of spatial information. A morphogen gradient could diffuse from the furrow or some other source, and over time, a given cell could experience increasing or decreasing levels of the morphogen. Thus, a spatial gradient could convey temporal information. Such a mechanism could work similarly to an intrinsic clock mechanism, with cells being instructed to express different transcription factors, and thus progress through a series of potential fates.

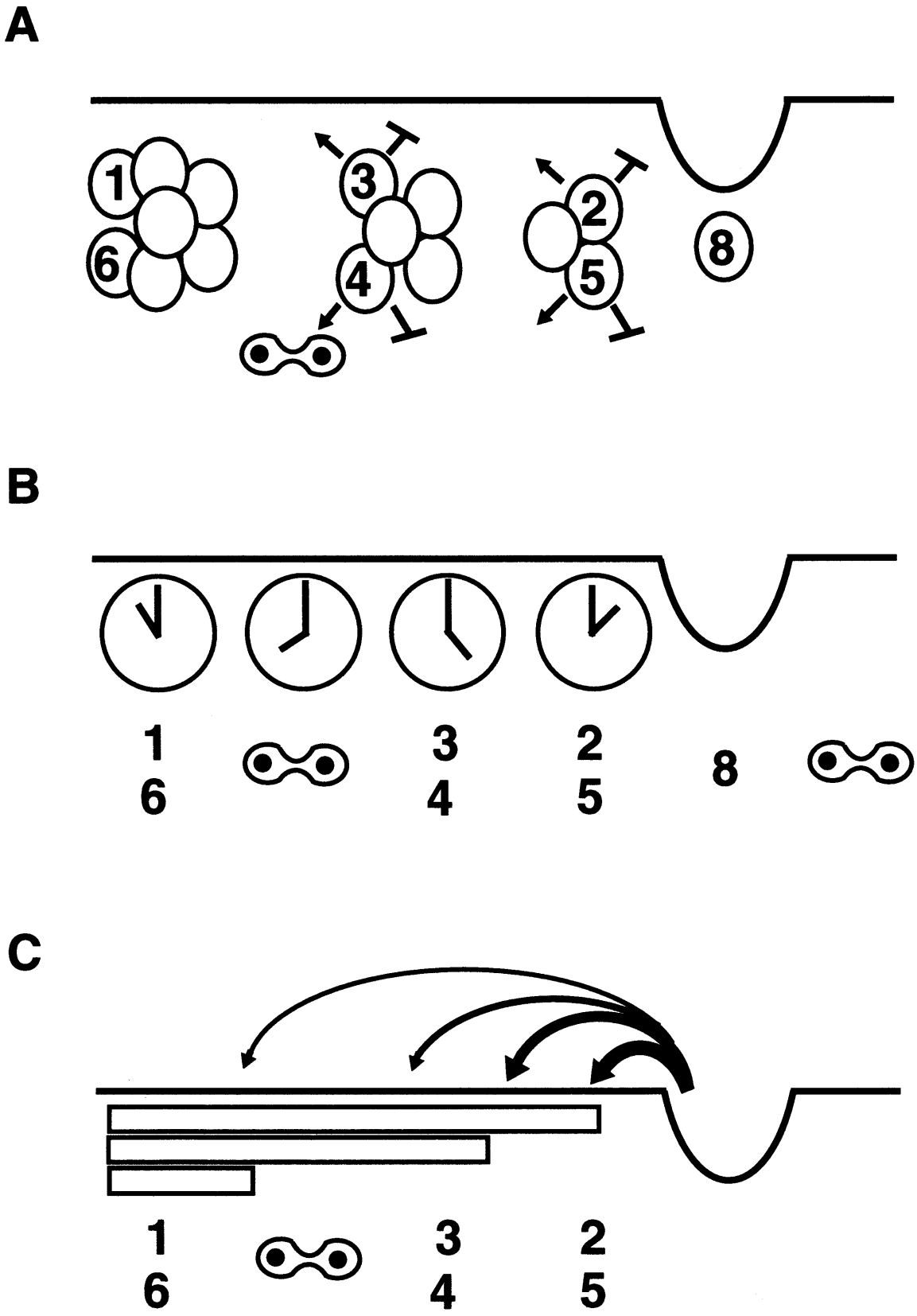

Fig. 5.

of expression is probably defined by the loss of Hairy, rather than by a specific morphogen concentration [51, 144].

Wg is expressed along the dorsal and ventral margins of the eye disc during furrow progression, and has a more restricted role than Hh or Dpp, opposing furrow reinitiation from the margin [18, 156–158]. Although Wg is not normally expressed in the center of the disc, ectopic expression there blocks furrow progression, by acting somewhere downstream of *dpp* transcription [158].

Furrow-associated morphogens also regulate the cell cycle synchronization that occurs at the furrow. Cells anterior to the furrow synchronize by arresting their furrows in the G1 phase, the phase in which cells typically respond to extracellular signals [8, 160]. In mutant conditions where G1 arrest is prevented, cells at the furrow cycle one more time before responding to differentiation signals, resulting in a very disorganized retinal array [8]. When TGF-β receptors are removed in mutant clones, G1 arrest at the furrow autonomously fails [7]. Ectopic expression of *dpp* in clones anterior to the furrow locally inhibits mitosis, further suggesting that one way in which Dpp signaling in the furrow contributes to pattern formation in the eye is by ensuring that cells arrest long enough to respond to differentiation signals synchronously [7, 155]. Eventually, cells lacking Dpp receptor components do arrest their cell cycles in the posterior part of the furrow, suggesting that redundant mechanisms ensure proper cell cycle synchronization at this important juncture [7].

Additional evidence that signals emanating from the furrow region establish a moving patterned field of developmental stages anterior to the furrow is the restricted zone of competence anterior to the furrow in which ectopic activation of the Hh signaling pathway can trigger ectopic furrows [145]. Although the mutant patches of tissue are generated early in larval life, they do not generate ectopic patterning until the endogenous furrow is within about 20 cell diameters, suggesting that cells must experience a certain developmental preparation, which only the endogenous furrow can induce, before patterning can occur.

Furrow progression is an early metamorphic event in the life history of the fly, and it must be co-ordinated with transformations that occur throughout the animal. Such co-ordination is achieved by the ecdysteroid hormones which are centrally produced in the fly, and which trigger events associated with metamorphosis in many tissues [161]. Removal of ecdysteroids, through genetic means or by in vitro culturing of eye discs, blocks furrow progression, and removal of ecdysteroid response elements in mutant discs or in mosaic clones disrupts furrow progression and subsequent ommatidial assembly [162–165; C. A. Brennan and K. Moses, unpublished data). How global hormonal signaling is integrated into local signaling networks in the eye disc is not known, but there is evidence that the furrow is a boundary between larval and metamorphic patterns of gene expression [C. A. Brennan and K. Moses, unpublished data).

In summary, there is strong evidence that long- and short-range signaling associated with the furrow serve to impose a slow schedule of expansion of retinal patterning across the eye disc. The disordered photoreceptor pattern that occurs behind the accelerated furrows in *hairy/emc* clones supports the notion that restricting the rate of furrow progression is important for proper pattern formation.

By long-range activation and short-range repression of Hairy expression which prevents Ato from expanding far anterior to the furrow, the signaling from the furrow indirectly restricts proneural potential to just anterior to the furrow. The furrow-associated signals also ensure that cells arrest their cycles in the furrow, granting time for local signals to refine Ato expression and allowing ommatidial assembly to be synchronized row by row (fig. 4B).

Many aspects of ommatidial assembly posterior to the furrow also seem to be very precisely timed. For example, gain or loss of Notch signaling has different effects at different times or at different distances from the furrow. The pairwise recruitment of photoreceptors to the cluster despite constant transcription of the *spi* gene could be due to temporally regulated processing of the signal or ability of surrounding cells to respond. After R8, R2, R5, R3, and R4 are determined, the last cell division among remaining unpatterned cells is synchronous. The prepattern of transcription factors that gives undetermined cells a potential identity is constantly shifting anteriorly. A number of genes such as *Delta*, *Star*, and *Bar* are transiently expressed in the furrow, and then reappear in selected cells a few hours later, where they are required for proper cell fate specification. Misexpression of *svp* in presumptive photoreceptors at the wrong time causes gross patterning defects. Boss expression in R8 is delayed until all the photoreceptors but R7 have been determined. Lozenge activity is delayed until after the final mitosis, when it becomes important for placing many of the later cell fate-determining transcription factors.

Little is known about how the tremendous number of individual events contributing to orderly ommatidial assembly are co-ordinated. Three general strategies for such co-ordination can be imagined (fig. 5). First, all cellular changes (at the transcriptional or post-transcriptional level) could be instructed by sequential, local interactions, ensuring, for example, that no Boss expression can occur until R1 and R6 are securely determined,

or that cofactors for Lz activity are repressed until after the second mitosis. A variation on this theme was proposed by Freeman [90], who suggested that cells could ratchet through a preprogrammed series of states, or potential fates, by repeated nonspecific signaling from neighboring cells. Another possibility is that cells could have an internal clock, perhaps measuring time since the last mitosis, allowing them to autonomously advance through a series of potential fates [166]. The third possibility is similar to the second, in that cells could independently know 'what time' it is, not intrinsically from the ticking of an internal clock, but from an exogenous field of temporal information, much as cells anterior to the furrow seem to be instructed when to initiate and when to terminate *hairy* expression by changing levels of morphogens. Of course, these three general possibilities are not mutually exclusive: ommatidial assembly could employ any combination of overlapping mechanisms.

Evidence for a strategy of the first kind is that one prepattern factor, *ro*, seems to regulate the expression of other genes required for proper ommatidial assembly (rather than just regulating structural genes such as rhodopsins). Rough represses *ato*, the R8-determining gene, in R2 and R5. Rough may also repress *svp*, an R3/4/1/6-determining gene, in R2 and R5 [167]. In R1 and R6, Rough is directly or indirectly required for BarH1 and H2 expression [132]. The phenotype of *Star* is greatly enhanced by loss of *ro* function, suggesting that Rough could regulate *Star* [106]. Expression of an enhancer detector in *rho* is lost in *ro* mutants, but since no genetic interaction between those two genes is seen, the relationship between *ro* and *rho* could be indirect [105]. Thus, regulated Rough expression likely not only specifies the fate of the cells in which it is expressed, but may also represent a component in a network of local interactions that ensures proper co-ordination of ommatidial assembly.

Other evidence suggests that postfurrow events are not all interdependent, or regulated by local interactions. Although cell cycles synchronized in G1 arrest anterior to the furrow, the final round of division among remaining unpatterned cells behind the furrow is apparently resynchronized, and under separate control [7, 40]. In mosaic clones lacking *da* function, the final round of cell division never occurs, although mitoses anterior to the furrow are unaffected [40]. Although consistent with roles for vertebrate *da* homologs in suppressing cell division [168], this result is strange in that by the time of the second mitosis, Daughterless has been excluded from those unpatterned cells, and is only expressed in R8. This suggests that prior expression of *da* is required for mitosis posterior to the furrow, which may be an example of intrinsic gene expression events serving to co-ordinate steps in ommatidial recruitment. However, this final division is not coupled to subsequent ommatidial differentiation. When this division is blocked, later patterning events still occur relatively normally: all cell types are specified, although there are not enough unpatterned cells available for completion of each ommatidium [169]. This could represent an example of cells independently responding to common external positional or temporal cues in different ways depending on their developmental state. It is not known whether positional information emanating from the furrow or some other common reference point co-ordinates postfurrow events in the same way as anterior events seem to be regulated. It would be difficult to discern any postfurrow functions for morphogens such as Dpp using mosaic clones, because mutant patches for TGF-β receptors have defects in patterning resulting from an inability to execute earlier steps such as co-ordinating G1 arrest. However, new technologies allow analysis of the requirements of postfurrow gene expression [15].

Also interesting is the role that systemic hormone signaling may play in co-ordinating postfurrow events. Mutant clones for Ultraspiracle (Usp), one partner in the heterodimeric ecdysone receptor complex, have aberrant cluster morphology, as do discs lacking Broad Complex proteins, required for amplification of the hormone signal within the cell [163, 164]. Although no ligand for the Svp nuclear receptor has been found, unlike many orphan nuclear receptors, Svp does require its ligand-binding domain to function [130]. Could global hormone titer regulate the activity of a localized prepattern transcription factor? Observations that Svp can disrupt the hormone-dependent ability of Usp to activate transcription in vitro, and that *usp* clones in the eye have defects in R7 formation, while in *svp* clones outer photoreceptors are transformed into R7s suggest that Svp may antagonize hormone-dependent activity [129, 170]. Much remains to be clarified about the role of hormone-dependent signaling on postfurrow ommatidial assembly, and whether it represents a timing mechanism.

Summary

Third-instar eye imaginal disc tissue can differentiate into photoreceptor clusters prior to the arrival of the furrow under certain mutant, misexpression, or explant conditions. However, when such precocious differentiation occurs, the normal precise array of retinal cell types is not achieved. This suggests that one important function of the furrow is to impose order on the differentiating retinal tissue. Signaling associated with the furrow appears to restrict the pace of differentiation events in at least two important ways. First, Hh-dependent expression of negatively acting HLH protein such as

Hairy far anterior to the furrow prevents Hh-dependent expression of Ato from expanding too fast anteriorly. Second, Dpp-dependent arrest of the cell cycle in G1 at the furrow ensures that cells are synchronized at this stage, and may be important to allow time for Notch-dependent interactions to occur and produce the proper pattern of R8 founders.

It is clear that extensive synchronization of differentiation events, such as the final mitosis and the successive rounds of recruitment of ommatidial members, continues far posterior to the furrow. Very little is known about how these events are temporally regulated. Although local interactions may co-ordinate some events, the distribution of developmental stages in distinct zones relative to the furrow raises the possibility that furrow-associated signaling provides spatial or temporal cues for events posterior as well as anterior to the furrow.

Furthermore, since Hh expression seems to be at the top of a signaling cascade driving furrow progression, it will be interesting to discover how Hh is regulated in the eye, and how its domain of expression is permitted to advance across the disc at the rate of one column every 2 h.

1 Wassarman D. A., Therrien M. and Rubin G. M. (1995) The Ras signaling pathway in *Drosophila*. Curr. Opin. Genet. Dev. **5:** 44–50

2 Strutt D. I., Wiersdorff V. and Mlodzik M. (1995) Regulation of furrow progression in the *Drosophila* eye by cAMP-dependent protein kinase A. Nature **373:** 705–709

3 Wiersdorff V., Lecuit T., Cohen S. M. and Mlodzik M. (1996) *Mad* acts downstream of Dpp receptors, revealing a differential requirement for *dpp* signaling in initiation and propagation of morphogenesis in the *Drosophila* eye. Development **122:** 2153–2162

4 Das P., Maduzia L. L., Wang H., Finelli A. L., Cho S.-H., Smith M. M. et al. (1998) The *Drosophila* gene *Medea* demonstrates the requirement for different classes of Smads in *dpp* signaling. Development **125:** 1519–1528

5 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

6 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

7 Horsfield J., Penton A., Secombe J., Hoffmann F. M. and Richardson H. (1998) *decapentaplegic* is required for arrest in G1 phase during *Drosophila* eye development. Development **125:** 5069–5078

8 Thomas B. J., Gunning D. A., Cho J. and Zipursky S. L. (1994) Cell cycle progression in the developing *Drosophila* eye: *roughex* encodes a novel protein required for the establishment of G1. Cell **77:** 1003–1014

9 Hay B. A., Wassarman D. A. and Rubin G. M. (1995) *Drosophila* homologs of baculovirus inhibitor of apoptosis proteins function to block cell death. Cell **83:** 1253–1262

10 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

11 Huang Z., Shilo B. Z. and Kunes S. (1998) A retinal axon fascicle uses *spitz*, an EGF receptor ligand, to construct a synaptic cartridge in the brain of *Drosophila*. Cell **95:** 693–703

12 Garrity P. A., Rao Y., Salecker I., McGlade J., Pawson T. and Zipursky S. L. (1996) *Drosophila* photoreceptor axon guidance and targeting requires the *dreadlocks* SH2/SH3 adapter protein. Cell **85:** 639–650

13 Papayannopoulos V., Tomlinson A., Panin V., Rauskolb C. and Irvine K. D. (1998) Dorsal-ventral signaling in the *Drosophila* eye. Science **281:** 2031–2034

14 Reifegerste R. and Moses K. (in press) The genetics of epithelial polarity and pattern in the *Drosophila* retina. Bioessays **21**

15 Pignoni F., Hu B., Zavitz K. H., Xiao J., Garrity P. A. and Zipursky S. L. (1997) The eye-specification proteins So and Eya form a complex and regulate multiple steps in *Drosophila* eye development. Cell **91:** 881–891

16 Halder G., Callaerts P., Flister S., Walldorf U., Kloter U. and Gehring W. J. (1998) Eyeless initiated the expression of both *sine oculis* and *eyes absent* during *Drosophila* compound eye development. Development **125:** 2181–2191

17 Chen R., Halder G., Zhang Z. and Mardon G. (1999) Signaling by the TGF-β homolog *decapentaplegic* functions reiteratively within the network of genes controlling retinal cell fate determination in *Drosophila*. Development **126:** 935–943

18 Hazelett D. J., Bourouis M., Walldorf U. and Treisman J. E. (1998) *decapentaplegic* and *wingless* are regulated by *eyes absent* and *eyegone* and interact to direct the pattern of retinal differentiation in the eye disc. Development **125:** 3741–3751

19 Halder G., Callaerts P. and Gehring W. J. (1995) New perspectives on eye evolution. Curr. Opin. Genet. Dev. **5:** 602–609

20 Xu P. X., Zhang X., Heaney S., Yoon A., Michelson A. M. and Maas R. L. (1999) Regulation of pax6 expression is conserved between mice and flies. Development **126:** 383–395

21 Wolff T. and Ready D. F. (1993) Pattern formation in the *Drosophila* retina. In: The Development of *Drosophila melanogaster*, vol. 2, pp. 1277–1325, Bate M. and Martinez-Arias A. (eds), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

22 Bowmaker J. K. (1991) The evolution of vertebrate visual pigments and photoreceptors. In: Evolution of the Eye and Visual System, vol. 2, pp. 63–81, Cronly-Dillon J. R. and Gregory R. L. (eds), CRC, Boston

23 Zuker C. S. (1996) The biology of vision of *Drosophila*. Proc. Natl. Acad. Sci. USA **93:** 571–576

24 Hardie R. C. (1985) Functional organization of the fly retina. Progr. Sensory Physiol. **5:** 1–79

25 Meinertzhagen I. A. (1991) Evolution of the cellular organization of the arthropod compound eye and optic lobe. In: Evolution of the Eye and Visual System, vol. 2, pp. 341–363, Cronly-Dillon, J. R. and Gregory R. L. (eds), CRC, Boston

26 Braitenberg V. (1967) Patterns of projection in the visual system of the fly. I. Retina-lamina projections. Exp. Brain Res. **3:** 271–298

27 Franceschini N. (1975) Sampling of the visual environment by the compound eye of the fly: fundamentals and applications. In: Photoreceptor Optics, pp. 98–125, Snyder A. W. and Menzel R. (eds), Springer, Heidelberg

28 Kunes S. and Steller H. (1993) Topography in the *Drosophila* visual system. Curr. Opin. Neurobiol. **3:** 53–59

29 Haynie J. L. and Bryant P. J. (1986) Development of the eye-antennal imaginal disc and morphogenesis of the adult head in *Drosophila melanogaster*. J. Exp. Zool. **237:** 293–308

30 Cohen S. M. (1993) Imaginal disc development. In: The Development of *Drosophila melanogaster*, vol. 2, pp. 747–842, Bate M. and Martinez-Arias A. (eds), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

31 Weismann A. (1864) Die nachembryonale Entwicklung der Musciden nach Beobachtungen an *Musca vomitoria* und *Sarcophaga carnaria*. Z. wiss. Zool. **14:** 187–336
32 Melamed J. and Trujillo-Cenóz O. (1975) The fine structure of the eye imaginal disks in muscoid flies. J. Ultrastruct. Res. **51:** 79–93
33 Campos-Ortega J. A. (1980) On compound eye development in *Drosophila melanogaster*. Curr. Topics Dev. Biol. **15:** 347–371
34 Ready D. F., Hanson T. E. and Benzer S. (1976) Development of the *Drosophila* retina, a neurocrystalline lattice. Dev. Biol. **53:** 217–240
35 Basler K. and Hafen E. (1989) Dynamics of *Drosophila* eye development and temporal requirements of sevenless expression. Development **107:** 723–731
36 Lawrence P. A. and Green S. M. (1979) Cell lineage in the developing retina of *Drosophila*. Dev. Biol. **71:** 142–152
37 Tomlinson A. and Ready D. F. (1987) Cell fate in the *Drosophila* ommatidium. Dev. Biol. **123:** 264–275
38 Caudy M., Vaessin H., Brand M., Tuma R., Jan L. Y. and Jan Y. N. (1988) *daughterless*, a gene essential for both neurogenesis and sex determination in *Drosophila* has sequence similarities to myc and the achaete-scute complex. Cell **55:** 1061–1067
39 Ellis H. M., Spann D. R. and Posakony J. W. (1990) *extramacrochaetae*, a negative regulator of sensory organ development in *Drosophila*, defines a new class of helix-loop-helix proteins. Cell **61:** 27–38
40 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
41 Jarman A. P., Sun Y., Jan L. Y. and Jan Y. N. (1995) Role of the proneural gene, *atonal*, in formation of the *Drosophila* chordotonal organs and photoreceptors. Development **121:** 2019–2030
42 Ghysen A. and Dambly-Chaudiere C. (1989) Genesis of the *Drosophila* peripheral nervous system. Trends Genet. **5:** 251–255
43 Jan Y. N. and Jan L. Y. (1994) Neuronal cell fate specification in *Drosophila*. Curr. Opin. Neurobiol. **4:** 8–13
44 Murre C., McCaw P. S., Vaessin H., Caudy M., Jan L. Y., Jan Y. N. et al. (1989) Interactions between heterologous helix-loop-helix proteins generate complexes that bind specifically to a common DNA sequence. Cell **58:** 537–544
45 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
46 Xu T. and Rubin G. (1993) Analysis of genetic mosaics in developing and adult *Drosophila* tissues. Development **117:** 1223–1237
47 Morgan T. H. (1914) Mosaics and gynandromorphs in *Drosophila*. Proc. Soc. Exp. Biol. Med. **11:** 171–172
48 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
49 Van Doren M., Bailey A. M., Esnayra J., Ede K. and Posakony J. W. (1994) Negative regulation of proneural gene activity: hairy is a direct transcriptional repressor of achaete. Genes Dev. **8:** 2729–2742
50 Fisher A. and Caudy M. (1998) The function of hairy-related bHLH repressor proteins in cell fate decisions. Bioessays **20:** 298–306
51 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
52 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
53 Wharton K. A., Johansen K. M., Xu T. and Artavanis-Tsakonas S. (1985) Nucleotide sequence from the neurogenic locus *notch* implies a gene product that shares homology with proteins containing EGF-like repeats. Cell **43:** 567–581
54 Kimble J. and Simpson P. (1997) The LIN-12/Notch signaling pathway and its regulation. Annu. Rev. Cell. Dev. Biol. **13:** 333–361
55 Rooke J. E. and Xu T. (1998) Positive and negative signals between interacting cells for establishing neural fate. Bioessays **20:** 209–214
56 Kidd S., Baylies M. K., Gasic G. P. and Young M. W. (1989) Structure and distribution of the Notch protein in developing *Drosophila*. Genes Dev. **3:** 1113–1129
57 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
58 Johansen K. M., Fehon R. G. and Artavanis-Tsakonas S. (1989) The *Notch* gene product is a glycoprotein expressed on the cell surface of both epidermal and neuronal precersor cells during *Drosophila* development. J. Cell. Biol. **109:** 2427–2440
59 Fehon R. G., Johansen K., Rebay I. and Artavanis-Tsakonas S. (1991) Complex cellular and subcellular regulation of *notch* expression during embryonic and imaginal development of *Drosophila*: implications for *notch* function. J. Cell. Biol. **113:** 657–669
60 Baker N. E. and Yu S.-Y. (1997) Proneural function of neurogenic genes in the developing *Drosophila* eye. Curr. Biol. **7:** 122–132
61 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
62 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
63 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
64 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
65 Simpson P. (1997) Notch signalling in development: on equivalence groups and asymmetric developmental potential. Curr. Opin. Genet. Dev. **7:** 537–542
66 Vässin H., Bremer K. A., Knust E. and Campos-Ortega J. A. (1987) The neurogenic gene *Delta* of *Drosophila melanogaster* is expressed in neurogenic territories and encodes a putative transmembrane protein with EGF-like repeats. EMBO J. **6:** 3431–3440
67 Kopczynski C. C., Alton A. K., Fechtel K., Kooh P. J. and Muskavitch M. A. (1988) *Delta*, a *Drosophila* neurogenic gene, is transcriptionally complex and encodes a protein related to blood coagulation factors and epidermal growth factor of vertebrates. Genes Dev. **2:** 1723–1735
68 Parks A. L., Turner F. R. and Muskavitch M. A. T. (1995) Relationships between complex Delta expression and the specification of retinal cell fates during *Drosophila* eye development. Mech. Dev. **50:** 201–216
69 Parody T. R. and Muskavitch M. A. (1993) The pleiotropic function of Delta during postembryonic development of *Drosophila melanogaster*. Genetics **135:** 527–539
70 Jan Y. N. and Jan L. Y. (1993) The peripheral nervous system. In: The Development of *Drosophila melanogaster*, vol. 2, pp. 1207–1244, Bate M. and Martinez-Arias A. (eds), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
71 Heitzler P. and Simpson P. (1991) The choice of cell fate in the epidermis of *Drosophila*. Cell **64:** 1083–1092
72 Baker N. E., Mlodzik M. and Rubin G. M. (1990) Spacing differentiation in the developing *Drosophila* eye: a fibrino-

gen-related lateral inhibitor encoded by *scabrous*. Science **250:** 1370–1376
73 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
74 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
75 Lee E.-C., Hu 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
76 Lee E. C. and Baker N. E. (1996) GP300SCA is not a high affinity Notch ligand. Biochem. Biophys. Res. Commun. **225:** 720–725
77 Rabinow L. and Birchler J. A. (1990) Interactions of *vestigial* and *scabrous* with the *Notch* locus of *Drosophila melanogaster*. Genetics **125:** 41–50
78 Rooke J., Pan D., Xu T. and Rubin G. M. (1996) Kuz, a conserved metalloprotease-disintegrin protein with two roles in *Drosophila* neurogenesis. Science **273:** 1227–1231
79 Qi H., Rand M. D., Wu X., Sestan N., Wang W., Rakic P. et al. (1999) Processing of the Notch ligand Delta by the metalloprotease Kuzbanian. Science **283:** 91–94
80 Sun X. and Artavanis-Tsakonas S. (1996) The intracellular deletions of Delta and Serrate define dominant negative forms of the *Drosophila* Notch ligands. Development **122:** 2465–2474
81 Sun X. and Artavanis-Tsakonas S. (1997) Secreted forms of Delta and Serrate define antagonists of Notch signaling in *Drosophila*. Development **124:** 3439–3448
82 Cagan R. L. and Ready D. F. (1989) The emergence of order in the *Drosophila* pupal retina. Dev. Biol. **136:** 346–362
83 Domínguez M., Wasserman J. D. and Freeman M. (1998) Multiple functions of the EGF receptor in *Drosophila* eye development. Curr. Biol. **8:** 1039–1048
84 Kumar J. P., Tio M., Hsiung F., Akopyan S., Gabay L., Seger R. et al. (1998) Dissecting the roles of the *Drosophila* EGF receptor in eye development and MAP kinase activation. Development **125:** 3875–3885
85 Lesokhin A., Yu S.-Y., Katz J. and Baker N. (1998) Several levels of EGF receptor signaling during photoreceptor specification in wild-type, *Ellipse*, and null mutant *Drosophila*. Dev. Biol. **205:** 129–144
86 Freeman M. (1996) Reiterative use of the EGF receptor triggers differentiation of all cell types in the *Drosophila* eye. Cell **87:** 651–660
87 Rutledge B. J., Zhang K., Bier E., Jan Y. N. and Perrimon N. (1992) The *Drosophila spitz* gene encodes a putative EGF-like growth factor involved in dorsal-ventral axis formation and neurogenesis. Genes Dev. **6:** 1503–1517
88 Tio M. and Moses K. (1997) The *Drosophila* TGFα homolog Spitz acts in photoreceptor recruitment in the developing retina. Development **124:** 343–351
89 Tio M., Ma C. and Moses K. (1994) *spitz*, a *Drosophila* homolog of transforming growth factor-α, is required in the founding photoreceptor cells of the compound eye facets. Mech. Dev. **48:** 13–23
90 Freeman M. (1997) Cell determination strategies in the *Drosophila* eye. Development **124:** 261–270
91 Kretzschmar D., Brunner A., Wiersdorff V., Pflugfelder G. O., Heisenberg M. and Schneuwly S. (1992) *Giant lens*, a gene involved in cell determination and axon guidance in the visual system of *Drosophila melanogaster*. EMBO J. **11:** 2531–2539
92 Freeman M., Klämbt C., Goodman C. S. and Rubin G. M. (1992) The *argos* gene encodes a diffusible factor that regulates cell fate decisions in the *Drosophila* eye. Cell **69:** 963–975
93 Schweitzer R., Howes R., Smith R., Shilo B.-Z. and Freeman M. (1995) Inhibition of *Drosophila* EGF receptor activation by the secreted protein Argos. Nature **376:** 699–702
94 Golembo M., Schweitzer R., Freeman M. and Ben-Zion S. (1996) *argos* transcription is induced by the *Drosophila* EGF receptor pathway to form an inhibitory feedback loop. Development **122:** 223–230
95 Freeman M. (1994) Misexpression of the *Drosophila argos* gene, a secreted regulator of cell determination. Development **120:** 2297–2304
96 Fischer-Vize J. A., Rubin G. M. and Lehmann R. (1992) The *fat facets* gene is required for *Drosophila* eye and embryo development. Development **116:** 985–1000
97 Huang Y., Baker R. T. and Fischer-Vize J. A. (1995) Control of cell fate by a deubiquitinating enzyme encoded by the *fat facets* gene. Science **270:** 1828–1831
98 Huang Y. and Fischer-Vize J. A. (1996) Undifferentiated cells in the developing *Drosophila* eye influence facet assembly and require the Fat facets ubiquitin-specific protease. Development **122:** 3207–3216
99 Isaksson A., Peverali F. A., Kockel L., Mlodzik M. and Bohmann D. (1997) The deubiquitination enzyme fat facets negatively regulates RTK/Ras/MAPK signalling during *Drosophila* eye development. Mech. Dev. **68:** 59–67
100 Schweitzer R., Shaharabany M., Seger R. and Shilo B.-Z. (1995) Secreted Spitz triggers the DER signaling pathway and is a limiting component in embryonic ventral ectoderm determination. Genes Dev. **9:** 1518–1529
101 Bier E., Jan L. Y. and Jan Y. N. (1990) *rhomboid*, a gene required for dorsoventral axis establishment and peripheral nervous system development in *Drosophila melanogaster*. Genes Dev. **4:** 190–203
102 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
103 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
104 Golembo M., Raz E. and Shilo B. Z. (1996) The *Drosophila* embryonic midline is the site of Spitz processing, and induces activation of the EGF receptor in the ventral ectoderm. Development **122:** 3363–3370
105 Freeman M., Kimmel B. E. and Rubin G. M. (1992) Identifying targets of the *rough* homeobox gene of *Drosophila*: evidence that *rhomboid* functions in eye development. Development **116:** 335–346
106 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
107 Heberlein U., Hariharan I. K. and Rubin G. M. (1993) *Star* is required for neuronal differentiation in the *Drosophila* retina and displays dosage-sensitive interactions with *Ras 1*. Dev. Biol. **160:** 51–63
108 Sturtevant M. A., Roark M., O'Neill J. W., Biehs B., Colley N. and Bier E. (1996) The *Drosophila* Rhomboid protein is concentrated in patches at the apical cell surface. Dev. Biol. **174:** 298–309
109 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
110 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
111 Hart A. C., Krämer 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
112 Krämer 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

113 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

114 Van Vactor D. L., 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 **67:** 1145–1155

115 Rubin G. M. (1989) Development of the *Drosophila* retina: inductive events studied at single cell resolution. Cell **57:** 519–520

116 Diaz-Benjumea F. J. and Hafen E. (1994) The *sevenless* signalling cassette mediates *Drosophila* EGF receptor function during epidermal development. Development **120:** 569–578

117 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

118 Dickson B., Sprenger F. and Hafen E. (1992) Prepattern in the developing *Drosophila* eye revealed by an activated torso-sevenless chimeric receptor. Genes Dev. **6:** 2327–2339

119 Bray S. (1998) A Notch affair. Cell **93:** 499–503

120 Kumar J. and Moses K. (1997) Transcription factors in eye development: a gorgeous mosaic? Genes Dev. **11:** 2023–2028

121 Dickson B. (1998) Photoreceptor development: breaking down the barriers. Curr. Biol. **8:** R90–R92

122 Dickson B. (1995) Nuclear factors in sevenless signalling. Trends Genet. **11:** 106–111

123 Cagan R. (1993) Cell fate specification in the developing *Drosophila* retina. Development [**Suppl**]: 19–28

124 Saint R., Kalionis B., Lockett T. J. and Elizur A. (1988) Pattern formation in the developing eye of *Drosophila melanogaster* is regulated by the homoeo-box gene, *rough*. Nature **334:** 151–154

125 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

126 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

127 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

128 Basler K., Yen D., Tomlinson A. and Hafen E. (1990) Reprogramming cell fate in the developing *Drosophila* retina: transformation of R7 cells by ectopic expression of *rough*. Genes Dev. **4:** 728–739

129 Mlodzik M., Hiromi Y., Weber U., Goodman C. S. and Rubin G. M. (1990) The *Drosophila seven-up* gene, a member of the steroid receptor superfamily, controls photoreceptor cell fates. Cell **60:** 211–224

130 Hiromi Y., Mlodzik M., West S. R., Rubin G. M. and Goodman C. S. (1993) Ectopic expression of *seven-up* causes cell fate changes during ommatidial assembly. Development **118:** 1123–1135

131 Kramer S., West S. R. and Hiromi Y. (1995) Cell fate control in the *Drosophila* retina by the orphan receptor seven-up: its role in the decisions mediated by the ras signaling pathway. Development **121:** 1361–1372

132 Higashijima S., Kojima T., Michiue T., Ishimaru S., Emori Y. and Saigo K. (1992) Dual *Bar* homeo box genes of *Drosophila* required in two photoreceptor cells, R1 and R6, and primary pigment cells for normal eye development. Genes Dev. **6:** 50–60

133 Hayashi T., Kojima T. and Saigo K. (1998) Specification of primary pigment cell and outer photoreceptor fates by *BarH1* homeobox gene in the developing *Drosophila* eye. Dev. Biol. **200:** 131–145

134 Weber U., Siegel V. and Mlodzik M. (1995) *pipsqueak* encodes a novel nuclear protein required downstream of *seven-up* for the development of photoreceptors R3 and R4. EMBO J. **14:** 6247–6257

135 Daga A., Karlovich C. A., Dumstrei K. and Banerjee U. (1996) Patterning of cells in the *Drosophila* eye by Lozenge, which shares homologous domains with AML1. Genes Dev. **10:** 1194–1205

136 Flores G. V., Daga A., Kalhor H. R. and Banerjee U. (1998) Lozenge is expressed in pluripotent precursor cells and prepatterns multiple cell types in the *Drosophila* eye through the control of cell-specific transcription factors. Development **125:** 3681–3687

137 Lebovitz R. M. and Ready D. F. (1986) Ommatidial development in *Drosophila* eye disc fragments. Dev. Biol. **17:** 663–671

138 Nellen D., Burke R., Struhl G. and Basler K. (1996) Direct and long-range action of a Dpp morphogen gradient. Cell **85:** 357–368

139 Zecca M., Basler K. and Struhl G. (1996) Direct and long-range action of a Wingless morphogen gradient. Cell **87:** 833–844

140 Struhl G., Barbash D. A. and Lawrence P. A. (1997) Hedgehog acts by distinct gradient and signal relay mechanisms to organise cell type and cell polarity in the *Drosophila* abdomen. Development **124:** 2155–2165

141 Dierick H. and Bejsovec A. (1999) Cellular mechanisms of Wingless/Wnt signal transduction. Curr. Top. Dev. Biol. **43:** 153–190

142 Strigini M. and Cohen S. M. (1997) A Hedgehog activity gradient contributes to AP axial patterning of the *Drosophila* wing. Development **124:** 4697–4705

143 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

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

145 Pan D. and Rubin G. (1995) cAMP-dependent protein kinase and *hedgehog* act antagonistically in regulating *decapentaplegic* transcription in *Drosophila* imaginal discs. Cell **80:** 543–552

146 Heberlein U., Wolff T. and Rubin G. M. (1993) 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

147 Perrimon N. (1995) Hedgehog and beyond. Cell **80:** 517–520

148 Burke R. and Basler K. (1997) Hedgehog signaling in *Drosophila* eye and limb development – conserved machinery, divergent roles? Curr. Opin. Neurobiol. **7:** 55–61

149 Heberlein U. and Moses K. (1995) Mechanisms of *Drosophila* retinal morphogenesis: the virtues of being progressive. Cell **81:** 987–990

150 Domínguez M. and Hafen E. (1997) Hedgehog directly controls initiation and propagation of retinal differentiation in the *Drosophila* eye. Genes Dev. **11:** 3254–3264

151 Strutt D. I. and Mlodzik M. (1997) Hedgehog is an indirect regulator of morphogenetic furrow progression in the *Drosophila* eye disc. Development **124:** 3233–3240

152 Burke R. and Basler K. (1996) Hedgehog-dependent patterning in the *Drosophila* eye can occur in the absence of Dpp signaling. Dev. Biol. **179:** 360–368

153 Penton A., Selleck S. B. and Hoffman F. M. (1997) Regulation of cell cycle synchronization by *decapentaplegic* during *Drosophila* eye development. Science **275:** 203–206

154 Chanut F. and Heberlein U. (1997) Role of *decapentaplegic* in initiation and progression of the morphogenetic furrow in the developing *Drosophila* retina. Development **124:** 559–567

155 Pignoni F. and Zipursky S. L. (1997) Induction of *Drosophila* eye development by Decapentaplegic. Development **124:** 271–278

156 Ma C. and Moses K. (1995) *wingless* and *patched* are negative regulators of the morphogenetic furrow and can affect tissue polarity in the developing *Drosophila* compound eye. Development **121:** 2279–2289

157 Royet J. and Finkelstein R. (1997) Establishing primordia in the *Drosophila* eye-antennal imaginal disc: the role of *decapentaplegic*, *wingless* and *hedgehog*. Development **124:** 4793–4800
158 Treisman J. E. and Rubin G. M. (1995) *wingless* inhibits morphogenetic furrow movement in the *Drosophila* eye disc. Development **121:** 3519–3527
159 Khalsa O., Yoon J.-W., Torres-Schumann S. and Wharton K. A. (1998) TGF-b/BMP superfamily members, Gbb-60A and Dpp, cooperate to provide pattern information and establish cell identity in the *Drosophila* wing. Development **125:** 2723–2734
160 Pardee A. B. (1989) G1 events and regulation of cell proliferation. Science **246:** 603–608
161 Thummel C. S. (1996) Flies on steroids – *Drosophila* metamorphosis and the mechanisms of steroid hormone action. Trends Genet. **12:** 306–310
162 Li C. and Meinertzhagen I. A. (1995) Conditions for the primary culture of eye imaginal discs from *Drosophila melanogaster*. J. Neurobiol. **28:** 363–380
163 Zelhof A. C., Ghbeish N., Tsai C., Evans R. M. and McKeown M. (1997) A role for Ultraspiracle, the *Drosophila* RXR, in morphogenetic furrow movement and photoreceptor cluster formation. Development **124:** 2499–2506
164 Brennan C. A., Ashburner M. and Moses K. (1998) Ecdysone pathway is required for furrow progression in the developing *Drosophila* eye. Development **125:** 2653–2664
165 Champlin D. T. and Truman J. W. (1998) Ecdysteroids govern two phases of eye development during metamorphosis of the moth, *Manduca sexta*. Development **125:** 2009–2018
166 Reh T. A. and Cagan R. L. (1994) Intrinsic and extrinsic signals in the developing vertebrate and fly eyes: viewing vertebrate and invertebrate eyes in the same light. Perspect. Dev. Neurobiol. **2:** 183–190
167 Treisman J. E. and Rubin G. M. (1996) Targets of glass regulation in the Drosophila eye disc. Mech. Dev. **56:** 17–24
168 Hassan B. and Vaessin H. (1997) Daughterless is required for the expression of cell cycle genes in peripheral nervous system precursors of *Drosophila* embryos. Dev. Genet. **21:** 117–122
169 Nooij J. C. de and Hariharan I. K. (1995) Uncoupling cell fate determination from patterned cell division in the *Drosophila* eye. Science **270:** 983–985
170 Zelhof A. C., Yao T.-P., Chen J. D., Evans R. M. and McKeown M. (1995) Seven-up inhibits Ultraspiracle-based signaling pathways in vitro and in vivo. Mol. Cell. Biol. **15:** 6736–6745