Specification of cell fate in the developing eye of Drosophila

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Summary

Determination of cell fate in the developing eye of Drosophila depends on cellular interactions. In the eye imaginal disc, an initially unpatterned epithelial sheath of cells, single cells are specified in regular intervals to become the R8 photoreceptor cells. Genes such as Notch and scabrous participate in this process suggesting that specification of ommatidial founder cells and the formation of bristles in the adult epidermis involve a similar mechanism known as lateral inhibition. The subsequent steps of ommatidial assembly involve a different mechanism: undetermined cells read their position based on the contacts they make with neighbors that have already begun to differentiate. The development of the R7 photoreceptor cell is best understood. The key role seems to be played by sevenless, a receptor tyrosine kinase on the surface of the R7 precursor. It transmits the positional information - most likely encoded by boss on the neighboring R8 cell membrane - into the cell via its tyrosine kinase that activates a signal transduction cascade. Two components of this cascade - Sos and sina - have been identified genetically. sina encodes a nuclear protein whose expression is not limited to R7. Constitutive activation of the sevenless kinase by overexpression results in the diversion of other ommatidial cells into the R7 pathway, suggesting that activation of the sevenless signalling pathway is sufficient to specify R7 development.

Key words: Drosophila, ommatidia, R7 photoreceptor cell, sevenless signalling pathway.

Introduction

Cell–cell interactions play an important role in the specification of cell fate in both vertebrates and invertebrates. In genetically well characterized organisms such as Drosophila and Caenorhabditis elegans, recent identification and molecular characterization of genes involved in these interactions have started to uncover the molecular mechanisms of position-dependent cell fate determination. In this article we will review the progress made towards the understanding of cell fate determination in the compound eye of Drosophila.

The developing eye of Drosophila is well suited to study position-dependent determination of cell fate because the different cell types develop independently of lineage restrictions (Ready et al. 1976; Lawrence and Green, 1979). Furthermore, in contrast to developmental decisions taken during early embryonic stages where often groups of cells or entire germ layers are induced to follow a certain developmental pathway, in the development of the eye, individual neighboring cells adopt distinct developmental fates. Due to the repetitive nature and the precise order with which patterning in the eye imaginal disc occurs, cell fate decisions can be analyzed at the single cell level (Tomlinson and Ready, 1987a). Furthermore in genetic mosaics the cellular requirements for genes involved in cell fate decisions can be determined with single cell resolution.

The eye consists of a hexagonal array of approximately 800 facets or ommatidia (Fig. 1A). Each ommatidium is composed of 8 photoreceptor cells and 12 accessory cells (Fig. 1B,C). The photoreceptor cells can be grouped into three functional classes (R1–R6, R7, and R8) based on morphology, axon projection pattern and spectral sensitivity. Each photoreceptor cell possesses a microvillar stack of membranes, called the rhabdomere, where the photopigments reside. The position and the size of the rhabdomere is one of the morphological features distinguishing the three different classes of photoreceptor cells. The rhabdomeres of the photoreceptors R1 to R6 form an asymmetric trapezoid. The rhabdomere of R7 is smaller than the R1–6 rhabdomeres and occupies a central position in the distal part of the ommatidium. The R8 rhabdomere is located below R7. The cluster of eight photoreceptor cells is surrounded by pigment cells that optically insulate the unit. Four cone cells lie above the photoreceptor cells and secrete the central part of the lens (Fig. 1B).

The stereotyped arrangement of cell types in the ommatidia is generated during the last larval and the
pupal stage. Patterning starts at the posterior margin of the eye imaginal disc, which prior to this stage consists of a single layer epithelium of dividing unpatterned cells (Ready et al. 1976). Closely associated with the initiation of pattern formation is a morphological indentation in the disc - the morphogenetic furrow - which moves across the disc epithelium in an anterior direction. In the furrow, individual cells spaced by approximately seven cells assume a neural fate and will become the R8 photoreceptor cells. These cells are the founder cells for each ommatidial cluster. The other ommatidial cells become integrated in a fixed sequence: first R2 and R5, followed by R3 and R4, R1 and R6, and finally R7 is added (Fig. 2A,B). At a later stage the cone cells follow and finally the pigment cells are added (Tomlinson and Ready, 1987a).

**Specification of R8 cells involves lateral inhibition**

The regularity with which the ommatidial units are spaced in the adult eye is initiated by the specification of R8 cells in the furrow. This process appears to be different from the specification of subsequent cell types. Whereas it is assumed that all other cell types develop as a consequence of their direct contacts with neighboring cells that have been determined earlier, specification of R8 cells occurs in the absence of any previously differentiated cells in the disc. Nevertheless they appear to be spaced at regular intervals as early as they express the neural antigens (Tomlinson and Ready, 1987b: Fig. 2A). Mutations in four different genes, scabrous (sca), retina-aberrant-in-pattern (rap), Notch, and Ellipse (Elp) have been shown to affect R8 cell specification. Initiation of cluster formation in the morphogenetic furrow is irregular in scabrous mutant discs (Baker et al. 1990). A similar phenotype is observed in rap mutants (Karpilov et al. 1989). Mosaic analysis with both sca and rap indicates that both genes are exclusively required in R8 cells for correct ommatidial assembly (Baker et al. 1990; Karpilov et al. 1989). Experiments with a temperature sensitive allele of Notch indicate that in the absence of functional Notch product in the morphogenetic furrow, too many precursor cells enter a neural pathway (Cagan and Ready, 1989). In contrast, dominant gain-of-function mutations in the gene of the Drosophila EGF receptor, called Ellipse (Elp), result in the opposite phenotype - only very few cells enter the neural pathway (Baker and Rubin, 1990). Notch encodes a cell surface protein with EGF-like repeats and is homologous to the lin-12 gene product in Caenorhabditis elegans (Wharton et al. 1985; Greenwald. 1985). Both Notch and lin-12 have been shown to be involved in a number of different developmental decisions that involve cell-cell interactions. In the differentiation of bristles in Drosophila, Notch appears to act as a receptor for an inhibitory signal sent out by the cell that has adopted the neural fate (Simpson. 1990). A similar function has been described for lin-12 in vulval development (Seydoux and Greenwald, 1989). sca encodes a putative secreted factor that is expressed ubiquitously in the furrow but becomes restricted to the R8 cells very rapidly (Mlodzik et al. 1990a). Genetic interactions between scabrous and a hypomorphic allele of Notch, split, suggest that these gene products might act in the same pathway. Similar to Notch, sca also affects the determination of bristles in the adult cuticle (Mlodzik et al. 1990a). It is therefore likely that the specification of R8 cells occurs by mechanisms similar to those described for bristle development (Simpson, 1990). Initially small differences in the amount of receptor and signal produced by groups of multipotent cells are increased by autoregulatory feedback loops such that the cell producing more signal will inhibit its neighbors from entering the neuronal pathway (Simpson, 1990). This inhibitory mechanism can act over more than one cell diameter if proteins, as is the case for sca, are diffusible. Lateral inhibition might at least in part be responsible for the regular spacing of the ommatidial units (Mlodzik et al. 1990a).

**Specification of the fate of the other photoreceptor cells depends on inductive interactions between neighboring cells**

In contrast to the specification of R8 cells which probably depends on signals passing over more than one cell diameter, the specification of subsequent cell types proceeds autonomously in each unit. In Elp mutant eyes where only few ommatidial units are formed, normal clusters form even when completely isolated from other clusters (Baker and Rubin, 1989). Tomlinson and Ready (1987a) proposed a model in which new cells read positional information encoded in the contacts they make with cells that have been determined previously. R2 and R5 contact only R8, whereas R3 and R4 are in contact with both R8 and R2 or R5, which at that time have already begun to differentiate. R7, the last photoreceptor cell, can be identified by its contacts with R8 and with R1 and R6 (Fig. 2A,B). The fate of the ommatidial cells might therefore be determined by a combinatorial code of cell contacts (Tomlinson and Ready, 1987a).

**Determination of the outer photoreceptor cells R1–R6**

So far two genes, seven-up (svp) and rough, have been identified that are involved in the specification of the fate of the R1–R6 cells. Mutations in rough lead to an early disruption of the assembly (Tomlinson et al. 1988). Whereas R2/5 initiate neural development normally in rough mutants the specification of R3/4 is impaired. Analysis of genetic mosaics indicates that the rough gene product is only required in R2 and R5 for correct ommatidial development (Tomlinson et al. 1988). Therefore rough appears to act on the signalling side of the R3/4 pathway. Molecular characterization of rough
indicated that it encodes a homeodomain protein and not a membrane-bound or secreted protein (Tomlinson et al. 1988). It has been proposed that rough controls the production of an inducing signal in R2 and R5 for the specification of the R3/4 cell fate. Recent studies using ectopic expression of rough, however, point to a more central role of rough in the specification of cell identity in the cells where it is expressed. When rough is expressed ectopically in the R7 precursor, this cell frequently develops into an outer photoreceptor cell type and not into an R7 cell (Basler et al. 1990; Kimmel et al. 1990). This indicates that rough specifies R2/5 cell identity and that the failure to gain R2/5 identity in rough mutants prevents the precursors for R3 and R4 from recognizing their position.

The svp gene encodes a nuclear protein with high homology to the family of steroid receptors (Mlodzik et al. 1990b). Mutations in the svp gene are lethal and the lethal embryos exhibit defects in the central nervous system. Mutant cell clones in the eye show incorrect differentiation of R3/4 and R1/6 into R7-like photoreceptors. It has been proposed that svp functions to suppress R7 cell fate in R3/4 and R1/6 (Mlodzik et al. 1990b). One form of svp-up protein has a conserved ligand-binding domain. It is unclear, however, whether its function in the specification of photoreceptor cell fate depends on ligand-binding, since svp similar to rough is expressed only in the cells where it is required. Therefore expression of svp and rough can be viewed as a first consequence of the determination of these cells.

**Determination of the R7 photoreceptor cell: a signalling pathway unfolds**

In contrast to the substantial disruption of ommatidial development observed in mutations affecting cell fate decisions during the early steps of assembly, mutations preventing R7 development mostly do not alter the recruitment of subsequent cells. Furthermore, since R7 cells contain specific u.v.-sensitive photopigments they can be identified biochemically (Zuker et al. 1987) and, based on their function as u.v. receptors, in a behavioral assay (Harris et al. 1976). This has permitted the isolation of mutations that specifically prevent the development of the R7 cell. So far four genes have been identified that affect this pathway – sevenless (sev), bride-of-sevenless (boss), Son-of-sevenless (Sos), and seven-in-absentia (sina) (Harris et al. 1976). Mosaic analyses indicate non-autonomy for boss (Reinke and Zipursky, 1988), but autonomy for the remaining three genes sev (Campos-Ortega et al. 1979), Sos (Rogge et al. 1991) and sina (Carthew and Rubin, 1990). This suggests that boss acts on the signalling side of the pathway whereas sev, Sos and sina function in the R7 precursor in the reception and interpretation of the positional information.

The boss gene has been cloned, sequenced and shown to encode a protein with seven putative membrane-spanning domains and a large extracellular domain. Although the boss protein sequence lacks significant homology with any known protein, its overall structure based on the hydropathy profile is similar to the G protein-coupled receptors (Hart et al. 1990). Its exclusive requirement in R8, together with the fact that it is a membrane bound protein, suggests that it might act directly as an inducing signal or that it indirectly controls the production of a signal (Reinke and Zipursky, 1988).

The sev gene encodes a receptor tyrosine kinase (Hafen et al. 1987; Basler and Hafen, 1988; Bowtell et al. 1988). The sev protein is transiently expressed in a subpopulation of ommatidial precursor cells but is exclusively required in R7 (Tomlinson et al. 1987). sev most likely acts as a receptor for an R7-inducing signal. Binding of the signal to the extracellular domain of sev could result in the activation of the tyrosine kinase by which an intracellular signal transduction cascade is activated.

Sos, isolated as a dominant suppressor of a hypomorphic sev allele, acts downstream of sev in the signal transduction cascade. Loss-of-function mutations of Sos are homozygous lethal, but certain surviving heteroallelic combinations can cause a sevenless-like phenotype, indicating that the wild-type Sos gene product participates in R7 development (Rogge et al. 1991).

Finally, sina encodes a nuclear protein that is expressed in a similar subpopulation of cells to sev (Carthew and Rubin, 1990). The lack of functional sina product in R7 prevents R7 formation. Its nature as a nuclear protein that is expressed in more than just the cells where it is required makes it a good candidate for a gene product that is modified by an activated signal transduction cascade. Although all these four genes affect R7 development, it has not yet been demonstrated whether these genes act in a single pathway.

**How is the R7 cell fate specified so accurately?**

The central R8 photoreceptor cell which produces an R7 inducing signal – possibly the boss protein – is contacted by all the other photoreceptor cells. Why then is the cell in the position of the R7 precursor the only cell that develops into an R7 cell? There are at least three alternative models by which the observed specificity could be accomplished. (1) In the first model which is based on the combinatorial model proposed by Tomlinson and Ready (1987a), more than one signal is required to specify R7 identity. In addition to a signal from R8 to R7, there might be another signal from R1/6 to R7 and only the combination of the two specifies R7 fate. (2) R7 cell fate is specified by only one signal from R8 but this signal is spatially restricted on the surface of the R8 cell such that it is only accessible for the R7 precursor. (3) Restriction of the signal is not spatial but temporal, such that it is not expressed on the surface of R8 before the R7 precursor becomes determined.

To address these questions we have investigated the role of the sev protein in the specification of cell fate. First we have tested the role of the tyrosine kinase
domain by changing the conserved lysine in the putative ATP-binding site of the catalytic domain into a methionine. sev function is completely abolished by this single amino acid change, suggesting that kinase activity is a critical component in the R7 determination (Basler and Hafen, 1988). To test whether the spatially and temporally restricted expression of the sev protein contributes to the decision as to where R7 cells are formed, sev has been expressed under the control of the heat shock promoter in all cells at different stages of development. The ubiquitous presence of the sev protein leads to the correct specification of R7 cells in a sev mutant background (Basler and Hafen, 1989; Bowtell et al. 1989). Therefore the choice as to where R7 cells form does not depend on the distribution of the receptor. The decision must either depend on the restricted presentation of the sev ligand, or other signals are required in addition to the activation of sev for the specification of R7 cells.

To distinguish between a combinatorial and a single–signal mechanism for R7 determination we sought to construct a sev gain-of-function mutation (SevSll) that is constitutively active, independent of ligand stimulation. We achieved this by overexpressing a sev protein truncated at the N terminus (sev-Sll). Since overexpression was accomplished by the duplication of the sev enhancer fragment that controls the temporal and spatial expression pattern of sev, the time when and the cells where this truncated sev protein is expressed were left unchanged. The shortened protein was produced at a higher rate than in wild type (Basler et al. 1991).

**Position-independent recruitment of supernumerary R7 photoreceptor cells by constitutive sevenless tyrosine kinase activity**

Introduction of the sev-Sll construct into sev mutant
Fig. 1. Structure of the compound eye of *Drosophila*. (A) Scanning electron micrograph of the left eye of a wild-type fly. (B) Schematic view of an ommatidial unit. A longitudinal section is shown on the left and cross sections at three different levels are shown on the right. Histological cross sections through the distal region of a wild-type eye (C) and a *sevenless* mutant eye (D) are shown. A, photoreceptor cell axons; AC, anterior cone cell; B, bristle; C, liquid-filled pseudocone; CZ, cone cells; EOC, equatorial cone cell; L, lens; M, basal membrane; PC, posterior cone cell; PLC, polar cone cell; PP, primary pigment cell; Rh, rhabdomere; SP, secondary pigment cells; TP, tertiary pigment cells; 1–8, photoreceptor cells R1–R8. Magnification, ×220 (A) and ×1000 (C), (D).
Fig. 3. Overexpression of a truncated sevenless protein (sev-S11) causes a dominant rough eye phenotype. Scanning electronmicrographs of left eyes of a sevenless (sev) fly, and transformant (SevS11) homozygous for the sev-S11 construct, or the sev-S11LysMet construct are shown. The eyes of the SevS11 transformants exhibit an irregular ommatidial pattern and are slightly smaller compared to the eyes of the sevenless parental strain. The transformants carrying the sev-S11LysMet construct that encodes a protein with an inactive kinase do not exhibit the rough eye phenotype. Anterior is to the left. Magnification, ×130.

flies produced a rough eye phenotype (Fig. 3). This phenotype is dependent on the amount of sev-S11 protein produced and on a functional tyrosine kinase: a variant of sev-S11 carrying only a single enhancer element did not produce the rough eye phenotype except when present in two copies in homozygous transformants. Furthermore, another variant form of sev-S11 that carried the lysine-to-methionine amino acid substitution in the catalytic domain (sev-S11LysMet) did not produce the rough eye phenotype.

Sections through the eyes of SevS11 transformants show the presence of more than the normal number of rhabdomeres per ommatidium (Fig. 4). On average each ommatidium contains 6 large rhabdomeres and 4 small rhabdomeres (Fig. 4C,G). Based on the size and position of the small rhabdomeres, the expression of an R7-specific rhodopsin, and based on the R7-dependent behavioral assay, we concluded that these cells are fully differentiated R7 cells. The increased activity of the sev kinase achieved by overexpression of the truncated sev-S11 protein therefore results in the recruitment of additional cells into the R7 photoreceptor cell pathway.

Using a monoclonal antibody (BP104) that specifically stains neuronal cells in Drosophila (Hortsch et al. 1990) to follow the ommatidial assembly in sev mutants and in SevS11 flies, we could demonstrate that all cells that express the sev-S11 gene enter a neuronal pathway. In particular, the mystery cells that express sev but are lost from the wild-type precluster start to express the neuronal marker and remain associated with the cluster in SevS11. The other cells that express sev but in wild-type do not become neuronal cells, are the cone cells. In SevS11 these do initiate neural development and can become R7 cells (Fig. 5). Our results indicate that
activation of the sev-S11 kinase is necessary and sufficient to specify R7 cell fate not only in the R7 precursor but also in other ommatidial cells.

The only cells whose fate is not noticeably changed by the activated sev construct are R3 and R4, since we detect an average of 6 cells with large rhabdomeres (Fig. 4C,G). It is possible that R3 and R4 express a mixed identity. Alternatively, expression of rough and seven-up in R3 and R4 might suppress the R7 pathway (Mlodzik et al. 1990b). Consistent with this hypothesis is the finding that ectopic expression of rough in R7 using the sev enhancer results in a complete transformation of the majority of the R7 cells into outer photoreceptor cells (Basler et al. 1990; Kimmel et al. 1990).

**Specification of R7 cell fate can be achieved by the activation of a single signalling pathway**

Since sev activity is sufficient to specify R7 cell fate in cells other than the R7 precursor, there is no necessity for an additional signal which in combination with sev activity specifies R7 cell fate. In wild type, activation of sev in any cell other than the R7 precursor must be prevented. The boss protein is required in R8 to specify R7 cell fate (Reinke and Zipursky, 1988). The fact that the multiple R7 cells in Sev^{ST1} are also formed in a boss~ background strongly suggests that boss and sev function in the same pathway (Basler et al. 1991). Furthermore since boss encodes a membrane-bound protein it is likely that the boss protein binds to sev (Fig. 6). How is it that in wild type, sev is only activated in the R7 precursor and not in the other photoreceptor cells that also contact R8? Either the boss signal is spatially restricted on R8 such that it is only presented to R7, or it is not expressed in R8 before R7 joins the cluster thereby preventing activation of sevenless in all other cells that contact R8.

Restriction of the availability of the sev ligand by temporal control of its expression seems more likely than invoking subcellular localization. It has been shown that the sequence with which photoreceptor cells express neuronal markers corresponds to the sequence with which they are integrated in the cluster. R8 is always the first cell in each cluster to express a certain marker and R7 is the last. If boss is expressed only relatively late in R8 development it might not yet be present on R8 when the mystery cells are in contact with R8. It is important to note that since R8 is the first cell to initiate photoreceptor cell development, the temporal control of boss expression alone could be sufficient to achieve the required specificity. Expression of boss in other photoreceptor cells at a later stage might be without consequences because all sev-expressing cells would have already become determined. Although boss protein can be detected in R8 in the eye discs, the level of boss mRNA detected on Northern blots is more than 100 times higher in heads than in imaginal discs (Hart et al. 1990). The high levels of boss mRNA in adult heads could indicate that boss primarily serves another function at a later stage. Maybe determination of cell fate by cell–cell interactions should not be viewed as an active induction of the undetermined cell by the differentiated cell, but rather that the undetermined cell interprets existing surface markers on neighboring cells as was originally proposed by Tomlinson and Ready (1987a).

**Receptor tyrosine kinases play an essential role in cell fate determination mediated by cell–cell interactions**

Constitutive activation of receptor tyrosine kinases has been studied so far primarily by virtue of their transforming capacity in tissue culture cells or by their oncogenic potential in tumor formation. Although the appearance of extra R7 cells in Sev^{ST1} flies might at first sight seem to be a consequence of cell proliferation, our analysis clearly shows that in Sev^{ST1} no additional cell divisions of the R7 precursor occur (Basler et al. 1991). Therefore, in contrast to the elevated tyrosine kinase activity of a growth factor receptor, constitutive
activation of sevenless does not lead to the proliferation of cells but to the transformation of cell fate.

A change in cell fate rather than proliferation is also the consequence of dominant mutations in the torso gene (Klingler et al. 1988). The torso gene product is another receptor tyrosine kinase (RTK) and it is required for the formation of the terminal anlagen of the embryo (Sprunger et al. 1989). The torso protein is expressed in all cells of the blastoderm but is activated only locally at the poles (Casanova and Struhl, 1989). Similarly, the gain-of-function mutation Elp of the Drosophila EGF-receptor prevents cells from entering a neural pathway rather than having an overt effect on cell proliferation (Baker and Rubin, 1989). This points to a more central role of RTKs in developmental decisions than merely the control of cell proliferation and physiological changes, as was assumed from studies of known vertebrate RTKs and from the association of RTKs with oncogenesis and cell transformation.

A genetic search for targets of the sevenless tyrosine kinase

The problem of how a signal is transmitted from the membrane to the nucleus is not restricted to developmental biology. Biochemical approaches to the identification of components of tyrosine kinase signalling pathways have turned out to be difficult, even of RTKs for which ligand and tissue culture systems are available. For the sev RTK pathway, members could in principle be identified through new mutations in which the R7 cell does not develop correctly. The gain-of-function mutation SevS11, however, permits one to carry out a much simpler revertant screen for its rough eye phenotype in order to uncover genes acting downstream of sev in the signalling cascade within the R7 precursor. Furthermore, since the rough eye phenotype is dosage-dependent it is possible that the inactivation of just one copy of a potential downstream gene would cause reversion. This would not only facilitate the genetic screen but it would also allow the identification of genes that are required in other pathways earlier in development. Such genes could not be detected in a screen for recessive mutations with a sevenless-like phenotype since their inactivation would be likely to cause lethality.

Using the combination of genetic and molecular techniques available in Drosophila, it is conceivable that most or all of the components of the sevenless-mediated signal transduction pathway can soon be identified, thereby leading to a detailed molecular model of cell fate determination.

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References


Development of Drosophila eye 129


