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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, 1987*a*). 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, 1987*a*).

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, 1987*b*; 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 at *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.* 1990*a*). 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.* 1990*a*). 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.* 1990*a*).

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 (1987*a*) 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, 1987*a*).

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.* 1990*b*). 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.* 1990*b*). One form of *seven-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 (1987*a*), 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

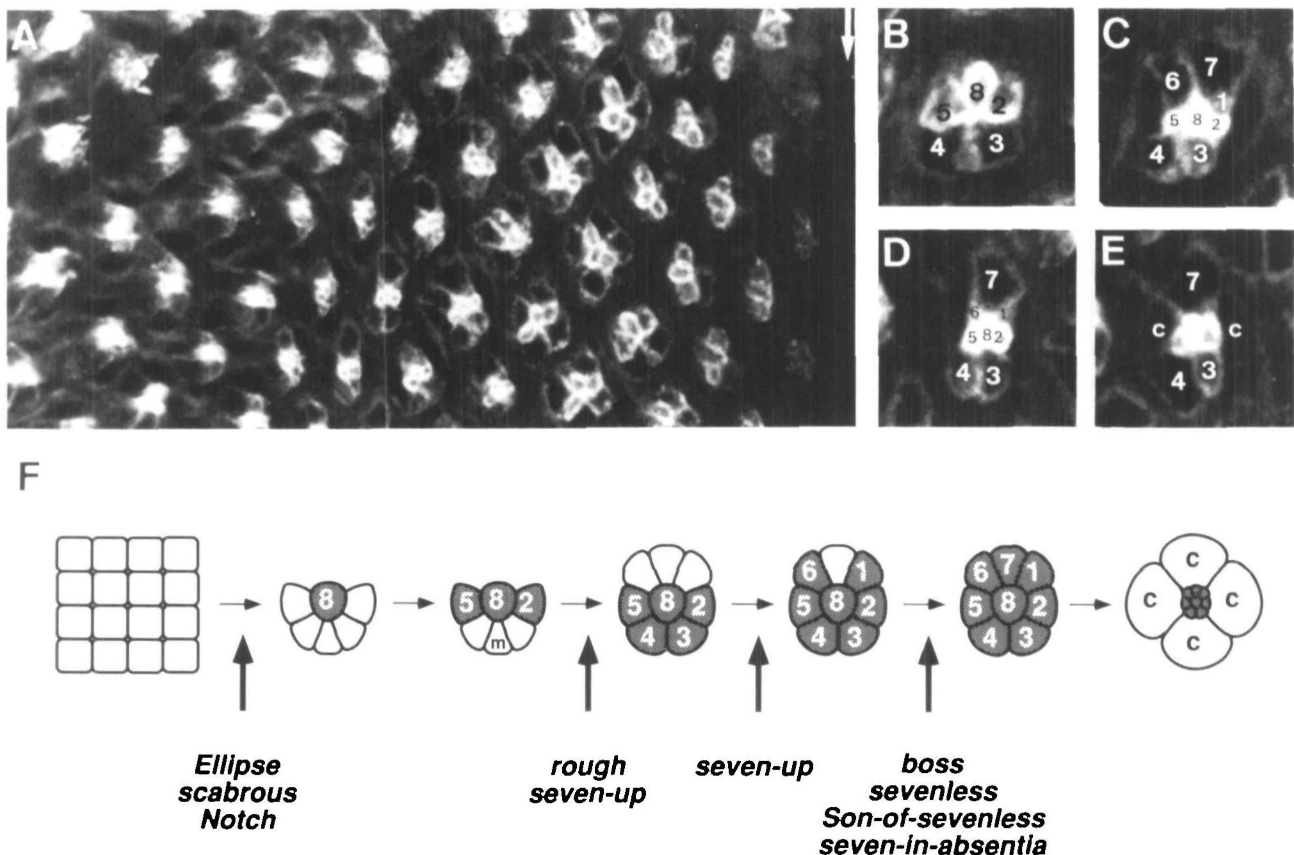


Fig. 2. Assembly of ommatidial units in the eye imaginal disc. (A) An optical section of a whole mount eye imaginal disc that has been stained with the neural specific monoclonal antibody BP-104. Anterior is to the right. The temporal sequence of ommatidial assembly is spatially displayed along the anterior–posterior axis. The arrow indicates the position of the morphogenetic furrow. (B–E) High magnification views of selected stages of ommatidial assembly. During disc development, the ommatidia undergo a 90° rotation. All clusters are shown in their final orientation such that anterior is to the right. (B) Five cells corresponding to R2, R3, R4, R5 and R8 are stained in column 5 behind the morphogenetic furrow. (C) In column 9 photoreceptors R1, R6 and R7 have initiated differentiation. (D) and (E) Two clusters from columns 11 and 12 are shown. Cells R1, R2, R5, R6 and R8 have moved basally in the disc such that only the apical projections are visible. R7 is still in the apical region. (F) Schematic representation of the assembly sequence of one ommatidial unit. Mutations that affect the different steps of the assembly are indicated. It should be noted that in contrast to the other genes, *Notch* function is required throughout the development of the ommatidial unit. 1–8, photoreceptor cells R1 to R8; m, mystery cell; c, cone cells. Magnification, ×900 (A) and ×1800 (B–E).

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 (Sev^{S11}) that is constitutively active, independent of ligand stimulation. We achieved this by overexpressing a *sev* protein truncated at the N terminus (*sev-S11*). 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-S11* construct into *sev* mutant

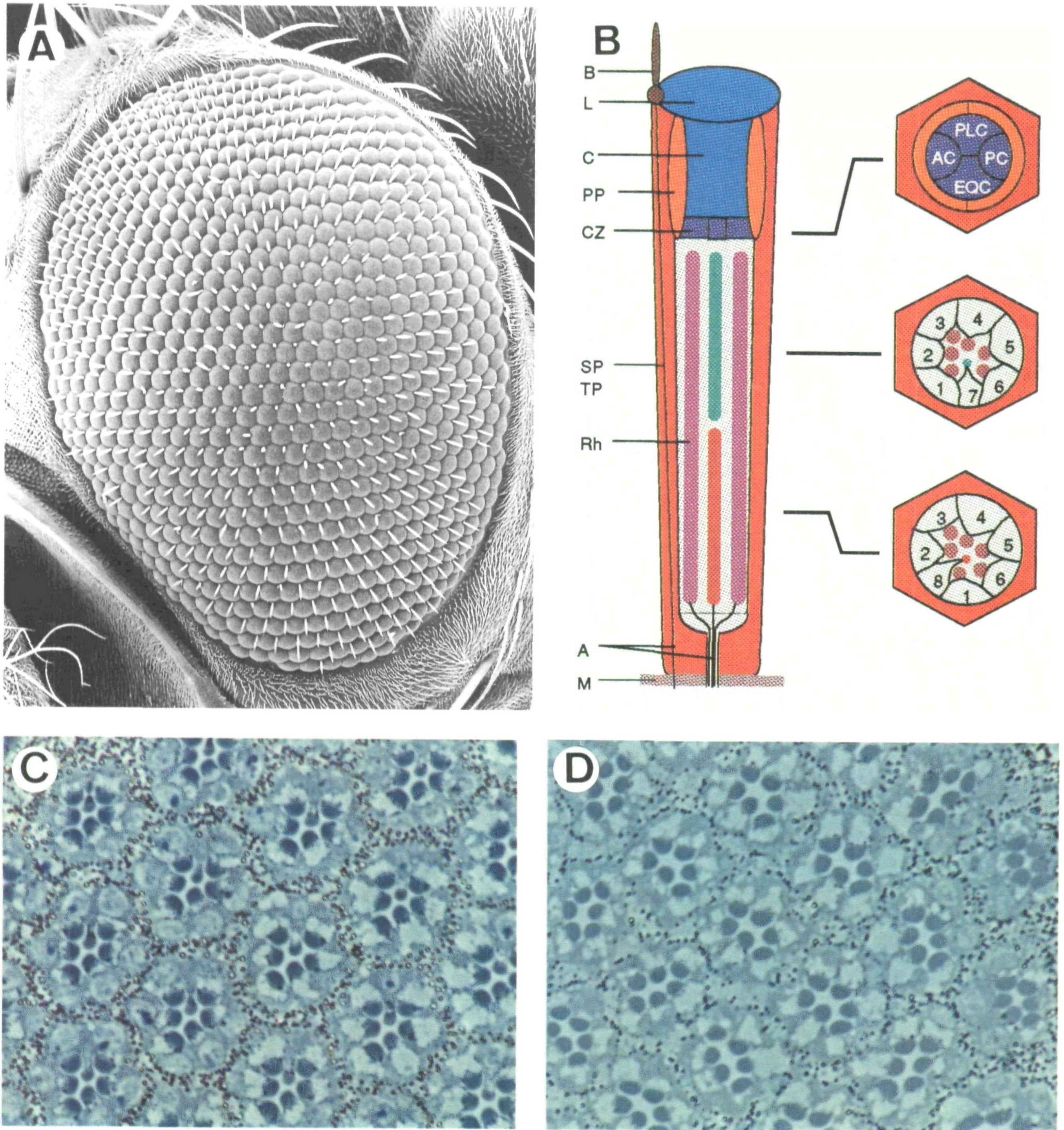
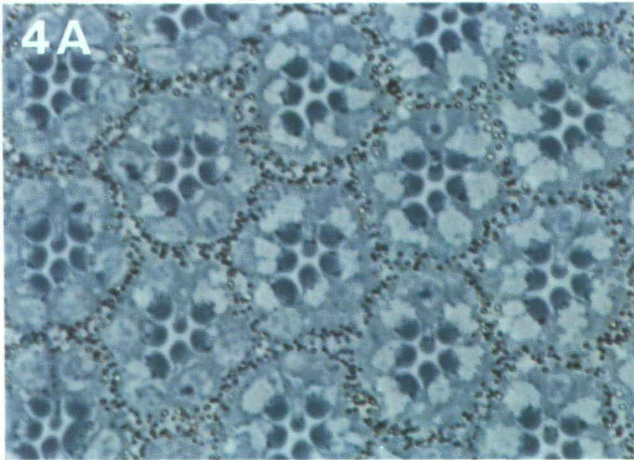


Fig. 1. Structure of the compound eye of *Drosophila*. (A) Scanning electron micrograph of the left eye of a wild-type fly. (B) Schematic view of an ommatidial unit. A longitudinal section is shown on the left and cross sections at three different levels are shown on the right. Histological cross sections 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; EQC, 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).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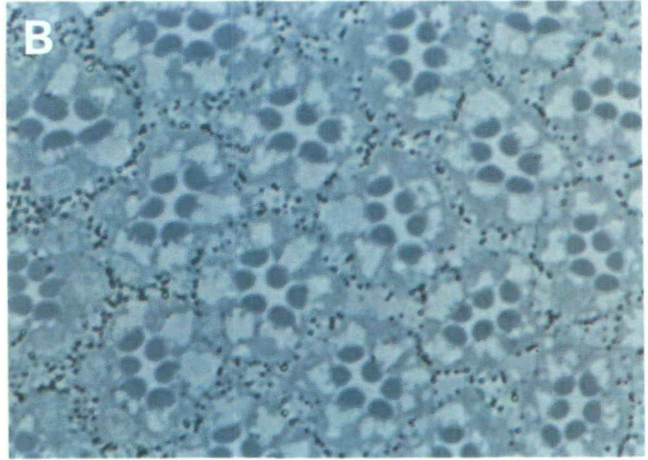
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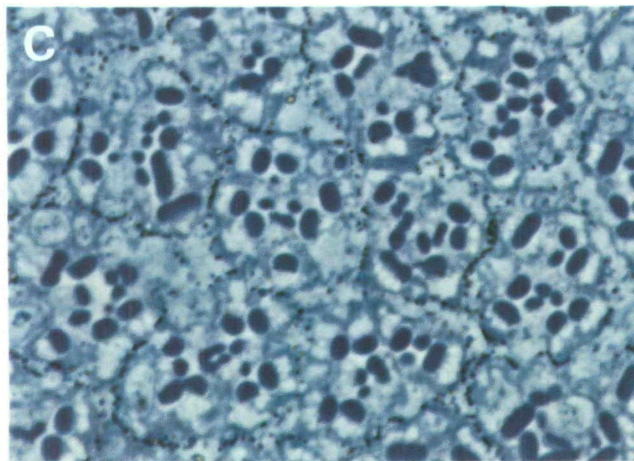
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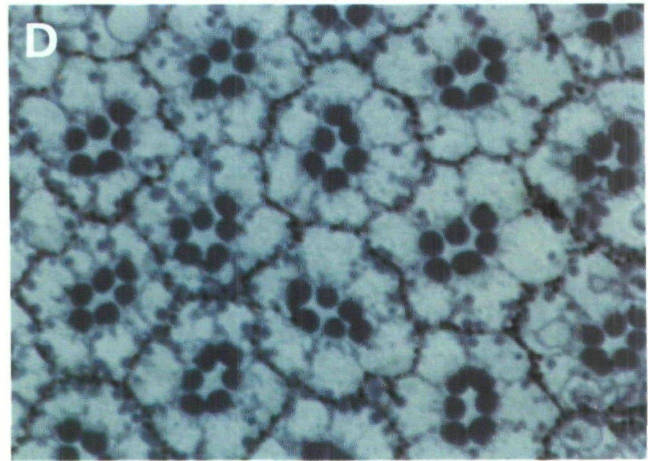
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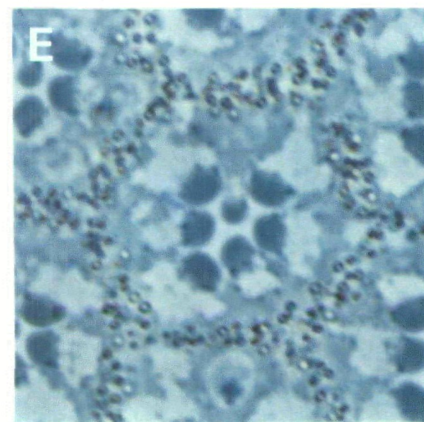
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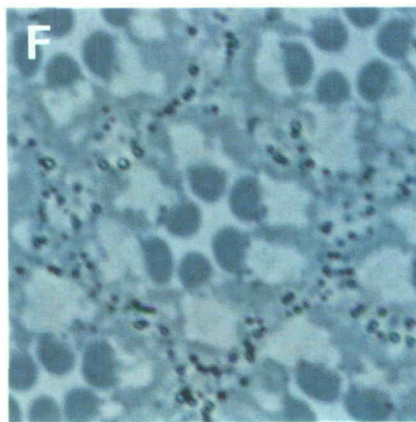
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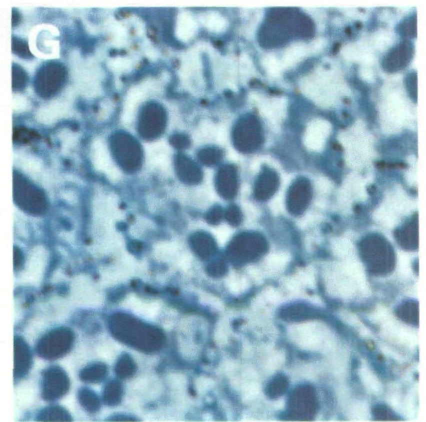
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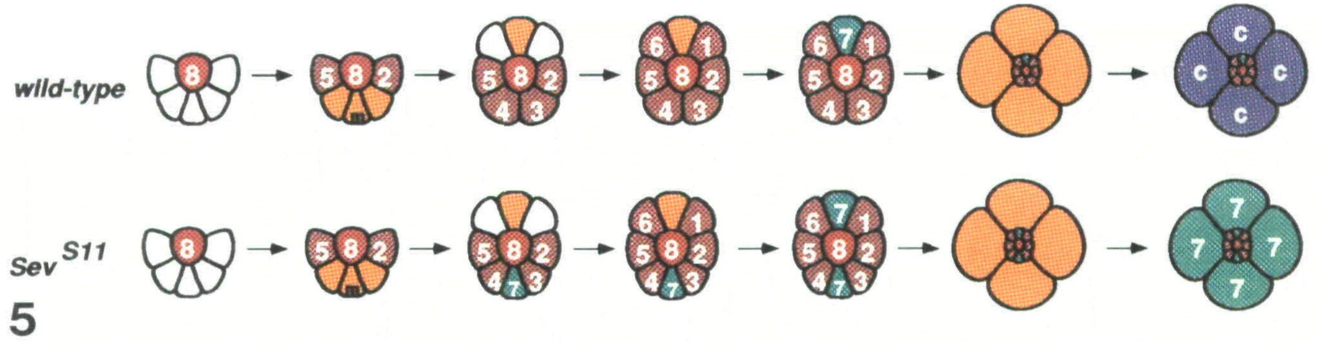
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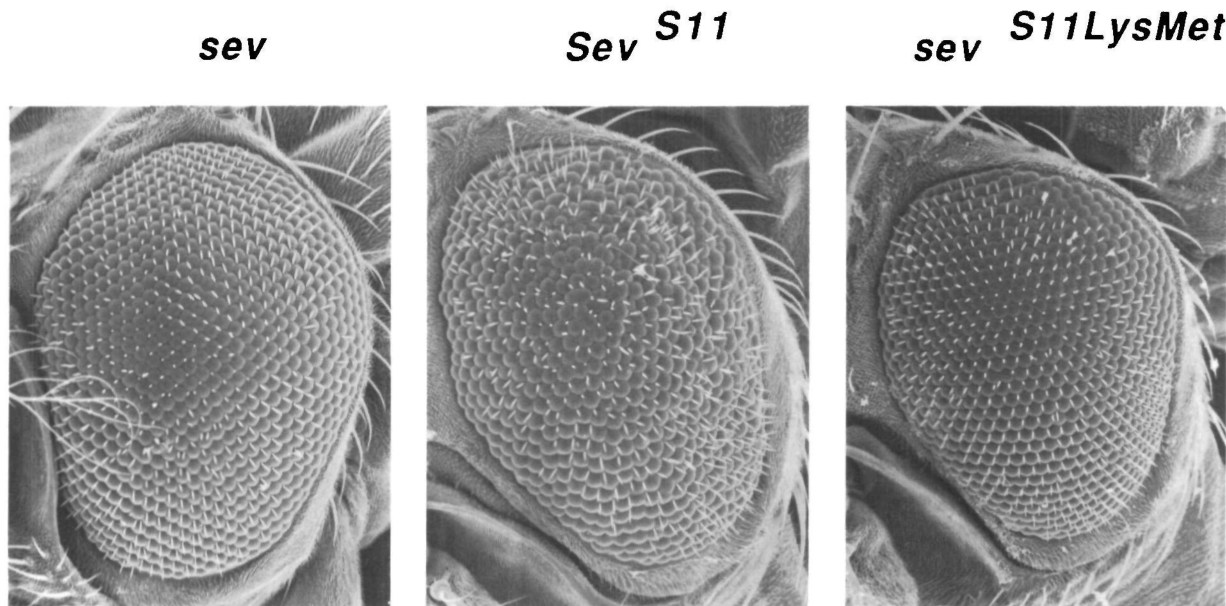

Fig. 3. Overexpression of a truncated *sevenless* protein (*sev-S11*) causes a dominant rough eye phenotype. Scanning electronmicrographs of left eyes of a *sevenless* (*sev*) fly, and transformant (Sev^{S11}) homozygous for the *sev-S11* construct, or the *sev-S11LysMet* construct are shown. The eyes of the Sev^{S11} 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 Sev^{S11} 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 Sev^{S11} 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 Sev^{S11}. The other cells that express *sev* but in wild-type do not become neuronal cells, are the cone cells. In Sev^{S11} these do initiate neural development and can become R7 cells (Fig. 5). Our results indicate that

Fig. 4. Sev^{S11} causes the formation of multiple R7 photoreceptor cells per ommatidium. Histological sections through wild type (A), *sevenless* sev^{d2} (B), sev^{d2}, Sev^{S11} (C) and $sev^{S11LysMet}$ (D) eyes are shown.
(E–G) Enlargements of single ommatidial units of wild-type, sev^{d2} and sev^{d2}, Sev^{S11} respectively. The R7 rhabdomere differs morphologically from the rhabdomeres of R1 to R6. It is smaller in diameter and occupies a central position in wild type (A and E). In *sevenless* the R7 cell is missing (B and F). In Sev^{S11} there are on average more than 6 photoreceptor cells visible in each ommatidium; many have small rhabdomeres (C and G). In $sev^{S11LysMet}$, as in the *sevenless* recipient, only 6 photoreceptor cells are visible (D). Anterior is to the right. Magnification, ×1000 (A–D) and ×2400 (E–G).

Fig. 5. Comparison of ommatidial assembly in wild type and in the gain-of-function mutant Sev^{S11}. Both the wild-type and the *sev-S11* proteins are expressed in the mystery cell, and in the progenitors for R3, R4, R7 and the cone cells. In wild type the *sev* kinase is only activated in the R7 precursor and therefore only this cell develops into an R7 cell. In Sev^{S11} the *sev* kinase is constitutively active. With the exception of R3 and R4 all cells that express the *sev-S11* protein can become R7 cells. The six R7 cells correspond to the maximal number of small rhabdomere cells seen in sections of Sev^{S11} eyes (compare Fig. 5C,G). c, cone cell; m, mystery cell; 1–8, photoreceptors R1–R8; orange shading, cells expressing either wild-type *sev* protein or *sev-S11* protein; red shading, R8 identity; purple shading, R1–6 identity; green shading, R7 identity; blue shading, cone cell identity.

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.* 1990*b*). 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^{S11} are also formed in a $boss^-$ background strongly suggests that *boss* and *sev* function in the same pathway (Basler at *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*

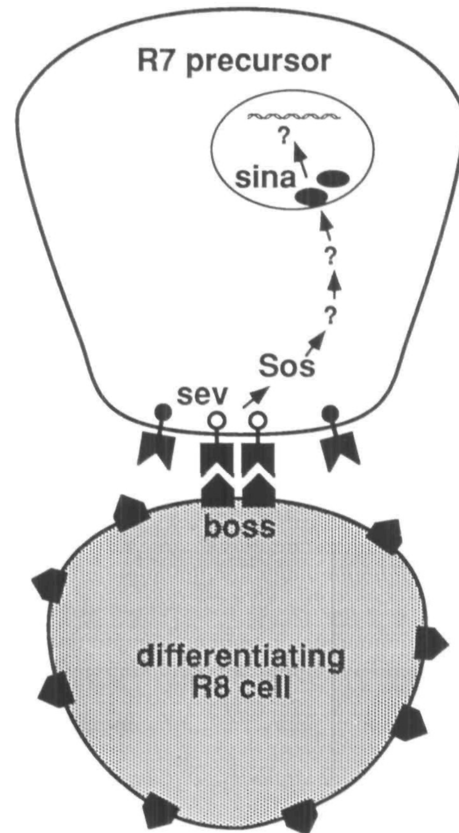

Fig. 6. Model for R7 cell fate determination: the undetermined R7 precursor cell expresses *sev* as a receptor for positional information. Binding of *sev* to the cell surface protein *boss* (which at that time is only present on the differentiating R8 cell) elicits *sev* tyrosine kinase activity. The signal is transmitted *via Sos* and an unknown number of intracellular components to the nucleus where the *sina* gene product is required to translate the information ultimately into altered gene expression.

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 (1987*a*).

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^{S11} flies might at first sight seem to be a consequence of cell proliferation, our analysis clearly shows that in Sev^{S11} 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 (Sprenger *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 identifation 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 Sev^{S11}, 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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