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Localization of the *sevenless* Protein, a Putative Receptor for Positional Information, in the Eye Imaginal Disc of Drosophila

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Summary

The Drosophila gene *sevenless* encodes a putative *trans*-membrane receptor required for the formation of one particular cell, the R7 photoreceptor, in each ommatidium of the compound eye. Mutations in this gene result in the cell normally destined to form the R7 cell forming a non-neuronal cell type instead. These observations have led to the proposal that the *sevenless* protein receives at least part of the positional information required for the R7 developmental pathway. We have generated antibodies specific for *sevenless* and have examined expression of the protein by light and electron microscopy. *sevenless* protein is present transiently at high levels in at least 9 cells in each developing ommatidium and is detectable several hours before any overt differentiation of R7. The protein is mostly localized at the apices of the cells, in microvilli, but is also found deeper in the tissue where certain cells contact the R8 cell. This finding suggests that R8 expresses a ligand for the *sevenless* protein.

Introduction

Development of the specialized tissues of multicellular organisms requires the differentiation of distinct cell types and their organization into integrated structures. The fate of a cell may be determined solely by lineage or may also require the reception and interpretation of positional information in its environment. A molecular description of the processes that allow cells to detect and respond to positional information is crucial to an understanding of cellular determination and differentiation.

The eye of D. melanogaster is well suited to the study of cellular determination in response to positional cues. The adult eye consists of several hundred identical subunits or ommatidia, each of which is a precise assembly of a few distinct cell types (Dietrich, 1909). Extensive mosaic analyses have failed to detect any lineage relationships between cell types in the developing retina (Ready et al., 1976; Lawrence and Green, 1979). Rather, progressive recruitment of undetermined cells in response to positional cues appears to be the primary mechanism of ommatidial assembly (Ready et al., 1976; Tomlinson, 1985; Tomlinson and Ready, 1987a). Aspects of this process can be readily observed in the eye imaginal disc of third instar larvae. Organization of the initially unpatterned epithelium of the disc is associated with the progression of the morphogenetic furrow anteriorly across the disc (Ready et al., 1976). Histological and immunochemical studies have demonstrated the progressive and stereotyped recruitment of cells posterior to the furrow into the cell clusters that will give rise to the adult ommatidia (Ready et al., 1976; Tomlinson, 1985; Tomlinson and Ready, 1987a). Each ommatidium is about 2 hr more mature than its anterior neighbor, so that by late third instar, over 24 hr of a smoothly graded sequence of development can be observed in a single disc preparation.

Analysis of mutants defective in ommatidial development provides a means of investigating the acquisition of cellular identity within the developing eye disc. The mutation *sevenless* causes the complete absence of the UV photoreceptor, R7, from each ommatidium with no other obvious aberration (Harris et al., 1976). Histological studies of eye discs from *sevenless* larvae have shown that a cell initially occupies the developmental position of the R7 cell, but fails to differentiate as a photoreceptor, becoming instead a lens-secreting cone cell (Tomlinson and Ready, 1986; Tomlinson and Ready, 1987b). Analyses of ommatidia mosaic for *sevenless* have shown that *sevenless* is cell-autonomous and that only the R7 cell appears to require *sevenless* function for its normal development (Harris et al., 1976; Campos-Ortega et al., 1979; Tomlinson and Ready, 1987b). These studies suggest that *sevenless* is involved either in reading the positional information that specifies the R7 cell or in the subsequent differentiation of this cell type. The *sevenless* gene has been isolated (Hafen et al., 1987; Banerjee et al., 1987), and its DNA sequence strongly suggests that it encodes a *trans*-membrane protein with an intracellular tyrosine kinase domain and a large extracellular domain (Hafen et al., 1987), related in its general structure to several hormone receptors including the insulin, EGF, and CSF-1 receptors (Ebina et al., 1985; Ullrich et al., 1984a, 1984b; Coussens et al., 1986). This structural homology led to the proposal (Hafen et al., 1987) that the *sevenless* protein receives and transduces information required for the R7 pathway by the binding of ligand to the extracellular domain and a consequent modulation of its tyrosine kinase activity.

Genetic analysis has shown that *sevenless* is required in the developing eye only for the formation of the R7 cell; however, it neither indicates what role the *sevenless* product plays, nor predicts its pattern of expression. If *sevenless* is indeed involved in reception of the positional information directing a cell to become an R7 cell, then we would expect *sevenless* to be expressed in a pool of undetermined cells prior to the specification of R7. In the simplest case *sevenless* would be expressed in all cells of the developing ommatidium, but its ligand would be presented only to R7, causing activation in only this cell. Conversely, *sevenless* might only be necessary for the execution of the R7 developmental pathway in a cell that had

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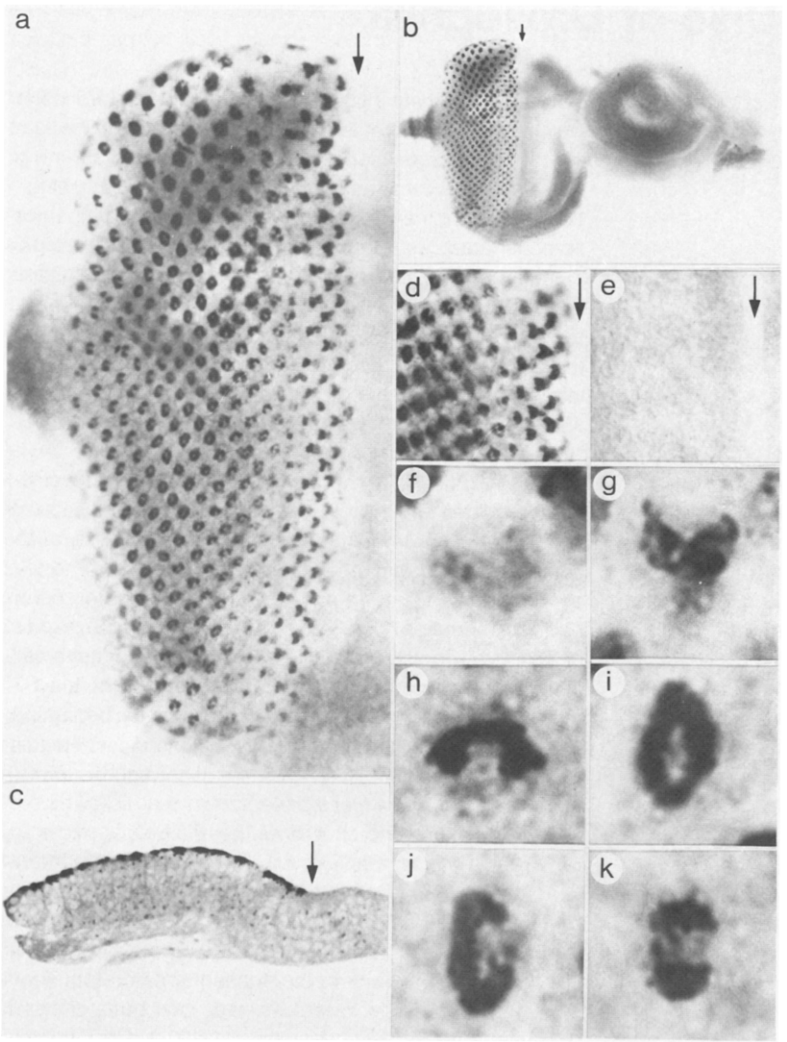

Figure 1. Light Micrographs of Eye Imaginal Discs from Third Instar Larvae Stained with *sevenless* Antisera

Peripodial membranes of discs have been removed. Arrows mark the center of the morphogenetic furrow. All except (d) are stained with the antiserum raised against the C-terminal peptide.

In (a–e) anterior is to the right. (a and b) Antibody staining of disc whole mounts; magnifications, ×600 and ×150, respectively. Staining begins in the morphogenetic furrow and changes progressively toward the posterior of the disc. (c) Longitudinal section through a stained disc showing the onset of staining in the morphogenetic furrow and its apical position within the disc. The stain is in the region of the cells' microvilli, which project up into the lumen of the disc between the developing epithelium and the peripodial membrane, which has been removed; magnification, ×525. (d) Staining of a whole mount disc with an antiserum raised to a second *sevenless* peptide (see Experimental Procedures). An identical staining pattern is seen; magnification, ×600. (e) No detectable signal is seen in sev^{P3} control discs; magnification, ×600.

High power images (×4000) of the progressive changes in staining pattern that occur are shown in (f–k). During disc development, the ommatidia undergo a 90° rotation. Initially in the furrow they are oriented directly posterior, but by at the back of the disc, they point toward the equator (see Tomlinson and Ready, 1987a). The shapes are shown in a standardized orientation, as though the rotation was complete, with anterior to the right. (f) The first staining seen is a diffuse butterfly shape. (g) Part of the butterfly darkens considerably. (h) The butterfly staining declines, and a horseshoe shape emerges. (i) The open end of the horseshoe is now filled, producing a complete ring of stain. (j) Reduction of stain in the anterior side of the circle produces another horseshoe shape. (k) Reduction in the posterior side now leaves two separated dots of stain.

already been instructed to form an R7 cell. In the simplest version of this hypothesis *sevenless* would be expressed only in the developing R7 cell. To investigate the role of the *sevenless* protein in R7 development, we have generated antisera specific for the *sevenless* protein and have used them to examine the pattern of *sevenless* expression in wild-type eye discs. We find that the *sevenless* protein is expressed in each developing ommatidium prior to differentiation of the R7 cell and is found transiently at high levels in at least 9 cells. This expression pattern is consistent with the proposal (Hafen et al., 1987) that *sevenless* directly reads positional information required to specify the R7 developmental pathway. However, *sevenless* expression is more highly regulated than this model requires; the absence of the protein from some cells and the transient pattern we see in expressing cells implies two levels of positional information: a restricted pattern of *sevenless* protein expression and a limitation of its activation to a subset of these expressing cells.

Results

Generation of Antisera and Light Microscope Analysis

Antisera were raised against two different synthetic peptides from the C-terminal region of the deduced *sevenless* protein sequence (Hafen et al., 1987) as described in Experimental Procedures. Light micrographs of an eye disc stained with an antibody directed against the C-terminal 13 amino acids of the *sevenless* protein are shown in Figure 1. Staining begins in the morphogenetic furrow and extends to the posterior of the disc (Figures 1a and 1b). Banerjee et al. (1987) raised an antibody against a β-galactosidase–*sevenless* fusion protein and reported a whole mount immunofluorescence staining pattern consistent with the pattern shown in Figure 1a. We found that stain was localized extremely apically within the epithelium where the microvilli of the cells project upward into the disc lumen (Figure 1c). An indistinguishable pattern of staining was seen when a second antiserum, raised

against a peptide corresponding to the 13 amino acids located between 35–47 residues from the *sevenless* C-terminus, was used (Figure 1d). Discs from larvae carrying the sev^{P3} mutation, a deletion allele that removes the sequences encoding both peptide antigens (Banerjee et al., 1987), showed a complete absence of staining (Figure 1e). The observations that the same pattern of staining was seen with antisera raised to two separate *sevenless* peptides and that staining was absent in sev^{P3} eye discs demonstrate that the staining we observed was specific for the *sevenless* protein.

From the morphogenetic furrow to the posterior of the disc, about 24 hr of ommatidial development is displayed spatially, with the earliest stages in the furrow itself and progressively more mature forms found posteriorly. The staining pattern changed from the furrow to the back of the disc, commencing with butterfly shapes that progressed to horseshoes, then complete rings, horseshoe shapes again, and, finally, to pairs of dots (Figures 1f–1k). In studies using antibodies to neuronal antigens (Tomlinson and Ready, 1987a), the identities of stained cells could be ascertained directly from such whole mount discs, but the *sevenless* protein was localized so apically in the cells that accurate cell identification in this preparation was not possible. However, it was clear that many cells within each developing ommatidium express the protein in a transitory manner, resulting in the highly dynamic pattern observed.

Electron Microscope Analysis

To establish the identities of the cells expressing the *sevenless* protein, immunocytochemistry was performed using electron microscopy. Because of the complexity of the staining pattern, we will describe our results with reference to previously published descriptions of ommatidial assembly deduced from electron microscopy of serial sections (Tomlinson, 1985; Tomlinson and Ready, 1987a). The earliest developmental stage at which the *sevenless* protein could be detected was at the precluster stage. The precluster was initially identified as a 5 cell unit composed of those cells destined to become photoreceptors R2, R3, R4, R5, and R8 (Ready et al., 1976). However, using cobalt sulphide staining, Tomlinson and Ready (1987a) detected another cell associated with the unit, the fate of which was unclear. At this stage we found *sevenless* expression in R3 and R4, the cell of unknown fate, and another cell of similar appearance (Figure 2a). We shall refer to these latter two cells as the mystery cells M1 and M2. No staining reproducibly above background was detected in R2, R5, or R8 (see Experimental Procedures). Together the cells R3, R4, M1, and M2 produced the hazy butterfly shape seen by light microscopy (Figure 1f). As precluster morphogenesis proceeded, first M2 and then M1 were lost from the unit and the staining in R3 and R4 intensified (Figure 2b), producing the darker butterfly shape of Figure 1g. The fates of M1 and M2 are unknown; as cell death is not evident at this stage (Tomlinson and Ready, unpublished data), we presume that they contribute to the pool of dividing cells (see below).

At this point division of cells surrounding the precluster occurs, generating all other cells required for the ommatidium (Ready et al., 1976). Cells are then systematically incorporated into the growing cell cluster, and their nuclei sequentially rise into the apical regions of the epithelium, producing a series of well-defined developmental stages (Tomlinson, 1985). The cells destined to become photoreceptors R1, R6, and R7 are incorporated first to form the immature 8 cell cluster, followed by the anterior and posterior pair of cone cells, and then the equatorial and polar cone cell pair (Tomlinson, 1985). Immediately after the cell divisions, a low but reproducible level of antibody staining was detected in most of the cells surrounding the cluster. As they became incorporated into the cluster, staining in the presumptive R1 and R6 cells fell to background level, while staining in other presumptive cell types dramatically increased. By the symmetrical cluster, a stage prior to the apical migration of the cone cell nuclei and characterized by the symmetrical arrangement of the photoreceptors, R7 and the anterior and posterior cone cells stained heavily (Figure 2c). Intense staining of the microvilli of these cells at this stage (Figure 2f) produced the horseshoe shape seen with light microscopy (Figure 1h). The symmetrical cluster progresses to the 2 cone cell stage as the nuclei of the anterior and posterior cone cells rise. As this occurred, the polar cone cell began to stain, the stain in R7 reduced, and the equatorial cone cell darkened, resulting in a ring of heavily stained cone cells surrounding the photoreceptor apical projections (Figure 2g; Figure 1i). As ommatidial morphogenesis proceeds, the nuclei of the equatorial and polar cells rise, producing the 4 cone cell stage and the apical projections of the anterior and posterior cone cells begin to close over the photoreceptors. Staining in the anterior cone cell reduced, resulting in another horseshoe shape composed of the stained equatorial, posterior and polar cone cells (Figure 2h; Figure 1j). Stain in the posterior cone cells then decreased, leaving only the polar and equatorial cone cells heavily stained (Figure 2i; Figure 1k). This is the most advanced stage reached prior to pupation. For reasons of technical difficulty we have not followed the ommatidial staining past this stage and we cannot rule out expression of *sevenless* protein later in the developmental sequence.

Within all cells expressing the *sevenless* antigen, the heaviest staining occurred in the microvilli at the apical surface of the epithelium, which lies below an extracellular matrix (Figure 3f). Staining in the microvilli accounted for the shapes seen with light microscopy (Figures 1f–1k). Deeper into the cell, staining was found in large membrane-bound vesicle-like structures (an example is seen in Figure 3b), which were found in all cells in which the *sevenless* protein was detected. Although the majority of these structures were found in the most apical regions, they were also present throughout the entire depth of the epithelium. A striking staining of plasma membranes occurred at the level of the adherens-type junctions in the first few microns of the tissue below the microvilli (see Figure 4). Here we saw an accumulation of stain within R3, R4, and R7 precisely at the position where they appose R8, but not where they abut other cells (Figures 3a–3e) or below the level of the adherens junctions. This localized staining follows the general sequence of *sevenless* ex-

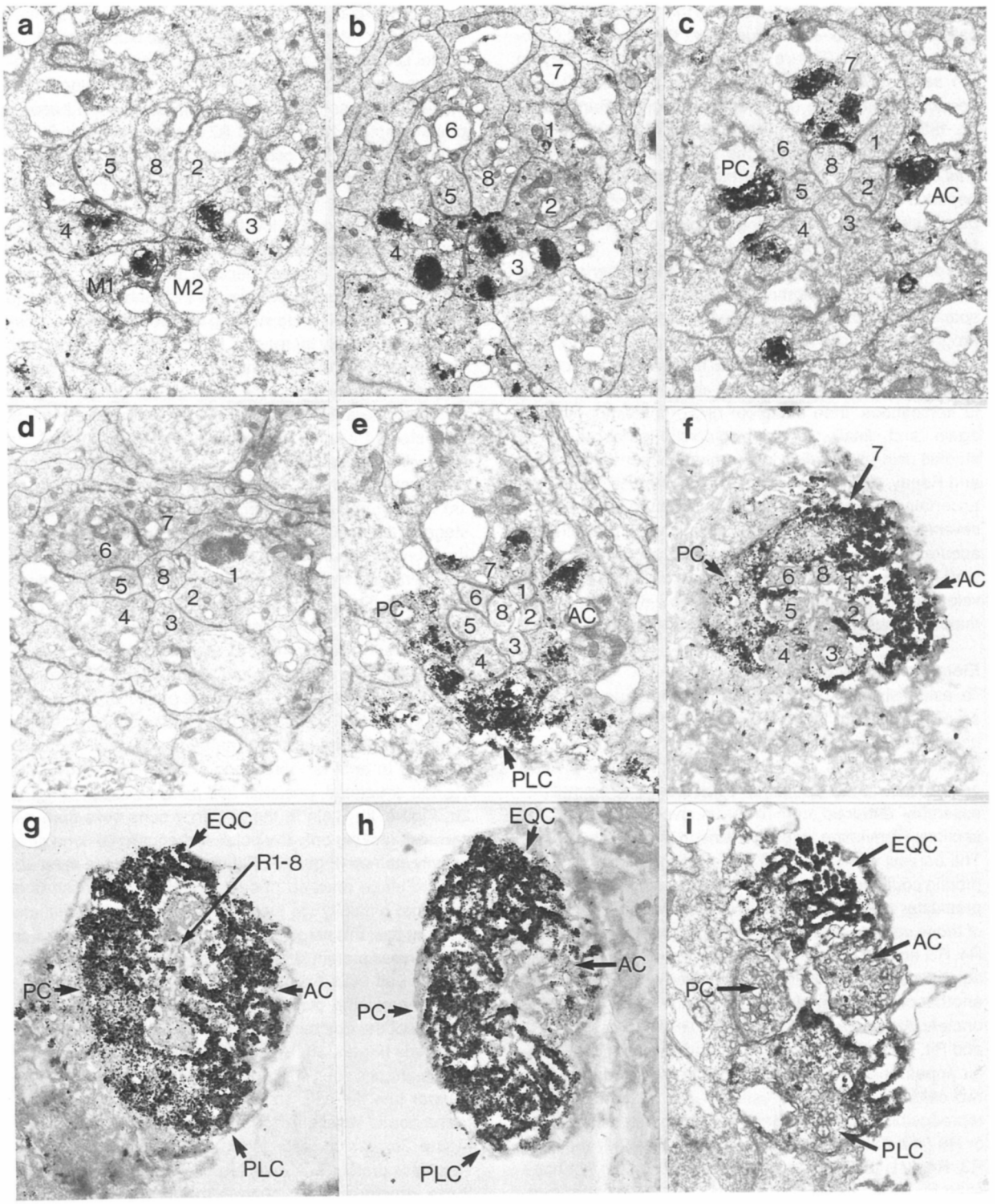

Figure 2. Electron Microscope Localization of *sevenless* Protein

The major staining occurs in the microvilli of the cells, but it is difficult to resolve individual cells at this level. (a–e) Sections below the microvilli in the epithelium where staining cells contain dark vesicular structures; magnification, ×7200. Higher magnification examples of staining within individual cells are shown in Figure 3. (f–i) Sections in the microvillus region of the epithelium illustrating the staining patterns seen in the light microscope; magnification, ×8800. Photoreceptors are numbered. AC, anterior cone cell; PC, posterior cone cell; EQC, equatorial cone cell; PLC, polar cone cell; M1 and M2, cells present in the precluster but lost shortly thereafter (see text). Anterior is to the right. (a) Staining is first detected in the early precluster in cells R3, R4, M1, and M2. R2, R5, and R8 show no staining detectable above background. M1 and M2 are arbitrarily labeled here, as we are not able to distinguish between them at this stage. (b) After the burst of mitoses, the immature 8 cell cluster is formed. M1 and M2 have been displaced from the cluster, and the staining in R3 and R4 has become more intense. Strong staining is seen where R3 and R4 contact R8. (c) The staining in R3 and R4 begins to decline by the late symmetrical cluster stage, and heavy staining occurs in R7 and the anterior and posterior cone cells. The staining within R7 is strongly associated with its contact with R8. (d) sev^{P3} control discs at the symmetrical cluster stage.

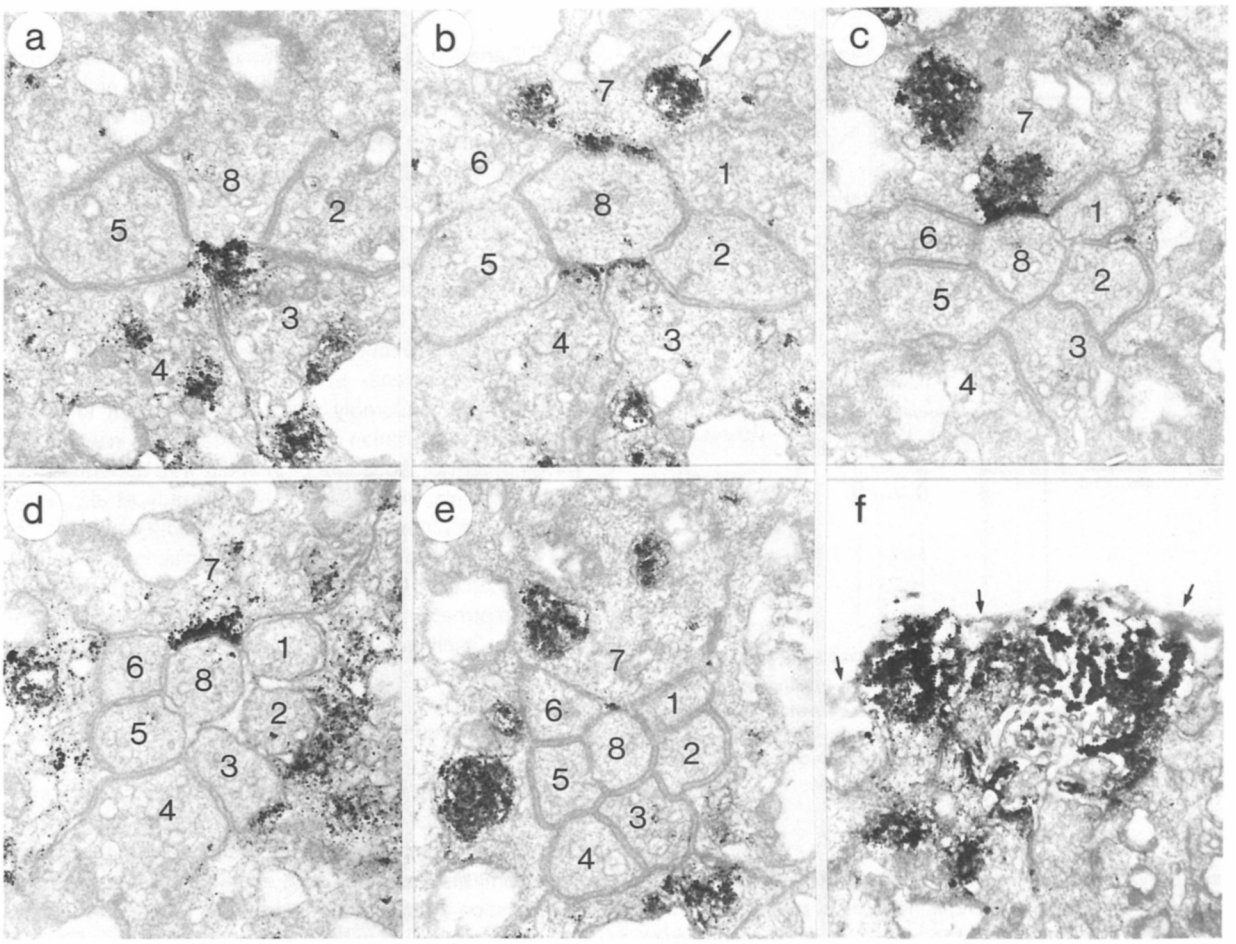

Figure 3. Intracellular Localization of *sevenless* Antibody

All electron micrographs are at a magnification of ×14,000. (a–e) Cross sections through developing ommatidia at the level of the adherens-type junctions and perpendicular to the plane of section shown in Figure 4. Anterior is to the right. Photoreceptors are numbered. (a) At the immature 8 cell cluster stage, the staining is found on the membranes of R3 and R4 only where they contact R8. Note that intense staining also occurs in the cytoplasm of R3, R4, and R7 (see [b–d]) directly adjacent to where membrane staining is observed. (b) At the early symmetrical cluster stage, staining occurs simultaneously in R7, R3, and R4 and is localized in each of these cells at their points of contact with R8. The arrow points to a membrane-bound vesicular structure that is strongly stained. Such structures are found in all cells in which expression is detected and account for the vast majority of intracellular staining. (c and d) Late symmetrical cluster stages in which staining in R3 and R4 has now reduced considerably and the stain in R7 where is contacts R8 has become more intense. (e) Staining of the R7 membrane where it contacts R8 persists late into the 2 cone cell stage. (f) Longitudinal section through the region of the microvilli. Note how the extracellular matrix (arrows) lies directly over the microvilli.

pression described for these cells, occurring first in R3 and R4 (Figure 3a), then for a brief period simultaneously in R3, R4, and R7 (Figure 3b), and finally in R7 alone (Figures 3c–3e).

Discussion

Several lines of evidence have led to the hypothesis that the *sevenless* protein is involved in the reception and transduction of information required for the R7 developmental pathway (Tomlinson and Ready, 1986, 1987b; Hafen et al., 1987). Using antibodies to the *sevenless* protein, we find a relatively complex pattern of expression in eye discs (summarized in Figure 5). This pattern is consistent with a role for *sevenless* in reception of signals involved in specifying the R7 pathway, but does not formally exclude the possibility that *sevenless* is simply required in the developing R7 cell after it has been instructed to its fate.

Expression of *sevenless* protein is first detected in the

No staining is seen at any stage or any level within the disc. (e) At the late 2 cone cell stage, staining is still selectively localized on the R7 membrane at the point of contact with R8. (f) At the early symmetrical cluster stage, the microvilli of R7 and the anterior and posterior cone cells stain heavily, producing a horseshoe shape. (g) The microvilli of the 4 cone cells are heavily stained and surround the unstained photoreceptors, producing the ring shape. (h) The cone cells close over the photoreceptors, and the anterior cone cell reduces in stain, producing a horseshoe shape. (i) Reduction of stain in the posterior cone cell leaves only the equatorial and polar cone cells staining. The 4 cone cells behave as 2 pairs during morphogenesis of the ommatidium, with the anterior/posterior pair developing ahead of the equatorial/polar pair (Tomlinson, 1985). It is therefore interesting that the staining in the anterior cone cell reduces many hours before that in the posterior cone cell. This behavior may reflect positional asymmetries being incorporated into the ommatidium as development proceeds; other examples include R4 breaking its symmetrical arrangement with R3 and the anterior movement of the cell body of R8 from the center of the ommatidium to a position between R1 and R2 (see Tomlinson, 1985).

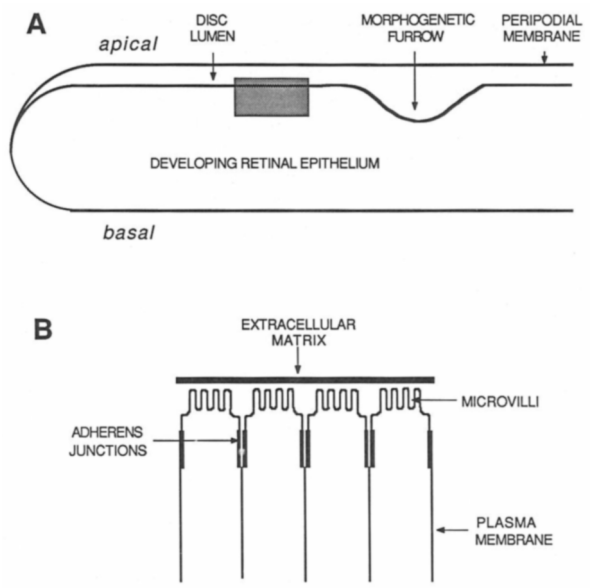

Figure 4. Schematic Diagram of Tissue Structure in the Eye Disc

(A) Longitudinal section of eye disc, anterior is to the right. (B) Details of cell structure in the region of the shaded box shown in (A). The adherens type junctions in the eye disc, described by Perry (1968), appear to be typical desmosomes anchoring the apical regions of the cells together and structurally stabilizing the epithelium.

morphogenetic furrow prior to the cell division that generates the presumptive R7 cell. The onset of expression in R3, R4, and other cells of the ommatidium closely follows the sequence of, but precedes, the maturation of these cells as revealed by their expression of neural antigens (Tomlinson and Ready, 1987a). In particular, *sevenless* staining is seen at high levels in the presumptive R7 cell some 8 hr before any overt differentiation of this cell is evident. However, unlike the staining revealed with antibodies to neuronal markers, the expression of *sevenless* is transient and only a proportion of photoreceptors express detectable levels; R2, R5, and R8 being the notable exceptions.

In situ hybridization to *sevenless* RNA reveals a pattern grossly similar to that seen with the antibody (Hafen et al., 1987; Banerjee et al., 1987). However, the resolution of this technique is insufficient to identify individual cells in the ommatidium. Therefore, it is unclear whether the sequential and transient expression of *sevenless* protein we observe reflects transcriptional control of *sevenless* expression or posttranscriptional control, for example, by receptor down regulation through interaction with other receptor systems (reviewed by Sibley et al., 1987). Moreover, the biological function of this transient pattern of expression remains unclear. It may reflect a window of competence to respond to inductive signals that closes when cell determination takes place.

Ommatidial assembly was initially thought to occur much like the formation of a crystalline lattice, in which the development of individual ommatidia was dependent on the preceding, more mature units (Ready et al., 1976). Subsequently, it was shown that ommatidia could assemble when isolated from more mature units, indicating that the ommatidium is a self-assembling unit, with the positional information specifying cell types local to the ommatidium itself (see Lebovitz and Ready, 1986). These and other observations led Tomlinson and Ready (1987a) to suggest that undetermined cells read signals expressed by their differentiating neighbors in order to determine their own fates. In this model, differentiating cells express cell-type-specific signals and undetermined cells receive a combination of signals dependent upon the cells they contact. Different signal combinations specify distinct developmental pathways, and absence of any one of the combination a particular cell normally receives would be expected to lead to a fate change for that cell. R7 is a unique cell type, and the presumptive R7 cell makes a unique set of cell contacts in the developing ommatidium; it is the only cell that contacts collectively R1, R6, and R8.

The *sevenless* ligand is a candidate for one of the signals in this combinatorial code; it could be associated with the membrane of the cells adjacent to R7 or be a short range diffusable molecule released from these cells. Asymmetries in the distribution of *sevenless* protein within R7 might indicate on which of the neighboring cells the ligand is expressed. In this regard it is intriguing that the *sevenless* protein is localized at the adherens junction contact of R7 with R8. In fact all three cells, R7, R3, and R4, that

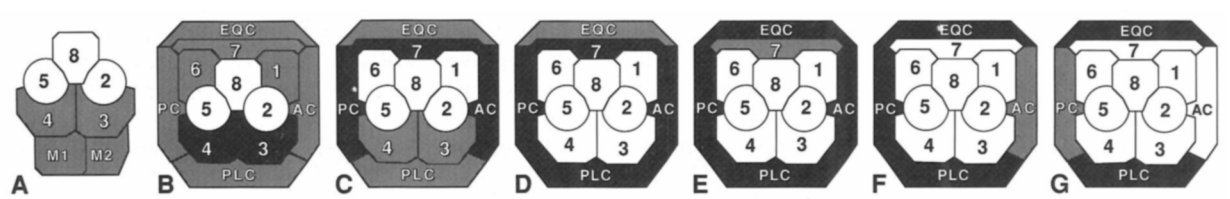

Figure 5. Schematic Representation of the Sequence of Cells Staining with Antisera Directed against the *sevenless* Protein

Photoreceptors are numbered; AC, anterior cone cell; PC, posterior cone cell; EQC, equatorial cone cell; PLC, polar cone cell; M1 and M2, cells present in the precluster but lost shortly thereafter (see text). Three levels of staining are shown: white, which signifies a level indistinguishable from background; grey, which represents a low but definite level to a moderately high level; black, which shows the cells heavily staining with the antibody. (A) Precluster stage at which R3, R4, M1, and M2 stain. Following the precluster stage, cell divisions occur, generating all other cells of the ommatidium. (B) Immature 8 cell stage. (C–E) Progression through the symmetrical cluster stage to the 2 cone cell stage. (F and G) Four cone cell stage. Cells are shown in the position they come to occupy rather than their position at that developmental point. For example, EQC and PLC cannot be identified at stage B, but they are derived from cells surrounding the cluster, all of which show a low level of stain at this stage.

express high levels of *sevenless* and contact R8 show this striking localization at their adherens junction contacts with R8, which suggests an interaction of the *sevenless* protein with a ligand expressed on cell R8. It is important to emphasize, however, that mosaic analyses clearly indicate that *sevenless* gene function is not required in R3 or R4, either for their own development or for that of the R7 cell (Harris et al., 1976; Campos-Ortega et al., 1979; Tomlinson and Ready, 1987b), and therefore the *sevenless* protein in R3 and R4 cannot be essential for the specification of these cells. If the localization of *sevenless* protein we observe in R7, R3, and R4 does reflect ligand binding, then we infer activation of *sevenless* protein in these three cells. This would imply that additional signals, not transduced by *sevenless*, are required for R7 specification, or that R3 and R4 are unresponsive to the *sevenless* signal perhaps because of their reception of other positional cues. The absence of detectable expression in R2, R5, and R8 is noteworthy. If the putative ligand for the *sevenless* protein is indeed expressed on R8, then the absence of expression on R8 would be expected since a receptor and its ligand are normally not expressed on the same cell.

While the majority of *sevenless* protein is found in microvilli at the extreme apex of the cells, the function of the protein in this location is unclear; it may represent the point at which *sevenless* protein is added to the cell surface or potentially another point where interaction with a ligand could occur. Conceivably such a ligand could be diffusible between microvilli or immobilized in the overlying extracellular matrix, as has been suggested in the case of stromal cells in mammalian bone marrow (Gordon et al., 1987).

In conclusion, the appearance of *sevenless* protein prior to the specification of R7 and its presence in many cells are consistent with a role of *sevenless* in receiving a signal required to induce the R7 developmental pathway. We believe that the identity of each cell in the developing ommatidium is specified by a combination of signals received from its neighbors and that *sevenless* mediates the reception of one of the signals required for R7 determination. It is unlikely that this signal alone is sufficient to specify the R7 developmental pathway; the *sevenless* protein's apparent activation in cells R3 and R4, suggested by the localization of the *sevenless* protein in these cells, does not lead to the R7 pathway. Furthermore, our data suggest that limitation of activation of *sevenless* protein to particular cells is accomplished by a combination of restricted temporal and spatial expression of the *sevenless* protein and a restricted distribution of its ligand. Our data suggest that this ligand is expressed by the R8 cell.

Experimental Procedures

Generation of Antisera

Two peptides corresponding to the carboxy-terminal 13 amino acids (DKQLYANEGVSRL) and a peptide commencing 47 amino acids from the carboxy-terminus (EFKVRFDGQPLEE) were synthesized by the Biomedical Resource Center, UCSF. Both peptides were synthesized with an N-terminal cysteine residue. The peptides were coupled via their amino groups to PPD (Statens Seruminstitut, Copenhagen, Denmark) using glutaraldehyde. Briefly, a 1:1 (w/w) ratio of PPD to peptide (both at 0.5 mg/ml) was coupled overnight in PBS after the addition of glutaraldehyde to 0.05%. The coupled material was precipitated with 4 vol of acetone, 0.01% HCl, and resuspended in 144 mM NaCl.

Female BALB/c mice, 6–7 weeks old, were immunized subcutaneously with 50 μg of coupled material emulsified in Freund's complete adjuvant (GIBCO Laboratories, N.Y.) and, 1 month later, boosted with 50 μg in Freund's incomplete adjuvant. In the case of the peptide EFKVRFDGQPLEE, a second boost was performed. Two weeks after the last boost, the mice were bled and serum was collected.

Immunostaining of Eye Discs

Staining of eye discs for both light and electron microscopy was performed as described in Tomlinson and Ready, (1987a) up to the incubation in the secondary antibody. Both antisera used were diluted 1/1000. Except for Figure 1d, all antibody staining shown used the antiserum directed against the C-terminal 13 amino acids. After the secondary antibody, discs were fixed in 2% glutaraldehyde for 30 min, washed in 0.1 M phosphate buffer for 15 min, and placed in the DAB oxidation solution until signal was visible. Discs were then transferred to 2% sodium acetate solution, and the oxidized DAB was then silver-gold-intensified as described by Lipostis et al. (1984). After the intensification procedures were completed, the discs were transferred to 0.1 M phosphate buffer for 10 min. Osmication and further treatments were as described in Tomlinson and Ready (1987a).

In wild-type discs, posterior to the morphogenetic furrow a low level of silver grains is seen in cells otherwise not staining. In sev^{P3} control discs and in wild-type discs, ahead of the morphogenetic furrow, this low level of staining is not observed. Since a heavy deposition of silver grains occurs in cells surrounding those showing this low level, we cannot rule out movement of grains during sectioning as being responsible for the weak signal seen in these cells, rather than very low level expression of the *sevenless* protein. We have therefore considered this low level of staining to be not significantly above background.

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