Prepattern in the developing *Drosophila* eye revealed by an activated torso–sevenless chimeric receptor

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Induction of the R7 photoreceptor cell fate in the developing eye of *Drosophila* depends on the activation of the sevenless receptor tyrosine kinase in the R7 precursor cell. The sevenless protein is expressed transiently in 8 of the 20 precursors of an ommatidium. Activation of the sevenless kinase in these eight cells indicates that six of them are competent to become R7 cells. To test the competence of all 20 ommatidial precursors in a temporally unrestricted manner we have used a constitutively activated sevenless kinase created by fusing the extracellular domain of a mutant torso protein, another *Drosophila* receptor tyrosine kinase, to the sevenless kinase. Our results show that competence to develop as neuronal cells in response to sevenless activity is spatially and temporally limited to the cells expressing sevenless. Therefore, the expression of sevenless marks a preexisting pattern of developmental potential in the disc epithelium.

[Key Words: Eye development; *Drosophila*; sevenless; torso; tyrosine kinase; chimeric receptor; heat shock; induction; prepattern]

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During the development of multicellular organisms, cell–cell interactions are important for the generation of the different cell types. In vertebrates, for example, mesodermal structures are only formed in response to the interaction between vegetal and animal cells (for review, see Melton 1991). Although recent molecular characterization of genes and gene products involved in inductive interactions has identified signals and receptors, little is known about the information content of the signals received and transmitted by the cells. That is, do all cells in a developing field have an equal potential to react to a given signal, or are they already predisposed to respond to a signal only in a certain way. For example, miniature embryos with complete body axes can be formed when tissue explants from the animal pole of *Xenopus* blastomeres are incubated in a homogenous solution of the mesoderm-inducing growth factor activin A (Sokol and Melton 1991). This suggests that cells in this tissue possess unequal potentials to respond to activin, which then acts as a mere trigger for the development of this preexisting pattern.

The specification of cell fate in the compound eye of *Drosophila* is a suitable system to study the information content of an inductive signal, as a detailed morphological and genetic description of ommatidial assembly is available (for review, see Hafen 1991; Rubin 1991). Ommatidial assembly begins with the specification of the R8 photoreceptor cell, followed by the pairwise integration of R2/5, R3/4, R1/6 and, finally, R7. In subsequent steps the cone cells and pigment cells are added to complete the ommatidial cluster (Tomlinson and Ready 1987). Cell fates are determined independent of cell lineage (Lawrence and Green 1979), and it has been proposed that cells already integrated in the cluster determine, by a process of induction, the fate of their uncommitted neighbors (Tomlinson and Ready 1987). All cells in the epithelium might initially have an equal potential to develop into any one of the different cell types. They would possess a complete set of receptors and signal transducers that allows them to respond in the appropriate manner to a given inductive signal. Alternatively, the potential of a cell might already be restricted to respond to only a subset of signals. The different models can best be tested in the specification of the R7 cell fate. Genetic and molecular studies have indicated that the induction of the R7 photoreceptor cell depends on the activation of the sevenless receptor tyrosine kinase in the R7 precursor by the membrane-bound boss protein expressed on the neighboring R8 cell (Krämer et al. 1991). The sevenless receptor is expressed on a subset of ommatidial precursor cells: R3 and R4, the mystery cells, R7, and the cone cells (Tomlinson et al. 1987). The constitutive activation of the sevenless kinase in all sevenless-expressing cells in the *Sev*Δ7 mutant results in the transformation of the mystery and cone cells into R7 cells (Basler et
al. 1991]. Similarly, ubiquitous expression of the boss protein results in the recruitment of the cone cells into the R7 pathway [Van Vactor et al. 1991]. In both experimental situations the potential to respond to sevenless activity can be tested only in the sevenless-expressing cells and only within the restricted time window during which the sevenless protein is present. Only by the uniform application of a given inductive signal can the potential of all cells be tested. Therefore, we sought to create a constitutively activated sevenless protein that could be expressed in a completely unrestricted manner under the transcriptional control of the heat-inducible hsp70 promoter. We achieved this by constructing hybrid receptors containing the mutant extracellular domain of a constitutively activated torso receptor tyrosine kinase and the cytoplasmic kinase domain of sevenless.

Expression of these chimeric receptors under the transcriptional control of the sevenless enhancer mimics the Sevnull phenotype. Spatially unrestricted expression of sevenless activity under heat shock control allowed us to probe the potential of all ommatidial precursor cells to respond to sevenless activity. Our results indicate that only a small subset of ommatidial precursor cells can develop into R7 cells in response to sevenless activity. Furthermore, even these cells are sensitive to sevenless activity only during the brief period when they normally choose their cell fate. The different responses of ommatidial precursor cells to sevenless activity suggests an inherent difference in their competence to respond to the sevenless signal.

**Results**

*Ligand-independent activation of the sevenless kinase in chimeric torso–sev receptors*

In Sevnull transformants, constitutive kinase activity was achieved by overexpression of a truncated sevenless protein by a duplicated sevenless enhancer. To obtain constitutive activation of the sevenless kinase independent of the sevenless enhancer, we constructed chimeric receptors in which the tyrosine kinase domain of constitutively activated torso proteins [F. Sprenger, M.M. Trosciar, and D. Morrison, in prep.] was replaced by that of sevenless. The torso gene of Drosophila encodes a receptor tyrosine kinase required for the specification of the unsegmented anterior and posterior terminal regions of the embryo [Klingler et al. 1988; Sprenger et al. 1989]. Two gain-of-function mutations that suppress the formation of thorax and abdomen have been described: torso4021 and torso4029 [Klingler et al. 1988]. These mutant phenotypes are the consequence of constitutively active torso proteins that contain single amino acid substitutions in their extracellular domains [F. Sprenger and C. Nüsslein-Volhard, in prep.]. The two gain-of-function mutations differ in their phenotypic strength; the embryonic phenotypes suggest that the torso4021 mutation results in a higher degree of ligand-independent activation of the torso kinase than does the torso4029 mutation [Klingler et al. 1988]. In addition, both alleles are cold sensitive [Szabad et al. 1989]. Anticipating that these activating mutations would also activate the sevenless kinase in torso–sevenless (torso–sev) chimeras, we assembled P-element constructs that encode the hybrid proteins depicted in Figure 1. The constructs contain sequences corresponding to the extracellular and transmembrane domains of either wild-type or mutant torso proteins fused to sequences coding for the sevenless tyrosine kinase domain. To test whether the chimeras were functional and constitutively active, we expressed the constructs under the control of the sevenless enhancer (sev). If the mutations led to an activation of the sevenless kinase, expression under the transcriptional control of the sev should mimic the “multiple R7” phenotype of Sevnull. The different constructs were injected into sevenless mutant embryos, and several transformed lines were obtained for each construct. Figure 2 shows scanning electron micrographs (SEMs) and histological sections of eyes of flies from representative lines for each construct. All lines transformed with the sev–torso4021–sev construct have smooth eyes in which each ommatidium contains six R1–R6-type photoreceptor cells with large rhabdomeres but no R7-type cell with a small rhabdomere in apical sections. This phenotype is indistinguishable from that of the sevenless recipient. The sev4021–sev and the sev–torsoY9–sev transformants have rough eyes. Interestingly, similar to the different strengths of the embryonic phenotypes of the two torso mutations, the phenotype of the sev–torso4021–sev transformants [Fig. 2C,G] is consistently stronger than that of the sev–torsoY9–sev transformants [Fig. 2B,F]. There are, on average, 3.8 R7-like cells with small apical rhab-
Figure 2. Chimeric torsoD-sev receptors expressed under the transcriptional control of the sevenless enhancer (sE) produce a sevenless gain-of-function phenotype. SEMs and histological sections of transformants expressing, under the transcriptional control of the sE, chimeric receptors containing the extracellular domains of either wild-type or mutant torso receptors fused to the sevenless tyrosine kinase domain. All transformants are mutant for the endogenous sevenless gene. sE-torsoY9-sev and sE-torsoD021-sev transformants are heterozygous for the insertion of the transgene, whereas the sE-torsoY7-sev and sE-torsoD021-sevLys-Met transformants contain two copies of the respective construct. (A, E) The chimera containing the wild-type torso extracellular domain (sE-torsoY9-sev) does not activate the sevenless kinase and does not alter the sevenless mutant phenotype. (B, F) Transformants expressing the sE-torsoY9-sev construct possess slightly rough eyes owing to the recruitment of some R7 cells. (C, G) Chimeras with the torsoD021 extracellular domain (sE-torsoD021-sev) have very rough eyes with multiple R7 cells in each ommatidium. (D, H) No R7 cells are produced in the transformant expressing a torsoD021-sevLys-Met chimera in which the sevenless kinase is inactivated by an amino acid substitution. Magnification, 70× in A-D and 610× in E-H.

Figure 3. Cold sensitivity of the sE-torsoY7-sev phenotype. Flies heterozygous for either the sE-torsoY9-sev (open bars) or the sE-torsoD021-sev (shaded bars) construct were raised at the indicated temperature, and tangential sections through the eyes of three adults scored for the number of R7-like cells present per ommatidium. A total of between 369 and 641 ommatidia were analyzed in each case. Error bars indicate ±2× S.E.M.
In contrast to Sev<sup>511</sup>, the rough eye phenotype of the torso–sev hybrids is observed in heterozygous transformants containing one copy of the construct driven by a single sevenless enhancer. In Sev<sup>511</sup> transformants, kinase activity is raised primarily by overexpression of the truncated protein. Activation of the sevenless kinase in the torso–sev chimeric receptors does not require overexpression but is achieved by the activating mutations in the torso domain of the chimeric receptors.

**Figure 4. Genetic interactions between sE-torso<sup>4021</sup>–sev, rough, and sina.** Histological sections of eyes of sevd<sup>22</sup>, sE–torso<sup>4021</sup>–sev transformants in different genetic backgrounds are shown. (A) sev<sup>22</sup>, sE–torso<sup>4021</sup>–sev: The constitutive activity of the sevenless kinase results in the recruitment of multiple R7 cells. (B) sev<sup>22</sup>, sE–torso<sup>4021</sup>–sev; sE–ro: In a sev<sup>+</sup> background, ectopic expression of rough (sE–ro), which specifies R1–R6 photoreceptor cell type, results in the transformation of the R7 cell into an R1–R6 photoreceptor cell. When sE–ro is combined with sE–torso<sup>4021</sup>–sev, the additional R7 cells are also transformed into R1–R6 cells. As in sev<sup>+</sup>, sE–ro, not all of the R7 cells are transformed into outer photoreceptor cells by rough expression. (C) sev<sup>22</sup>, sE–torso<sup>4021</sup>–sev; sina: The formation of most of the R7 cells is dependent on sina. (D) sev<sup>22</sup>, sE–torso<sup>4021</sup>–sev; sE–ro; sina: The rough-induced transformation of the R7 cells into R1–R6 cells also depends on sina, suggesting that sina is involved together with sevenless in the primary decision between photoreceptor and nonphotoreceptor cell fate. Although the number of R7-like cells is drastically reduced in a sina background, still some cells with small rhabdomeres in apical sections remain. Expression of the R7-specific marker construct Rh3–β–gal (Fortini and Rubin 1990, Basler et al. 1991) was used to demonstrate that the remaining small rhabdomeres in apical sections belong to differentiated R7 cells (data not shown). Because sina is not expressed in mystery cells (Carthew and Rubin 1990), it is likely that the sevenless-induced transformation of these cells into R7 cells is independent of sina and that these remaining R7 cells correspond to transformed mystery cells.

**initial decision to become a photoreceptor cell, which occurs independently of the decision specifying photoreceptor cell subtype.**

**Ommatidial precursors have unequal potentials to respond to sevenless activity**

Ommatidial assembly does not occur synchronously throughout the eye disc but starts at the posterior margin and progresses anteriorly (Ready et al. 1976). Closely as-
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sociated with the anterior boundary of ommatidial assembly is a morphological depression, called the morphogenetic furrow. Cells anterior to the furrow are unpatterned and randomly organized, whereas cells posterior to the furrow become incorporated into the ommatidial clusters in a fixed sequence (Tomlinson and Ready 1987). The progressive movement of the morphogenetic furrow anteriorly across the eye disc results in a spatial array of the various stages of ommatidial assembly along the anteroposterior axis of the disc. Each column consists of ommatidia ~1.5 hr older than their more anteriorly located neighbors (Basler and Hafen 1989a). Thus, with a single heat shock during late third-instar larval development, ommatidia at different stages of development will be confronted simultaneously with sevenless activity. The consequences for their subsequent development can then be deduced from the ommatidial pattern displayed along the anteroposterior axis of the adult eye. From this pattern it can then be inferred at what developmental stages and for how long the various ommatidial precursor cells are sensitive to sevenless kinase activity. We therefore made a further construct, hsp-torso4021-sev, with which sevenless activity could be induced ubiquitously by heat shock activation of the hsp70 promoter.

Larvae of the hsp-torso4021-sev transformant line were heat shocked for 1 hr at 37°C around the time of puparium formation. The eyes of five eclosed flies were serially sectioned, and the number of cells with large and small rhabdomeres in apical sections was recorded for each ommatidium. Figure 5 shows an apical section through the anterior portion of such an eye. Tracings of the ommatidial arrangement in sections of three different eyes are shown in Figure 6. The various combinations of cells with small and large rhabdomeres have been color coded. Because all of the experiments were performed in a sevenless background, only the six large rhabdomeres of the R1–R6 cells should be detectable in apical sections through unaffected ommatidia (uncolored in Fig. 6). Ommatidia affected by the induction of sevenless activity should show a deviation from this composition. As expected, the composition of the ommatidial units varies along the anteroposterior axis. Ommatidia at the anterior margin of the eye are unaffected by sevenless activity and contain six outer photoreceptor cells and no R7-like cells. Cells giving rise to these ommatidial columns were still located anterior to the furrow at the time of heat shock. Similarly, ommatidia at the posterior margin of the eye are also devoid of R7 cells. These represent older ommatidia in the posterior part of the eye disc in which the photoreceptors and the cone cells had already been specified at the time of heat shock. Between these unaffected marginal areas is a broad stripe in which the ommatidia exhibit a variable number of outer (R1–R6) and inner (R7) photoreceptor cells. Ommatidia in the anterior of the stripe often have fewer than six outer photoreceptor cells (Fig. 6, green), whereas ommatidia in the posterior region of the stripe usually contain six outer photoreceptor cells and multiple R7-like cells (Fig. 6, orange and red). The distribution of the different combinations of outer and inner photoreceptor cells was remarkably similar in all five individuals.

Ommatidia in a single column in the middle of the stripe contain six outer photoreceptor cells, and a central R7 cell, and exhibit the same dorsoventral polarity as wild-type ommatidia (Fig. 6, dark green). The position of these ommatidia along the anteroposterior axis corre-

![Image of ommatidial arrangement](https://genesdev.cshlp.org/content/7/7/2331/F1.large.jpg)

**Figure 5.** Temporally restricted ubiquitous expression of the activated sevenless kinase reveals the different potential of ommatidial precursor cells. Third-instar larvae of the transformant containing the hsp-torso4021-sev construct were heat-shocked once for 1 hr around puparium formation. **(Top)** An apical section through the anterior portion of the eye of a fly that developed from such a heat-shocked larva is shown. Anterior is to the left. Owing to the developmental gradient of ommatidial assembly along the anteroposterior axis, ommatidia developing in the anterior portion of the eye have been confronted with sevenless activity at an earlier developmental stage than ommatidia in the posterior part of the eye. A schematic representation of the wild-type assembly sequence of ommatidial units is shown **below.** The addition of pigment cells occurs only after puparium formation and is not indicated. The horizontal bars indicate the developmental stage of the ommatidia at the time of induction of sevenless activity.
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Figure 6. Tracings of the ommatidial arrangements in apical sections of three different eyes similar to the one shown in Fig. 5. Anterior is to the left. The different combinations of photoreceptor cells with large and small rhabdomeres are color coded. The interpretation of which cells have been transformed is given in parentheses; it is based on the position of the ommatidia along the anteroposterior axis of the eye and, hence, their developmental stage at the time of heat shock. Furthermore, we have shown previously that induction of a wild-type sevenless cDNA at the time of puparium formation results in the specification of R7 cells in ommatidial columns, 10–14 columns from the anterior margin. white (6/0), unaffected ommatidia with six outer photoreceptors and no R7 cells; light green (≤5/0–1), reduced number of outer photoreceptor cells; light blue (7–8/0), mystery cells transformed into R1–R6; medium blue (6/1), mystery cell transformed into R7; dark blue (7/1), ommatidia containing both an additional R1–R6 and an R7 cell owing to the recruitment of either two mystery cells or one mystery cell and an R7 precursor; dark green (6/1), ommatidia with a single R7 cell in the correct dorsoventral orientation derived from the recruitment of the R7 precursor; light orange (6/2), ommatidia containing two R7 cells derived from two cone cells or the R7 precursor and a cone cell; orange (6/3), ommatidia containing three R7 cells derived from three cone cells or an R7 cell and two cone cells; red (6/4), clusters containing four R7 cells derived from the recruitment of all cone cells; yellow (6/1), ommatidia containing a single R7 cell derived from the recruitment of the last cone cell; gray, ommatidia that could not be classified because of fusion of two adjacent ommatidial clusters.
responses to the position of the stripe of wild-type ommatidia produced when we induced the wild-type sevenless protein at the time of puparium formation (Basler and Hafen 1989a). Therefore, we assume that these clusters have been confronted with the activated sevenless kinase at the normal time of R7 specification, thereby rescuing the sevenless mutant phenotype. We have used this band of wild-type ommatidia as a reference for the interpretation of the phenotypes observed in the other ommatidia. Ommatidia located anterior to the stripe of wild-type ommatidia had been at a stage prior to R7 specification at the time of heat shock, whereas ommatidia posterior to the band of rescued ommatidia had been at a more advanced stage of assembly. For example, posterior to these wild-type ommatidia are ommatidia containing more than one R7 cell. In these regions, sevenless activity has been presented at the time of cone cell specification. Whereas the wild-type protein rescues a stripe of ommatidia approximately four columns wide, the activated torso4021–sev hybrid rescues only a single ommatidium column. This could be the result of rapid degradation of the activated protein. However, Western analysis shows that the hybrid protein is still present at high levels 12 hr after heat shock induction (data not shown). Furthermore, ommatidia can be seen in which all four cone cells have been transformed to R7 cells. Because the four cone cells are determined over a period of 4–5 hr, the hybrid protein must remain active for at least this length of time. More likely, the perdurance of the activated hybrid results in the subsequent additional transformation of the cone cells in many of the ommatidia in which the R7 precursor had been rescued. In this case, the rescue event escapes detection. Taking this into account, the area in which R7 cells have been rescued may extend to column 14.

We also detect ommatidia containing R7-like cells several columns anterior to the rescued ommatidia (Fig. 6, medium blue). The R7-like cells are most likely derived from the mystery cells, which in wild-type are only transiently associated with the five-cell precluster ~8 hr before the specification of R7 cells. Examination of eye discs after induction of the hsp–torso4021–sev construct indicates that some mystery cells do initiate neuronal development, as revealed by the expression of the neuronal antigen encoded by the elav gene (Fig. 7B, small arrows, Robinow and White 1991).

It would seem, however, that not all of the neuralized mystery cells ultimately differentiate as R7 cells, because ommatidia with extra R1–R6 type photoreceptor cells (seven instead of six photoreceptor cells with large rhabdomeres, Fig. 6, light blue) can also be detected in this region. Ommatidia with seven outer photoreceptor cells in addition to multiple R7-like cells were also found in Sev511 flies. The origin of these outer photoreceptor cells, however, was difficult to trace in these flies because there are no early markers to detect differences between developing R7 cells or R1–R6 cells (Basler et al. 1991). The fact that these additional outer photoreceptor cells occur only anterior to the stripe of ommatidia with rescued R7 cells suggests that these cells cannot be derived from either the normal R7 precursors or from the cone cells. Therefore, it is very likely that both the R7 cells and the additional R1–R6 cells observed in this region derive from the mystery cells. Of a total of 153 mystery cells that had been recruited as photoreceptors in five eyes, 108 developed as R1–R6 type cells and 45 developed as R7-like cells.

Ommatidia in the most anterior regions of the stripe often possess a reduced number of outer rhabdomeres. This defect was not seen when torso4021–sev was expressed under the sevenless enhancer or in Sev511 flies (Basler et al. 1991). In the sevenless-expressing cells significantly less torso4021–sev protein is produced under heat shock control alone than under the control of the sevenless enhancer, as judged by immunohistochemical staining of eye discs (data not shown). It is therefore unlikely that the reduced number of photoreceptors is simply the result of higher levels of sevenless kinase activity in these cells. It is more likely that the reduction in the
number of photoreceptor cells is caused by the presence of sevenless activity in cells that do not normally express sevenless. Although it is not possible to decide, on the basis of morphological criteria, which of the R1–R6 cells are missing, the position of these clusters anterior to those containing transformed mystery cells suggests that R8 and/or R2 and R5 cannot fully differentiate in the presence of sevenless activity. Staining of eye discs with an antiserum against the neuron-specific nuclear protein elav [Robinow and White 1991] 6 hr after induction of sevenless activity revealed the aberrant assembly of the ommatidial clusters (Fig. 7). In particular, the five-cell precluster consisting of R2, R3, R4, R5, and R8 frequently does not form properly; instead, only four cells initiate neuronal development behind the morphogenetic furrow. [Fig. 7B, open arrows].

sevenless activity suppresses pigment cell development

About 10 hr after puparium formation, all ommatidia have reached the cone cell stage. Ommatidial assembly is completed by the selection of the pigment cells from the remaining precursor cells. Unlike the previous stages in eye development, completion of the ommatidial pattern, which involves a process of programmed cell death, occurs uniformly across the disc (Wolff and Ready 1991). To investigate the effects of ectopic sevenless activity on pigment cell development, a single 1 hr heat shock was applied to pupae collected at 4-hr intervals up to 44 hr after puparium formation at 25°C. The heat shock induction of sevenless activity was lethal to pupae up to 8 hr after puparium formation. Flies heat-shocked between 8 and 16 hr after puparium formation exhibit rough eyes with large white patches (Fig. 8A). The fusion of the lenses of adjacent ommatidia is caused by the loss of pigment cells [Fig. 8B]. The arrangement of the photoreceptor cell cluster, however, is normal for a sevenless mutant. In no case did we observe additional photoreceptor cells. It appears therefore that ectopic sevenless activity in the developing pigment cells is incompatible with their normal development but cannot transform these cells into photoreceptor cells. Furthermore, the absence of additional photoreceptor cells indicates that cells undergoing differentiation as non-neuronal cells cannot be reprogrammed to initiate neuronal development.

Discussion

Activation of a heterologous kinase in chimeric receptors

The two gain-of-function torso alleles torso<sup>4021</sup> and torso<sup>Y9</sup> encode proteins with single amino acid substitutions in their extracellular domains. Introduction of either of these two mutations into a wild-type torso cDNA produces a protein capable of suppressing segmentation and inducing the formation of terminal structures when injected as RNA into the central regions of preblastoderm embryos [Sprenger and Nüsslein-Volhard 1992].

Here, we demonstrate that the same mutations also cause a constitutive activation of the heterologous sevenless kinase in the chimeric torso–sev receptors. The torso<sup>4021</sup> mutation produces a significantly stronger embryonic phenotype than the torso<sup>Y9</sup> mutation. Similarly, more R7-like cells are produced in the eyes of sE–torso<sup>4021</sup>–sev transformants than in those of sE–torso<sup>Y9</sup>–sev transformants, indicating that the relative degree of activation by the two mutations is similar for both kinase domains. Furthermore, the activities of both the gain-of-function torso receptors and the torso–sev chimeric receptors exhibit the same cold sensitivity. There-
fore, the sevenless and the torso kinase domains react similarly to these activating point mutations. It is not clear how these different amino acid substitutions result in activation of the kinase. Assuming that receptor dimerization is a necessary step in kinase activation, it is possible that these mutations result in a conformational change in the torso extracellular domain that favors dimerization even in the absence of ligand. Our observation that the torso4021 and torsoY9 extracellular domains can activate a heterologous kinase could provide a means to create dominant gain-of-function mutations in other receptor tyrosine kinase genes.

Information content of the signal mediated by sevenless

The only detectable phenotype of loss-of-function mutations in either sevenless or boss is the transformation of a single photoreceptor cell into a non-neuronal cone cell. Because both the inducing signal, the boss protein, and the sevenless receptor appear to be exclusively dedicated to the correct specification of this one cell type, it has been assumed that this signal is sufficient to instruct an undetermined cell to become a R7 photoreceptor cell. Ectopic activation of the sevenless kinase in the cone cells, either in the Sev387 mutant or by ectopic expression of boss, is sufficient to induce the R7 cell fate in these cells (Basler et al. 1991; Van Vactor et al. 1991). In some cases, however, sevenless activity can be uncoupled from the R7 cell fate, suggesting that the primary role of sevenless is to induce photoreceptor cell development (Rubin 1991). For example, ectopic expression of rough in the R7 precursor causes this cell to adopt an R1–R6 identity (Basler et al. 1990; Kimmel et al. 1990). The decision to become a photoreceptor cell, however, still depends on sevenless activity. Similarly, ectopic rough expression in the cone cells results in a transformation into R1–R6-type cells only if sevenless activity, provided by the sE–torso4021–sev construct, is present in these cells (Fig. 4B). The transformation of the cone cells not only depends on sevenless activity, but also on sina (Fig. 4C,D). This suggests that both sevenless and sina are involved in the initial decision between non-neuronal and neuronal development. Because the development of the other photoreceptor cells is independent of sevenless, boss, or sina, specification of neuronal development in these cells must occur by a different mechanism. It has been shown recently that the transmission of the sevenless signal depends on the activation of a ubiquitous signal-transducing protein, Ras1 (Simon et al. 1991; Fortini et al. 1992). It is likely that in the other photoreceptor precursors Ras1 is activated by a different receptor system (Rubin 1991). We have demonstrated recently that the cell fate transformations observed with the activated sevenless kinase can also be obtained by expressing the activated torso kinase under the control of the sE [B. Dickson, F. Sprenger, and E. Hafen, in prep.]. It appears, therefore, that there is little specificity in the signal mediated by the tyrosine kinase and that the nature of the response of the cell depends more on the developmental history of the cell than on the identity of the signaling kinase.

Different ommatidial precursors exhibit a different, temporally limited response to sevenless activity

A pulse of ubiquitous expression of the activated torso4021–sev kinase results in differential responses by the various ommatidial precursors. Cells anterior to the morphogenetic furrow are refractory to sevenless activity. Similarly, sevenless activity does not interfere with ommatidial spacing in the furrow, a process that requires the Drosophila epidermal growth factor receptor homolog (DER), another receptor tyrosine kinase. Gain-of-function mutations in DER, called Ellipse, cause a reduction in the number of ommatidial units that initiate development (Baker and Rubin 1989). Although some components involved in transduction of the signal from the receptor, such as Sos and Ras1, are shared between sevenless and DER (Rogge et al. 1991; Simon et al. 1991), ommatidial spacing in the heat-shocked hsp–torso4021–sev transformants is normal. It appears that the activated sevenless kinase cannot interfere with the activity of the DER signaling pathway.

Ectopic sevenless activity in cells of the precluster results in a reduced number of outer photoreceptor cells (Fig. 6, green). Sevenless activity in R2 and R5 might interfere with the correct specification of R2/5 subtype, such that either R3/4 or R1/6 can no longer be recruited. Consistent with this interpretation is the fact that upon induction of sevenless activity, the precluster does not form properly as visualized by staining with the anti-elav antiserum. Instead of five cells, often only four cells initiate neuronal development (Fig. 7B, open arrows). It appears that sevenless activity in these cells is incompatible with the correct specification of cell fate.

The ability to spatially separate the responses of different ommatidial precursor cells along the anteroposterior axis by a single pulse of sevenless activity was especially informative for the fate of the mystery cells. We have shown previously that in Sev387 transformants, the mystery cells, positioned between R3 and R4, can initiate neuronal development in response to sevenless activity (Basler et al. 1991). Here, we have shown that the mystery cells can initiate either R7 or R1–R6 development in response to sevenless activity. It is possible that the acquisition of an R1–R6 identity by the mystery cells in hsp–torso4021–sev is dependent on rough function in R3 and R4. A similar nonautonomous dependence on rough expression in neighboring cells (R2 and R5) is observed for the development of R3 and R4: In mosaic ommatidia, rough– R3/4 precursor cells can develop as outer photoreceptor cells only when R2 and R5 are rough+ (Tomlinson et al. 1988). The observation that the mystery cells can adopt either an R7 or an R1–R6 fate in response to sevenless activity further supports the hypothesis that sevenless activation triggers neuronal development independent of photoreceptor subtype.

The development of both R3/4 and R1/6 appears to be unaffected by sevenless activity. Because these cells ini-
tiate neuronal development independently of sevenless activity, ectopic sevenless activity is unlikely to result in a transformation of fate in these cells. The only detectable transformation in our assay would be the adoption of an R7 cell fate by one of these cells. However, we do not observe any ommatidia with additional R7 cells and a concomitant reduction in the number of outer photoreceptors in this region of the eye. These results are consistent with previous findings that these cells appear unchanged in \textit{Sev}^{s11} or in \textit{hsp-boss} mutants (Basler et al. 1991; Van Vactor et al. 1991). In these cells activation of Ras1 is presumably achieved by another pathway, in which case the signal from the sevenless kinase would be redundant. Furthermore, the R3/4 cells express the genes \textit{rough} and \textit{seven-up}, both of which have been shown to suppress R7 development (Basler et al. 1990; Kimmel et al. 1990; Mlodzik et al. 1990).

Induction of sevenless activity at the time of the determination of the R7 precursor and the cone cells causes these cells to develop as R7 cells. In contrast, induction of sevenless activity during pupal development results in the absence of pigment cells. The failure of pigment cells to develop is observed when sevenless activity is induced between 8 and 16 hr of pupal development. This period coincides with the recruitment of pigment cells into the ommatidial lattice and precedes the time where supernumary cells are eliminated by programmed cell death, which peaks at \( \sim 28 \) hr of development at 25°C (Wolff and Ready 1991). The notion that sevenless activity interferes with the determination of pigment cells rather than with the process of cell death or differentiation is supported further by the fact that the responsive period coincides with the period during which these cells are sensitive to temperature shifts with a \textit{Notch}^{ts} allele (Cagan and Ready 1989).

The clear distribution of ommatidial phenotypes along the anteroposterior axis demonstrates that cells can respond to sevenless activity only during a brief period. Previous experiments involving either the expression of the wild-type \textit{sevenless} cDNA under the heat shock promoter \( (\text{hsp-sev}) \), or the analysis of temperature-sensitive mutations of \textit{sevenless} indicated that the R7 precursor can respond to the presence of the sevenless protein only during a short period (Basler and Hafen 1989b; Bowtell et al. 1989; Mullins and Rubin 1991). This could be due to the fact that either the ligand required for sevenless activation is only presented during this short time or that R7 precursor cells can respond to the sevenless activity only for a limited time. Our results support the latter hypothesis. As in the case of ubiquitous induction of the wild-type sevenless protein, heat shock induction of \textit{torso}^{s021} -\textit{sev} rescues R7 cells only in a narrow region \( \sim 12 \) columns from the anterior margin of the eye. The stripe of rescued R7 cells, as well as the stripes in which cone cell and mystery cell transformations are observed, indicate the regions within the disc epithelium in which cells are competent to respond to sevenless activity. These regions correspond to a period of only a few hours for each of the three cell types. Therefore, independent of the distribution of the ligand and the receptor, cells can respond to sevenless activity only during a limited period. Transient competence to respond to an inducing signal has been noted for a long time in other systems. For example, the competence of \textit{Xenopus} animal cap cells to respond to mesoderm induction is limited to a few hours during development (Gurdon 1992). It is unclear, however, whether the competence to form mesoderm is limited by the presence of the receptor, components in the signal transduction cascade, or the ability to interpret the signal. In the case of sevenless-mediated induction, competence appears to be limited down-stream of the receptor.

**Only sevenless-expressing cells are competent for neuronal induction by sevenless activation**

The only cells competent to enter a neuronal pathway in response to sevenless activity express sevenless in wild type (Fig. 9). Furthermore, even their period of competence to respond to sevenless activity coincides very closely with the stages when they normally express \textit{sevenless}. In contrast, in cells where \textit{sevenless} is not normally expressed, such as in R2/5, R8, and the pigment cells, sevenless activity appears to be harmful because we observe either a reduction in the number of outer photoreceptor cells or a failure of pigment cells to develop in the presence of sevenless activity. Therefore, \textit{sevenless} expression in the mystery cells and in the R7 and cone cell precursors marks a preexisting pattern of cells with equivalent potentials [an equivalence group]. It is interesting to note that in the honey bee \textit{Apis mellifera}, which contains two UV-sensitive R7-like photoreceptor cells, the additional R7-like cell develops in a position analogous to that of the mystery cells in \textit{Drosophila}.
phila (Eisen and Youssef 1980, Ready 1989), suggesting that these cells form a similar equivalence group in Apis.

In view of the correlation between sevenless expression and the competence to respond to its activation, it seems probable that other components required for the transduction of the signal are expressed coordinately with sevenless. Consistent with this idea is the observation that sina is expressed in a pattern very similar to sevenless (Carthew and Rubin 1990). The distribution of the sina protein alone, however, cannot account for this difference, because the mystery cells, which do not express sina, belong to the R7 equivalence group.

In summary, our results suggest that before the normal activation of the sevenless kinase, a preexisting pattern dictates that only a small group of cells will transiently become competent to respond to this signal. The understanding of how this prepattern is established before any known commitment will be important for understanding how cell type diversity is generated. It is likely that the identification of genes controlling the sevenless expression pattern might provide valuable insight into this problem.

Materials and methods

DNA constructs

Sequences encoding the torso extracellular domain were amplified by polymerase chain reaction (PCR) from the plasmids pBtor, pBY9, and p84021 (Sprenger and Nüsslein-Volhard 1992) using primers with the following sequences: 5'-GAGGTAC- preceded by an EcoRI linker {GGTACC} and followed by an SmaI site. Both mutations result in the same base pair change, rather than the T--* A transition in the original Y9 allele, was introduced into the plasmid pBY9 to create the sina construct.

For the analyses described here, representative transformant lines were chosen for each construct. The SEM photographs and eye sections presented in Figure 2 are from the following lines: sE–torsoY9−sev, STY9S.1 (heterozygous insertion on the third chromosome), sE–torsoY6−sev, STY9S.1 (heterozygous insertion on the third chromosome), sE–torso4~sev, ST4021S.4 (heterozygous insertion on the second chromosome); and sE–torsoLys4~sev, ST4021SKM.1 (homozygous insertion on the third chromosome). For the temperature-dependence analysis presented in Figure 3, the same sE–torsoY9−sev transformant was used (STY9S.1), but a different sE–torso4~sev transformant (ST4021S.1) was used, showing a marginally weaker eye phenotype and also carrying a homozygous viable insertion on the second chromosome. Heat shock analysis using the hsp–torso4~sev construct was performed on the line HT4021S.2, obtained by mobilization of the original insertion as described above. These flies carry the hsp–torso4~sev construct as a homozygous lethal insertion at 94A on the third chromosome. Heterozygotes were used in which this chromosome was balanced over the TM3 chromosome.

Transgenic flies carrying either the sE–ro (original designation was sev–hsp–rough) or Rb3–bal constructs were as described previously (Basler et al. 1990, 1991). All analyses were carried out in either a w¹¹⁸, sev¹⁶ or w¹¹⁸, sev¹⁶, sina¹⁶ background.

SEM, histology and β-galactosidase staining

SEM and β-galactosidase staining were performed as described previously (Basler et al. 1991). Eye discs were stained with a

For all four fusions, separate transformation constructs were prepared in duplicate using the products of two independent PCR reactions. Multiple transformant lines were obtained for all constructs, and because duplicate constructs showed no phenotypic variation when expressed under the sevenless enhancer, we conclude that no significant sequence variation has been unwittingly introduced by the PCR amplification.
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1 : 25 dilution of affinity-purified rat polyclonal anti-elav antiserum (gift of K. White, Brandeis University, Waltham, MA), followed by an FITC-conjugated secondary antibody (Southern Biotech, Inc.) according to the protocol described in Gaul et al. [1992]. Semithin sections of adult eyes were prepared as described previously [Basler et al. 1991] and stained for light microscopy with either toluidine blue or a 1 : 1 mixture of toluidine blue and methylene blue.

Heat shock conditions

Single heat shocks were applied in sealed Drosophila culture tubes submerged in a water bath at 37°C for 1 hr. After heat shock treatment, development was allowed to continue at 25°C.

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