Neuronal development in the *Drosophila* retina: The *sextra* gene defines an inhibitory component in the developmental pathway of R7 photoreceptor cells

(eye development/sevenless)

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ABSTRACT Mutations in a gene called *sextra* (*sxt*) have been isolated. Loss of one copy of *sxt* promotes R7 photoreceptor cell development in a genetically sensitized background, while loss of both copies results in precursors of non-neuronal cone cells transforming into R7 cells. The requirement for *sxt* function is cell-autonomous. The transformation of cone-cell precursors into R7 cells occurs independently of the *sevenless* signal. However, the R7 precursor becomes neuronal in an *sxt/sxt* mutant only in a wild-type *sevenless* background. The genetic analysis of *sxt* suggests that it plays an inhibitory role, preventing cone cells from becoming neuronal. Additionally, *sxt* functions in R7 precursors, but the *sevenless* signal is essential for specification of this fate, since loss of *sextra* alone is unable to impart a neural fate to this cell.

Characterization of a set of genes controlling R7 development has provided much insight into the process of induction and signal transduction (1–4). Genetic and molecular analysis of *sevenless* (*sev*) (5–8) and bride of *sevenless* (*boss*) (9, 10) has demonstrated that R8 induces a neighboring cell to take on the R7 fate (9). In *boss* or *sev* mutants, R7 cells are missing within the eye. While the *boss* protein, a membrane-bound ligand, is required in the inducing R8 cell for normal R7 development (11), the *sev* gene encodes a tyrosine kinase receptor required in the R7 precursor for it to assume an R7 fate (12, 13). The *boss* protein has been shown to bind directly to the *sevenless* protein (11), initiating a molecular cascade that causes development of the R7 neuron.

Recently, two more genes, Son of *sevenless* (*Sos*) and *Dras1*, have been shown to participate in this signal-transduction pathway (14–16). Both gain- and loss-of-function mutations in *Sos* affect the development of R7 cells. *Sos* functions downstream of *sevenless* and the *Drosophila* epidermal growth factor receptor and encodes the *Drosophila* homolog of CDC25 of *Saccharomyces cerevisiae*. The CDC25 product has been shown to be an activator of Ras in yeast (17). It is likely that *Sos* functions as an activator of *Drosophila* Ras1.

A dominant mutation in *Sos* (called *Sos^{JC2}*) suppresses the phenotype of a specific allele (*sev^{EA}*) of *sevenless* (14). While *sev^{EA}* flies lack all R7 cells, in *sev^{EA}/sev^{EA};Sos^{JC2}/+* flies, R7 cells develop in a small fraction of the ommatidia. The *sev^{EA}* product is likely to have residual tyrosine kinase activity, and the *Sos^{JC2}* product compensates for the partial loss of this kinase activity by hyperactivating the downstream molecule Ras (14, 16). This is a sensitized system where the fraction of ommatidia in which R7 develops is critically dependent on the dosage of other genes in the *sevenless* pathway. For example, in a *sev^{EA}/sev^{EA};Sos^{JC2}/+* mutant background, no

R7 cells develop when one copy of *boss* or of *Dras1* is eliminated.

In this paper we characterize a gene called *sextra* (*sxt*), which also affects the development of R7 cells. However, unlike *sev⁺*, *boss⁺*, *Sos⁺*, and *Dras1⁺*, which promote the development of R7 cells, the wild-type function of *sxt* in the eye is to repress R7 fate.

MATERIALS AND METHODS

Genetic Analysis. The *sxt^{BJ61}* allele was isolated in a *P*-element mutagenesis scheme essentially following Bier *et al.* (18), except that a *P(ry⁺)* element was used.

For mosaic analysis, females carrying a *P[w⁺]* insertion at 78C/D were mated to either *sxt^{BJ61}/sxt^{BJ61}* or *sxt^{BJ61},boss¹/sxt^{BJ61},boss¹* males. Progeny from these crosses were irradiated with γ -rays (1200 rads; 1 rad = 0.01 Gy) between 24 and 48 hr of development. Mosaic eyes were generated at a frequency of ≈ 0.01 .

Histology. Preparation of samples for transmission electron microscopy (TEM) was essentially as described (19), except that uranyl acetate staining *en bloc* was omitted. The sections were stained for 30 min with uranyl acetate and 10 min with lead citrate and were analyzed on a Phillips 300 electron microscope operating at 60 kV. For light-level microscopy, the fixation conditions were modified as described (9). To facilitate scoring of pigment granules, flies were exposed to bright light for 10 min before dissection. Cobalt sulfide staining was done as described (4).

Immunohistochemistry. Adult heads were dissected into halves and fixed for 1 hr in 0.8% glutaraldehyde in phosphate-buffered saline. The retinas were transferred to fresh fixative, dissected out of the surrounding cuticle, allowed to fix for another 30 min, stained with antibodies (8), and embedded for sectioning (9). Eye imaginal discs were stained with monoclonal antibody (mAb) 22C10 and processed for TEM (11).

RESULTS

Loss of One Copy of *sxt* Enhances *Sos^{JC2}*. R7 cells develop in 17% of the ommatidia in *sev^{EA}/sev^{EA};Sos^{JC2}/+* flies (Table 1). This double mutant serves as a starting point for identification of other genes involved in R7 development. Loss of one wild-type copy of *boss* or *Dras1* reduces the suppression level to 0%. These genes have been shown to function as positive regulators of the pathway (11, 15, 16). Mutations in *boss* or *Dras1* are normally recessive; only in this sensitized system does a 2-fold reduction in their activity have an effect on R7 development.

Mutations in *sxt* enhance the *sev^{EA}/sev^{EA};Sos^{JC2}/+* phenotype. Loss of a single copy of *sxt* causes R7 cells to develop

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Abbreviation: mAb, monoclonal antibody.

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Table 1. Levels of suppression

Genotype	Ommatidia	
	% containing R7 cells	No. scored
<i>sev^{E4}/sev^{E4};Sos^{JC2}/+</i>	17	1772
<i>sev^{E4}/sev^{E4};sxt^{BJ61}/+</i>	0	2259
<i>sev^{E4}/sev^{E4};Sos^{JC2}/+;<i>boss</i>¹/+</i>	0	2337
<i>sev^{E4}/sev^{E4};Sos^{JC2}/+;<i>Df</i>(<i>Dras1</i>)/+</i>	0	2175
<i>sev^{E4}/sev^{E4};Sos^{JC2}/+;<i>sxt</i>^{BJ61}/+</i>	49	1934

Individual ommatidia were scored for the presence of R7 by using the optical technique of pseudopupil (ref. 5 and references therein). The *boss*¹ mutation is a null allele. Since a *Dras1* point mutation was not available, *Df*(3R)by62, a deficiency including this locus, was used.

in 49% of the ommatidia (Table 1), a substantial increase over the 17% level with two wild-type copies of *sxt*. In contrast, loss-of-function mutations in all other genes identified in this assay reduce the number of R7 cells that develop. This suggests an inhibitory role of *sxt* in the R7 developmental pathway.

The ommatidia seen in *sev^{E4}/sev^{E4};Sos^{JC2}/+;*sxt*/+* flies never contain more than the one R7 cell, and this cell always occupies its wild-type position between R1 and R6. As shown later, the recessive phenotype of *sxt* is to create additional R7 cells—hence the name *sextra* (for “seven extra”).

Supernumerary R7 Cells Develop in *sxt/sxt* Eyes. In tangential sections of wild-type eyes, the rhabdomeres of outer photoreceptors R1–R6 are large and form a trapezoidal pattern in each ommatidium. These appear in both proximal and distal sections (Fig. 1 A and B) because they extend the entire length of the ommatidium. The rhabdomeres of R7 and R8 project centrally and are smaller in size. The R7 rhabdomere is found distally (Fig. 1A), whereas R8 extends

FIG. 1. Transmission electron micrographs of adult eyes. (A) Distal section through a wild-type ommatidium. Dark structures on the photoreceptor (R) cells are membrane specializations called rhabdomeres. Numbers 1–7 correspond to cells R1–R7. (B) Proximal section of same ommatidium as in A. The rhabdomere of R8 is visible at this level. (C) Distal section through an *sxt^{BJ61}/sxt^{BJ61}* eye, showing multiple central photoreceptors. (D) Proximal section of same ommatidium as in C, showing a normal R8. (Bars = 2 μ m.)

proximally (Fig. 1B). The *sxt* mutation was made homozygous in order to assess its recessive phenotype. Sections of *sxt/sxt* eyes show that every ommatidium contains multiple centrally projecting rhabdomeres (Fig. 1C). Morphologically, these are similar to R7 rhabdomeres in that they are small and project centrally and distally.

Serial reconstruction of 61 ommatidia at the electron microscopic level showed that the development of R1–R6 is not affected in *sxt*. In 35 of the 61 ommatidia reconstructed, the rhabdomeres of R1–R6 were found to be displaced, failing to extend the entire length of the ommatidium. However, in no case did an ommatidium contain fewer than six outer photoreceptor cells. In every ommatidium, a single rhabdomere was found to project proximally, in the position of R8 (Fig. 1D). The number of R7-like cells in each ommatidium ranged from 2 to 6, although the majority contained either 3 or 4. The average number was 3.8.

To ascertain the identity of the extra cells, *sxt^{BJ61}* was crossed into a *ninaE* mutant background. The *ninaE* mutation causes the rhabdomeres of the outer photoreceptor cells (R1–R6) to degenerate, leaving the rhabdomeres of R7 and R8 intact (Fig. 2A). In *sxt^{BJ61};ninaE* double mutants, the rhabdomeres of the extra R cells do not degenerate (Fig. 2B). This implies that the extra cells have the identity of either R7 or R8.

To distinguish between these two possibilities, the type of opsin expressed in the extra cells was determined. In *Drosophila*, two opsins, Rh3 and Rh4, are specific to R7 (20). A reporter gene controlled by the Rh4 promoter is expressed in a subset of R7 cells in wild-type eyes (Fig. 2C). In *sxt^{BJ61}* a similar fraction of the extra central cells express this R7-specific marker (Fig. 2D). This demonstrates that the central cells are of the R7 type.

FIG. 2. Identity of the extra R cells in *sxt/sxt*. (Upper) Transmission electron micrographs. (A) Distal section through the eye of a fly carrying two copies of the *ninaE* deficiency *Df*(3L)I17e. The flies were aged for 5 days. Outer cell rhabdomeres have completely degenerated. The single rhabdomere visible in each ommatidium belongs to the R7 cell. (B) Distal section through the eye of an *sxt^{BJ61},Df(3L)I17e/sxt^{BJ61},Df(3L)I17e* fly. The rhabdomeres of the extra cells do not degenerate in this background. (Lower) Light microscope sections of adult eyes. (C) Wild-type fly carrying the *lacZ* gene driven by the Rh4 promoter, stained with an anti- β -galactosidase antibody. A fraction of the R7 cells express the Rh4 promoter-driven reporter gene. (D) An *sxt^{BJ61}/sxt^{BJ61}* fly carrying the *lacZ* gene driven by the Rh4 promoter, stained with an anti- β -galactosidase antibody. A fraction of the R7 cells, including the extra central cells, stain positively with the antibody against the reporter gene product. (Bars = 5 μ m.)

The extra R7 phenotype is strictly recessive in that *sxt/+* flies have wild-type eyes. Due to the change in the internal morphology of the *sxt/sxt* flies, the external appearance of the eye is irregular or "rough." This external phenotype facilitated the mapping of the *sxt* locus.

Genetic Mapping. The *sxt^{BJ61}* mutation was isolated in a *P*-element-induced mutagenesis and was mapped between the hairy and scarlet loci by standard genetic recombination techniques. Consistent with this mapping, a *P* element was detected by *in situ* hybridization on band 67C of the third chromosome (data not shown). The *sxt^{BJ61}* mutation was mapped to the deficiency *Df(3L)AC1*, which deletes bands 67A through 67D. The phenotype of *sxt^{BJ61}/Df(3L)AC1* is identical to that of *sxt^{BJ61}/sxt^{BJ61}* at the light microscope level, in that the eyes are rough and extra R7 cells are seen, suggesting that *sxt^{BJ61}* is a null allele. Further, this same phenotype is seen when any one of 15 different imprecise excision alleles is placed over either *sxt^{BJ61}* or *Df(3L)AC1*. Thirty-five precise excisions of the *P* element in *sxt^{BJ61}* were isolated. In each case, the *sxt* phenotype reverted to wild type, demonstrating that the *P*-element insertion was responsible for the *sxt* mutation.

Developmental Profile. To view early events in ommatidial development, wild-type and *sxt* eye discs were stained with cobalt sulfide. In the wild-type disc, cobalt sulfide highlights the initial formation of six- or seven-cell preclusters at the morphogenetic furrow. This is followed by the loss of one or two "mystery" cells (21), giving rise to a normal five-cell precluster consisting of R2, R3, R4, R5, and R8 (Fig. 3A). In *sxt* mutants, early development proceeds normally through the five-cell precluster stage (Fig. 3B). This is to be expected, since the fate of R8 and the outer cells is unaffected in this mutant.

To study later events, wild-type and *sxt* eye discs were stained with mAb 22C10, which recognizes a neural-specific antigen expressed by photoreceptor cells. The stained discs were sectioned tangentially and analyzed by electron microscopy. In wild-type development, R1, R6, and finally R7 add to 5-cell preclusters, giving rise to mature 8-cell clusters. This is followed by the addition of 4 non-neuronal cone cells. Cone cells in wild-type flies never stain with mAb 22C10 (Fig. 3C and E). In *sxt*, R1, R6, and R7 add normally. However, cells in the position of cone-cell precursors begin expressing antigen 22C10, revealing their transformation to a neural fate (Fig. 3D). Thus, the extra phenotype results from the transformation of cone-cell precursors into R7 cells. The cone-cell precursors are added to the developing extra ommatidium in a strikingly ordered sequence (Fig. 3F; see also Fig. 5C). The anterior and posterior cone cells are the first to show transformation. This results in a 10-cell neural cluster in which cells are arranged in a stereotyped fashion. After a lag of several hours, a second pair of anterior and posterior cone-cell precursors are added, in addition to an equatorial and a polar cone-cell precursor. Some of these cells will express antigen 22C10 as well. This is in contrast to the transformation seen with ectopic *boss* expression (22), where development proceeds as in wild-type up to 28 hr, and only then do the cone-cell precursors assume R7 fates.

Dependence on *sev* and *boss*. In *sxt*, as in wild type, the membrane-bound *boss* protein is restricted to R8 (data not shown). The cone-cell precursors that are converted into R7 in *sxt* do not contact R8 and therefore are not exposed to the ligand, yet they do become R7 cells. This implies that the development of the cone-cell precursors as R7 cells should be *boss*-independent. To test this genetically, double mutant combinations of *sxt* were made with *boss* as well as with *sev*. While flies mutant for *sev* or *boss* always lack R7 cells (Fig. 4B and C), the double mutants *sev/sev;sxt/sxt* and *sxt,boss/sxt,boss* are similar in phenotype to *sxt/sxt* in that they display multiple R7 cells (Fig. 4D–F).

FIG. 3. Ommatidial assembly in *sxt* mutant eye discs. (A and B) Cobalt sulfide staining of third-instar larval eye discs. (Bars = 10 μ m.) (A) Wild type. Cobalt sulfide stains apical membranes of differentiating cells. Staining commences as a dark band at the morphogenetic furrow. Initial seven-cell clusters (arrow) resolve into five-cell preclusters of R8, R2, R5, R3, and R4 (arrowhead). (B) *sxt^{BJ61}/sxt^{BJ61}*. Pattern formation in *sxt* mutant discs proceeds normally through the stages described in A. (C–E) Transmission electron micrographs of eye disc stained with mAb 22C10. (Bars = 0.5 μ m.) (C) Developing wild-type ommatidium at the two-cone-cell stage. The anterior (AC) and posterior (PC) cone cells add to the cluster after the eight R cells have differentiated. Staining with mAb 22C10 can be seen in photoreceptor cells. The non-neuronal cone cells do not stain with this neural-specific antibody. (D) *sxt^{BJ61}/sxt^{BJ61}* ommatidium at a stage comparable to that shown in C. In this ommatidium, the cell in the position of the anterior cone cell (star) expresses the 22C10 neural antigen. (E and G) Wild-type ommatidium at the four-cone-cell stage. The membranes of the transmission electron micrograph in E have been traced in G for clarity. The cluster at this stage has 12 cells, R1–R8 and 4 cone cells. (F and H) *sxt^{BJ61}/sxt^{BJ61}* ommatidium at a stage comparable to that shown in E and G. This cluster contains 14 cells: R1–R8, 3 cone-cell precursors converted into R7 cells (stars), and 3 other cells that do not stain with mAb 22C10 and are presumed to be cone cells.

Two different experiments were performed to determine whether the development of all R7-like cells in *sxt* mutants is *boss*- and *sev*-independent. Adult eyes of *sev^{d2}/sev^{d2};sxt/sxt* flies were sectioned, and 63 ommatidia were serially reconstructed at the EM level. The average number of R7 cells

FIG. 4. Light microscope tangential sections through adult eyes. (A) Wild type. In this distal section, the rhabdomere projecting centrally in each ommatidium belongs to R7. (B and C) R7 cells are missing in *sev*^{d2}/*sev*^{d2} (B) and *boss*¹/*boss*¹ (C) mutant eyes. (D–F) Multiple R7 cells develop in *sxt*^{BJ61}/*sxt*^{BJ61} (D), *sev*^{d2}/*sev*^{d2}; *sxt*^{BJ61}/*sxt*^{BJ61} (E), and *sxt*^{BJ61}, *boss*¹/*sxt*^{BJ61}, *boss*¹ (F) mutants. *sev*^{d2} and *boss*¹ are null alleles at the respective loci (6, 10). (Bars = 5 μ m.)

developing per cluster was found to be 2.8. This number is different from the average of 3.8 seen in *sxt/sxt* flies, suggesting that 1 cell per ommatidium is *sev*-dependent. Furthermore, when *sev/sev*; *sxt/sxt* and *boss/sxt*; *boss/sxt* eye discs were stained using an elav antibody, which stains neuronal nuclei, no staining could be detected in the cell in the position of the R7 precursors at the light microscopic level. To determine whether the development of the R7 precursor into a neuron is *sev*-dependent in these double mutants, we scored developing *sxt, boss/sxt, boss* eye discs for the development of the ommatidium. The cell in the position of R7 did not stain with mAb 22C10 in a *sxt, boss/sxt, boss* double mutant (Fig. 5). Of the 70 ommatidia that we scored at the EM level, one was found to show staining of the R7 precursor, while the other 69 precursors did not stain. Ommatidia were scored at a stage in which cone-cell precursors always express antigen 22C10 in a *sxt/sxt* mutant. We therefore conclude that the development of the R7 precursor is *sev*- and *boss*-dependent in a *sxt*^{BJ61} mutant background, whereas the cone-cell precursors are transformed into an R7 fate independently of *sev*.

The transformation of the cone cells results in functional R7 neurons since, like wild-type flies, *sev/sev*; *sxt/sxt* and *sxt, boss/sxt, boss* double mutants choose UV light over visible light in a color choice paradigm (Table 2).

***sxt* Function Is Cell-Autonomous.** A mosaic patch of white cells of genotype *sxt/sxt* was generated in an otherwise red eye of genotype *sxt/+*. Along the boundary of the patch, mosaic ommatidia consisting of a mixture of red and white cells were screened. The aim was to determine which cells within an ommatidium must be white (i.e., *sxt/sxt*) for R7 cells to develop properly. This experiment was performed in two different ways.

First, mosaic patches were generated in a *boss*[−] background to eliminate the inductive signal. Pigment granules in R7-containing mosaic ommatidia were scored. In 92 such ommatidia, all R7 cells were white (i.e., *sxt/sxt*). Thus the cells that take on R7 fate must be mutant for *sxt*. This implies that *sxt* function is autonomously required in precursor cells choosing between cone and R7 cell fates.

The second mosaic experiment was done in a *boss*⁺ (i.e., wild-type) background. In this case, a cell in the position of the R7 precursor receives the *boss*- and *sevenless*-mediated signal, and therefore even when it is *sxt*⁺ (i.e., red), it would become R7. However, if *sxt* is indeed autonomous, in no case

should there be more than one red R7 cell in any ommatidium. This was indeed found to be the case. A total of 55 mosaic ommatidia containing multiple R7 cells were scored. Of these, 46 ommatidia had all white R7 cells. Each of the remaining 9 ommatidia contained a single red R7 cell, and no ommatidia were found with more than one red R7 cell.

FIG. 5. Development of the R7 precursor in an *sxt, boss/sxt, boss* double mutant. (A) mAb 22C10 staining of an ommatidium in this double mutant. (B) The cells shown in A have been traced for clarity. Staining can be seen in the cone-cell precursors (stars), while the R7 precursor (X) is clearly unstained. C, cone cells. (Bar = 1.0 μ m.) (C) Schematic representation of ommatidial assembly. Shading represents mAb 22C10 staining. Unlike wild type, the anterior and posterior cone-cell precursors (ccp) stain in *sxt* and *sxt, boss/sxt, boss* mutants. Four more cone-cell precursors are added later (stars), some of which transform into additional R7 cells. The R7 precursor always stains in *sxt*, but virtually never stains in *sxt, boss/sxt, boss* (X). Hours refer to the time since initial cluster formation.

Table 2. Color choice data

Genotype	No. to UV (A)	No. to visible (B)	Choice index, (A - B)/(A + B)
Wild type	93	5	+0.90
<i>sev^{E4}/sev^{E4}</i>	6	105	-0.89
<i>boss¹/boss¹</i>	1	94	-0.98
<i>sev^{E4}/sev^{E4};sxt^{BJ61}/sxt^{BJ61}</i>	96	3	+0.94
<i>sxt^{BJ61},boss¹/sxt^{BJ61},boss¹</i>	94	6	+0.88

Flies were allowed to choose between visible (550 nm) and UV (350 nm) light in a color choice test apparatus (6). Thirty flies were tested at a time; 20 sec was allowed for the test, with a gentle tapping at the end of 10 sec. Each group of flies was tested three consecutive times.

No requirement for *sxt* was found in any of the other cell types in the ommatidium. In the two experiments described above, a total of 147 mutant mosaic ommatidia were serially reconstructed and were scored for the pigment phenotype of the outer cells and R8. In 64 of these ommatidia, the R8 cells were red (i.e., *sxt*⁺), and in the rest of the cases they were white. This implies that the genotype of *sxt* in R8 is irrelevant for the development of R7 cells. Similarly, the outer cells in these ommatidia displayed no specific requirement for the *sxt* genotype. The identity of an individual outer cell cannot be determined in the ommatidia that have extra R7 cells, since the normal trapezoidal pattern is often disrupted. However, in 13 of the ommatidia all six outer cells were red, implying that extra R7 cells can develop when all outer cells are wild-type for *sxt*. Finally, 4 ommatidia were found in which R1-R6 and R8 were red (i.e., *sxt*⁺) and all the R7 cells were white (i.e., *sxt/sxt*).

When taken together, the above results imply that wild-type *sxt*⁺ function is required only in the cells that have the potential to become R7, including the cone-cell precursors.

DISCUSSION

In a fly that is otherwise wild-type, the *sxt* mutation is recessive, i.e., a mutant phenotype is seen only in *sxt/sxt* flies. This phenotype results from the conversion of cone-cell precursors into R7, leading to supernumerary R7 cells in each ommatidium.

The effect of *sxt* on the development of R7, rather than cone-cell precursors, is more apparent in a *sev^{E4}/sev^{E4};Sos^{JC2}/+* genetic background. In this sensitized system, the eye is neither completely wild type nor entirely lacking R7 cells, and loss of a single wild-type copy of *sxt* affects the number of R7 cells that develop. Thus, a significantly larger number of R7 cells are seen in *sev^{E4}/sev^{E4};Sos^{JC2}/+;sxt/+* flies than in flies of the *sev^{E4}/sev^{E4};Sos^{JC2}/+* genotype. However, in this case, no more than one R7 cell ever develops in a single ommatidium and cone-cell precursors are never converted to R7.

A combination of these two results suggests that the normal function of *sxt* is to inhibit a set of competent precursor cells from assuming R7 fate. In wild type, this inhibition is presumably overridden in the R7 precursor by the boss- and sevenless-mediated signal. This model is consistent with the observations that nonspecific expression of boss (22) and overexpression of an activated form of sevenless (23) lead to phenotypes that are similar to that seen in *sxt/sxt* flies. Presumably, in these cases, ectopic activation of the receptor can overcome the inhibitory effects of *sxt* in the cone-cell precursors.

Unexpectedly, the R7 precursor in a *sextra* mutant fails to become a neuron unless the sevenless signal is present, even though the cone-cell precursors transform into R7 independently of sevenless. Clearly, there is an early difference between the R7 and the cone-cell precursors. This difference

may be due to the developmental timing of the cells or may result from the signals they receive. The role of *sxt* in the cone cells appears to be to inhibit their development as R7 cells. The failure of *sxt^{BJ61}* to show complete epistasis to *sev* and *boss*, however, leaves the role of *sxt* in the development of the R7 cell unclear. One possible explanation of these results is that *sxt^{BJ61}* is not a null allele of the *sxt* locus. Given the consistent failure of the R7 precursor cell to develop as a neuron in an *sxt,boss/sxt,boss* double mutant, a more likely explanation is that *sextra* is not directly acted upon by the sevenless receptor.

Note Added in Proof. The *mip* mutation isolated independently by G. Buckles, Z. Smith, and F. Katz and the *Gap1* mutation isolated by U. Gaul, G. Mardon, and G. Rubin fail to complement *sxt*.

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