Development 119, 377-395 (1993)
Printed in Great Britain © The Company of Biologists Limited 1993

The *strawberry notch* gene functions with *Notch* in common developmental pathways

Catherine A. Coyle-Thompson and Utpal Banerjee*

Department of Biology, Molecular Biology Institute, and Brain Research Institute, University of California, Los Angeles, CA 90024, USA

*Author for correspondence

SUMMARY

Genetic and phenotypic analysis of *strawberry notch* suggests that its gene product is required during embryogenesis and oogenesis, and for the development of the eye, wing and leg. Several lines of evidence suggest that *strawberry notch* participates together with *Notch* in many common pathways. A number of *strawberry notch* mutant phenotypes are similar to those of *Notch* mutants and can be rescued by an extra copy of wild-type *Notch*. In addition, mutations in *strawberry notch* interact strongly with *Notch* mutants in a tissue-specific manner. Mutations in the *strawberry notch* and *Notch* loci also show very similar interactions with genes like *Hairless, Delta, groucho, Serrate,* and *deltex* that have all been proposed to participate in *Notch* related pathways. The genetic evidence presented here suggests that *strawberry notch* participates with members of the *Notch* pathway in facilitating developmentally relevant cell-cell communications.

Key words: *strawberry notch, Notch*, cell-cell interaction, *Drosophila* gene

INTRODUCTION

Developmental analysis of virtually all neuronal tissues in *Drosophila* has shown that the interaction of a cell with its microenvironment is the primary mechanism of establishing cell fate. This has been particularly well established in the embryo (reviewed in Campos-Ortega and Jan, 1991; Artavanis-Tsakonas and Simpson, 1991; Cabrera, 1992) and in the developing eye (reviewed in Rubin, 1991; Banerjee and Zipursky, 1990). Two different classes of interactions seem to be involved. The first group comprises molecules that are used at defined stages of development for the exchange of specific intercellular signals. For example, the products of the genes participating in the *sevenless* pathway mediate specific developmental cues that are important for the establishment of the R7 neuronal identity in the eye (Kramer et al., 1991; Rogge et al., 1991, 1992; Basler et al., 1991; Simon et al., 1991; Bonfini et al., 1992; Dickson et al., 1992; Gaul et al., 1992). The second class of interactions have been termed 'permissive' (Cagan and Ready, 1989a). The function of permissive molecules is to make the cell receptive to specific signals, whether inhibitory or inductive. The *Notch* gene product is an excellent example of a molecule that has a permissive role (Artavanis-Tsakonas and Simpson, 1991; Cagan and Ready, 1989a). During embryonic neurogenesis, a lack of the *Notch* gene product prevents the process of lateral inhibition and leads to an overproduction of neurons. However, in the eye disc, where both lateral inhibition and induction play clear, separable roles, and in the development of sensory bristles on the thorax, a temperature-sensitive mutation in *Notch* can cause either a gain, or a loss of cells depending on the time of development when *Notch* gene function is disrupted (Shellenbarger and Mohler, 1978; Cagan and Ready, 1989a; Hartenstein and Posakony, 1990). *Notch* functions in the determination of many tissues, including the development of the oocyte (Ruohola et al., 1991; Xu et al., 1992), muscle cells (Corbin et al., 1991), adult retina (Cagan and Ready, 1989a) and sensory bristles (Hartenstein and Posakony, 1990). In each of these systems, differentiation is dependent upon cell-cell interactions, with *Notch* enabling cells to communicate with each other through different pathways. It is not clear whether the *Notch*-mediated pathways involve the transduction of a signal, or the creation of proper contacts between cells allowing signals to be transduced through other molecules.

The classification of *Notch*, along with *mastermind, neuralized, big brain, Enhancer of split* and *Delta* as neurogenic genes (Lehmann et al., 1981, 1983) is based upon the similarity of the mutant embryonic phenotypes. It is not clear however, that these genes all act in the same pathway. Recent evidence indicates that *Notch, Delta, mastermind, Enhancer of split, Serrate, groucho* and *deltex* may participate in a common scheme of cell-cell interactions (Artavanis-Tsakonas and Simpson, 1991). Two members of this group, *Ser* and *dx*, are not neurogenic genes (Fleming et al., 1990; Thomas et al., 1991; Xu and Artavanis-Tsakonas, 1990), yet they interact prominently with *Notch*.

Clearly, the *Notch*-mediated pathways involve many genes including some that are not classical neurogenics.

The *Notch* gene encodes a 288×10^3 M_r transmembrane protein with an extracellular domain primarily composed of EGF-like repeats (Wharton et al., 1985; Kidd et al., 1986). Similar repeats have been found in the products of the *Delta* (Fehon et al., 1990; Vässin et al., 1987; Kopczynski et al., 1988) and *Serrate* (Fleming et al., 1990; Thomas et al., 1991) genes, which bind to Notch directly when expressed in cell lines. Rebay et al. (1991) have suggested that Delta and Serrate could compete in binding to Notch. If this competition occurs in vivo, it could provide a mechanism for the selection of a particular cell fate. Unlike *Delta* and *Serrate*, the *deltex* gene product is likely to be cytoplasmic (Diederich and Artavanis-Tsakonas, personal communication; Artavanis-Tsakonas and Simpson, 1991), while the *mastermind* (Yedvobnick et al., 1988; Weigel et al., 1987) and *Enhancer of split* (Klämbt et al., 1989; Delidakis et al., 1991; Delidakis and Artavanis-Tsakonas, 1992) products are nuclear and are likely to participate in transcriptional processes.

In this paper, we describe the phenotypic analysis and genetic interactions of *strawberry notch* (*sno*). This gene is different from, and not allelic to *Notch*. It has been mapped to the 11D/E band of the *X* chromosome (Lefevre and Peterson, 1972), while *Notch* maps to the 3C7 band of the *X* chromosome. Mutations in *sno* share many phenotypes with mutations in *Notch* and their functions can be further correlated based on genetic interactions. Thus, an extra copy of *Notch* suppresses many of the *sno* phenotypes, while a loss of *Notch* function greatly enhances these same phenotypes. In this paper, we investigate the role of *sno* in the determination of cell fate and propose that *sno* functions in conjunction with *Notch* in permissively allowing a cell to interact with its neighbors.

MATERIALS AND METHODS

Drosophila lines

The *sno* alleles were isolated by EMS mutagenesis by G. Lefevre and M. M. Green and were kindly given to us for this study. The nd^1, nd^2, Ax^{E2} and Ax^{9b2} alleles of *Notch* (Shellenbarger and Mohler, 1975; Foster, 1975 and Portin, 1975), a cosmid transformant including a wild-type copy of *Notch (Cos 479* Ramos et al., 1989) and the neu^{IF65}, mam^{IL115}, bib^{ID05}, $E(spl)^{BX22}$, $groucho^{E73}$ and H^{99} mutations were gifts of S. Artavanis-Tsakonas. N^{ts1} was donated by R. Cagan and is described by Shellenbarger and Mohler, 1975. *Ser* (Belt, 1971), and the $E(spl)^{R1}$ and $E(spl)^{X72}$ deficiencies (Lehmann et al., 1983; Preiss et al., 1988) were gifts of E. Knust. The $boss^{16}$ deficiency was a gift of L. Zipursky. The Dl^{9P39} and Dl^{6b37} flies were provided by M. Muskavitch. *Dp(1;Y) 1* was provided by B. Baker. The *spl, dx,* Dl^3 and $E(spl)^D$ mutations were kindly provided by Kathy Matthews at the Indiana Stock Center and are described in Lindsley and Zimm (1992).

Recombination mapping

The initial mapping of the *sno* locus to the 11D/E region on the *X* chromosome was done in G. Lefevre's laboratory. The *sno* locus was placed 0.5 map unit distal to *wy* (Lefevre and Peterson, 1972). We have mapped the temperature-sensitive allele more closely by mating *w sn sno f car* males and *v fw wy* females. The resulting heterozygous females were mated to *v fw wy* males. Recombinants between *fw* and *wy* were analyzed for *sno* phenotypes**.** Using standard recombination methods, sno^{71e3} was placed between *fw* and *wy*, 1.5 cM from *fw* and 0.5 cM from *wy*.

The lethal allele sno^{EF531} was crossed to sno^{71e3} and the sno^{EF531}/sno^{71e3} females have a strong *sno* phenotype. The lethality was recombination mapped between *fw* and *wy* using a *v fw wy g* chromosome. Thus the lethality and the sno^{ts} phenotypes mapped to the same region, and failed to complement each other. We concluded that the temperature-sensitive and lethal mutations are alleles of *sno.*

The recombinant *sno* chromosomes were analyzed for the presence of any modifiers or other mutations that would affect the *sno* phenotype. When the distal region of the *X* chromosome was replaced through to *furrowed* (11A),and the proximal region of the chromosome was replaced from *wavy* (11E) to the centromere, no phenotypic change was observed for either the temperature-sensitive or lethal alleles of *sno.* In addition, the distal and proximal recombinant chromosomes resulting from removing *sno* were viable and fertile.

Complementation mapping

The sno^{71e3} males were mated to *Df(1) C246/FM6* (11D-E;12A1-2), *Df(1) N12/FM6* (11D1-2; 11F1-2) and *Df(1)* wy^{26}*/FM7* (11B17-C1; 11E9). Each of these deficiencies failed to complement the *sno* phenotype. The sno^{71e3} males were also mated to *Df(1) JA26/FM7* (11A1; 11D-E) and *Dp(1;Y) 1* females (*C(1)DX, y pn v/* y^+*YDp 1:* this duplication rescues *Df(1) C246* and does not rescue *Df(1) N12* or *Df(1)* wy^{26}. Thus, the 11D/E to 12A region of the *X* chromosome is duplicated in the *Dp 1* chromosome). The deficiency complemented the *sno* phenotype and the duplication rescued *sno* phenotypes and the lethality of $sno^{EF531.}$ Based on the complementation mapping, the *sno* locus was defined as between the 11D/E and the 11E9 salivary gland chromosome bands.

Generation of recombinants

The interactions between *sno* and alleles of *N* and *dx* were studied by making double-mutant combinations. Recombinants were balanced with *FM7* and the mutant sons from the lines were analyzed. In every case, the recombinants were checked for the presence of both *sno* and the desired allele of *Notch* or *deltex.* In each combination, the effects of the additional mutations on the chromosome were also analyzed. Neither *white (w), singed (sn)* nor *forked (f)* nor *carnation (car)* was observed to enhance or suppress the effect of either *sno* or of any other mutations or their combinations used in this analysis.

The *spl sno* combination was generated by recombination between a *spl* and a *w sn sno f car* chromosome. A *spl sno f car* recombinant was obtained. Similarly, nd^1 *sno* was obtained from w^a nd^1 and *w sn sno f car.* A *wa* nd^1 *sno f car* recombinant was obtained. Ax^{E2} *sno* was generated from Ax^{E2} *sn* and *w sno f car* and an Ax^{E2} *sno f car* recombinant was recovered. Ax^{9b2} *sn sno* was obtained from *y* Ax^{9b2} *sn* and *w sn sno f car.* Thus *y* Ax^{9b2} *sn sno f car* was constructed. N^{ts1} *sno* was generated from N^{ts1} *rb* and *w sn sno f car.* A N^{ts1} *rb sno f car* recombinant was isolated; and the *dx sno* combination was generated from *w dx* and *sno f car* chromosomes. A *w dx sno f car* combination was constructed.

Crosses

The following crosses were made with heterozygous sno^{71e3} females so that the maternal effect was the same for all the comparisons. The mutations mated to *sno* were all loss of function alleles with the exception of $E(spl)^D$, *Ser* and gro^{E73}.

Notch

w sn sno f car/FM7 × N^{55e11}*/ Dp (1;2)* w^{+51b7}/+ (*Dp* N^+*=Dp(1;2)* w^{+51b7}=3C1/2; 3D6; 52E; Lefevre, 1952)
sno f car/FM7 × *Cos 479/TM6B*

Hairless

sno f car/FM7 × H^{99} *e/TM3*

Delta

sno f car/FM7 × Dl^{3}*/TM3*
sno f car/FM7 × Dl^{6b37}*/TM6C*
sno f car/FM7 × Dl^{9p39}*/TM6B*

E(spl)C and *groucho*

sno f car/FM7 × $E(spl)^{D}$ *e* ($E(spl)^{D}$ has been described by Welshons 1956; Klämbt et al., 1989).
sno f car/FM7 × $E(spl)^{R1}$*/Tm6B* ($E(spl)^{R1}$ = In (3R) 96F2; 96F12-14; 99C, deficient for 96F12-14; Lehmann et al., 1983; Preiss et al., 1988).
sno f car/FM7 × $E(spl)^{X72}$*/TM6B* ($E(spl)^{X72}$ = Df(3R)96F5-97B1; Preiss et al., 1988).
sno f car/FM7 × gro^{E73}*/TM6B* (gro^{E73} is a lethal point mutation in *groucho* which has been described as antimorphic; Preiss et al., 1988; Delidakis et al., 1991).
sno f car/FM7 × $E(spl)^{BX22}$*/TM3* ($E(spl)^{BX22}$ is an inversion and deletion; Preiss et al., 1988).
sno f car/FM7 × *Df (3) boss*^{16}*/TM3* ($boss^{16}$ = Df(3R) 96F5/7-12/13 $boss^{16}$ also has an insertion in the *groucho* transcript; Hart et al., 1990; Schrons et al., 1992).

Other neurogenics

sno f car/FM7 × bib^{ID05}*/CyO*
sno f car/FM7 × mam^{IL115}*/CyO*
sno f car/FM7 × neu^{IF65}*/TM6B*

Serrate

sno f car/FM7 × *Ser/Ser*

Phenotypic analysis

The crosses to analyze the embryonic phenotype of the lethal allele was *v fw* sno^{EF531}*/FM7* × *FM7/Y*. The parents were placed in a yeasted bottle for two days and transferred to egg collection chambers. Eggs were collected for 2 hour intervals for antibody staining, or collected overnight for cuticle preparations. Cuticle preparations were also made from the progeny of these two crosses: *w* sno^{EF531}*/FM7* × *FM7/Y* and sno^{EF531}*/FM7* × *FM7/Y* and the results were the same. The cross to analyze embryonic phenotypes of the temperature-sensitive allele was sno^{71e3} *f car/Y* × *C(1) DX, y w f*.

Cuticle preparations of embryos

Cuticle preparations were made following Wieschaus and Nüsslein-Volhard (1986). The collected eggs were incubated for 48-72 hours, dechorionated and fixed in a 1:4 glycerol acetic acid mixture for 1 hour at 60°C. The warm embryos were lifted with a paint brush and placed into cold Hoyers medium on a slide. The warm embryos sank into the Hoyers within a few minutes. The mounted embryos were cleared overnight at 60°C.

Antibody staining of embryos

Embryos were collected over 2 hour intervals and aged for 13-15 hours. After washing with ddH_2O, the embryos were dechorionated with bleach. Immunohistochemical staining was performed as described by Patel et al. (1989). Embryos were incubated overnight at 4°C in mAb 22C10 diluted 1:1 in PBTS (1× PBS, 0.2% BSA, 0.1% Triton X-100, 5% goat serum (GIBCO). The HRP-conjugated goat anti-mouse secondary antibody (Cappel) was used. Embryos were incubated in rabbit anti-β-galactosidase primary antibody (Cappel) which was diluted 1:500. The HRP-conjugated goat anti-rabbit secondary antibody (Cappel) was used.

Embryos were incubated in the mouse anti-actin primary antibody (Amersham) which was diluted 1:200. The FITC-conjugated goat anti-mouse secondary antibody (Cappel) was used.

DAPI staining of embryos

Embryos were collected and aged for 0-3 hours, 2-4 hours, 7-9 hours and 13-15 hours. The embryos were fixed and stained with the anti-β-galactosidase antibody to distinguish hemizygous progeny from heterozygotes following Patel et al. (1989). The embryos were then washed in PBT (1× PBS, 0.2%BSA, 0.1% Triton X-100). A stock solution of 1.0 mg/ml DAPI in 0.18M Tris buffer pH 7.4 was made. This solution was diluted with PBT to a final concentration of 1 μg/ml and added to the washed embryos. The embryos were incubated for 10 minutes, washed in PBT for 30 minutes and mounted in 50% glycerol.

Cobalt sulfide staining of larval eye discs

Cobalt sulfide-stained preparations were made essentially following the protocol of Cagan and Ready (1989a). Late third instar larval eye discs with mouth parts attached, were dissected from the larvae in 1× PBS on a Petri dish filled with Sylgard (DOW). A small drop of 2% glutaraldehyde was put into the solution containing the eye discs. After a few seconds the discs were transferred to a drop of 2% glutaraldehyde in ddH_2O for 5 minutes and then transferred to 2% cobalt nitrate in ddH_2O. The peripodial membrane was removed while the discs were in the cobalt nitrate using fine insect pins (Original Emil Carls insect pins, size 00). The mouth parts were carefully pierced with an insect pin and the discs were rinsed for less than 30 seconds in ddH_2O and transferred to a drop of freshly made 1% ammonium sulfide solution. The color reaction was monitored until the discs were dark. They were then transferred to ddH_2O, washed three times and mounted in AQUA-MOUNT (Lerner).

Analysis of wing and leg morphology

Wings and legs were removed with fine forceps and immersed into AQUA-MOUNT. Bubbles were carefully removed with forceps. Pressure was used on a coverslip to flatten the wings. The slides were sealed with nail polish.

Scanning electron microscopy

Flies were mounted in colloidal silver paste (Ted Pella), dried for a day, desiccated overnight, and then sputter coated with gold/platinum. Adult eyes were analyzed on an ISI DS-130 Scanning Electron Microscope at 20 kV.

Sections of adult eyes

Heads were removed from the bodies using razor blades and a longitudinal cut was made between the eyes to separate them. The tissue was fixed in 1% paraformaldehyde and 1% glutaraldehyde in 1× PBS for 1 hour at room temperature, osmicated in 1% osmium tetroxide in 1× PBS for 1 hour at room temperature, dehydrated in a series of 5 minute incubations in 50%, 70%, 80%, 90%, 95% and 100%, and again in 100% ethanol solutions. The tissue was then transferred into propylene oxide for 2× 10 minutes, infiltrated overnight in a 1:1 mixture of Propylene oxide and resin, and subsequently desiccated for 4 hours. The eyes were then embedded in pure resin at 60°C for 3 days.

Staining of ovaries with DAPI

The sno^{71e3}*/FM7* stock was raised at 19°C. Virgin homozygous *sno* females were collected, mated to wild-type males, incubated at 25°C for 4-5 days. The ovaries were dissected in 0.7% NaCl and fixed for 4 minutes in 5% glutaraldehyde, stained for 3 hours in a solution of 1 μg/ml of DAPI in 0.18 M Tris pH 7.4, rinsed twice in 1× PBS, incubated in fresh 1× PBS overnight and mounted in 50% glycerol/PBS.

Generation of germ-line clones

The maternal effect of *sno* was analysed using the dominant female-sterile ovo^{D} mutation (Perrimon and Gans, 1983). Wild-type or *v fw* sno^{EF531}*/FM7* females were mated to ovo^{D} males. The

progeny were γ-irradiated (0.8 Rads) at 24 to 48 hours and incubated at 23°C. The eclosed females were collected, mated and the vials examined for the presence of eggs.

The distal region containing *v fw* was crossed off and is viable and fertile. In addition, the proximal region from *wy* was also removed and is viable and fertile as well. Thus the infertility is due to the *sno* locus mapping between *fw* (11A) and *wy* (11E).

Temperature-sensitive period

The sno^{71e3} temperature-sensitive period was determined by mating sno^{71e3} *f car* males to *C(1) DX, y w f* females at 19°C. The progeny were incubated at 19°C and then shifted to 25°C at 28, 57, 93, 186, 207, 365 or 387 hours. The eclosed males were analyzed for rough eye, notched wing and fused tarsal leg segment phenotypes. In a reciprocal experiment, the progeny were incubated at 25°C and then shifted to 19°C at 24, 48, 72, 96, 114, 127, or 200 hours.

RESULTS

Phenotypic analysis of *strawberry notch*

The strongest mutant allele of *strawberry notch, EF531,* is a recessive embryonic lethal at all temperatures. In addition, three heat-sensitive alleles, *71e1*, *71e2*, *71e3,* and one cold-sensitive allele, *76a,* exist. At 23°C these alleles are viable and show several temperature-sensitive phenotypes. Phenotypic analysis of the temperature-sensitive alleles has revealed that *sno* functions in many tissues during development. For the sake of consistency in this paper, unless otherwise indicated, all comparisons involving temperature-sensitive alleles are shown using hemizygous sno^{71e3} males.

Embryonic phenotype

Wild-type embryos can be analyzed using several cuticular structures as markers. For example, the cephalopharygeal skeleton and the Filzkörpers are found at the anterior and posterior ends respectively and the anterior part of each segment is bordered by ventral denticles (Fig. 1A; described in Campos-Ortega and Hartenstein, 1985). Embryos hemizygous for sno^{EF531} show severe cuticular and nervous system defects. In 96.5% of the lethal embryos (n=1053) most cuticular structures fail to develop (Fig. 1B,C); occasionally random patches of denticles or misplaced Filzkörpers are seen. When present, the Filzkörper remnants are usually at 25-50% egg length, rather than at the posterior pole, suggesting that germband retraction is not completed.

In about 2.5% of the embryos collected, the denticle bands develop, but germband retraction fails to occur, and 1% even have a retracted germband, but head involution fails to occur resulting in defective head structures. It is likely that this small degree of variability is due to a maternal component of *sno* (see later). However, we cannot rule out the possibility that this 1% class is unrelated to *sno* and is due to the genetic background of the embryo collection.

The embryos were stained with the neural-specific antibody mAb 22C10 (Zipursky et al., 1984) to analyze the development of the nervous system. In wild-type embryos, this antibody stains the regular array of central and peripheral neurons (Fig. 1D). In contrast, staining 13-15 hour *sno* embryos with mAb 22C10 revealed defects in the nervous system, which correspond well in their frequency and severity to the cuticular phenotypes described above. Greater than 95% of the mutant embryos show no staining, implying that they do not develop an organized nervous system (Fig. 1E), while in the remaining small percentage of embryos, a disorganized or sparsely developing nervous system is seen (not shown). At 30°C, the embryos from the temperature-sensitive allele sno^{71e3} show identical cuticular and nervous system defects as the lethal allele sno^{EF531}.

The organization of the nuclei of 13-15 hour embryos was analyzed by staining with DAPI. Wild-type embryos have a retracted germband at this stage. In the *sno* mutant collection germband retraction fails to occur. The organized array of nuclei normally seen in wild type is also defective in *sno*. Instead, scattered patches of nuclei are seen in the embryo (Fig. 1F). This defect in the organization of the nuclei can also be seen in 7-9 hour collections (Fig. 1G). This collection corresponds to the germband extended stage for wild type.

Embryos at the syncytial blastoderm and cellular blastoderm stages (0-4 hours) were stained with DAPI to determine the earliest times at which *sno* mutant defects can be observed. In a small fraction of embryos, defects in nuclear organization can be seen in the late syncytial blastoderm stage when the nuclei have migrated to the periphery (not shown). This defect becomes obvious in cellularized embryos (Fig. 1I). Large gaps are seen in the pattern of cells when compared with wild type (Fig. 1H). The regular array of actin rings seen in wild type (Fig. 1J) is also disrupted in *sno* (Fig. 1K). We conclude that *sno* mutations cause defects at least as early as the cellular blastoderm stage. The defects are not limited to the ventral neurogenic part of the embryo and occur earlier than those observed in neurogenic mutations. Therefore, the initial defects in *sno* can be observed very early in development, with continued cell death and disorganization at later stages.

Eye phenotype

The wild-type *Drosophila* eye is made up of 800 facets arranged in a hexagonal array which gives rise to a smooth and regular surface (Ready et al., 1976) (Fig. 2A). Disruptions in this pattern generates an eye that is rough and irregular. At 23°C, sno^{71e3} flies have rough eyes (Fig. 2B). The external morphology looks particularly disrupted since eye bristles, normally present at alternate corners of each facet, are often misplaced and sometimes duplicated. At 19°C, the eyes are not as rough and the ommatidia are in a hexagonal array (Fig. 2C).

The uniformity of the size and shape of the wild-type ommatidium is due to the proper development of the cone, pigment and photoreceptor cells in the eye. Mutant retinae were tangentially sectioned and compared with wild type (Fig. 2E-G), to analyze the nature of the defect at the cellular level in a *sno* eye. In wild-type apical sections, membrane specializations called rhabdomeres can be seen on seven photoreceptor (R) cells. The rhabdomeres of R1-6 surround the smaller and centrally projecting rhabdomere of R7 (Figs 2E, 3B). The rhabdomere of the eighth photoreceptor R8 is located basally and centrally, underneath the R7 rhabdomere, and is not seen in the plane of this section. In sno^{71e3} (Fig. 2F) the ommatidial organization deviates from

wild type, and many of the clusters contain abnormal numbers of R cells. 100 ommatidia were serially reconstructed through the tangential sections to quantify this defect. At 23°C, 40% of the ommatidia were found to be wild type, 30% have lost one or more R cells, and the remaining 30% contained an extra R cell. The rhabdomere of this extra cell resembles that of an outer R1-R6 class, and it occupies a stereotypical position between that of R3 and R4 (Figs 2F, 3C). Serial reconstruction showed that ommatidia with the extra photoreceptor do have basally located R8 cells, and therefore contain a total of nine R cells. The phenotype at 27°C is not qualitatively different from that at 23°C except the disruption of ommatidial assembly is more severe. Virtually all ommatidia are affected (not shown). Tangential sections through the eyes of flies raised at 19°C showed that, at this temperature, the ommatidia are essentially wild type except that the recruitment of the extra photoreceptor is still evident (Fig. 2G).

Developmental origins of the mutant ommatidia in *sno*

Late third instar larval eye discs from *sno* mutants and wild-type flies were stained with cobalt sulfide to analyze the recruitment of cells in the developing eye disc. This procedure highlights the apical membranes of the photoreceptor cells and outlines the developing clusters (Tomlinson and Ready, 1987; Melamed and Trujillo-Cenoz, 1975). In wild-type discs, the initial cluster that separates out from the furrow usually has six or seven cells. One or two of these cells, referred to as 'mystery cells', are eliminated from the cluster so that the precluster is reduced to five cells. Three additional cells are later added to complete the 8-cell photoreceptor cluster (Tomlinson and Ready, 1987). Mutant sno^{71e3} eye discs developing at 23°C were stained with cobalt sulfide. At the 5-cell precluster stage, 20% of the ommatidia in *sno* contained six R cells (Fig. 3A). The extra cell is positioned between R3 and R4 where a mystery cell would have been in an earlier column. This suggests that, in the mutant, a mystery cell fails to leave the precluster giving rise to a 6-cell precluster, and finally a 9-cell cluster in the adult (Fig. 3B,C). Thus, the wild-type *sno* gene product is necessary for proper elimination of the mystery cell.

While the recruitment of the extra R cell could be rationalized in terms of an early defect in the developing precluster, this was not the case for the missing photoreceptor cells. We failed to detect any loss of photoreceptor cells in cobalt sulfide-stained discs, yet in the adult, a significant proportion of the ommatidia are missing photoreceptor cells. This defect, in fact, occurs later in pupal development. In wild type, the photoreceptor cells of the retina are supported at their basal surface by a fenestrated membrane. The feet of the secondary and tertiary pigment and cone cells help form this membrane (Cagan and Ready, 1989b). In *sno*, the development of this membrane is defective. As shown in Fig. 3E, a secondary consequence of this defect is that some of the photoreceptor cells drop through the fenestrated membrane into the brain. Therefore, the loss of R cells is not due to improper determination of R cell fate, but may be due to a defect in cone or pigment cell recruitment. This can, in fact, be directly observed in *sno* pupal eye discs.

Pupal eye discs were stained with cobalt sulfide to study the organization of the developing cone and pigment cells. In wild type, four cone cells are surrounded by two primary pigment cells in each ommatidium. The hexagonal shape of the ommatidium is further delineated by the secondary and tertiary pigment cells as well as the bristle cells that complete the cluster. Each face of the hexagon consists of three different cells. The two corners are occupied by a bristle cell and a tertiary pigment cell respectively, while a secondary pigment cell is in the center (Cagan and Ready, 1989a). In sno^{71e3}, altered numbers of bristle, cone and pigment cells can be observed (Fig. 3F). At 23°C, 2-5% of the ommatidia contain five cone cells instead of four. More commonly, extra bristle and pigment cells are seen (Fig. 3F). This defect may cause a poorly structured fenestrated membrane to form and the subsequent loss of R cells into the lamina.

Wing and leg phenotypes

The wings of sno^{71e3}, raised at 23°C, are compared with wild type in Fig. 2I-K. The mutant has a notched blade and thick veins which end in large deltas (Fig. 2J). The distal portion of the wing blade is always notched. In addition, the anterior margin can be notched as well (Compare Figs 2J with 4J). The notching and thick vein phenotypes are temperature sensitive. At 27°C, the phenotype is more severe (not shown), while at 19°C the wing blade is not notched, the veins are not thick and the deltas are much reduced (Fig. 2K).

In wild type, five tarsal segments can be seen in each leg (Fig. 2M). Each segment is clearly distinguishable with constrictions or joints separating them. The fifth segment is the most distal with the tarsal claws attached. In sno^{71e3}, the fourth and fifth tarsal segments of the leg are fused (Fig. 2N). This phenotype is 80% penetrant at 23°C. In a small number of legs, the second and third tarsal segments are fused as well. The legs are wild type at 19°C (Fig. 2O).

Temperature-sensitive periods

The temperature dependence of the eye and wing phenotypes in *sno* were analyzed to determine the critical periods for *sno* function. Male sno^{71e3} embryos were collected at 19°C and then shifted to 25°C at different developmental stages (see Materials and Methods). Adults were scored for the rough eye, notched wing and thick wing vein phenotypes. When shifted to 25°C at any time between 0 and 186 hours after egg laying, the adult flies expressed a fully mutant eye and wing phenotype. However, when shifted after 365 hours beyond egg laying, the eclosed flies did not show the temperature-sensitive phenotypes. Individuals shifted between 186 and 365 hours show eye and wing phenotypes intermediate to the 19°C and 25°C phenotypes. This suggests that the temperature-sensitive period is between 186 and 365 hours after egg deposition. At 19°C this corresponds to the prepupal to pupal stages (Shellenbarger and Mohler, 1978). In reciprocal experiments, sno^{71e3} embryos were collected at 25°C and shifted to 19°C. The temperature-sensitive period was determined to be between 115 and 215 hours. At 25°C, this again corresponds to the prepupal to pupal period (Bodenstein, 1950). The temperature-sensitive period for *sno* function coincides with the time when eye and wing morphogenesis take place. This further supports a developmental role for *sno*.

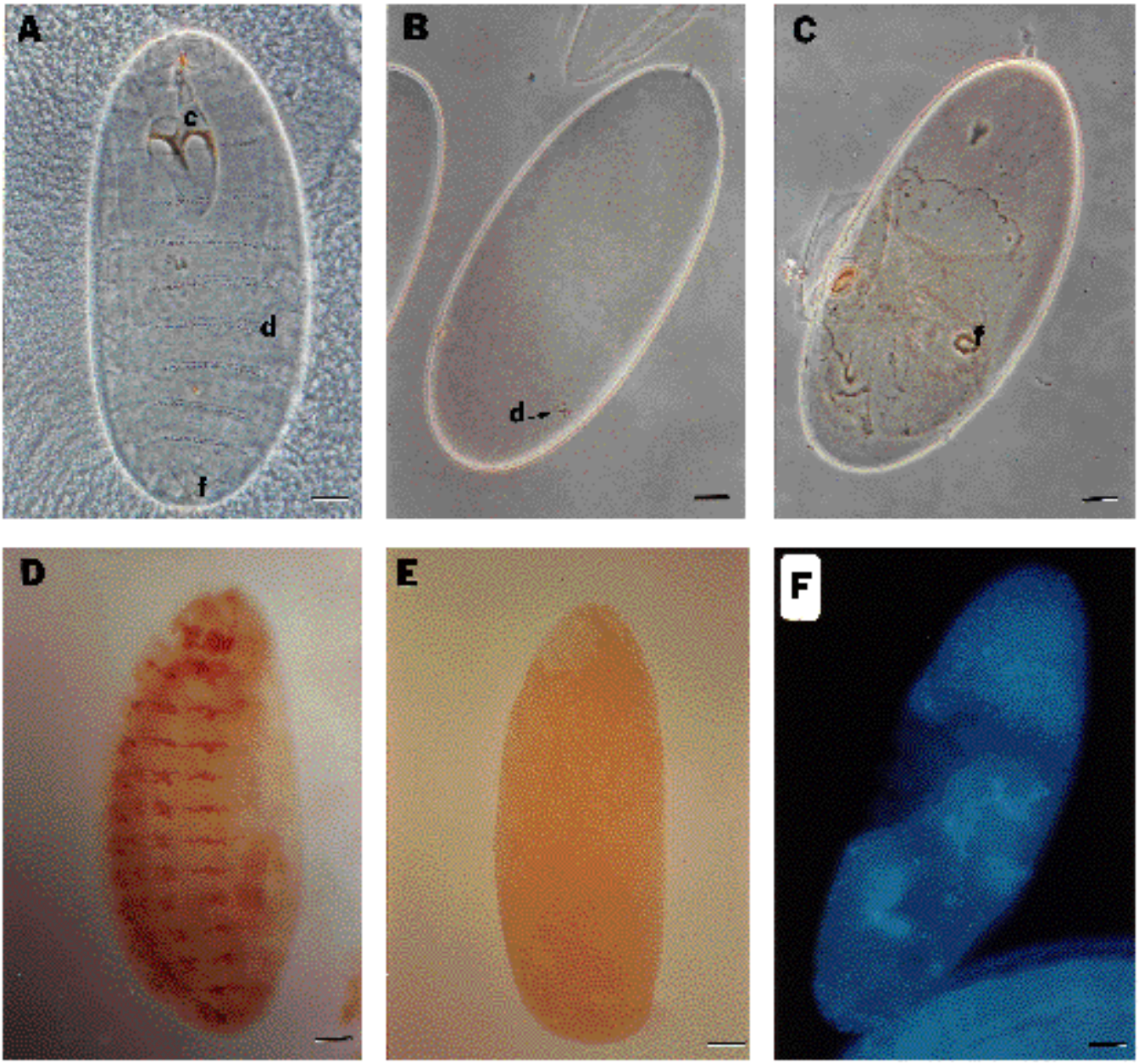
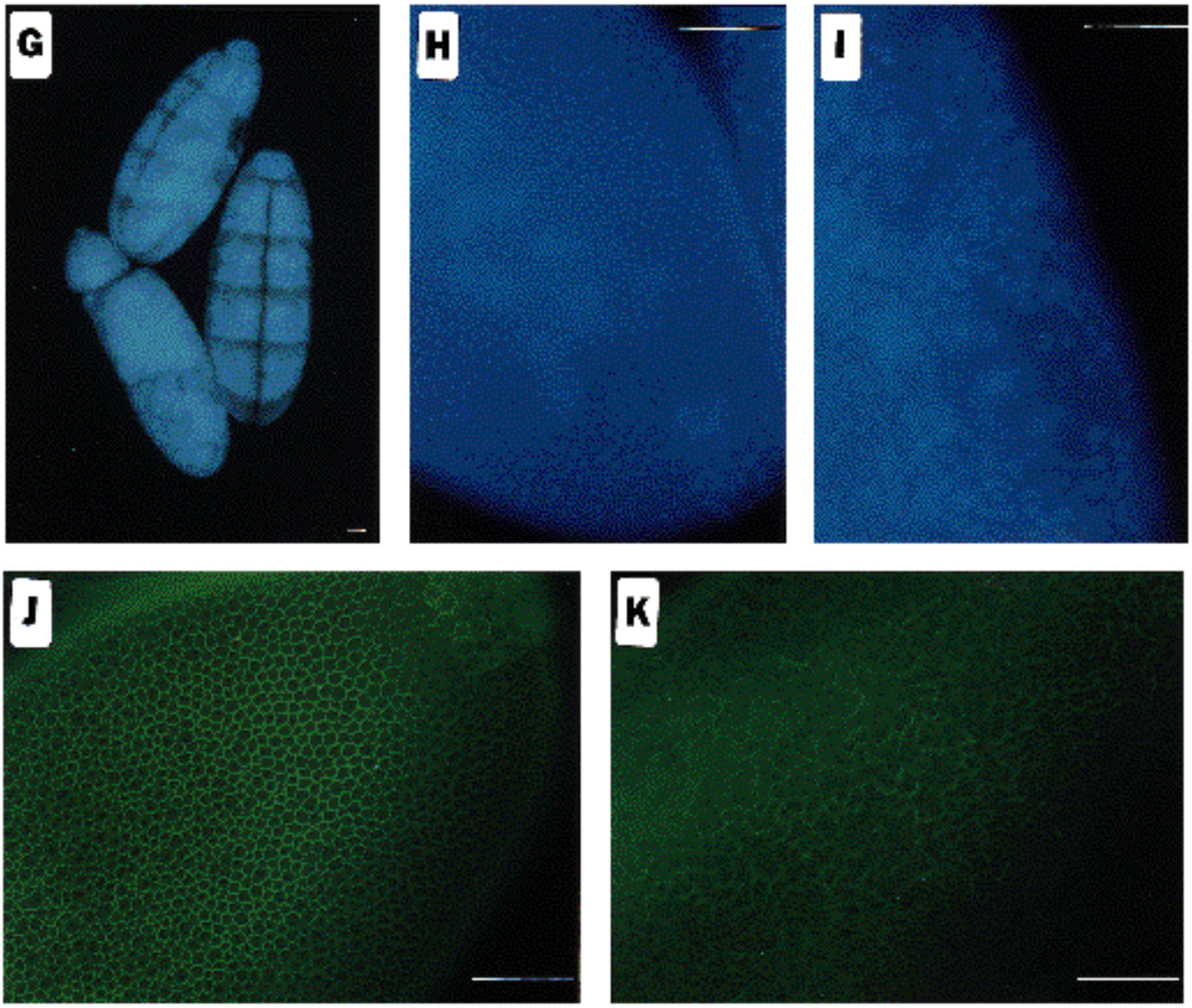


Fig. 1. Embryonic phenotype of the lethal *sno*^{EF531} allele. Bars=50 μm. (A) Cuticle of a wild-type embryo. The cephalopharyngeal skeleton (c) is in the anterior. The bands of ventral denticles (d) delineate the segmentation pattern, and the Filzkörper (f) is in the posterior end of the embryo. (B,C) Cuticles of two representative examples of *sno*^{EF531} embryos. The cephalopharyngeal skeleton, other anterior structures and the regular array of denticle bands, seen in wild type, are missing. Small patches of denticles (d) can sometimes be seen. Filzkörpers (f), when present, are at 25 to 50% egg length. (D-E) 13-15 hour embryos stained with the neuronal-specific mAb 22C10. (D) Wild-type embryo. A regular array of neurons is observed. (E) *sno*^{EF531} embryo. Neuronal development is not observed. (F-I) Embryos stained with DAPI. (F) 13 to 15 hour *sno*^{EF531} embryo. The nuclei lack organization and are in scattered patches. (G) 7-9 hour embryos stained with DAPI and the anti-β-galactosidase antibody. The balancer embryos contain a *ftz-lacZ* element and stain with the anti-β-galactosidase antibody. The disorganization of the nuclei and the overall morphology of the *sno* embryo is clearly visible. (H,I) Embryos at the late cellular blastoderm stage (2-4 hour) stained with DAPI. (H) Wild type. The nuclei are arranged at the surface of the embryo and pole cells have formed at this stage. (I) *sno*^{EF531}. The nuclei are disorganized and often missing in large patches indicating an early defect in cellular organization in the embryo. (J,K) Embryos at the late cellular blastoderm stage (2-4 hours) stained with an anti-actin antibody. (J) Wild type. An organized array of the actin rings around the nuclei is seen. (K) *sno*^{EF531}. The actin rings are disorganized.

Maternal phenotype

We analyzed the maternal effect of *sno* using a method that involves the dominant female-sterile mutation *ovo*^{D} (Perrimon and Gans, 1983). When present in one copy, the *ovo*^{D} mutation gives rise to females that are sterile and fail to produce eggs. These *ovo*^{D} females have a defective germ



line, but the somatic process of oogenesis is functional. Therefore, only when wild-type germ-line clones are induced in a fly that is otherwise ovo^{D}/ovo^{+}, is a mosaic fly able to produce eggs.

In a control experiment, males of an ovo^{D}/Y genotype were mated to wild-type females and the heterozygous ovo^{D}/ovo^{+} larvae were irradiated to promote mitotic recombination events in the germ line. Of the 1180 females collected, 69 produced eggs. This represents a 6% mitotic clone frequency. In a parallel experiment, ovo^{D}/Y males were mated to $sno^{EF531}/FM7$ females and the larvae in the next generation were similarly irradiated. A total of 3800 irradiated sno^{EF531}/ovo^{D} females were collected. Based on the above 6% control clone frequency, we expected at least 150 of these females to have mosaic germ lines that are homozygous for sno^{EF531}. Any eggs produced by such mothers would not have the maternal component of the *sno* gene product. In our experiment, none of these 3800 females produced eggs. We conclude that *sno* is required in the germ line for oogenesis. This result is consistent with the fact that sno^{71e3} females are poorly fertile at 23°C and completely sterile at 27°C.

Ovaries of 20 sno^{71e3} homozygous females raised at 25°C were analyzed by DAPI staining to look for defects in their development. The control wild-type females, examined by DAPI staining, showed that each pair of ovaries contained an average of 40-50 ovarioles (not shown). Each of the ovarioles contains cystoblasts which have a specific polarity and are at different developmental stages (King, 1970). In sno^{71e3} females at 25°C, the number of ovarioles was reduced by 25% to an average of 30 per female. In 20% of the *sno* females either only a single ovary was present or both ovaries together had as few as 12 ovarioles. In addition to the changes in the numbers of ovarioles, certain reproducible defects were observed in 12% of the developing ovarioles (n=395). Even at this permissive temperature, every ovary scored contained examples in which ovarioles with dying cystoblasts, cystoblasts with a reversed polarity, or cystoblasts with increased numbers of nurse cell nuclei were seen (Fig.

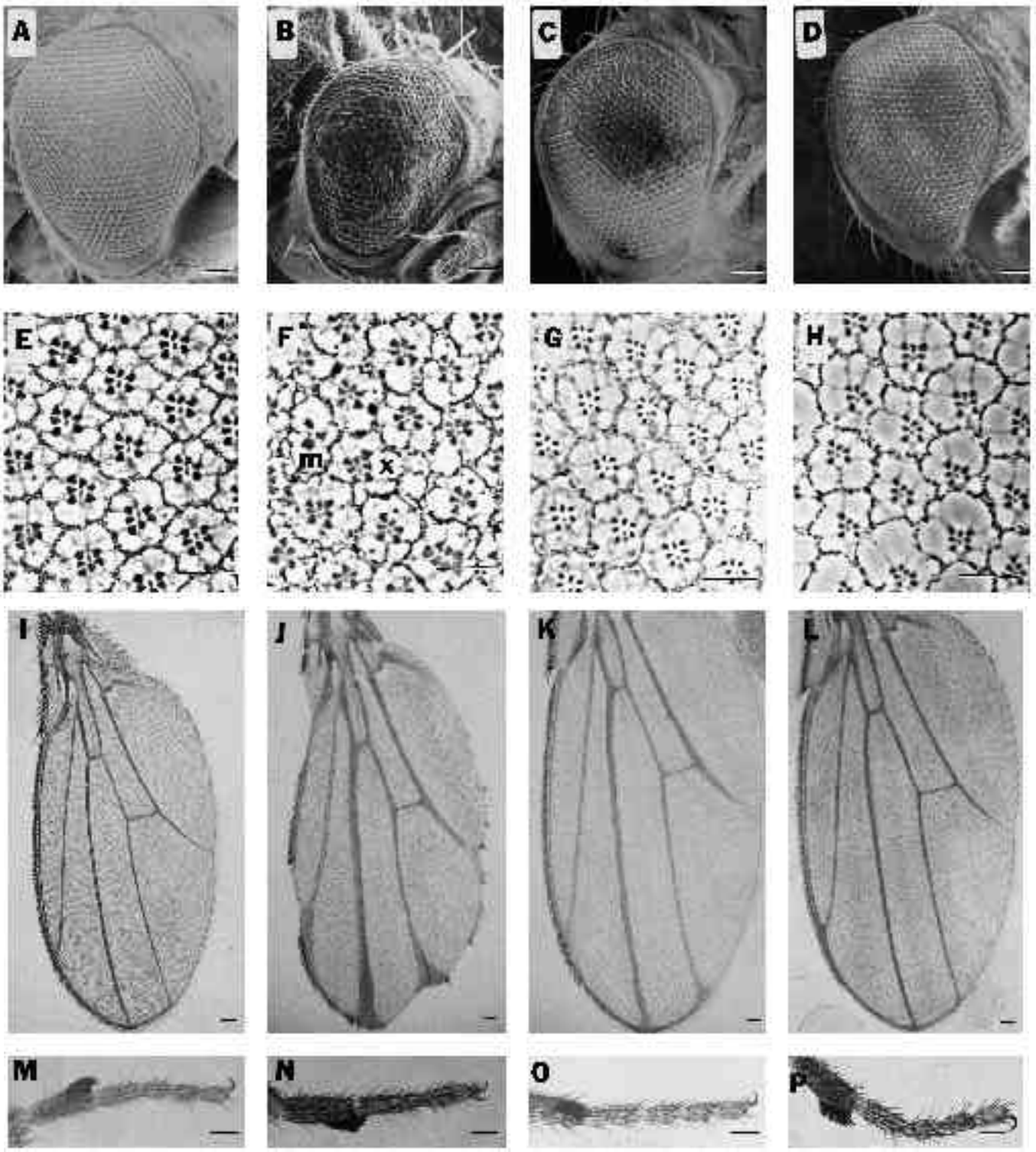


Fig. 2

3G). This is similar to the observed defects in *Notch* mutant ovaries (Xu et al., 1992).

Interactions of *sno* with duplications of *Notch*

We have confirmed and extended the earlier observations of Lefevre and Peterson (1972) that *Dp(1;2)w*$^{+51b7}$ (*Dp N*$^{+}$), a duplication of the *Notch* locus, rescues many of the phenotypes of *sno*. Since this duplication contains many genes in addition to *Notch* (Lefevre, 1952), we mated the *sno* mutant into a genetic background containing a cosmid transformant encoding the wild-type *Notch* transcript (Cos 479, see Ramos et al., 1989). The results of these experiments were

Fig. 2. Phenotypes of the temperature-sensitive sno^{71e3} allele and their rescue by an extra copy of *Notch*. (A-D) Scanning electron micrographs of adult eyes. Bars=50 μm. (A) Wild type. The facets are in a uniform hexagonal array. (B) sno^{71e3}/Y raised at 23°C. The eye is rough and the facets are not in a uniform hexagonal array. (C) sno^{71e3}/Y raised at 19°C. The rough eye phenotype is temperature sensitive and at 19°C the external morphology is not rough. (D) sno^{71e3}/Y; *Cos 479*/+ raised at 23°C. The extra copy of the *Notch* gene provided by Cos479 rescues the rough eye phenotype of *sno*. The sno^{71e3}/Y and sno^{71e3}/Y; *Cos479*/+ flies shown are siblings from the same cross. (E-H) Light microscope photographs of distal tangential sections through the adult eye. The dark structures are the rhabdomeres of the photoreceptor cells. Bars=10 μm. (E) Wild type. The ommatidia are in a hexagonal array. The rhabdomeres of R1-6 surround the central R7 rhabdomere. (F) sno^{71e3}/Y raised at 23°C. The hexagonal array of the ommatidia is disrupted. Some of the ommatidia are missing R cells (m), while others have an extra R cell (x). (G) sno^{71e3}/Y raised at 19°C. The loss of photoreceptor cells is temperature sensitive and at 19°C R cells are not missing. The extra photoreceptor cell phenotype is not temperature sensitive and at 19°C an extra R cell is present in 18% of the ommatidia. (H) sno^{71e3}/Y; *Cos479*/+ raised at 23°C. The loss of photoreceptor cell phenotype is sensitive to the level of *Notch* function and is suppressed when an extra copy of *Notch* is included. (I-L) Light microscope photographs of the adult wings. Bars=50 μm. (I) Wild type. (J) sno^{71e3}/Y raised at 23°C. The peripheral wing margin is notched. The wing veins are thick and join the margin with large deltas. (K) sno^{71e3}/Y raised at 19°C. The notching and thick vein phenotypes are temperature sensitive. The peripheral margin is not notched at 19°C. The thickness of the veins and the size of the deltas are reduced. (L) sno^{71e3}/Y; *Cos 479*/+ raised at 23°C. The notching, thick veins and large delta phenotypes are sensitive to the level of *Notch* function. The peripheral margin is not notched, the veins are not thick, and the deltas are reduced in size in a *sno* mutant carrying an extra copy of *Notch*. (M-P) Light microscope photographs of adult tarsal leg segments. Bars=50 μm. (M) Wild type. The five tarsal segments are clearly separated by joints. (N) sno^{71e3}/Y raised at 23°C. The two most distal tarsal segments (fourth and fifth) are fused. (O) sno^{71e3}/Y raised at 19°C. The fused tarsal segment phenotype is temperature sensitive and at 19°C the leg is wild type. (P) sno^{71e3}/Y; *Cos479*/+ raised at 23°C. An extra copy of *Notch* completely rescues the fused segment phenotype of *sno*.

identical to those obtained with the duplication of *Notch*. In Fig. 2, the phenotypes of sno^{71e3}/Y flies are compared with those of sno^{71e3}/Y; *Cos 479*/+. In the presence of an extra copy of *Notch*, the ommatidia are of uniform size and the normal hexagonal array is restored (Fig. 2D). Tangential sections through the eye show the loss of photoreceptor cell phenotype is suppressed, suggesting that extra *Notch* can compensate for a loss of *sno* function. However, the mystery cell is still recruited as a photoreceptor in flies carrying the duplication (Fig. 2H). An extra copy of *Notch* also rescues the leg phenotype (Fig. 2P), as well as the thick vein and notched blade phenotypes of the wing (Fig. 2L). The deltas at the end of the wing veins are much reduced though not completely eliminated (Fig. 2L). The sno^{71e3}/Y brothers without the cosmid of *Notch* show all *sno* mutant phenotypes. The lethality of either the sno^{EF531} allele or the temperature-sensitive alleles at non-permissive temperatures is not suppressed. In addition to the rescue of most *sno* phenotypes by a duplication of *Notch*, we also found that *sno* mutations rescued a phenotype resulting from *Notch* overexpression. Flies carrying an extra copy of *Notch* show a subtle wing phenotype described in the literature as 'confluens' (Schultz, 1941; Lefevre et al., 1953, Welshons, 1965). Extra wing vein material and the thickening of regions of the longitudinal and cross veins are observed (Fig. 7B). The *sno/Y; Dp* w^{+51b7}/+ wings do not have this confluens phenotype (Fig. 7E). Therefore, reduction in *sno* levels counters the effects of elevated levels of *Notch*, just as extra *Notch* compensates for a loss in *sno* function.

It is important to point out that *sno* and *Notch* are different genes. *Notch* maps to the 3C7 region of the *X* chromosome, while recombination mapping places *sno* between *fw* and *wy*. Complementation tests show that *sno* maps to the 11D/E band, based on its inclusion within *Dp(1;Y) 1*, *Df(1) C246*, and *Df(1) N12*, and its exclusion from *Df(1) JA26* (see Materials and Methods for details).

Interactions of *sno* with mutant alleles of *Notch*

N^{ts1}

The rescue of *sno* by an extra copy of *Notch* suggested that *sno* might interact with mutant alleles of *Notch*. The $Notch^{ts1}$ (N^{ts1}) allele is viable and wild type below 23°C. However at nonpermissive temperatures, N^{ts1} exhibits all the pleiotropy of the *Notch* locus (Shellenbarger and Mohler, 1975). When N^{ts1} flies are shifted to non-permissive temperatures during the larval and pupal periods, rough eyes, small wings, notched wings, fused tarsal segments and thick wing veins are observed (Shellenbarger, 1971). Thus the phenotypes of sno^{ts} are phenocopied in N^{ts1}. Furthermore, the double mutant, N^{ts1} sno^{71e3}/Y, is lethal with no escapers at any temperature. The N^{ts1} mutation is likely to result in a reduction of *Notch* activity even at the permissive temperature. A mutation in *sno* is likely to further reduce the level of this *Notch*-related function below a threshold, resulting in lethality.

split

The *split* (*spl*) allele of *Notch* has a rough eye phenotype while the wings and legs are wild type. As shown in Fig. 4B,E the hexagonal array of ommatidia is disrupted in *spl*. The ommatidia are unequal in size and the bristles between some of the facets are duplicated. Sections through a *spl* eye (Fig. 4H) show ommatidia with abnormal numbers of photoreceptor cells. This *spl* eye phenotype is synergistically enhanced in combination with *sno*. The *spl* sno^{71e3}/Y double mutant has an extremely rough eye which is severely reduced in size (Fig. 4C,F). The ommatidia are unequal in size with large gaps between them. Many extra bristles are present in clumps between the ommatidia. The effect is more dramatic at a cellular level, as revealed in tangential sections of the eye. While both *spl* and sno^{71e3} have some alterations in photoreceptor numbers in their ommatidia at 23°C, the double mutant ommatidium is very severely defective (Fig. 4I). Large clusters of pigment cells can be seen between highly aberrant clusters of photoreceptor cells. In the double mutant, only the eye phenotype is synergistically affected. The wing and leg phenotypes are not distinguishable from that of *sno* alone (Fig. 4J,L).

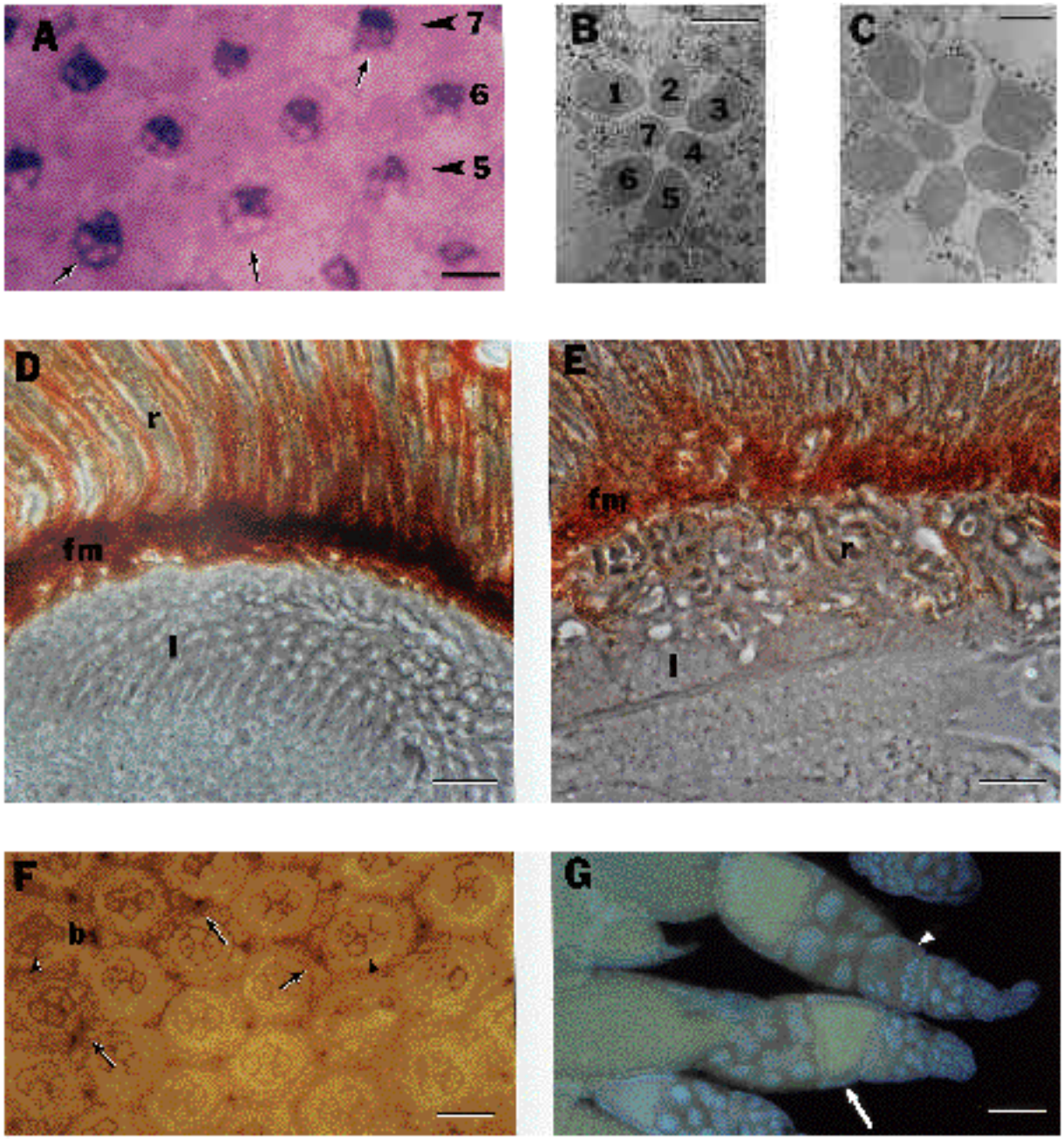


Fig. 3. Developmental origins of the *sno*^{71e3} eye phenotypes and female sterility. All flies were raised at 23°C. Bars=10 μm. (A) Cobalt sulfide-stained late third-instar larval eye disc. Columns 5-7 are shown (arrowheads and numbers). Posterior is up and the morphogenetic furrow (not shown) is four columns below the picture. Normal five cell preclusters and mutant six cell preclusters (arrows) are observed in columns 5, 6 and 7 where the mystery cell is normally absent in wild type (Tomlinson and Ready, 1987). (B,C) Transmission electron micrographs of examples of ommatidia from adult *sno*^{71e3} eyes. (Bars=1 μm.) Both ommatidia are from the same eye and apical tangential sections through the ommatidia are shown. At this level R7 is visible. (B) A normal ommatidium. The rhabdomeres of R1-6 surround R7. (C) Ommatidium with an extra photoreceptor cell. This additional cell is between R3 and R4 and is a mystery cell that has been fated to be a photoreceptor neuron in the mutant. (D,E) Transverse sections through the adult eye. Bars=10 μm. (D) Wild type. The photoreceptor cells (r) are supported by a fenestrated membrane (fm) at their base. This membrane separates the lamina (l) of the brain from the eye. (E) *sno*^{71e3}. Photoreceptor cells (r) can be found beneath the fenestrated membrane within the lamina. (F) Cobalt sulfide-stained pupal eye disc from *sno*. Bar=10 μm. Incorrect numbers of the pigment, cone and bristle cells are recruited in the pupal eye disc. Bristle cells (b) can be duplicated and some fail to migrate to the proper corner. An extra cone cell can be seen in some ommatidia (arrowhead). Extra pigment cells are also seen. Arrows indicate duplicated rows of pigment cells between clusters. (G) Light microscope photographs of ovarioles stained with DAPI. Ovarioles with cystoblasts dying (arrowhead) or with a reversed polarity of the oocyte within the egg chamber (arrow) are seen in *sno*^{71e3}.

notchoid

In contrast to *spl*, the *notchoid*^{1} (*nd*^{1}) allele of *Notch* has a wild-type eye and leg, while the wings are slightly notched (Fig. 5B,E). The *nd*^{1} *sno*^{71e3}/*Y* double mutant, shown in Fig. 5C,F shows a synergistic enhancement of this wing phenotype, while the eye and leg phenotypes resemble that of *sno* alone. The wing blade is severely notched, with most of the peripheral margin missing. The wing veins end in large deltas, which are larger in size than those of *sno* alone. The viability of these double mutant flies is very poor, with all adults dying at, or within hours of eclosion. The *nd*^{1} *sno*^{71e3}/*Y* flies raised at 19°C have improved viability, however the wing phenotype is similar to that at 23°C (not shown). As with *spl*, the phenotypic effects of the interaction between *nd* and *sno* are limited to a tissue in which the mutant effects of *nd* are seen. The synergistic interactions of *spl* and *sno* in the eye and *nd* and *sno* in the wing suggest that *sno* and *Notch* function cooperatively in many different tissues during development.

Abruptex

Another class of viable mutations at the *Notch* locus is *Abruptex* (*Ax*) (Foster, 1975; Xu et al., 1990). These alleles are dominant mutations with interrupted wing veins (Fig. 6C,E). In *Ax*^{E2}*sno*^{71e3} double mutants, the interrupted wing vein phenotype of *Ax*^{E2} is suppressed, but the wing still resembles that seen in a *sno*^{71e3} mutant (Fig. 6D). In contrast, the *Ax*^{9b2}*sno*^{71e3} double mutant displays a mutual suppression of both the *Ax*^{9B2} and the *sno* wing phenotypes (Fig. 6F). The wing looks essentially wild type, with no interruptions of the wing veins or notching of the blade. The interactions seen between *Ax* alleles and *sno* exactly mimics the reported interactions between *Ax* and *Notch*. The *Ax*^{E2}/*N* heteroallelic combination suppresses the interrupted wing vein phenotype, while the *Ax*^{9b2}/*N* combination mutually suppresses both the *Ax* and the *Notch* phenotypes (Foster, 1975; Portin, 1975), reflecting once again that loss of *sno* function has an effect similar to that obtained with a loss of *Notch* function.

Since duplications of *Notch* interact with *sno*, we also determined if a duplication of the *sno* gene has an effect on mutant alleles of *Notch*. The duplication *Dp (1;Y) 1* includes the *sno* locus, and it does not rescue or enhance *spl*, *nd* or *Ax*.

Interactions between *sno* and genes related to *Notch*

Hairless

The *Hairless*^{99} mutation has an interrupted wing vein phenotype similar to *Ax* (Fig. 6G) (Bridges and Morgan, 1923). As shown in Fig. 6H, the *sno*^{71e3}/*Y; H*^{99}/+ double mutant wing is essentially wild type. Not only does *sno* suppress the incomplete wing veins of *Hairless*, but *Hairless* also suppresses the notched blade and thick veins seen in *sno*. This mutual suppression of *sno* and *Hairless* is consistent with the suggestion that *Hairless* is a negative regulator of *Notch* function (Bang and Posakony, 1992). In a *Hairless* mutant, Notch activity would thus be expected to be high, and *sno* phenotypes therefore suppressed in the same way as with a duplication of *Notch*. As with the tissue-specific *Notch* alleles, the interaction between *Hairless* and *sno* is limited to the specific tissue in which mutations in both *Hairless* and *sno* have phenotypes. *Hairless* fails to rescue the lethality of the *N*^{ts1} *sno* double mutant and the eye phenotype of the *spl sno* double mutant.

Delta

The hallmark of *Delta* mutants is the presence of triangular expansions (deltas) at the ends of the wing veins (Bridges and Morgan, 1923; Bridges and Brehme, 1944). Mutations in *sno* also have this phenotype (Fig. 7B), but we did not detect any enhancement of the delta phenotype in double mutant flies (Fig. 7F). However, another subtle phenotype seen in *Delta* is *sno* dependent. *Delta* mutants have the same confluens phenotype (Fig. 7E) that is associated with duplications of *Notch* (Fig. 7C). Fig. 7F shows that this confluens is suppressed in the *sno*^{71e3}/*Y; Dl*^{3}/+ double mutant combination. The same result was obtained with *Dl*^{6b37} and *Dl*^{9p39} alleles (not shown). It is likely that confluens results from local reductions in the level of Delta-Notch complexes resulting in an effect that is equivalent to the expression of excess free Notch. This is supported by the evidence that the confluens due to *Delta* is also suppressed in *Notch*/+; *Delta*/+ double mutants (Vässin et al., 1985; Alton et al., 1989; Godt, 1990). We conclude that the phenotypic interaction between *sno* and *Delta* indirectly reflects an interaction between *sno* and *Notch.*

groucho

The 96F11-14 region of the *Drosophila* chromosome contains the *Enhancer of split complex* (*E(spl)C*) encoding at least 7 different helix-loop-helix proteins (Klämbt et al., 1989; Knust et al., 1992; Delidakis and Artavanis-Tsakonas, 1992) and the *groucho* locus (Delidakis et al., 1991). The *groucho* gene encodes a nuclear protein with homology to protein-protein interacting domains of β transducin (Hartley et al., 1988; Delidakis et al., 1991). The *groucho* gene has been shown to function in the process of neurogenesis (Knust et al., 1987; Hartley et al., 1988; Delidakis et al., 1991; Schrons et al., 1992) and in photoreceptor development (Fischer-Vise et al., 1992). We have used a number of mutant strains from the region of the *E(spl)C* and *groucho* to identify a possible interaction with *sno*. The *sno*^{71e3} mutation was combined with one copy of *E(spl)*^{R1,} *E(spl)*^{X72}, *E(spl)*^{Bx22}, *Df(3) boss*^{16} and the *groucho* point mutation *gro*^{E73} (See Materials and Methods). In each case, flies hemizygous for *sno*^{71e3} and heterozygous for the deletion or *gro*^{E73} are lethal with a small number of escapers. These escapers have severely rough eyes (not shown) and die within hours of eclosion.

The above analysis has revealed that *gro* interacts dominantly with *sno* since the point mutation and the deletions used all affect *gro* (see Materials and Methods). However, it does not rule out that the *E(spl)C* also has additional effects on *sno*. We crossed *sno* with the *E(spl)*^{D} chromosome. The wing, but not the eye, phenotype of this double mutant is enhanced over *sno* (not shown). However, this wing effect is not due to the *Enhancer of split* gene encoded by the m8 transcript since a transformant line m8^{D} carrying the m8 mutation of *E(spl)*^{D} (Klämbt et al., 1989) does not

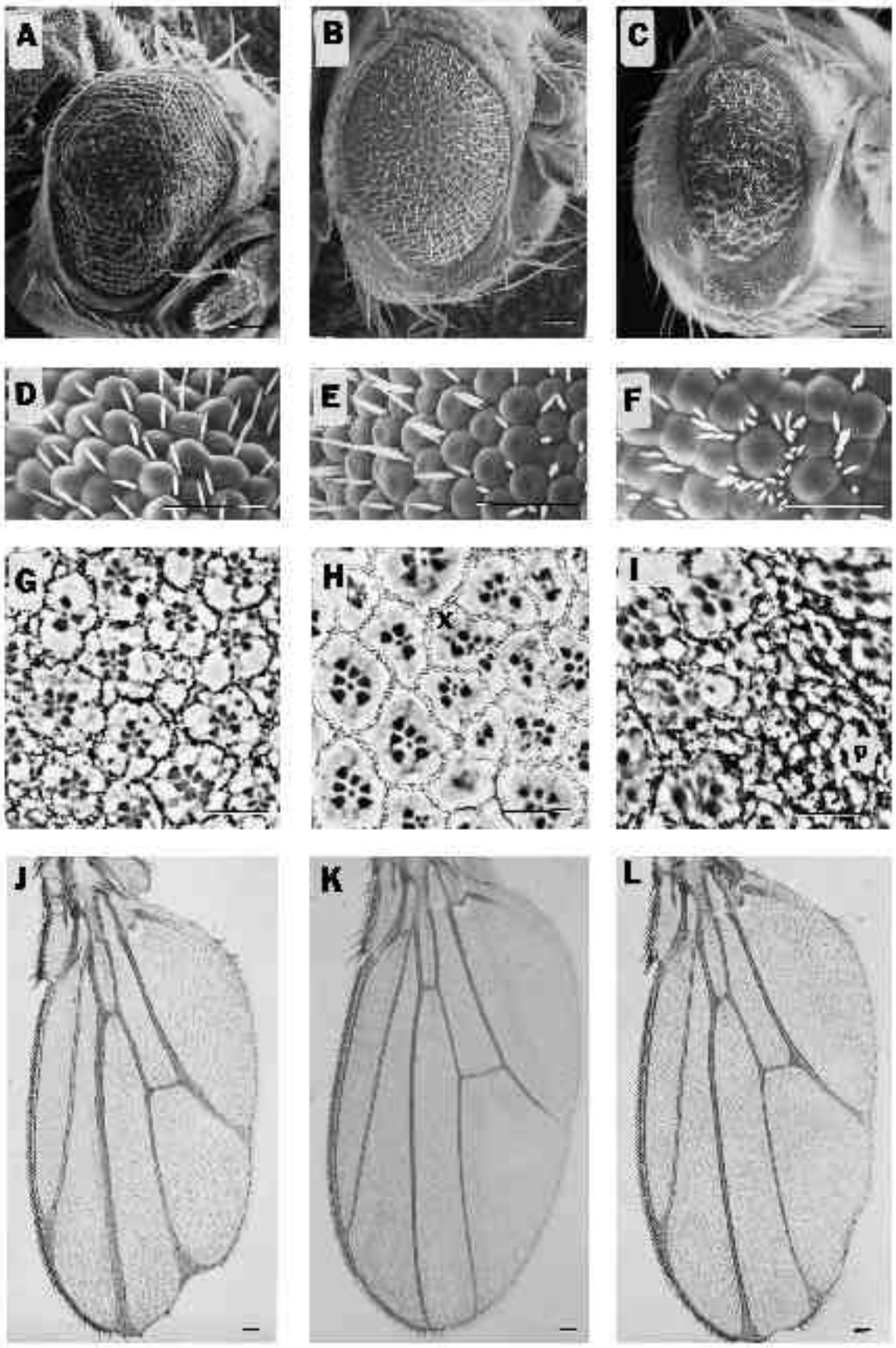


Fig. 4

Fig. 4. Interaction of *spl* with sno^{71e3}. All flies were raised at 23°C. (A-F) Scanning electron micrographs of the adult eye. Bars=50 μm. (A,D) sno^{71e3}/Y. The surface of the eye is rough and the facets are not in a uniform hexagonal array. Extra bristles are present between some of the ommatidia. (B,E) *spl/Y*. The surface of the eye is rough and eye bristles are often duplicated. (C,F) *spl* sno^{71e3}*/Y*. The rough eye is synergistically enhanced. The eye is reduced in size, fewer ommatidia are present and tufts of bristles occupy the clefts between the facets. (G-I) Light microscope photographs of distal tangential sections through the retina of the adult eye. Bars=10 μm. (G) sno^{71e3}*/Y*. Normal ommatidia are present, as well as those missing R cells and those with an extra R cell. (H) *spl/Y*. Ommatidia looking normal, as well as those missing R cells, or with extra central R cells can be seen. (I) *spl* sno^{71e3}*/Y*. The *spl sno* eye is very severely affected. The defect is more than additive. Very few wild-type ommatidia are present. Large patches of pigment cells (p) are seen between the ommatidia. (J-L) Light microscope photographs of the adult wing. Bars=50 μm. (J) sno^{71e3}*/Y*. (K) *spl/Y*. The wing is wild type. (L) *spl* sno^{71e3}. The *spl* sno^{71e3} wing is similar to that of *sno*.

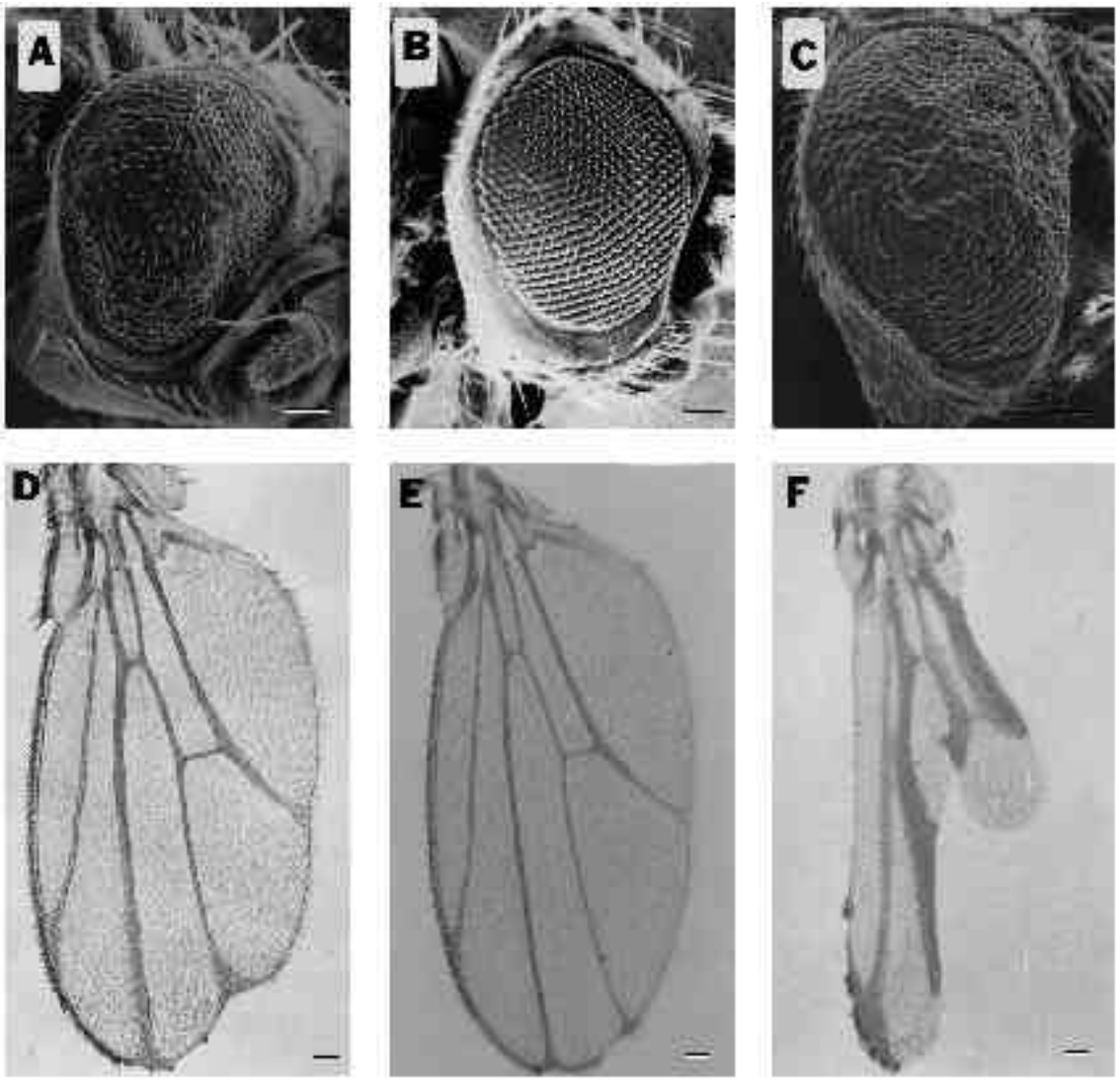


Fig. 5. Interaction between *nd* and *sno*. All flies were raised at 23°C. Bars=50 μm. (A-C) Scanning electron micrographs of the adult eye. (A) sno^{71e3}/Y. (B) nd^{1}*/Y*. The ommatidial array resembles wild type. (C) nd^{1} sno^{71e3}*/Y* The eye phenotype is not different from that of sno^{71e3}. (D-F) Light microscope photographs of the adult wings. (D) sno^{71e3}*/Y*. (E) nd^{1}*/Y*. The periphery of the wing blade is slightly notched. (F) nd^{1} sno^{71e3}*/Y*. The wing phenotype of the double mutant combination is synergistically enhanced over that of *nd* and *sno*. The majority of the periphery is notched. The margin and the LI wing vein are almost completely missing. The wing veins are thicker than in *sno* and end in large deltas.

have this effect. In addition to the m8 defect, the $E(spl)^{D}$ chromosome has defects in the *groucho* gene (Klämbt et al., 1989). We therefore consider it likely that this wing phenotype once again reflects an interaction between *sno* and *groucho*.

Other neurogenic genes

The fact that *sno* interacts with loss-of-function mutations in *groucho*, *Hairless* and *Delta*, when only one copy is deleted, led us to look for interactions of *sno* with *mam*, *neu* and *bib* (Lehmann et al., 1981, 1983). The $sno^{71e3}/Y;mam^{IL115}/+$, the

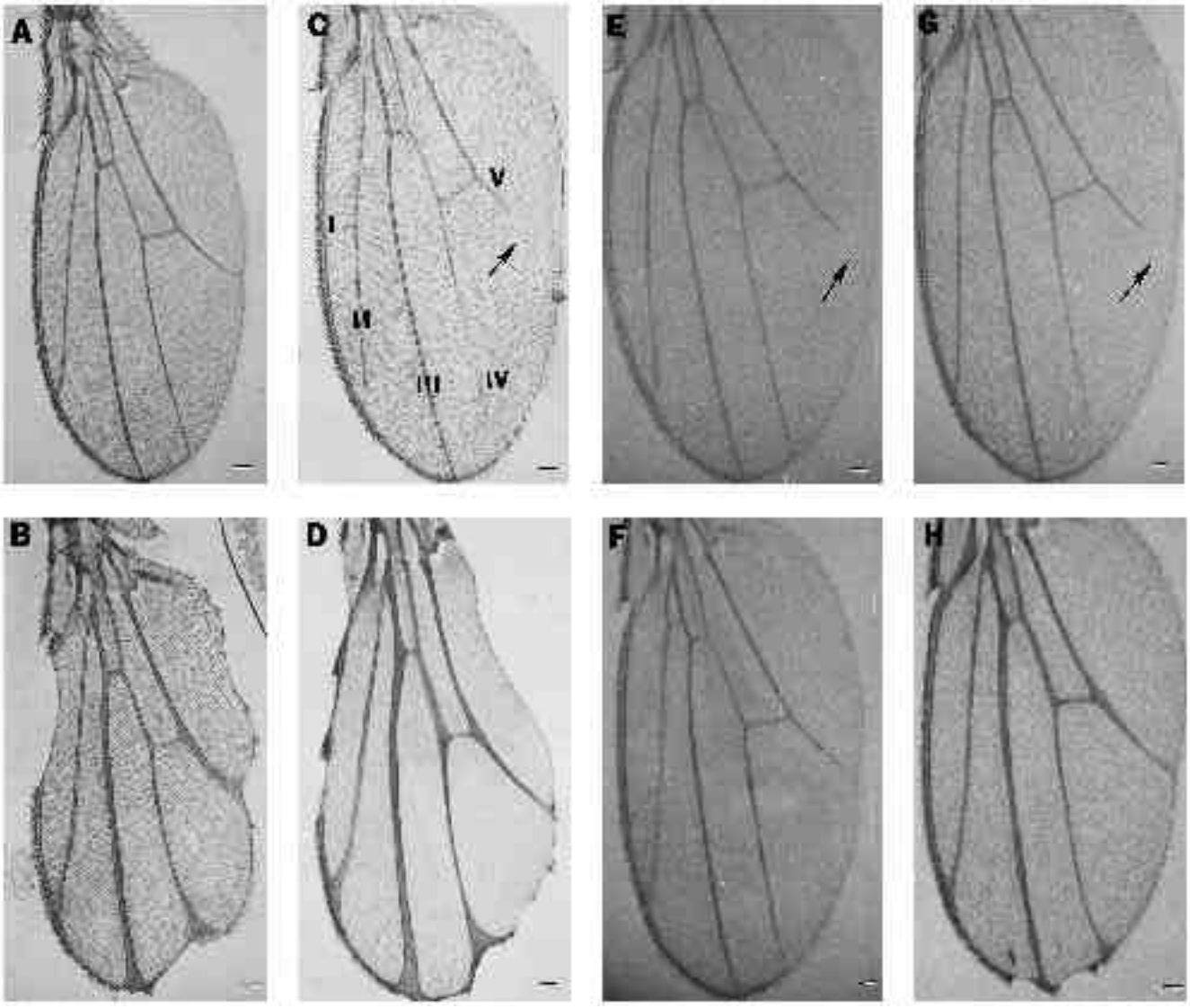


Fig. 6. Interactions of *sno* with *Ax* and *H*. All flies were raised at 23°C. Light microscope photographs of the adult wing. Bars=50 μm. (A) Wild type. (B) sno^{71e3}/Y. (C) Ax^{E2}/Y. The wing veins are interrupted (arrow). The LII, LIV and LV veins do not join the margin of the wing blade. (D) Ax^{E2} sno^{71e3}/Y. The wing phenotype is similar to sno^{71e3}. The Ax^{E2} interrupted wing vein phenotype is suppressed. (E) Ax^{9b2}/Y. The wing veins are interrupted with LV not reaching the margin (arrow). The LII and LIII wing veins are also interrupted in other examples of these wings. (F) Ax^{9b2} sno^{71e3}/Y. In this combination, the Ax^{9b2} and the *sno* wing phenotypes are mutually suppressed. (G) +/Y; H^{99e}/+. Wings of H^{99e} are virtually indistinguishable from *Ax*. (H) sno^{71e3}/Y; H^{99e}/+. A partial mutual suppression of both the *sno* and the *H* wing phenotypes is seen.

sno^{71e3}/Y; neu^{IF65}/+ and the sno^{71e3}/Y; bib^{ID05}/+ flies all resemble sno^{71e3}/Y alone (not shown). Therefore, using the above alleles, there is no detectable dominant interaction between *sno* and *mam, neu* and *bib.*

deltex

Mutations at the *deltex* locus (Xu and Artavanis-Tsakonas, 1990; Gorman and Girton, 1992) share several genetic features with *sno*. The wing veins of *deltex* flies end in small deltas (Fig. 8G), and in 10% of cases the wing blade is notched. The *dx* eyes and legs are wild type. Xu and Artavanis-Tsakonas (1990) have shown that duplications of *Notch* rescue the phenotypes of *dx.* Since this was reminiscent of the effect of duplications of *Notch* on *sno,* we made double mutant combinations of *dx* and *sno*. The *dx* sno^{71e3}/Y double mutant is pupal lethal at all temperatures with a very small number of escapers that die within hours of eclosion. While these escapers have eyes that are similar to *sno,* their wings are severely affected (Fig. 8H). Most of the wing blade, including the peripheral margin, is missing. In many cases, wing veins are absent. The ones remaining, are thick and end in deltas. The dorsal and ventral surfaces of the wings fail to fuse resulting in a blistered appearance. The strong interaction between *dx* and *sno* can also be observed in the adult legs. All leg tarsal segments are fused into one in the *dx* sno^{71e3}/Y escapers (Fig. 8K).

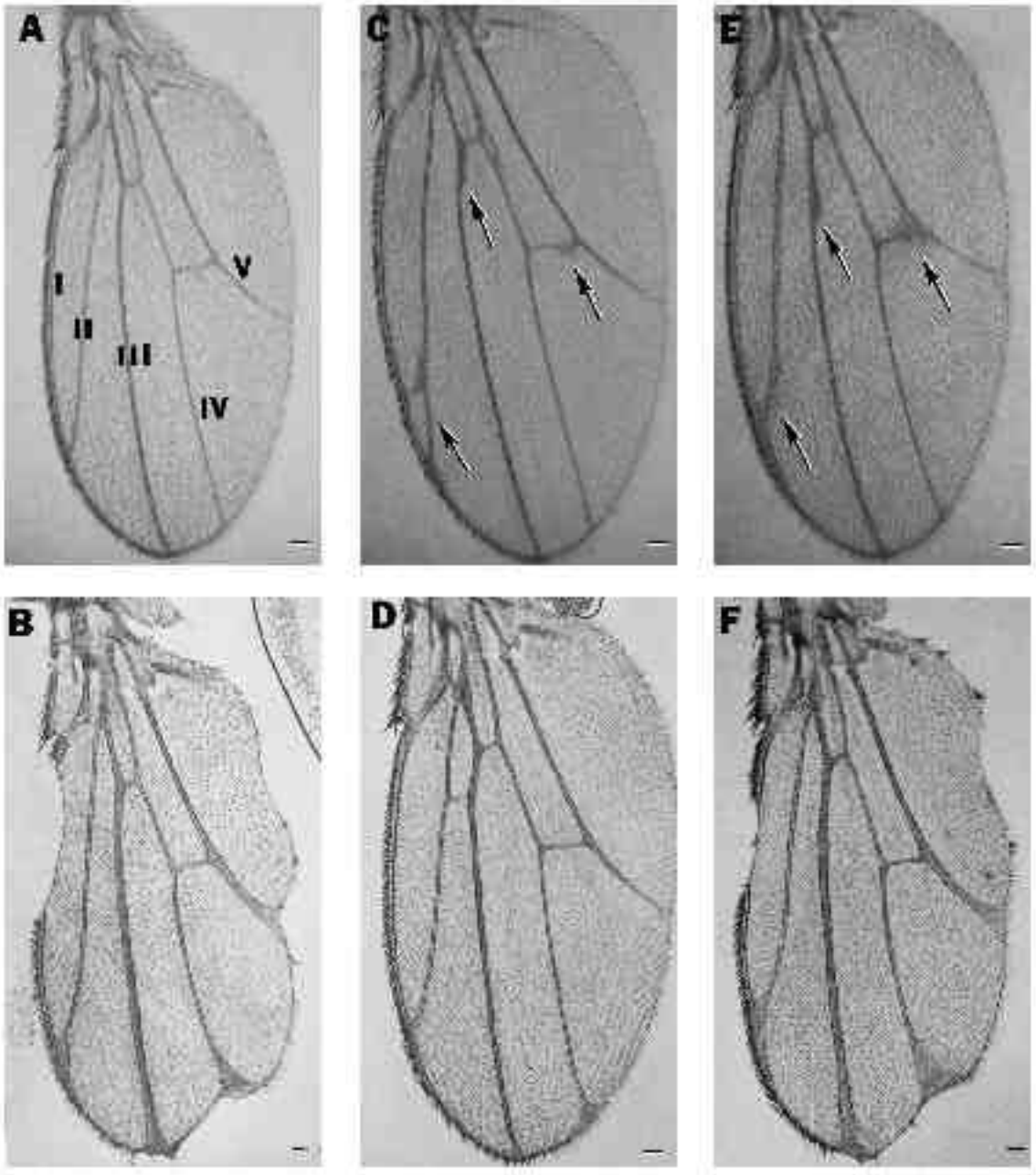


Fig. 7. Suppression of confluens by *sno*. Light microscope photographs of adult wings of flies raised at 23°C. Bars=50 μm. (A) Wild type. (B) sno^{71e3}/Y. (C) $+/Y$; $Dp\ N^{+}/+$. An additional copy of *Notch* gives rise to extra wing vein material leading to a confluens phenotype (arrows). This is most noticeable at the LII and LIII veins and at the anterior and posterior cross veins (arrow). (D)sno^{71e3}/Y; $DpN^{+}/+$. A mutual suppression of both the *sno* phenotype and the DpN^{+} confluens phenotype is observed. (E) $+/Y$; $Dl^{3}/+$. The confluens phenotype of $Dl^{3}/+$ is most apparent at LII, LIII, and the anterior and posterior cross veins (arrows). The wing veins also end in deltas. (F) sno^{71e3}/Y; $Dl^{3}/+$. The $Dl^{3}/+$ confluens phenotype is suppressed; however, the notching, thick veins and deltas are similar in severity to sno^{71e3}.

The pupal lethality of $dx\ sno^{71e3}$ is rescued by an extra copy of *Notch*. The $dx\ sno^{71e3}/Y$; $Dp\ N^{+}/+$ flies also have less severe wing and completely wild-type leg phenotypes (Fig. 8I,L). The strong genetic interaction between *sno*, *deltex* and *Notch* suggests that these genes function together in common pathways.

Serrate

Like *deltex*, the *Serrate* (*Ser*, also called *Beaded-Serrate*, Bd^{S}) mutation shares many genetic features with *sno*. Flies mutant for *Ser* are viable with a dominant notched wing phenotype (Fig. 8B) while their eyes and legs are wild type (Belt, 1971; Dexter, 1914). *Ser* interacts strongly with *Notch* mutations (Fleming et al., 1990; Thomas et al., 1991), and the *Serrate* gene product, expressed in cell lines, has been shown to bind directly to the extracellular domains of Notch (Rebay et al., 1991). The sno^{71e3}/Y; $Ser/+$ double mutant is viable but has a severely enhanced wing phenotype (Fig.

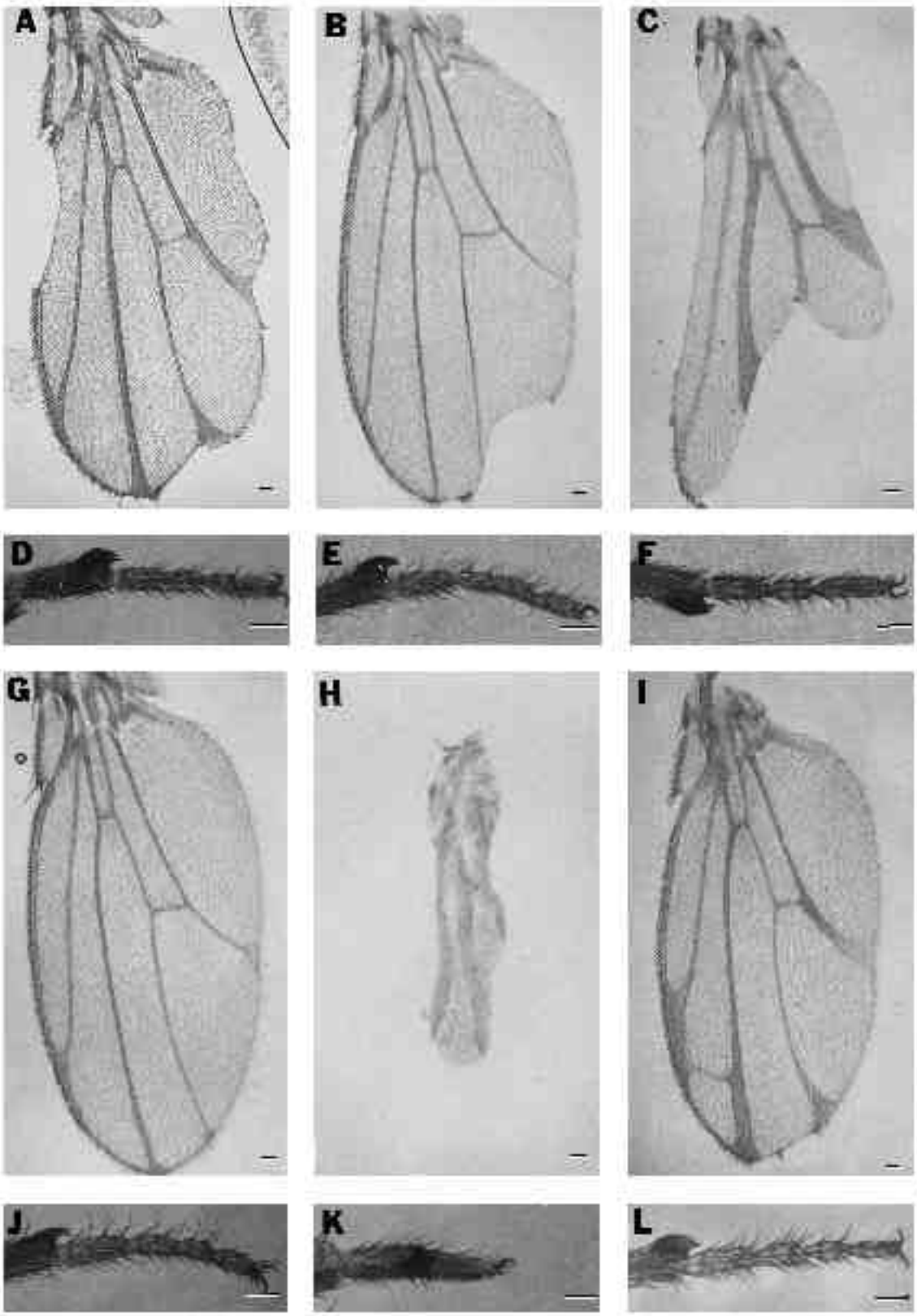


Fig. 8

Fig. 8. Interactions of *sno* with *dx* and *Ser*. All flies shown here were raised at 23°C. (A-C) Light microscope photographs of the adult wing. Bars=50 μm. (A) sno^{71e3}/Y. (B) *+/Y; Ser/+*. The peripheral wing blade is notched. (C) sno^{71e3}/Y; *Ser/+*. The wing phenotype is synergistically enhanced over the single mutant phenotypes. (D-F) Light microscope photographs of the adult leg tarsal segments. Bars=50 μm. (D) sno^{71e3}/Y. (E) *+/Y; Ser/+*. The leg phenotype is wild type. (F) sno^{71e3}/Y; *Ser/+*. The leg phenotype is not different from that of *sno*. (G-I) Light microscope photographs of the adult wing. Bars=50 μm. (G) *dx/Y*. The wing veins end in deltas. (H) *dx* sno^{71e3}/Y. This combination is essentially lethal. Shown here is the wing phenotype of a rare escaper. The blade is severely notched with the peripheral margin missing. The wing veins are thick. (I) *dx* sno^{71e3}/Y; *Dp* $N^{+}/+$. An extra copy of the *Notch* gene rescues the lethality of the *dx sno* combination. The severity of the wing phenotype is reduced. (J-L) Light microscope photographs of the adult leg tarsal segments. Bars=50 μm. (J) *dx/Y*. The leg resembles wild type. (K) *dx* sno^{71e3}/Y. The tarsal segments are fused into a single segment. (L) *dx* sno^{71e3}/Y; *Dp* $N^{+}/+$. An extra copy of *Notch* completely suppresses the *dx sno* fused tarsal segment phenotype. The tarsal segments resemble wild type.

8C). Virtually all of the periphery of the wing is notched and the wing veins are thicker than in *sno* alone and end in enhanced deltas.

DISCUSSION

Like *Notch*, the *strawberry notch* gene product is required during many developmental stages including oogenesis, embryogenesis and imaginal development of the eye, wing and leg. This suggests a common role for these two genes in the morphogenesis of diverse sets of tissues. The embryonic phenotype shows that *sno* is required early in embryogenesis, prior to germband retraction. When *sno* is missing, the embryos fail to complete germband retraction and head involution, and do not develop a nervous system or external cuticular structures. This defect is different from the neural hyperplasia seen in *Notch* embryos (Poulson, 1937, 1939), perhaps because the earliest defect in *sno* occurs prior to the process of neuroblast segregation. The early requirement for *sno* is consistent with its strong maternal component. No eggs are produced when the *sno* gene product is missing in the germ line.

The eye, wing and leg phenotypes of the temperature-sensitive alleles have proven most important for the genetic analysis of *sno*. Detailed examination of these phenotypes allowed us to propose that *sno* functions in processes that require the interaction of a cell with its neighbor. For example, in a *sno* mutant, a mystery cell fails to leave the precluster presumably because it has not received the correct signal to do so. In certain instances, mutations in *sno* can lead to indirect phenotypic effects as in the loss of photoreceptors due to a primary defect in the recruitment of the cone and pigment cells. This loss of photoreceptor cells into the lamina has also been observed in N^{ts1} and *spl* (R. Cagan, personal communication).

During development and differentiation, many cues are required to make the transition from one cell type to another. In the embryo, the delamination of the presumptive neuroblasts from the epithelial sheet of cells requires distinct morphological transitions. Similarly, the ommatidial assembly and the formation of legs and wings from the imaginal discs all require changes in cell morphology. A prerequisite for such morphological changes is the proper exchange of developmental signals. The *Notch* gene product is involved in these processes, permissively allowing cells to transmit and receive messages that are important for the determination of their fates. Our data suggest that the *strawberry notch* gene product functions in this system. Many of the *sno* phenotypes are identical to those observed in *Notch* mutants (eg. notched wing, rough eye, loss of R cells into the lamina, fused tarsal leg segments, and defects in oogenesis). This is not simply coincidental, since all such *sno* phenotypes can be rescued by increasing the dose of *Notch*. Similarly, defects caused by excessive levels of *Notch* can be rescued by mutations in *sno*. The fact that certain phenotypes of *sno*, like the lethality and the recruitment of a mystery cell as an extra photoreceptor, are not rescued by extra *Notch*, could reflect a requirement of *sno* outside of the *Notch* system. Alternatively, the increased level of *Notch* in a duplication simply may not be sufficient to overcome a loss of *sno* in every instance. Furthermore, the relationship between *sno* and *Notch* seems to be directional, since extra *sno* gene product cannot compensate for the loss of *Notch* in any of the *Notch* alleles that were analyzed as part of this study.

The functional levels of *Notch* within a tissue may be altered in a variety of ways. Mutations in *Notch* can either reduce (as in N^{ts1}, *spl* and *nd*) or increase (as in Ax^{9b2}) *Notch* function. In addition, *Notch* function can be increased by adding an extra copy with a duplication, by mutating the *Hairless* gene (Bang and Posakony 1992) or by eliminating *Delta*. These genetic backgrounds provide insights into the function of *Notch* in different tissues. In each of these genetic backgrounds, we see interactions with *sno* in a consistent manner. An increase in *Notch* function can offset defects due to a mutation in *sno* and a decrease in *Notch* function enhances these defects.

An important observation from our studies is that *sno* synergistically affects *Notch* phenotypes in a tissue-specific manner. The $Notch^{ts1}$ allele, for example, affects all known tissues in which *Notch* has been proposed to function (Shellenbarger and Mohler, 1975). Consequently, double mutants of N^{ts1} and sno^{71e3} are lethal at all temperatures. In contrast, *split* affects the eye, and *notchoid* the wing. In double mutant combinations with *sno*, the phenotypes in these respective tissues are enhanced. This suggests a role of *sno* in many independent *Notch*-related pathways.

Several similarities are apparent in the way *sno* and *Notch* interact with other developmentally relevant genes. For example, *nd/Y; Ser/+* (Fleming et al., 1990), *sno/Y; Ser/+* and *nd sno* all have virtually identical strongly notched wing phenotypes. Similarly, the *nd dx* (Xu and Artavanis-Tsakonas, 1990) and *dx sno* combinations are both lethal. Not only do these double mutant combinations show similar phenotypes, but duplications of *Notch* rescue the phenotypes of *sno*, *dx* (Xu and Artavanis-Tsakonas, 1990) and *Ser* (Fleming et al., 1990), and the lethality of the *dx sno* combination. These effects suggest common pathways involving both *Notch* and *sno* and the above genes in different tissues during development. The *sno* gene product is expected to

have a very widespread function, similar to that of *Notch* in its pleiotropy. In the tissues in which these two genes work in concert with one another, their function is cooperative.

Historically, the neurogenic genes were defined for the dramatic effect their mutations have on the development of the central nervous system (Lehmann et al., 1981, 1983). Despite their phenotypic similarities, it is not clear that these genes all work in the same pathway. It has been suggested that the pleiotropic function of *Notch* may involve many pathways, each including Notch as a receptor, but not always including the same partners (Artavanis-Tsakonas and Simpson, 1991). It is therefore not surprising to find examples of genes that are non-neurogenic (like *Serrate* and *deltex*), as well as those that are classically defined as neurogenic (e.g., *E(spl)C* and *Delta*) that interact strongly with *Notch*. These genes have been collectively called the 'Notch group' (Artavanis-Tsakonas and Simpson, 1991). The genetic analysis presented here suggests that *sno* is also a member of this *Notch* group of genes.

We would like to dedicate this paper to the memory of George Lefevre who first isolated and studied the *strawberry notch* mutation. We would like to thank Bruce Baker, Elizabeth Knust, José Campos-Ortega, Spyros Artavanis-Tsakonas and Mark Muskavitch for their helpful suggestions and for sending the fly stocks for our use, Robert Diederich and Spyros Artavanis-Tsakonas for communicating unpublished results on *deltex*, Ross Cagan for his help with the cobalt sulfide protocol, Seymour Benzer for the mAb 22C10, John Nambu for suggestions on the immunohistochemical staining of embryos, Sarah Cramton for suggestions on the DAPI staining of ovaries and the members of the Banerjee laboratory for their critical reading of the manuscript. Part of this work was supported by a McKnight Scholars' award to U. B., an Alfred P. Sloan Foundation fellowship to U. B., a National Institute of Health grant (FDP USHHS 1 R01 EY08152-01A1) to U. B., a National Science Foundation grant (IBN 9219696) to U. B., and a Genetics Training grant (USPHS NRSA GM-007104) to C. A. C-T.

REFERENCES

Alton, A. K., Fechtel, K., Kopczynski, C. C., Shepard, S. B., Kooh, P. J. and Muskavitch, M. A. T. (1989). Molecular genetics of *Delta*, a locus required for ectodermal differentiation in *Drosophila*. *Dev. Genet.* **10,** 261-272.

Artavanis-Tsakonas, S. and Simpson, P. (1991). Choosing a cell fate: a view from the *Notch* locus. *Trends Genet.* **7,** 403-408.

Banerjee, U. and Zipursky, S. L. (1990). The role of cell-cell interaction in the development of the *Drosophila* visual system. *Neuron* **4,** 177-187.

Bang, A. G. and Posakony, J. W. (1992). The *Drosophila* gene *Hairless* encodes a novel basic protein that controls alternative cell fates in adult sensory organ development. *Genes Dev.* **6,** 1752-1769.

Basler, K., Christen, B. and Hafen, E. (1991). Ligand-independent activation of the sevenless receptor tyrosine kinase changes the fate of cells in the developing *Drosophila* eye. *Cell* **64,** 1069-1081.

Belt, A. L. (1971). A non-lethal allele of *Serrate? Drosophila Information Serv.* **46,** 116.

Bodenstein, D. (1950). The postembryonic development of *Drosophila*. In *Biology of Drosophila* (ed. M. Demerec), pp 275-367.

Bonfini, L., Karlovich, C. A., Dasgupta, C. and Banerjee, U. (1992). The *Son of sevenless* gene product: a putative activator of Ras. *Science* **255,** 603-606.

Bridges, C. B. and Brehme, K. (1944). Mutants of *Drosophila melanogaster*. *Carnegie Institution of Washington Publication* **552**.

Bridges, C. B. and Morgan, T. H. (1923). The third-chromosome group of mutant characters of *Drosophila melanogaster*. *Carnegie Institution of Washington Publication* **327**.

Cabrera, C. V. (1992). The generation of cell diversity during early neurogenesis in *Drosophila*. *Development* **115,** 893-901.

Cagan, R. L. and Ready, D. F. (1989a). *Notch* is required for successive cell divisions in the developing *Drosophila* retina. *Genes Dev.* **3,** 1099-1112.

Cagan, R. L., and Ready, D. F. (1989b). The emergence of order in the *Drosophila* pupal retina. *Dev. Biol.* **136,** 346-362.

Campos-Ortega, J. A. and Hartenstein, V. (1985). *The Embryonic Development of* Drosophila melanogaster. New York: Springer-Verlag.

Campos-Ortega, J. A. and Jan,Y. N. (1991). Genetic and molecular bases of neurogenesis in *Drosophila melanogaster*. *Ann. Rev. Neurosci.* **14,** 399-420.

Corbin V., Michelson A. M., Abmayr S. M., Neel V., Alcamo E., Maniatis T., and Young M. W. (1991). A role for the *Drosophila* neurogenic genes in mesoderm differentiation. *Cell* **67,** 311-323.

Delidakis, C., Preiss, A., Hartley, D. A. and Artavanis-Tsakonas, S. (1991). Two genetically and molecularly distinct functions involved in early neurogenesis reside within the *Enhancer of split* locus of *Drosophila melanogaster*. *Genetics* **129,** 803-823.

Delidakis, C. and Artavanis-Tsakonas, S. (1992). The *Enhancer of split [E(spl)]* locus of *Drosophila* encodes seven independent helix-loop-helix proteins. *Proc. Natn. Acad. Sci. USA* **89,** 8731-8735.

Dexter, J. S. (1914). The analysis of a case of continuous variation in *Drosophila* by a study of its linkage relations. *American Naturalist* **48,** 712-758.

Dickson, B., Sprenger, F., Morrison, D. and Hafen, E. (1992). Raf functions downstream of Ras1 in the Sevenless signal transduction pathway. *Nature* **360,** 600-603.

Fehon, R. G., Kooh, P. J., Rebay, I., Regan, C. L., Xu, T., Muskavitch, M. A. T., and Artavanis-Tsakonas, S. (1990). Molecular interactions between the protein products of the neurogenic loci *Notch* and *Delta*, two EGF-homologous genes in *Drosophila*. *Cell* **61,** 523-534.

Fischer-Vize, J. A., Vize, P. D. and Rubin, G. M. (1992). A unique mutation in the *Enhancer of split* gene complex affects the fates of the mystery cells in the developing *Drosophila* eye. *Development* **115,** 89-101.

Fleming, R. J., Scottgale, T. N., Diederich, R. J. and Artavanis-Tsakonas, S. (1990). The *Serrate* gene encodes a putative EGF-like transmembrane protein essential for proper ectodermal development in *Drosophila melanogaster*. *Genes Dev.* **4,** 2188-2201.

Foster, G. G. (1975). Negative complementation at the *Notch* locus of *Drosophila melanogaster*. *Genetics* **81,** 99-120.

Gaul, U., Mardon, G. and Rubin, G. (1992). A putative ras GTPase activating protein acts as a negative regulator of signalling by the sevenless receptor tyrosine kinase. *Cell* **68,** 1007-1019.

Godt, D. (1990). Wechselwirkungen zwischenden neurogenen genen bei *Drosophila melanogaster*. PhD thesis der Universitat zu Köln.

Gorman, M. J. and Girton, J. R. (1992). A genetic analysis of *deltex* and its interaction with the *Notch* locus in *Drosophila melanogaster*. *Genetics* **131,** 99-112.

Hart, A. C., Kramer, H., Van Vactor, D. L., Paidhungat, M. and Zipursky, S. L. (1990). Induction of cell fate in the *Drosophila* retina: the *bride of sevenless* protein is predicted to contain a large extracellular domain and seven transmembrane segments. *Genes Dev.* **4**, 1835-1847.

Hartenstein, V. and Posakony, J.W. (1990). A dual function of the *Notch* gene in *Drosophila* sensillum development. *Dev. Biol.* **142,** 13-30.

Hartley, D. A., Preiss, A. and Artavanis-Tsakonas, S. (1988). A deduced gene product from the *Drosophila* neurogenic locus, *Enhancer of split,* shows homology to mammalian G-protein β subunit. *Cell* **55,** 785-795.

Kidd, S., Kelly, M. R. and Young, M. W. (1986). Sequence of the *Notch* locus of *Drosophila melanogaster*: relationship of the encoded protein to mammalian clotting and growth factors. *Mol. Cell. Biol.* **6,** 3094-3108.

King, R. C. (1970). *Ovarian Development in Drosophila melanogaster*. New York: Academic Press.

Klämbt, C., Knust, E., Tietze, K. and Campos-Ortega, J. A. (1989). Closely related transcripts encoded by the neurogenic gene complex *Enhancer of split* of *Drosophila melanogaster*. *EMBO J.* **8,** 203-210.

Knust, E., Schrons, H., Grawe, F. and Campos-Ortega, J. A. (1992). Seven genes of the *Enhancer of split* complex of *Drosophila melanogaster* encode helix-loop-helix proteins. *Genetics* **132,** 505-518.

Knust, E., Tietze, K. and Campos-Ortega, J. A. (1987). Molecular

analysis of the neurogenic locus *Enhancer of split* of *Drosophila melanogaster*. *EMBO J.* **6,** 4113-4123.

Kopczynski, C. C., Alton, A. K., Fechtel, K., Kooh, P. J. and Muskavitch, M. A. T. (1988). *Delta* a *Drosophila* neurogenic gene, is transcriptionally complex and encodes a protein related to blood coagulation factors and epidermal growth factor of vertebrates. *Genes Dev.* **2,** 1723-1735.

Kramer, H., Cagan, R. L. and Zipursky, S. L. (1991). Interaction of *bride of sevenless* membrane bound ligand and the *sevenless* tyrosine kinase receptor. *Nature* **352,** 207-212.

Lefevre, G. (1952). Report of G. Lefevre. *Drosophila Information Serv.* **26,** 66.

Lefevre, G., Ratty, F. J. and Hanks, G, D. (1953). Frequency of *Notch* mutations induced in normal, duplicated, and inverted *X*-Chromosomes of *Drosophila melanogaster*. *Genetics* **38,** 345-359.

Lefevre, G. and Peterson, K. (1972). An unusual *Notch* mimic: *glossy-like*. *Drosophila Information Serv.* **48,** 126-127.

Lehmann, R., Dietrich, U., Jiménez, F. and Campos-Ortega, J. A. (1981). Mutations of early neurogenesis in *Drosophila*. *Wilhelm Roux's Arch. Dev. Biol.* **190,** 226-229.

Lehmann, R., Jiménez, F., Dietrich, U. and Campos-Ortega, J. A. (1983). On the phenotype and development of mutants of early neurogenesis in *Drosophila melanogaster*. *Wilhelm Roux's Arch. Dev. Biol.* **192,** 62-74.

Lindsley, D. and Zimm, G. (1992). *The Genome of* Drosophila melanogaster. San Diego, CA: Academic Press Inc.

Melamed, J., and Trujillo-Cenoz, O. (1975). The fine structure of the eye imaginal disc in *muscoid* flies. *J. Ultrastruc. Res.* **51,** 79-93.

Patel, N. H., Martin-Blanco, E., Coleman, K. G., Poole, S. J., Ellis, M. C., Kornberg, T. B. and Goodman, C. S. (1989). Expression of *engrailed* proteins in Arthropods, Annelids, and Chordates. *Cell* **58,** 955-968.

Perrimon, N. and Gans, M. (1983). Clonal analysis of the tissue specificity of recessive female-sterile mutations of *Drosophila melanogaster* using a dominant female-sterile mutation *Fs(1)K1237*. *Dev. Biol.* **100,** 365-373.

Portin, P. (1975). Allelic negative complementation at the *Abruptex* locus of *Drosophila melanogaster*. *Genetics* **81,** 121-133.

Poulson, D. F. (1937). Chromosomal deficiencies and the embryonic development of *Drosophila melanogaster*. *Proc. Natn. Acad. Sci. USA* **23,** 133-137.

Poulson, D. F. (1939). Effects of *Notch* deficiencies. *Drosophila Information Serv.* **12,** 64.

Preiss, A., Hartley, D. and Artavanis-Tsakonas, S. (1988). The molecular genetics of *Enhancer of split*, a gene required for embryonic neural development in *Drosophila*. *EMBO J.* **7,** 3917-3927.

Ramos, R. G. P., Grimwade, B. G., Wharton, K. A., Scottgale, T. N. and Artavanis-Tsakonas, S. (1989). Physical and functional definition of the *DrosophilaNotch* locus by *P*-Element transformation. *Genetics* **123,** 337-348.

Rebay, I., Fleming, R. J., Fehon, R. G., Cherbas, P. and Artavanis-Tsakonas, S. (1991). Specific EGF repeats of Notch mediate interactions with Delta and Serrate: Implications for Notch as a multifunctional receptor. *Cell* **67,** 687-700.

Ready, D. F., Hanson, T. E. and Benzer, S. (1976). Development of the *Drosophila* retina, a neurocrystalline lattice. *Dev. Biol.* **53,** 217-240.

Rogge, R. D., Karlovich, C. A. and Banerjee, U. (1991). Genetic dissection of a neurodevelopmental pathway: *Son of sevenless* functions downstream of the *sevenless* and EGF receptor tyrosine kinases. *Cell* **64,** 39-48.

Rogge, R. D., Majumdar, A., Cagan, R., Dulaney, T. and Banerjee, U. (1992). Neural development in the *Drosophila* retina: The *sextra* gene defines an inhibitory component in the R7 developmental pathway. *Proc. Natn. Acad. Sci. USA* **89**.

Rubin, G. (1991). Signal transduction and the fate of the R7 photoreceptor in *Drosophila*. *Trends in Genetics* **7,** 372-377.

Ruohola, H., Bremer, K.A., Baker, D., Swedlow, J.R., Jan, L.Y. and Jan, Y.N. (1991). Role of neurogenic genes in establishment of follicle cell fate and oocyte polarity during oogenesis in *Drosophila*. *Cell* **66,** 433-450.

Schrons, H., Knust, E. and Campos-Ortega, J. A. (1992). The *Enhancer of split* Complex and adjacent genes in the 96F region of *Drosophila melanogaster* are required for segregation of neural and epidermal progenitor cells. *Genetics* **132,** 481-503.

Schultz, J. (1941). Confluens, a tandem duplication of the *Notch* region. *Drosophila Information Serv.* **14,** 54-55.

Shellenbarger, D. L. (1971). A temperature sensitive *Notch* mutant of *Drosophila melanogaster*. *Genetics* **68,** s61-62.

Shellenbarger, D. L. and Mohler, J. D. (1975). Temperature-sensitive mutations of the *Notch* locus in *Drosophila melanogaster*. *Genetics* **81,** 143-162.

Shellenbarger, D. L. and Mohler, J. D. (1978). Temperature-sensitive periods and autonomy of pleiotropic effects of *l(1) N^{ts1}*, a conditional *Notch* lethal in *Drosophila*. *Dev. Biol.* **62,** 432-446.

Simon, M. A., Bowtell, D. A., Dodson, G. S., Laverty, T. R. and Rubin, G. R. (1991). Ras1 and a putative guanine exchange factor perform crucial steps in signalling by the Sevenless protein tyrosine kinase. *Cell* **67,** 701-716.

Thomas, U., Speicher, S. A., and Knust, E. (1991). The *Drosophila* gene *Serrate* encodes an EGF-like transmembrane protein with a complex expression pattern in embryos and wing discs. *Development* **111,** 749-761.

Tomlinson, A. and Ready, D. F. (1987). Neuronal differentiation in the *Drosophila* ommatidium. *Dev. Biol.* **120,** 366-376.

Vässin, H., Vielmetter, J. and Campos-Ortega, J. A. (1985). Genetic interactions in early neurogenesis of *Drosophila melanogaster*. *J. Neurogenetics* **2**, 291-308.

Vässin, H., Bremer, K. A., Knust, E. and Campos-Ortega, J. A. (1987). The neurogenic gene *Delta* of *Drosophila melanogaster* is expressed in neurogenic territories and encodes a putative transmembrane protein with EGF-like repeats. *EMBO J.* **6,** 3431-3340.

Weigel, D., Knust, E., and Campos-Ortega, J. A. (1987). Molecular organization of *mastermind*, a neurogenic gene of *Drosophila melanogaster*. *Molecular and General Genetics* **207,** 374-384.

Welshons, W. (1956). Dosage experiments with *split* mutants in the presence of an *enhancer of split*. *Drosophila Information Serv.* **30,** 157-158.

Welshons, W. J. (1965). Analysis of a gene in *Drosophila*. *Science* **150,** 1122-1129.

Wharton, K. A., Johansen, K. M., Xu, T. and Artavanis-Tsakonas, S. (1985). Nucleotide sequence from the neurogenic locus *Notch* implies a gene product that shares homology with proteins containing EGF-like repeats. *Cell* **43,** 567-581.

Wieschaus, E. and Nüsslein-Volhard, C. (1986). Looking at embryos. In Drosophila *a Practical Approach*, (ed. D. B. Roberts) pp. 199-227. Oxford: IRL Press.

Xu, T. and Artavanis-Tsakonas, S. (1990). *deltex*, a locus interacting with the neurogenic genes, *Notch, Delta*, and *mastermind* in *Drosophila melanogaster*. *Genetics* **126,** 665-677.

Xu, T., Rebay, I., Fleming, R. J., Scottgale, T. N. and Artavanis-Tsakonas, S. (1990). The *Notch* locus and the genetic circuitry involved in early *Drosophila* neurogenesis. *Genes Dev.* **4,** 464-475.

Xu, T., Caron, L. A., Fehon, R. F. and Artavanis-Tsakonas, S. (1992). The involvement of the *Notch* locus in *Drosophila* oogenesis. *Development* **115,** 913-922.

Yedvobnick, B., Smoller, D., Young, P. and Mills, D. (1988). Molecular analysis of the neurogenic locus *mastermind* of *Drosophila melanogaster*. *Genetics* **118,** 483-497.

Zipursky, S. L., Venkatesh, T. R., Teplow, D. B. and Benzer, S. (1984). Neuronal development in the *Drosophila* retina: Monoclonal antibodies as molecular probes. *Cell* **36,** 15-26.

(Accepted 12 July 1993)