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

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### 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 288x10^3 M, 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 (Lefèvre 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. Lefèvre and M. Green and were kindly given to us for this study. The nd^1, nd^2, Ax^E2 and Ax^962 alleles of Notch (Shellenberger and Mohler, 1975; Foster, 1975 and Portin, 1975), a cosmids transformant including a wild-type copy of Notch (Cos 479 Ramos et al., 1989) and the neur^P65, manim^R15, bldP005, E(spl) ^D22, grouchoe^73 and h^99 mutations were gifts of S. Artavanis-Tsakonas. N^01 was donated by R. Cagan and is described by Shellenberger and Mohler, 1975. Ser (Belt, 1971), and the E(spl)^B1 and E(spl)^P272 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 Df(1) P^39 and Df(6) P^87 flies were provided by M. Muskavitch. Dp(1;1Y) 1 was provided by B. Baker. The spl, ds, D^P and E(spl)^P 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. Lefèvre’s laboratory. The sno locus was placed 0.5 map unit distal to wy (Lefèvre and Peterson, 1972). We have mapped the temperature-sensitive allele more closely by mating w sn sno f car males and v f w wy females. The resulting heterozygous females were mated to v f w wy males. Recombinants between f w and wy were analyzed for sno phenotypes. Using standard recombination methods, sno^71^ was placed between f w and wy, 1.5 cM from f w and 0.5 cM from wy.

The lethal allele sno^EF531 was crossed to sno^71^ and the sno^EF531/sno^71^ females have a strong sno phenotype. The lethality was recombination mapped between f w and wy using a v f w wy g chromosome. Thus the lethality and the sno^P phenotype 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^71^ and sno^EF531 males were mated to Df(1) C246/FM6 (11D-E;12A1-2), Df(1) N12/FM6 (11D1-2; 11F1-2) and Df(1) wy^P26/FM7 (11B17-C1; 11E9). Each of these deficiencies failed to complement the sno phenotype. The sno^71^ and sno^EF531 males were also mated to Df(1) JA26/FM7 (11A1; 11D-E) and Dp(1;Y) 1 females (C(1)DI X, p m f YDp 1: this duplication rescues Df(1) C246 and does not rescue Df(1) N12 or Df(1) wy^P26. 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^d 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^962 sn sno was obtained from y Ax^962 sn and w sn sno f car. Thus y Ax^962 sn sno f car was constructed. N^01 sno was generated from N^01 rh and w sn sno f car. A N^01 rh 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^71^ 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)^P, Ser and gro^E73.

Notch

w sn sno f car/FM7 × N^55c11/Dp (1;2) w^+ 1167^+ (Dp N^+ = Dp (1;2) w^+ 1167^+ ; 3C1/2; 3D6; 52E; Lefèvre, 1952) sno f car/FM7 × Cos 479/TM6B
Hairless
sno f car/FM7 × H^99_ e/TM3

Delta
sno f car/FM7 × Dl^{1/TM3}
sno f car/FM7 × D^{R57}/TM6C
sno f car/FM7 × D^{R57}/TM6B

E(spl)C and grouch
sno f car/FM7 × E(spl)P e (E(spl)P) has been described by Welshons 1956; Klimb et al., 1989.
sno f car/FM7 × D(spl)P/Tm6B (E(spl)P) = In (3R) 96F2; 96F12-14; 99C, deficient for 96F12-14; Lehmann et al., 1983; Preiss et al., 1988).
sno f car/FM7 × E(spl)X^{77}/TM6B (E(spl)X^{77} = Df(3R)96F5-97B1; Preiss et al., 1988).
sno f car/FM7 × gro^F73/TM6B (gro^F73) is a lethal point mutation in grouch which has been described as antimorphic; Preiss et al., 1988; Delidakis et al., 1991).
sno f car/FM7 × E(spl)Y^{X22}/TM3 (E(spl)Y^{X22} is an inversion and deletion; Preiss et al., 1988).
sno f car/FM7 × Df (3) boss^Y^{TM3} (boss^Y^{TM3} = Df(3R) 96F5/7-12/13
boss^f6 also has an insertion in the grouch transcript; Hart et al., 1990; Schrons et al., 1992).

Other neurogenics
sno f car/FM7 × bI^{DOS}/Cy,O
sno f car/FM7 × marl^{H115}/Cy,O
sno f car/FM7 × neu^{F65}/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^{EF51}/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^{EF51}/FM7 × FM7/Y and sno^{EF51}/FM7 × FM7/Y and the results were the same. The cross to analyze embryonic phenotypes of the temperature-sensitive allele was sno^{71c} 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 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 ddH2O, 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 according to 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 ddH2O for 5 minutes and then transferred to 2% cobalt nitrate in ddH2O. 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 ddH2O 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 ddH2O, 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^{71c}/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^{EF51}/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 sno71e2 temperature-sensitive period was determined by mating sno71e2f ear 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, 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 sno71e2 males.

**Embryonic phenotype**

Wild-type embryos can be analyzed using several cuticular structures as markers. For example, the cephalopharyngeal 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 snoEF531 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 sno71e3 show identical cuticular and nervous system defects as the lethal allele snoEF531.

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. 1J). 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 mutant defects 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, sno71e3 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 retinai 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 sno71e3 (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 sno71e3 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 sno71e3, 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 sno71e3, 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 sno71e3, 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 sno71e3 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 (Shellenberger and Mohler, 1978). In reciprocal experiments, sno71e3 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.
Maternal phenotype

We analyzed the maternal effect of sno using a method that involves the dominant female-sterile mutation ovoD (Perrimon and Gans, 1983). When present in one copy, the ovoD mutation gives rise to females that are sterile and fail to produce eggs. These ovoD females have a defective germ
线，但有丝分裂过程中的卵母细胞是功能正常的。因此，只有当野生型生殖细胞克隆在一只由 otherwise ovo^+/ovo^+ 飞行时，才能产生杂交昆虫。

在对照实验中，野生型雄性 ovo^+/Y 与野生型雌性交配，然后将杂合体 ovo^+/ovo^+ 携带的后代辐射照射，以促进生殖细胞的有丝分裂重组事件。从 1180 只雌性中收集了 69 只产生了卵子。这代表了 6% 的有丝分裂克隆频率。在平行的实验中，ovo^+/Y 雄性与 sno^{EF531}/FM7 女性交配，并将下一代的后代进行照射。总共收集了 3800 只照射的 sno^{EF531}/ovo^+ 女性。基于以上 6% 的控制克隆频率，我们预计至少有 150 只这些女性会携带使有丝分裂克隆频率。任何这样的母亲产生的卵子都不会有 sno^+ 基因的母系成分。在我们的实验中，3800 只雌性中没有产生卵子。我们得出结论，sno^+ 基因对于生殖细胞的生育是必要的。这一结果与 sno^71e3 女性在 23°C 下生育能力差且在 27°C 下完全不育的事实一致。

卵巢 sno^71e3 女性在 25°C 下被 DAPI 染色，以观察其发育缺陷。对照野生型雌性通过 DAPI 染色显示，每对卵巢平均含有 40-50 个卵泡。每个卵泡包含具有特定极性的卵泡母细胞，并处于不同的发育阶段（King, 1970）。在 sno^71e3 女性中，25°C 下，卵巢数量减少 25% 降至平均 30 个。在 20% 的 sno^71e3 雌性中只有一只卵巢存在，或者两只卵巢共有的卵巢数量减少到 12 个。除数量变化外，12% 的发育卵泡可以观察到某些可重复的缺陷（n = 395）。即使在这一允许的温度下，每个卵巢都包含的例子中，卵泡母细胞的死亡，卵泡母细胞的极性反转，或卵泡母细胞的核内层细胞核增多（Fig.
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 $Dp(1;2)w^{51b^7}$ ($Dp N^+$), 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 sno71e3 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) sno71e3/Y raised at 23°C. The eye is rough and the facets are not in a uniform hexagonal array. (C) sno71e3/Y raised at 19°C. The rough eye phenotype is temperature sensitive and at 19°C the external morphology is not rough. (D) sno71e3/Y; Cos479/+ raised at 23°C. The extra copy of the Notch gene provided by Cos479 rescues the rough eye phenotype of sno. The sno71e3/Y and sno71e3/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 mutant carrying an extra copy of Notch. The five tarsal segments are clearly separated by joints. Bars=10 µm. (F) sno71e3/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) sno71e3/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) sno71e3/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) sno71e3/Y raised at 23°C. The peripheral margin is notched. The wing veins are thick and join the margin with large deltas. (K) sno71e3/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) sno71e3/Y; Cos479/+ 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) sno71e3/Y raised at 23°C. The two most distal tarsal segments (fourth and fifth) are fused. (O) sno71e3/Y raised at 19°C. The fused tarsal segment phenotype is temperature sensitive and at 19°C the leg is wild type. (P) sno71e3/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 sno71e3/Y flies are compared with those of sno71e3/Y; Cos479/+ . 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 sno71e3/Y 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 mutant carrying an extra copy of Notch. The five tarsal segments are clearly separated by joints. Bars=10 µm. (F) sno71e3/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) sno71e3/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) sno71e3/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) sno71e3/Y raised at 23°C. The peripheral margin is notched. The wing veins are thick and join the margin with large deltas. (K) sno71e3/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) sno71e3/Y; Cos479/+ 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) sno71e3/Y raised at 23°C. The two most distal tarsal segments (fourth and fifth) are fused. (O) sno71e3/Y raised at 19°C. The fused tarsal segment phenotype is temperature sensitive and at 19°C the leg is wild type. (P) sno71e3/Y; Cos479/+ raised at 23°C. An extra copy of Notch completely rescues the fused segment phenotype of sno.

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 wy51b7/+ 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) I, 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 Nts1

The rescue of sno by an extra copy of Notch suggested that sno might interact with mutant alleles of Notch. The Notchts1 (Nts1) allele is viable and wild type below 23°C. However at nonpermissive temperatures, Nts1 exhibits all the pleiotropy of the Notch locus (Shellenbarger and Mohler, 1975). When Nts1 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 are phenocopied in Nts1. Furthermore, the double mutant, Nts1 sno71e3/Y, is lethal with no escapers at any temperature. The Nts1 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 sno71e3/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 sno71e3 have some alterations in photoreceptor numbers in their ommatidia at 25°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\textsuperscript{7le3} eye phenotypes and female sterility. All flies were raised at 23°C. Bars=10 \( \mu \)m. (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\textsuperscript{7le3} eyes. (Bars=1 \( \mu \)m.) 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 \( \mu \)m. (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\textsuperscript{7le3}. Photoreceptor cells (r) can be found beneath the fenestrated membrane within the lamina. (F) Cobalt sulfide-stained pupal eye disc from sno. Bar=10 \( \mu \)m. 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\textsuperscript{7le3}.
notchoid
In contrast to spl, the notchoid (<i>nd</i>) allele of Notch has a wild-type eye and leg, while the wings are slightly notchcd (Fig. 5B,E). The <i>nd</i> <i>sno</i> <i>1</i> <i>E</i> double mutant, shown in Fig. 5C,F shows a synergistic enhancement of this wing phenotype, while the eye and leg phenotypes resemble that of <i>sno</i> alone. The wing blade is severely notchcd, with most of the peripheral margin missing. The wing veins end in large deltas, which are larger in size than those of <i>sno</i> alone. The viability of these double mutant flies is very poor, with all adults dying at, or within hours of eclosion. The <i>sno</i> mutation between <i>spl</i> and <i>nd</i> is consistent with the suggestion that <i>Hairless</i> is a negative regulator of Notch function (Bang and Posakony, 1992). In a <i>Hairless</i> mutant, Notch activity would thus be expected to be high, and <i>sno</i> phenotypes therefore suppressed in the same way as with a duplication of <i>Notch</i>. As with the tissue-specific Notch alleles, the interaction between <i>Hairless</i> and <i>sno</i> is limited to the specific tissue in which mutations in both <i>Hairless</i> and <i>sno</i> have phenotypes. <i>Hairless</i> fails to rescue the lethality of the <i>N</i> <i>s</i> <i>nd</i> <i>E</i> double mutant and the eye phenotype of the <i>spl</i> <i>sno</i> 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 <i>sno</i> 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 <i>Delta</i> is <i>sno</i> dependent. <i>Delta</i> 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 <i>sno</i> <i>1</i> <i>E</i>/ + double mutant combination. The same result was obtained with <i>D</i> <i>s</i> <i>71e3</i> and <i>D</i> <i>s</i> <i>9p39</i> 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 <i>Delta</i> is also suppressed in <i>Notch</i>+/<i>Delta</i>+/ double mutants (Vässin et al., 1985; Alton et al., 1989; Godt, 1990). We conclude that the phenotypic interaction between <i>sno</i> and <i>Delta</i> indirectly reflects an interaction between <i>sno</i> and Notch.

groucho
The 96F11–14 region of the <i>Drosophila</i> chromosome contains the Enhancer of split complex (<i>E(spl)</i>) encoding at least 7 different helix-loop-helix proteins (Klämbt et al., 1989; Knust et al., 1992; Delidakis and Artavanis-Tsakonas, 1992) and the <i>groucho</i> locus (Delidakis et al., 1991). The <i>groucho</i> gene encodes a nuclear protein with homology to protein-protein interacting domains of β transducin (Hartley et al., 1988; Delidakis et al., 1991). The <i>groucho</i> gene has been shown to function in the process of neurogenesis (Knust et al., 1987; Hartley et al., 1988; Delidakis et al., 1991; Schnors 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 <i>E(spl)</i> and <i>groucho</i> to identify a possible interaction with <i>sno</i>. The <i>sno</i> <i>1</i> <i>E</i> mutation was combined with one copy of <i>E(spl)</i> <i>Y</i>, <i>E(spl)</i> <i>Y</i> <i>T2</i>, <i>E(spl)</i> <i>P</i> <i>B</i> <i>2</i>, <i>D</i> <i>f</i> <i>3</i> <i>boss</i> <i>6</i> and the <i>groucho</i> point mutation <i>gro</i> <i>E73</i> (See Materials and Methods). In each case, flies hemizygous for <i>sno</i> <i>1</i> <i>E</i> and heterozygous for the deletion or <i>gro</i> <i>E73</i> 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 <i>gro</i> interacts dominantly with <i>sno</i> since the point mutation and the deletions used all affect <i>gro</i> (see Materials and Methods). However, it does not rule out that the <i>E(spl)</i> also has additional effects on <i>sno</i>. We crossed <i>sno</i> with the <i>E(spl)</i> <i>P</i> chromosome. The wing, but not the eye, phenotype of this double mutant is enhanced over <i>sno</i> (not shown). However, this wing effect is not due to the Enhancer of split gene encoded by the <i>m8</i> transcript since a transformant line <i>m8</i> carrying the <i>m8</i> mutation of <i>E(spl)</i> <i>P</i> (Klämbt et al., 1989) does not

**Interactions between <i>sno</i> and genes related to Notch**

**Hairless**
The <i>Hairless</i> <i>99</i> mutation has an interrupted wing vein phenotype similar to <i>Ax</i> (Fig. 6G) (Bridges and Morgan, 1923). As shown in Fig. 6H, the <i>sno</i> <i>1</i> <i>E</i>; <i>H</i> <i>99</i>/ + double mutant wing is essentially wild type. Not only does <i>sno</i> suppress the incomplete wing veins of <i>Hairless</i>, but <i>Hairless</i> also suppresses the notched blade and thick veins seen in <i>sno</i>. This mutual suppression of <i>sno</i> and <i>Hairless</i> is consistent with the suggestion that <i>Hairless</i> is a negative regulator of Notch function (Bang and Posakony, 1992). In a <i>Hairless</i> mutant, Notch activity would thus be expected to be high, and <i>sno</i> phenotypes therefore suppressed in the same way as with a duplication of <i>Notch</i>. As with the
Fig. 4
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 hib (Lehmann et al., 1981, 1983). The sno^{71e3}/Y;mam^{ll12}/+, the
sno\textsuperscript{71ec3}/Y; neu\textsuperscript{1F65}/+ and the sno\textsuperscript{71ec3}/Y; bib\textsuperscript{1ID05}/+ flies all resemble sno\textsuperscript{71ec3}/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 deltax locus (Xu and Artavanis-Tsakonas, 1990; Gorman and Girton, 1992) share several genetic features with sno. The wing veins of deltax 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\textsuperscript{71ec3}/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\textsuperscript{71ec3}/Y escapers (Fig. 8K).
The pupal lethality of dx sno71e3/Y is rescued by an extra copy of Notch. The dx sno71e3/Y; Dp N+/+ flies also have less severe wing and completely wild-type leg phenotypes (Fig. 8II). 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, BdS) 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 sno71e3/Y; Ser/+ double mutant is viable but has a severely enhanced wing phenotype (Fig.

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**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) sno71e3/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)sno71e3/Y; DpN+/+. A mutual suppression of both the sno phenotype and the DpN+ confluens phenotype is observed. (E) +/Y; Df3+/+. The confluens phenotype of Df3+/+ is most apparent at LII, LIII, and the anterior and posterior cross veins (arrows). The wing veins also end in deltas. (F) sno71e3/Y; Df3+/+. The Df3+/+ confluens phenotype is suppressed; however, the notching, thick veins and deltas are similar in severity to sno71e3.
Fig. 8
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 germ band retraction. When sno is missing, the embryos fail to complete germ band 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 (e.g. 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^ts02) 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 N^ts1 allele, for example, affects all known tissues in which Notch has been proposed to function (Shellenberger and Mohler, 1975). Consequently, double mutants of N^ts1 and sno^ts1 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 delta) 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.

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REFERENCES


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


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