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Senseless, a Zn Finger Transcription Factor, Is Necessary and Sufficient for Sensory Organ Development in *Drosophila*

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Summary

The *senseless* (*sens*) gene is required for proper development of most cell types of the embryonic and adult peripheral nervous system (PNS) of *Drosophila*. Sens is a nuclear protein with four Zn fingers that is expressed and required in the sensory organ precursors (SOP) for proper proneural gene expression. Ectopic expression of Sens in many ectodermal cells causes induction of PNS external sensory organ formation and is able to recreate an ectopic proneural field. Hence, *sens* is both necessary and sufficient for PNS development. Our data indicate that proneural genes activate *sens* expression. Sens is then in turn required to further activate and maintain proneural gene expression. This feedback mechanism is essential for selective enhancement and maintenance of proneural gene expression in the SOPs.

Introduction

The fly PNS consists of individual neurons and organs that provide information such as heat, pressure, and proprioreception to the central nervous system (CNS). A PNS organ typically consists of four cells that are derived from two or three divisions of an SOP cell (Brewster and Bodmer, 1995). Studies of the development of the *Drosophila* PNS have provided many insights into the molecular mechanisms by which cells become fated and differentiate (Modolell, 1997). The onset of PNS development is marked by the expression of proneural genes of the *achaete-scute* complex (AS-C), *atonal*, or *amos* in clusters of ectodermal cells, the proneural clusters. The SOP is then selected from a small group of cells within the proneural cluster, the proneural field, that accumulates higher levels of proneural proteins than neighboring cells (Cubas et al., 1991; Skeath and Carroll, 1991; Van Doren et al., 1992; Jarman et al., 1993; Culi and Modolell, 1998; Goulding et al., 2000; Huang et al., 2000). Eventually, the SOP accumulates the highest levels of proneural proteins. The proneural basic helix loop helix (bHLH) proteins (Scute, Achaete, Asense, Atonal, and Amos) then implement the neuronal differentiation pathway as functional heterodimers with the Daughterless protein (Murre et al., 1989). The upregulation of proneural gene expression in the SOP and the downregulation in adjacent ectodermal cells is mediated through a signaling pathway in which the genes of the Notch pathway, including *Notch* (*N*), *Delta* (*Dl*), *Suppressor of Hairless* [*Su(H)*], and the *Enhancer of split* complex genes [*E(spl)*], play a pivotal role. Loss-of-function mutations in these genes cause most or all cells of the proneural cluster to assume the SOP fate (Dietrich and Campos-Ortega, 1984; Schweisguth et al., 1996; Artavanis-Tsakonas et al., 1999). Although it has been proposed that accumulation of proneural gene products in the SOP via Notch signaling is stochastic, the SOP always occupies a very similar position in the cluster (Cubas and Modolell, 1992). Modolell and colleagues (Cubas and Modolell 1992; Culi and Modolell, 1998) have therefore proposed that the prepatterning factors predetermine a small subset of cells in the proneural cluster. One of these cells will become the SOP by accumulating more proneural protein than its neighbors and will produce more Delta protein (Artavanis-Tsakonas and Simpson, 1991; Hinz et al., 1994). Although the upregulation of proneural gene expression is thought to be a prerequisite for sensory organ development, it is thought that several other unidentified proteins are required for the process (Culi and Modolell, 1998).

Here we describe the identification and characterization of a novel proneural-like protein. We show that severe loss-of-function mutations in *sens* abolish the further upregulation and maintenance of proneural gene expression in the SOPs. Interestingly, ectopic expression of Sens induces ectopic PNS organs, causing large tufts of bristles. Sens is both necessary and sufficient for PNS organ development but seems to require the activity of proneural proteins. *sens* encodes a transcription factor that enhances and maintains proneural gene expression in the SOP and is able to create a proneural cluster when ectopically expressed. Sens is therefore an essential component of the proneural Notch signaling pathway required for proper SOP differentiation.

Results

Genetics of *sens*: *Lyra* Alleles Are Gain-of-Function Mutations of *sens*

The *sens* gene was previously shown to affect the development of the PNS and three alleles have been described: two ethylmethane sulfonate (EMS)–induced alleles ($sens^{M256}$ and $sens^{l235}$) and one allele induced by P element dysgenesis ($sens^{1228/4}$) (Salzberg et al., 1994, 1997). Unfortunately, the P element associated with the $sens^{1228/4}$ chromosome mapped at a different cytological position (85D) than $sens^{M256}$ and $sens^{l235}$ (3–41 cM). We found that $sens^{M256}$ and $sens^{l235}$ failed to complement $Lyra^1$, a deletion (*Df(3L)Ly*^{1}*, 70A2-3;A5-6)*. Although $Lyra^1$ embryos are homozygous lethal, a single copy of the $Lyra^1$ mutation causes a dominant loss of the anterior and posterior wing margin (Lindsley and Zimm, 1992). An EMS screen over the $Lyra^1$ deficiency permitted isolation of other alleles of *sens*: *E1*, *E2*, *E53*, *E54*, *E58*, and *E69* (H. Irick, personal communication to Flybase). Most

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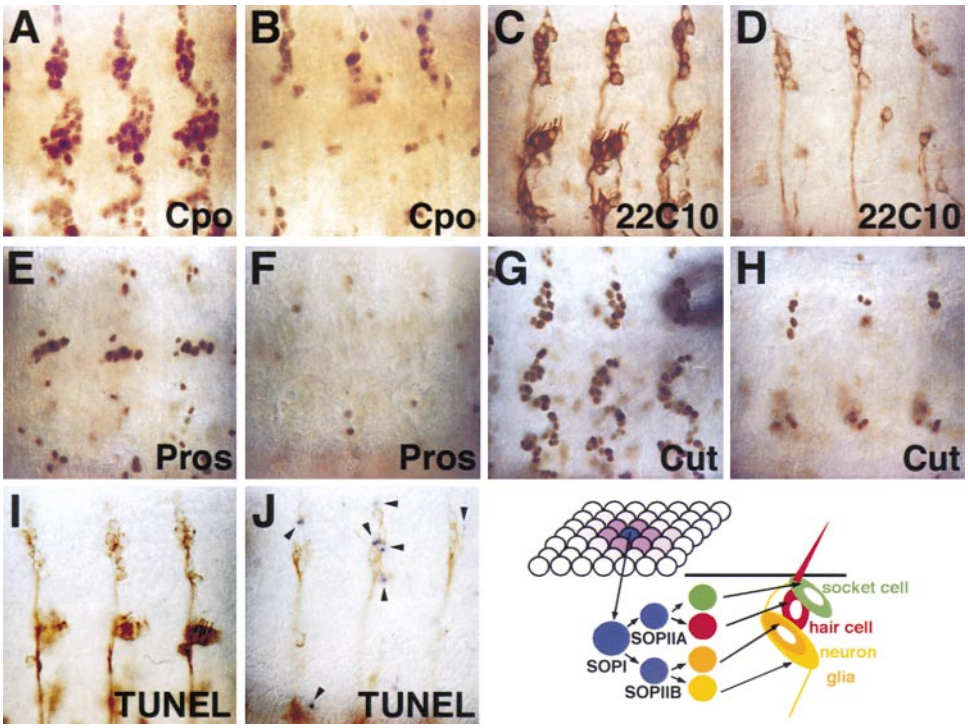

Figure 1. Mutations in *sens* Cause Loss of Most Embryonic PNS Cells

Each panel shows the dorsal and lateral cluster of three abdominal segments of the PNS of stage 16 embryos. Anterior is to the left, dorsal is up. (A), (C), (E), (G), and (I) are wild-type embryos. (B), (D), (F), (H), and (J) are *sens* mutant embryos ($sens^{M255}/Df(3L)1228/4$).

(A and B) Anti–Couch Potato staining shows loss of staining of numerous cells of the PNS.

(C and D) mAb22C10 staining reveals loss of neurons of all sensory organs.

(E and F) Anti-Prospero staining shows extensive loss of glia and support cells of sense organs.

(G and H) Anti-Cut staining reveals massive loss of cells of external sensory organs.

(I and J) TUNEL staining shows many apoptotic cells (purple dots with arrows). Brown staining corresponds to neurons revealed with mAb22C10.

The model inset depicts the putative cell lineage for embryonic external sensory organs.

alleles cause similar phenotypes, but much of the phenotypic analysis was carried out with allele *E2*.

As none of the *sens* alleles causes a dominant wing phenotype, the phenotype associated with $Lyra^{1}$ may map elsewhere. However, the following data support the notion that *Lyra* mutations correspond to gain-of-function mutations of *sens*. First, the dominant phenotype associated with $Lyra^{1}$ could not be recombined onto a *sens* mutant chromosome, indicating that both mutations map at the same site. Second, an X ray–induced revertant of $Lyra^{SX67}$, a *Lyra* allele that complements all *sens* mutations, is homozygous lethal and fails to complement all *sens* alleles. Third, *Lyra* mutations have breakpoints in or near the *sens* gene. Fourth, *Lyra* mutations cause ectopic expression of *sens* precisely in those portions of wing imaginal discs that normally give rise to tissues that are lost in *Lyra* mutants (R. N., L. A. A., and H. J. B, unpublished data). Finally, additional copies of $Lyra^{+}$ in *Lyra* mutants do not alter the wing phenotype (Abbott and Sprey, 1990). These and other data provide strong evidence that *Lyra* mutations cause their phenotype by ectopically expressing *sens*.

Loss of *sens* Affects the Development of Embryonic PNS Organs

The embryonic PNS of *Drosophila* consists of three types of sensory transducers: the multiple dendritic (md) neurons, the external sensory organs (es), and the chordotonal organs (Jan and Jan, 1993). The latter two types of organs consist of four cells: a neuron, a glial cell, and two support cells (see inset in Figure 1). These originate from the SOPI that produces the SOPII a and b. As shown in Figures 1A and 1B, mutations in *sens* cause an extensive loss of many cells of the embryonic PNS, as revealed by staining with anti–Couch Potato, a marker that labels all nuclei of the PNS cells. Labeling with mAb22C10, a neural-specific antibody (Figures 1C and 1D), shows that most neurons are absent. The few neurons that remain are mostly of the md type, but the md neurons that are lost are dependent on *scute*, *atonal*, and *amos* (data not shown). As shown in Figures 1E and 1F, staining with anti-Prospero, a marker for PNS glial cells, reveals a very severe loss of glial cells. Finally, staining with anti-Cut shows a significant loss of es support cells (Figures 1G and 1H). In summary, these data show that all types of PNS cells in *sens* mutants are affected and that most cells are absent in mature stage 16 embryos.

Salzberg et al. (1994) have shown that the SOPI and SOPII cells are present in *sens* mutant embryos. Hence, there are two possible alternatives to explain the phenotype: the SOPII cells fail to divide, or the SOPII cells and/or their progeny are eliminated by apoptosis. As shown in Figures 1I and 1J, double labeling with terminal deoxynucleotidyl transferase (TUNEL) and 22C10 shows dying cells in the PNS of stage 16 *sens* embryos but

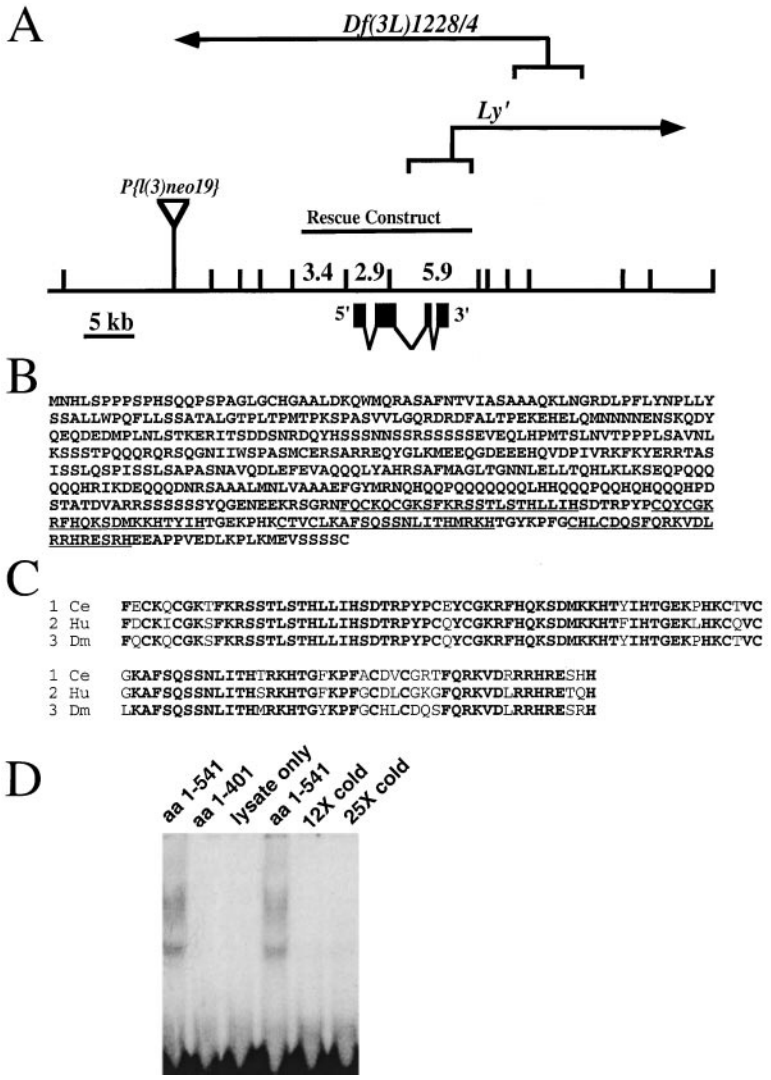

Figure 2. *sens* Encodes a Protein with Four Zn Fingers

(A) Genomic walk at the *sens* locus. The walk was initiated using a sequence tag of *P{l(3)neo19}*. The breakpoints of *Df(3L)1228/4* and *Ly*^{1} are shown. The gene contains four exons. The 11 kB rescue construct fully rescues the embryonic lethality and PNS phenotype of *sens* mutants.

(B) Sens protein sequence. The C2H2 zinc fingers are underlined. The protein sequence shows no significant homology to other proteins outside the Zn finger domain.

(C) Comparison of the last four zinc fingers of the *C. elegans* Pag-3 protein and the human Gfi-1 protein with those of the Sens. These four Zn fingers in these three proteins are extremely similar.

(D) Gel mobility shift assay using in vitro transcribed and translated proteins in the presence of poly(dI-dC). Oligos are shown in Experimental Procedures. Lanes 1–3 contain 5 μl of lysate, and lanes 4–6 contain 2 μl of lysate.

not in wild-type control embryos (Abrams et al., 1993). Similar data were obtained by in situ hybridization with *grim*, a marker for apoptotic cells in *Drosophila* (data not shown) (Chen et al., 1996). As we did not observe alterations in the expression of *dacapo*, a cyclin-dependent kinase inhibitor expressed in terminally dividing cells of the PNS (data not shown) (de Nooij et al., 1996; Lane et al., 1996), we conclude that the severe loss of PNS cells in late embryos is due to apoptosis of SOPIIs and/or differentiating cells of the PNS.

sens Encodes a Zn Finger Protein with Homology to Pag-3 and Gfi-1

The *sens* gene was isolated by positional cloning. As shown in Figure 2, the *sens*$^{1228/4}$ mutation corresponds to a small deficiency that fails to complement an unrelated P element–induced mutation (*l(3)neo19*; Spradling et al., 1999), suggesting that this P element is inserted in proximity of *sens*. We screened a genomic library with DNA adjacent to *l(3)neo19* and initiated a genomic walk (Figure 2). Since the breakpoint of *Lyra*^{1} may be associated with *sens* mutations, we screened embryonic cDNA libraries with the 5.9 kb genomic fragment that harbors one of the *Lyra*^{1} breakpoints (Figure 2). This allowed isolation of a cDNA that identifies a transcript of ~2.8 kb on Northern blots (data not shown). To demonstrate that this cDNA corresponds to the *sens* gene, we determined the structure of the gene by sequencing both the genomic area and cDNA (GenBank accession numbers AF212313 and AF212312). This allowed us to establish that an 11 kB fragment shown in Figure 2 does not contain significant open reading frames other than those encoded by *sens*. This genomic fragment fully rescued the lethality of seven *sens* mutations in trans to each other, demonstrating that the cDNA corresponds to the *sens* gene.

The Sens protein, deduced from the sequence, contains 541 amino acids and a nuclear localization signal (PIVRKFK; Figure 2B). The only domain that shows homology to known proteins is at the carboxy-terminal part of the protein between amino acids 410 and 518. This domain contains 4 Zn fingers of the 2Cys + 2His type that are homologous to Zn fingers found in the *C. elegans* Pag-3 protein (87% identity; Jia et al., 1997) and the vertebrate Gfi-1 protein (87% identity; Gilks et al., 1993). The Pag-3 protein has been shown to contain five Zn fingers and to affect neuronal lineages of motor neurons, interneurons, and touch neurons in *C. elegans*

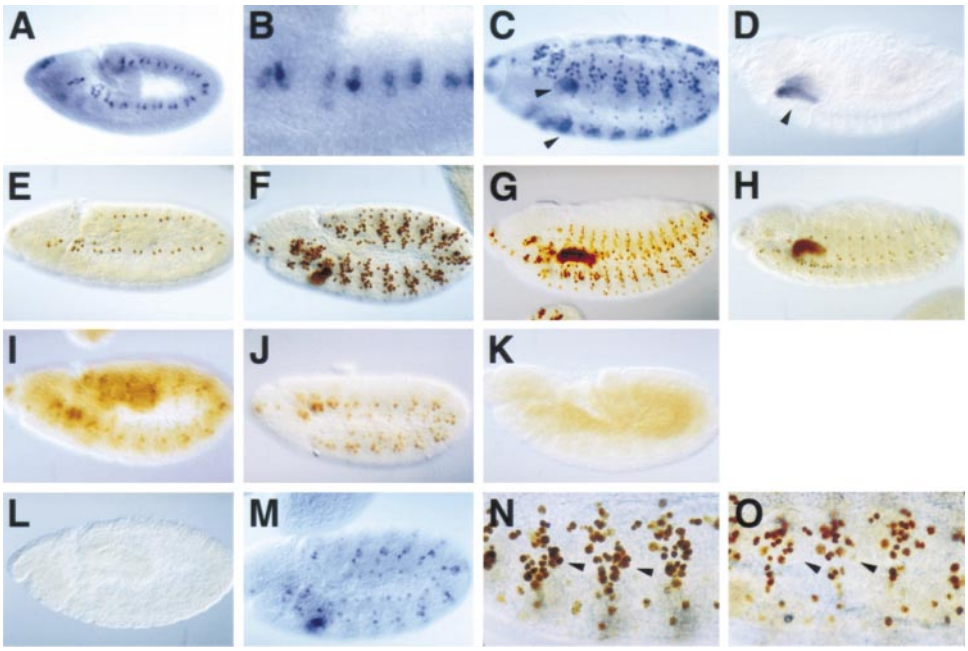

Figure 3. Sens Is Expressed in the Embryonic PNS

(A–D and M) In situ hybridizations to whole-mount embryos. (E–L and N–O) Sens protein expression patterns in wild-type and mutant embryos.
(A) Stage 10 embryo shows that *sens* mRNA is expressed in small clusters of cells in the lateral area.
(B) Enlargement of a portion of the embryo shown in (A) to illustrate that mRNA is expressed in clusters of three to five cells including the A and P precursor cells (Ghysen and O'Kane, 1989).
(C) Stage 11 embryo. The mRNA expression reaches its peak and most SOPs are labeled. Note the two patches on the ventral side that correspond to the anlagen of the salivary glands (arrowheads).
(D) Stage 13 embryo. During stage 12, mRNA expression in almost all cells is lost. The only tissues that still contain mRNA are the salivary glands.
(E) Protein expression in a stage 10 embryo. Note expression in the nuclei of the A and P cells.
(F) Protein expression in stage 11 embryo is confined to SOPs and salivary gland anlagen.
(G) Expression in stage 13 embryos shows that the early differentiating cells of the PNS are expressing Sens.
(H) Stage 14 embryo. Note that there are still some cells that express the protein. Sens expression mostly disappears in late stages 12 and 13.
(I) Stage 11 $sens^{M256}/sens^{l235}$ embryo. Note the dramatic reduction in protein levels when compared to (F). The remaining protein is not localized to the nucleus.
(J) A stage 11 $sens^{E2}/sens^{E2}$ embryo. The expression levels are much reduced but some protein seems to localize to the nuclei.
(K) A stage 12.5 *Df(3L)1228/4*/*Df(3L)1228/4* embryo that lacks *sens*. No immunoreactivity is observed in any cell or tissue showing that the antibody is specific.
(L) A stage 11 *Df(2L)J27/Df(2L)J27* embryo lacks the *daughterless* gene, and no Sens protein is detected.
(M) A stage 11 *Df(1)sc-B57/Df(1)sc-B57* embryo. Many fewer cells express mRNA when compared to (C).
(N) Wild-type stage 10–11 embryo stained for Sens, abdominal segments 5–7.
(O) Same as in (M) but ato^1 mutant. The arrowheads in (N) and (O) point to the position of the chordotonal SOPIs.

(Jia et al., 1996, 1997). The Gfi-1 protein contains six Zn fingers, binds DNA, and is a transcriptional repressor in T lymphocytes (Grimes et al., 1996; Zweidler-McKay et al., 1996). Loss of *pag-3* causes lineage defects in worms (Jia et al., 1996), whereas activation of Gfi-1 in T lymphocytes causes interleukin-2-independent proliferation (Gilks et al., 1993) and T cell leukemia in mice (Schmidt et al., 1998; Li et al., 1999; Hansen et al., 2000).

Given the sequence similarities, we tested whether Sens was able to bind the Gfi-1 consensus sequence (TAAATCAC core sequence; Zweidler-McKay et al., 1996). As shown in Figure 2D, full-length Sens is able to bind this oligonucleotide in an electromobility shift assay (EMSA) in the presence of poly(dI-dC). Sens protein that lacks the 4 C2H2 domain fails to bind, and cold nucleotides efficiently compete for binding of the full-length protein. In addition, we also tested whether Sens was able to bind to a sequence with a single base mutation in the core (AAAT**G**A). No binding was observed (data not shown), indicating that Sens is a DNA binding protein with a similar specificity as Gfi-1.

Sens Is Expressed in PNS Cells

To determine where the *sens* gene is expressed, we carried out in situ hybridization experiments. *sens* mRNA is first expressed in stage 10 embryos in small clusters of 2–4 ectodermal cells (Figures 3A and 3B). One of the cells in each cluster is the presumptive A or P SOP (Ghysen and O'Kane, 1989; Kania et al., 1993). This expression quickly refines to ectodermal cells that will give rise to SOP A and P cells. The message then rapidly accumulates in many SOPIs and IIs during stage 11 (Figure 3C). The mRNA is most abundant in the SOPI and barely detectable or absent in the progeny of the SOPII. During germ band retraction, the remaining *sens* mRNA disappears, and by stage 13 the mRNA is only detected in the salivary glands (Figures 3C [arrowhead]

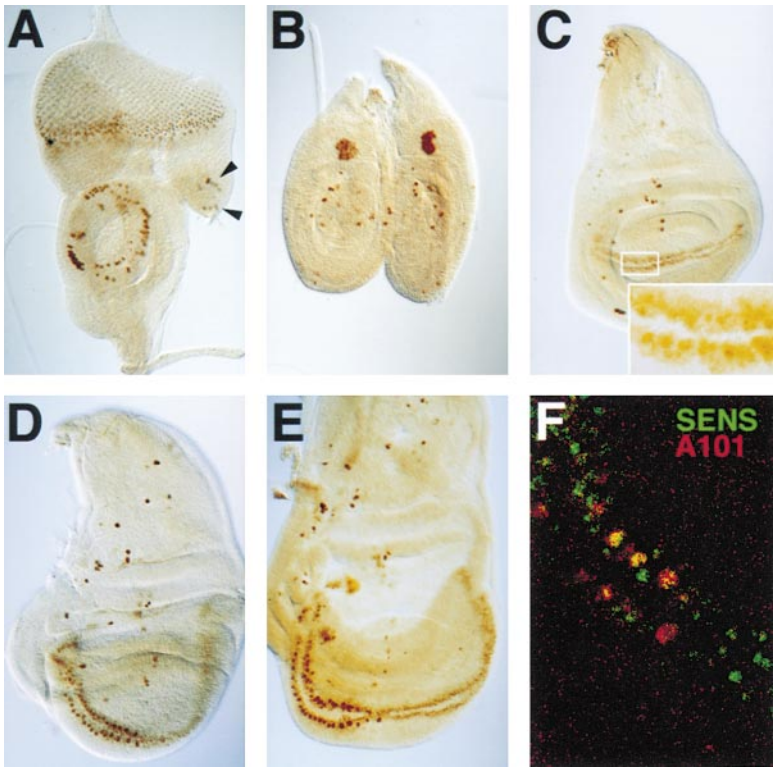

Figure 4. Sens Protein Expression in Wild-Type Imaginal Wing Discs

(A) Expression in eye-antennal disc. The ring of cells in the antennal disc corresponds to the SOPs of the chordotonal organs of the Johnston organ.

(B–F) Discs are shown anterior to the left and dorsal up. Not all positive cells are visible in any of the discs.

(B) Leg disc, notice the large clusters of chordotonal organ precursors.

(C) Early third instar imaginal wing disc. Sens is expressed in the entire wing margin and specific SOPs.

(D) Expression in a late third instar wing disc. Expression is confined to SOPs but is also expressed in the precursors of the bristles of the posterior wing margin that are not innervated.

(E) Wing disc at puparium formation.

(F) Third instar wing disc of an A101 *lacZ* enhancer detector larva double labeled for Sens and β-galactosidase. At this stage, A101 *lacZ* is expressed in chemosensory bristles only (Blair, 1992).

and 3D). In summary, our observations suggest that *sens* is most actively transcribed in the SOPI and that other cells of the PNS inherit the message or transcribe the gene at significantly lower levels than SOPIs.

Staining of embryos with anti-Sens antibodies showed a similar expression pattern to the mRNA (Figures 3E–3H). With exception of salivary glands and few ectodermal cells, the protein is confined to proneural fields of some SOPs (data not shown), nuclei of SOPs (Figures 3E and 3F), and differentiating cells of the PNS (Figures 3G and 3H). Protein expression is initiated during stage 10, is maximal during stage 11 and 12 (Figure 3F), and begins to vanish in differentiated embryos (Figures 3G and 3H). Double-labeling experiments with P element enhancer detector *A37* show that almost all SOPs that express *lacZ* in *A37* embryos (Ghysen and O'Kane, 1989; Kania et al., 1993) express Sens (data not shown). In contrast to the mRNA, the protein is present in differentiating cells of the PNS. The anti-Sens antibody is specific, as mutant embryos homozygous for *Df(3L)1228/4* lack *sens* message and protein (Figure 3K). All EMS-induced mutations express the protein, but the levels are either severely reduced or the protein fails to localize to the nucleus in mutants (Figures 3I and 3J). In summary, Sens is a nuclear protein whose expression is almost exclusively restricted to precursors and early differentiating cells of the PNS.

Sens Expression Is Dependent on Proneural Genes and *daughterless*

The above data show that Sens is expressed in some cells of proneural clusters and SOPs, when and where proneural genes are expressed. We therefore determined whether Sens expression is dependent on proneural activity by staining embryos that lack *daughterless* (*da*) or *atonal* or the genes of the AS-C ($Df(1)sc^{B57}$, a deficiency of *ac*, *sc*, *l'sc*, and *ase*). Embryos that lack *da* exhibit a loss of all PNS cells (Caudy et al., 1988), except the SOPIs (Ip et al., 1994; Vaessin et al., 1994). The Daughterless protein has been shown to form heterodimers with many proneural proteins, and this dimerization is essential for neuronal determination or differentiation of many SOP lineages. As shown in Figure 3L, embryos homozygous for a deficiency that removes *da* (*Df(2L)J27*) and da^1 (data not shown) fail to express Sens protein or mRNA (data not shown). Similarly, embryos that lack genes of the AS-C fail to express Sens (compare Figure 3M to 3C) in all the PNS cells that are affected by loss of the AS-C (Gonzalez et al., 1989; Dominguez and Campuzano, 1993). Finally, homozygous *atonal* (ato^1) mutant embryos fail to express Sens in chordotonal SOPs (arrows in Figures 3N and 3O; data not shown) except in those derived from P cells (data not shown) (Jarman et al., 1993). Interestingly, the P cell is an SOP that gives rise to the only embryonic chordotonal organ that is not dependent on the activity of the *atonal* gene (zur Lage et al., 1997).

Sens Is Expressed in SOPs of Imaginal Discs

To establish if Sens is expressed in imaginal tissues, we stained discs with the anti-Sens antibody. As shown in Figure 4A, Sens is expressed in eye-antennal discs in the R8 photoreceptors, two small clusters of cells in the lateral portion of the disc (arrowheads), and the chordotonal organs of Johnston organs. This pattern is similar to that reported for the A101 enhancer detector, an SOP marker (Huang et al., 1991; Jarman et al., 1993). In leg discs (Figure 4B), Sens is expressed in the precursors of the femoral chordotonal organ, as well as in other external sensory SOPs. In wing discs (Figures 4C–4F), Sens expression is very dynamic. Sens is first expressed in some ectodermal cells surrounding the cells

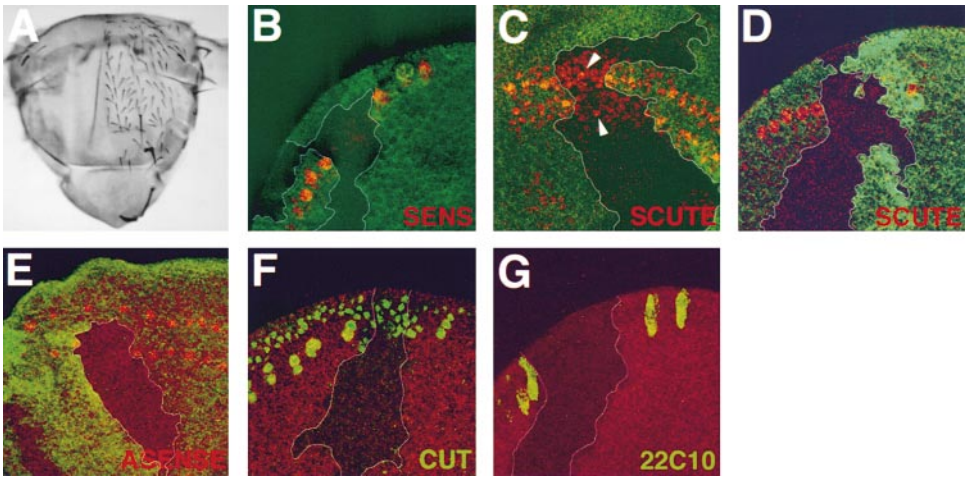

Figure 5. Sens Is Required for Enhanced Expression of Proneural Proteins in the SOPs

(A) A large *sens*^{E2} clone of about 50% of thorax. All bristles in the clone are lacking.

(B–G) Portions of imaginal wing discs containing *sens*^{E2}/*sens*^{E2} clones that straddle the rows of cells of the anterior wing margin and fail to express β-galactosidase.

(B) Cells in a mutant clone in a disc just after puparium formation do not express or express very low levels of a nonnuclear mutant Sens protein.

(C) Mutant clone in a disc just prior to puparium formation stained with anti-Scute. Note that expression in the ectodermal cells of the proneural cluster is not affected. Occasionally, a cell in the mutant clone expresses higher levels of Scute. However, most presumptive SOPs do not or poorly accumulate Scute protein.

(D) Imaginal disc just after puparium formation prepared the same fashion as in (C). Note that in contrast to (C), there is no Scute expression in the cells in the clone.

(E) Imaginal disc just prior to puparium formation stained with anti-Asense. There is no Asense expression in the clone.

(F) Imaginal disc 3–6 hr after puparium formation stained with anti-Cut. The Cut protein is lacking in the SOPIIs of mutant clones, but other ectodermal cells express the Cut protein in the clone.

(G) Imaginal disc 3–6 hr after puparium formation stained with mAb22C10. No staining is observed in the clone.

that will become fated as SOPs (Figure 4C, inset). The expression levels in these ectodermal cells are much lower than in the SOPs, and the protein is not confined to the nuclei. This is most easily illustrated for SOPs of the bristles of wing margins but is also observed in other areas. Individual cells within these clusters, the presumptive SOPs, start to accumulate higher levels of Sens protein. Double labeling for anti-β-galactosidase and anti-Sens in wing discs that carry the A101 SOP marker reveals that the onset of Sens expression precedes that of β-galactosidase (Figure 4F). Both β-galactosidase and Sens are often coexpressed (data not shown), except in the SOPs of the bristles of the posterior wing margin where Sens is expressed prior to A101 *lacZ* (Blair, 1992). To our knowledge, no proneural gene is expressed in the noninnervated bristles of the posterior wing margin, although they are similar to other es organs (Jack et al., 1991).

To determine whether proneural gene expression is required for Sens expression in imaginal discs, we stained eye-antennal discs of *atonal* mutants for Sens protein. Eye-antennal imaginal discs of *ato*^{1} are devoid of Sens expression, and in the absence of *sens*, photoreceptor development is aberrant (B. Frankfort, R. N., H. J. B., and G. Mardon, unpublished data). Similarly, wing discs of *achaete* mutants [*In(1)y*^{3PC} *sc*^{8R}] lack most SOPs and Sens expression (data not shown). In summary, Sens expression is essentially confined to cells of the PNS and is dependent on proneural gene expression. We did not observe Sens expression in the CNS of embryos or larvae.

Sens Is Required for Enhancing and Maintaining Proneural Activity in SOPs

To determine the phenotypic consequences of the loss of Sens protein function in imaginal discs, we performed a clonal analysis with the *sens*^{E2} mutation using gamma irradiation and the Minute technique (Morata and Ripoll, 1975), as well as the FRT/FLP technique (Xu and Rubin, 1993). The phenotypes of *sens*^{E2}/*sens*^{E2} and *sens*^{E2}/*sens*$^{1228/4}$ embryos are similar, suggesting that *sens*^{E2} is a severe loss of function or null allele (*sens*^{E2} contains a stop codon in the middle of the coding sequence). In addition, *sens*^{E2}/*sens*^{E2} embryos and flies can be fully rescued by the transgene, showing that this chromosome does not carry other obvious viable or lethal mutations. As shown in Figure 5A, mutant clones lack bristles, sockets, and microchaetae. This loss of bristles and sockets is confined to the mutant cells, indicating a cell-autonomous requirement for *sens* (data not shown).

In imaginal discs, *sens*^{E2} clones contain low levels of mutant cytoplasmic Sens protein several hours prior to puparium formation. However, they contain undetectable levels of Sens protein at and after puparium formation (Figure 5B). Mutant *sens*^{E2} clones are marked by the absence of β-galactosidase driven by the *arm-lacZ* transgene (Vincent et al., 1994). After puparium formation, mutant clones in imaginal discs fail to express the Scute protein (Figure 5D). However, mutant clones in less mature wing discs do contain Scute protein in proneural clusters, showing that Sens is not required for Scute expression in ectodermal cells (Figure 5C). In addition, some newborn SOPs in mutant clones contain

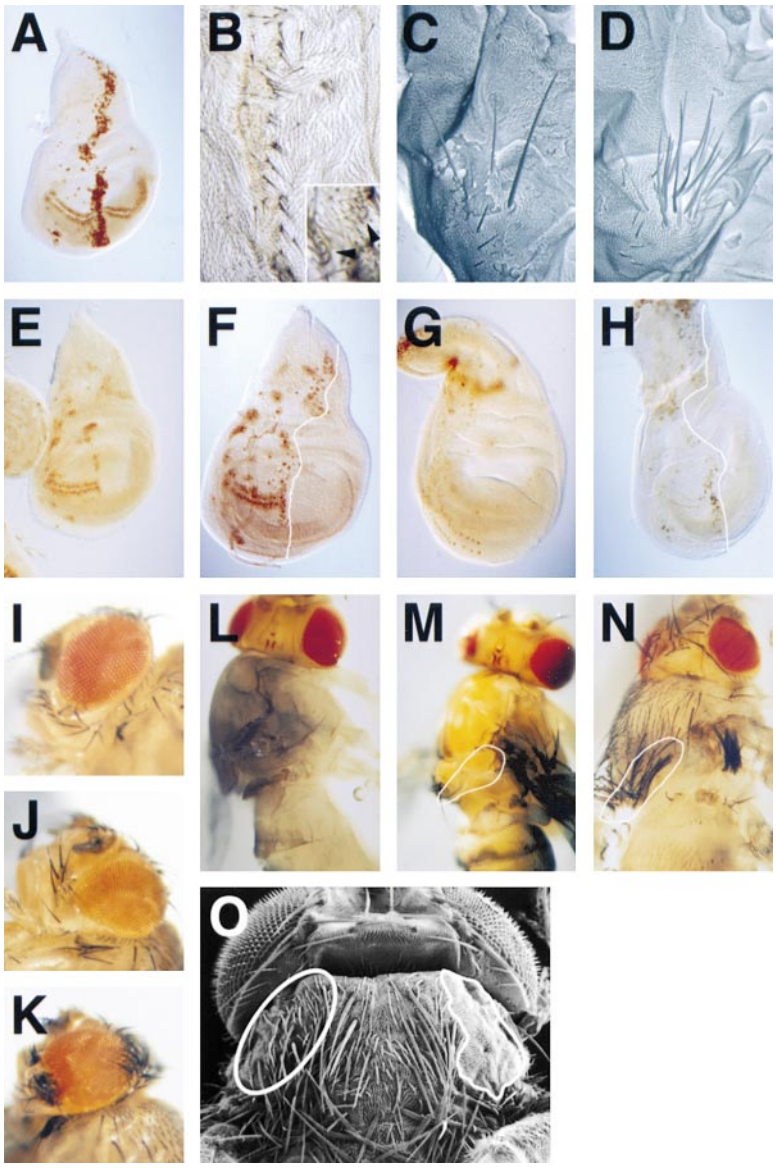

Figure 6. Ectopic Expression of Sens Recruits Epidermal Cells into a Neuronal Fate

All discs are shown anterior to the left and dorsal up. Wing is shown anterior to the left.
(A) Wing disc of a *dpp-GAL4/UAS-sens (C5)* stained with anti-Sens antibody. The protein is abundantly expressed along the anterior–posterior boundary as well as in many SOPs.
(B) Wing of a *dpp-GAL4/UAS-sens (C5)* fly. Note the numerous extra bristles and sensilla companiforma (inset with arrowheads) just posterior to the position of the third vein.
(C and D) (C) Scanning electron microscopy (SEM) of wild-type sternopleural bristles and (D) SEM of numerous extra sternopleural bristles caused by ectopic expression in a *dpp-GAL4/UAS-sens (C1)* leg disc. This is a mild phenotype when compared to other *UAS-sens* constructs.
(E) Wild-type imaginal disc stained with anti-Scute.
(F) A *UAS-sens(C5)/dpp-GAL4* wing disc stained with anti-Scute. Note the ectopic expression along the anterior–posterior boundary of Scute induced by ectopic Sens expression.
(G) Wild-type wing disc stained with anti-Asense.
(H) *UAS-sens(C5)/dpp-GAL4* wing disc stained with anti-Asense. Note the ectopic expression along the anterior–posterior boundary of Asense.
(I) *UAS-scute; dpp-GAL4* fly. Expression of Scute in the eye-antennal disc causes no or few extra bristles.
(J) A *UAS-sens (C1); dpp-GAL4* fly. The weakest *UAS-sens* transgenic construct in imaginal discs causes a few extra bristles at the base of the eye.
(K) A *UAS-sens(C1); UAS-scute; dpp-GAL4* fly. Combined expression of Sens and Scute under the control of the *dpp* enhancer causes numerous extra bristles to appear in positions where few or none were previously observed, e.g., dorsal and ventral portion of the head and numerous other areas (data not shown).
(L) A fly lacking both *achaete* and *scute* function is almost devoid of bristles.
(M) *UAS-sens(C1)* expressed under *dpp-GAL4*-driver in the $ac^{-}sc^{-}$ background. Note that there are only very few bristles when compared to a control fly shown in (N).
(O) SEM picture of a *yw hs-FLP; UAS-scute/apterous-GAL4; sens^{E2} FRT80b/arm-lacZ, FRT80b* fly carrying a *sens* mutant clone on the right dorsal portion of the thorax. Note the many supernumary micro- and macrochaetae on the thorax except in the clone. The portion on the left side is circled for comparison.

higher levels of Scute than surrounding cells of the proneural field. However, these are consistently lower levels of Scute than in neighboring SOPIs (Figure 5C, arrowheads; data not shown). The levels of Scute in mutant SOPIs remain low and are not maintained. No Scute expression is observed at puparium formation (Figure 5D). We conclude that Sens protein is required to further enhance and maintain expression of Scute in SOPs.

The specification of external sensory organs of anterior wing margin has been shown to be dependent not only on Scute and Achaete, but also on Asense (Brand et al., 1993; Dominguez and Campuzano, 1993). We therefore stained SOPs of anterior wing margin with anti-Asense. As shown in Figure 5E, Asense is not expressed in mutant SOPs, showing that Asense expression is also dependent on Sens. We were also unable to detect Cut expression in the SOPIIs, although expression of Cut in other cells of the margin is unaffected (Figure 5F). Similarly, expression of the antigen recognized by mAb22C10 in early differentiating cells of mutant clones is absent (Figure 5G). In summary, our data suggest that SOPIs may at least in part be specified in *sens* mutant clones as some express higher levels of Scute than the other cells of the proneural cluster. However, loss of Sens activity then causes a failure to further upregulate and maintain proneural gene expression leading to cell loss in the adult imago.

Sens Is Sufficient to Induce PNS Organ Development

If Sens is required for proneural expression, ectopic expression of Sens may induce proneural gene expres-

sion. As shown in Figure 6A, ectopic expression of Sens using the *dpp-GAL4* driver causes robust expression of the protein in the expected wing stripe. This expression causes the formation of numerous bristles and sensilla campaniforma (arrowheads, inset) in the adult wing in proximity of the third wing vein where *dpp* is normally expressed (Figure 6B). Similarly, ectopic expression of Sens in the leg disc causes many supernumerary bristles in the sternopleural area (Figures 6C and 6D) as well as in more distal portions of the leg (data not shown). Ectopic bristles were observed with all *UAS-sens* reporters. Some *UAS-sens* transgenes driven by *dpp-GAL4* cause very severe tufting in the adult notum, wings, and legs, and loss of tissues in other portions of imaginal discs, e.g., wing margins and distal leg structures (data not shown). We conclude that ectopic expression of Sens is sufficient to initiate ectopic external sensory organ development.

To determine the molecular cascade underlying the formation of the extra external sensory organs, we stained wing discs of *UAS-sens*; *dpp-GAL4* larvae with anti-Scute (Figures 6E–6F) and anti-Asense (Figures 6G and 6H) antibodies. Ectopic Sens expression causes ectopic activation of Scute and Asense. Hence, Sens is able to activate proneural gene expression. This provides a molecular basis for the generation of additional external sensory organs, since ectopic proneural gene expression has previously been shown to be sufficient to induce ectopic PNS organ formation (Campuzano et al., 1986; Balcells et al., 1988; Brand et al., 1993; Jarman et al., 1993; Ben-Arie et al., 2000).

If Sens induces proneural gene expression and proneural genes are required for Sens production, a superadditive or synergistic interaction between *sens* and proneural genes may occur. We therefore expressed the weakest *UAS-sens* transgene (*C1*) in combination with an *UAS-scute* and an *UAS-atonal* transgene under the control of *dpp-Gal4*. As shown in Figure 6I, overexpression of Scute or Atonal (data not shown) alone causes a relatively mild phenotype with relatively few extra bristles. Scute expression induces Sens expression, but the expression levels of Sens are lower than those induced by *dpp-Gal4; UAS-sens* (data not shown). Ectopic expression of Sens with the *dpp-Gal4* driver causes a stronger phenotype when compared to ectopic expression of Scute (Figure 6J) or Atonal (data not shown). However, simultaneous overexpression of Sens and Scute (Figure 6K) or Atonal (data not shown) causes severe tufting, including in many areas where Scute, Atonal, or Sens when expressed individually do not normally cause ectopic bristles. These areas do correspond to areas where the *dpp-Gal4* driver has previously been shown to be expressed (Chanut and Heberlein, 1997; Chen et al., 1997). We therefore conclude that *sens* and the proneural genes can interact in a synergistic fashion.

The data so far imply that Sens is dependent on proneural gene expression and that proneural gene expression can be induced by Sens. This raises another issue: can overexpression of Sens in the absence of proneural genes produce external sensory organs? Since *sens* expression depends on the expression of many proneural genes and since Sens is able to induce ectopic expression of several proneural proteins, removal of one or several proneural genes may not be fully effective, i.e., all proneural genes should be removed to test this hypothesis. As shown in Figure 6L, flies that are mutant for *achaete* and *scute* (*Df(1)sc10-1*) are almost devoid of micro- and macrochaetae. Overexpression of Sens *(C8)* in *Df(1)sc10-1* flies causes few ectopic bristles (Figure 6M) when compared to a wild-type background (Figure 6N), suggesting that Sens requires the presence of proneural genes.

To determine whether the proneural genes require Sens, we overexpressed Scute in the dorsal portion of the wing disc by driving *UAS-scute* with *apterous-GAL4* (Calleja et al., 1996) and induced mutant clones that lack *sens* in these discs. Flies that are *UAS-scute; apterous-GAL4* exhibit numerous thoracic extra bristles (Figure 6O, e.g., left ring). We obtained six flies that have large clones, and in each fly we observe a complete loss of all micro- and macrochaetae in the clone (Figure 6O, right clone). These data clearly suggest that Sens is required for bristle development, even when Scute is overexpressed.

Ectopic Sens Expression Establishes Proneural Domains

Our data indicate that ectopic expression of Sens is a more potent inducer of supernumerary PNS organs than is ectopic expression of proneural genes using the same drivers. For example, ectopic expression of Sens at the anterior–posterior border (the *dpp* domain between future wing veins 3 and 4) causes numerous bristles and sensilla campaniforma along the length of the wing blade (Figure 6B), except in the most proximal portion, the wing hinge region. In some areas, the width of the field contains as many as five adjacent PNS organs. As shown in Figures 7A and 7B, Sens is able to induce Scute in the ventral and the dorsal area of the wing pouch as well as in the dorsal portion of the disc. However, the area of the wing hinge region is much less sensitive to overexpression of Sens (circled in Figure 7B). This suggests that Sens does not have the same inductive capacity in all cells and also further supports the idea that Sens requires proneural activity to induce PNS organs.

Induction of Sens does not only induce Scute and Asense. As shown in Figures 7C and 7D, Sens expression using the *dpp-GAL4* driver alters Delta expression. The stripe labeled 3 in the wild-type wing disc (Figure 7C), the domain that normally gives rise to the third wing vein, is altered in Sens-overexpressing discs. Overexpression of Sens induces Delta expression ectopically in the *dpp* domain, broadening and intensifying the endogenous Delta domain. In addition, we observe a consistent reduction of expression in the fourth wing vein domain. We surmise that this ectopic Delta expression is mediated by Scute/Asense overexpression (Hinz et al., 1994).

To determine the relationship between Sens expression and the proteins of the Enhancer of Split complex, we stained wild-type discs for both proteins (Jennings et al., 1994). As shown in Figure 7E, there is little overlap between the two nuclear proteins. Cells that express Sens are intermingled with E(spl) expressing cells, but the majority of cells that express Sens do not express E(spl) (Jennings et al., 1995). Similar observations were also made with *E(spl)m8-lacZ* and with *E(spl)m4-lacZ* (Kramatschek and Campos-Ortega, 1994; Singson et al., 1994; Bailey and Posakony, 1995). These data indicate that Sens expression in cells fated to develop into SOPs

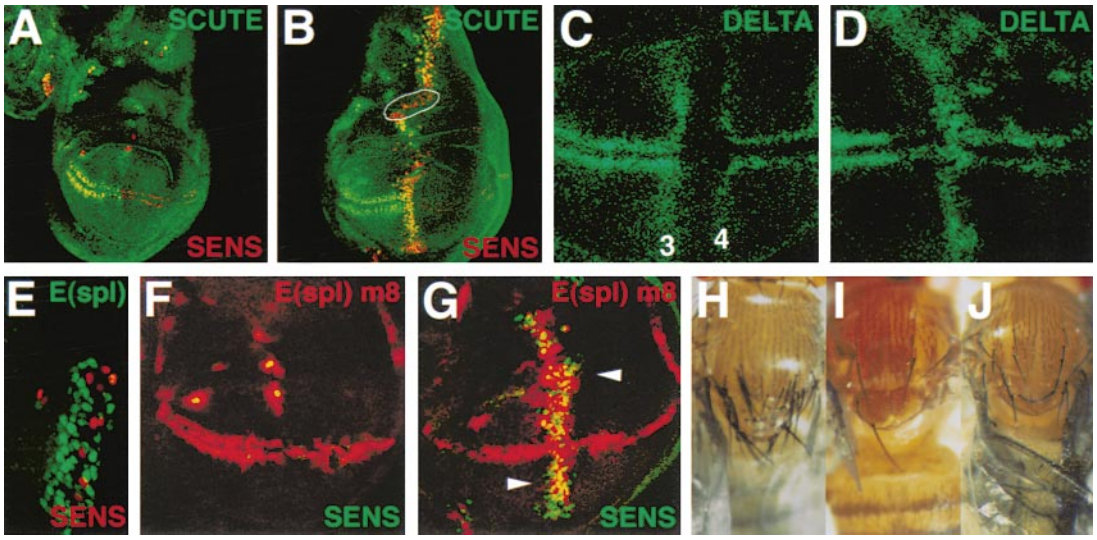

Figure 7. Ectopic Expression of Sens Creates Proneural Domains

(A) Expression of Scute and Sens in a wild-type imaginal disc.

(B) Expression of Scute and Sens in a *dpp-GAL4; UAS-sens* disc. Sens induces Scute in numerous cells of the domain but not in all cells. Ectopic expression of Sens in and near the wing hinge region is ineffective in inducing Scute (circled domain) as well as other proteins (data not shown).

(C and D) (C) Core of the wing pouch region of a wild-type disc and (D) *dpp-GAL4; UAS-sens* disc stained for Delta. Sens overexpression induces Delta expression in a domain that is just posterior to the third wing vein and reduces Delta expression in the adjacent domain that normally gives rise to the third vein.

(E) Expression of Sens protein and E(spl) protein in a large proneural domain of a wild-type haltere disc. Note that both proteins are most often not colocalized.

(F) Pouch region of a wing disc. Expression of Sens in a proneural cluster expressing cytoplasmic *E(spl)m8-lacZ*. When the cells that express Sens enlarge and express more protein, β-galactosidase expression fades. The cells are yellow because of the low magnification.

(G) Pouch region of a *E(spl)m8-lacZ*; *dpp-GAL4; UAS-sens* wing disc stained for β-galactosidase and Sens. Note that the domain of β-galactosidase has expanded vastly when compared to (F). Cells that do not express Sens express significantly higher levels of *E(spl)m8-lacZ*.

(H) *dpp-GAL4; UAS-sens* flies exhibit numerous extra bristles in the domain where *dpp* is expressed.

(I) *dpp-GAL4; UAS-E(spl)m8* causes a mild reduction in the number of bristles.

(J) *UAS-E(spl)m8; UAS-sens*; *dpp-GAL4* thorax. Overexpression of *E(spl)m8* very strongly inhibits the effects of overexpression of *sens* and causes loss of some bristles.

is concomitant with the presence of E(spl) proteins, but that elevation of Sens expression and cell enlargement during SOP specification accompanies a rapid removal of the E(spl) protein (Jennings et al., 1994, 1995; Lecourtois and Schweisguth, 1995). These data are also in agreement with the proposition that E(spl) is a negative regulator of proneural gene expression and that its downregulation permits SOP development (Jennings et al., 1994; Oellers et al., 1994; Culi and Modolell, 1998).

Ectopic expression of Sens may not only activate the proneural genes and Delta but may recreate an ectopic proneural field. Expression of several E(spl) proteins depends on the presence of the proneural genes (Kramatschek and Campos-Ortega, 1994; Singson et al., 1994). We therefore overexpressed Sens using the *dpp-GAL4* driver in *E(spl)m8-lacZ* and *E(spl)m4-lacZ* imaginal discs. As shown in Figure 7F, wild-type discs contain proneural clusters that express cytoplasmic β-galactosidase [E(spl)] in which few cells are Sens positive (the cells are yellow because of the low magnification; at high magnification Sens-positive cells have low levels of β-galactosidase; Figure 7E). Overexpression of Sens causes a strong induction of β-galactosidase staining associated with *E(spl)m4-lacZ* (data not shown) and *E(spl)m8-lacZ* (Figure 7G). This induction is not restricted to cells in which Sens is expressed but can be detected in adjacent cells as well. This indicates that Sens can induce in a cell-nonautonomous fashion E(spl) expression, probably by activating Delta expression (Hinz et al., 1994). A more detailed cellular analysis shows that when Sens expression is elevated in a particular cell, β-galactosidase levels are consistently low or absent (data not shown). We infer that ectopic Sens leads to expression of the essential components required to establish a proneural domain in some areas of the wing discs. This ability is most likely mediated by its ability to activate the proneural genes (Hinz et al., 1994). The wing hinge region is, however, refractory to induction of Scute, Delta, and E(spl) upon overexpression of Sens (Figure 7B; data not shown).

Since ectopic Sens is able to induce E(spl) expression and since elevated Sens levels are associated with low levels or absence of E(spl) protein during SOP specification in normal and ectopic conditions, we wondered how ectopic expression of both proteins in the same cells would affect PNS organ development. Since overexpression of E(spl) causes a loss of external sensory organs (Tata and Hartley, 1993; Nakao and Campos-Ortega, 1996), the component that is most downstream in the pathway should be epistatic to the more upstream component. Figure 7H shows the dorsal portion of the thorax of a *dpp-GAL4; UAS-sens* fly with extra bristles.

Note the loss of scutellar bristles in Figure 7I in a *dpp-GAL4; UAS-E(spl)m8* fly. As shown in Figure 7J, coexpression of both proteins always leads to a very strong reduction in supernumerary bristles in most areas, occasionally loss of bristles. Hence, ectopic E(spl) is epistatic to ectopic Sens in the pathway that specifies the SOP.

Discussion

The *sens* Gene Is Required for PNS Differentiation in Embryos and Adults

Loss of activity of *sens* causes aberrant expression of all PNS markers tested in late embryos (Figure 1). Some markers, such as Prospero and Asense, are expressed aberrantly early (data not shown) as well as late in embryogenesis. Although loss of *sens* affects all cells of the embryonic PNS, it affects multiple dendritic neurons less than the external and chordotonal sensory organs. The loss of PNS cells in embryos seems to be at least in part mediated by cell death. In embryos, Sens is therefore required for terminal differentiation and/or viability of most or all PNS cells. Hence, the phenotype associated with loss of Sens is similar but less severe than that associated with loss of proneural genes (e.g., Hassan and Vaessin, 1997). This may be due to another homolog of *sens* that is expressed in some cells of the PNS and CNS (R. N. and H. J. B., unpublished data).

Clonal analyses show that loss of *sens* during imaginal disc development leads to loss of bristles, socket cells, and neurons. Unlike in embryos, this loss is paired with a loss of all markers tested in SOPs or their daughters (Asense, Scute, Cut, mAb22C10, and Prospero). This phenotype is more severe than in embryos, suggesting a more prominent requirement for Sens in larval SOPs than in embryos. The phenotype associated with loss of *sens* in wing imaginal disc is similar to the phenotype associated with the loss of *scute* and *achaete*. In addition, clonal analysis indicates that *sens* is required in cells in which it is expressed. We therefore conclude that *sens* is a cell autonomous factor required for an early event in PNS differentiation. Unlike the proneural genes, the requirement for *sens* is not restricted to a type or subpopulation of sensory organs; rather, it is required for most PNS organs and cells.

sens Encodes a Nuclear Zn Finger Protein Expressed in PNS Lineage Precursors

Sens encodes a putative transcription factor with four C2H2 Zn fingers. Very similar Zn fingers are present in the *C. elegans* Pag-3 (Jia et al., 1997) and the rat Gfi-1 protein (Gilks et al., 1993). The Gfi-1 protein has been shown to be a protooncogene (Gilks et al., 1993; Schmidt et al., 1998; Li et al., 1999; Hansen et al., 2000) that functions as a transcriptional repressor (Grimes et al., 1996). It has six Zn fingers, of which only the third, fourth, and fifth C2H2 domains are required for DNA binding and activity (Grimes et al., 1996; Zweidler-McKay et al., 1996). Interestingly, these three domains correspond to the first, second, and third C2H2 domains of Sens. These three domains share 91% identity to the corresponding domains of Gfi-1, suggesting that both proteins have the same DNA binding specificity. This was confirmed by EMSA. Hence, by analogy to Gfi-1, Sens may act as a transcriptional repressor. However, Sens does not contain a SNAG repressor domain found in vertebrate Gfi-1 (Grimes et al., 1996), suggesting that it may act differently than Gfi-1, i.e., as a repressor, an activator, or both. Interestingly, a sequence that fully matches the Gfi-1/Sens consensus sequence (TAAATCAC) is present once ~2 kb upstream of the *scute* and *asense* transcription start sites and about 700 bp upstream of *achaete* as well as upstream from *E(spl)m8*.

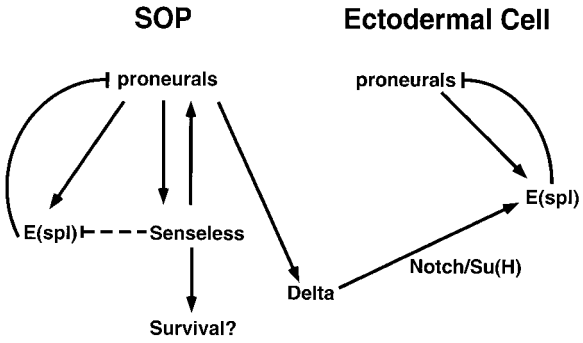

Figure 8. Model of Sens Action

We propose that proneural proteins reach a certain threshold in the SOP that activates *sens* transcription and protein synthesis. The Sens protein then in turn activates proneural gene transcription. This could be achieved in two ways: by direct transciptional activation of the proneurals and/or possibly by blocking transcription of *E(spl)* genes. Ectopic expression of the proneurals leads to the production of Delta, which signals to neighboring ectodermal cells and activates *E(spl)* transcription. *E(spl)* in turn represses proneural gene expression. Sens must play another important function as overexpression of the Scute in the absence of *sens* is unable to induce ectopic es organ formation. We surmise that it may be required for the viability of the cells.

In the embryonic PNS nervous system, Sens is expressed at low levels in some cells surrounding the SOPIs and at high levels in SOPIs, SOPIIs, and differentiating progeny. In imaginal discs, Sens expression is first observed at low levels in ectodermal cells in proximity of many SOPs. This domain may correspond to the proneural field, which has been shown to accumulate higher levels of Achaete-Scute expression than other cells of proneural cluster (Cubas et al., 1991; Culi and Modolell, 1998). However, levels of Sens protein expression are dramatically enhanced in the SOPIs. Sens is expressed prior to a typical SOP marker, A101 *lacZ* (Huang et al., 1991), but enhanced expression of Sens in the presumptive SOP is often coincident with A101 *lacZ* expression.

Ectopic Sens Induces PNS Formation

Ectopic expression of Sens causes many ectodermal imaginal cells to take the SOP fate. This implies that Sens is sufficient to induce external sensory organ formation. Our data also indicate that Sens is downstream of proneural gene activity, as their presence is required for its transcription. Sens protein is not required for proneural gene expression in proneural clusters, but its presence in the SOPs is necessary and sufficient to enhance and maintain proneural expression to specify proper neuronal fate of SOPs. This model is supported by numerous observations. First, Sens expression is dependent on proneural gene expression. Second, ectopic expression of Sens induces proneural gene expression. Third, ectopic proneural gene expression induces Sens expression. Fourth, Sens and the proneural genes *scute* and *atonal* interact in a synergistic fashion.

Fifth, loss of Sens leads to a failure to enhance and maintain proneural protein expression in the SOPs. Sixth, the ability of Sens to recruit ectodermal cells into the SOP pathway is severely impaired in *achaete* and *scute* mutants. These observations also provide a molecular basis for the observations that ectopic expression of different proneural genes, including Atonal, leads to the production of a variety of es organs. They may all activate Sens, which in turn activates different proneural genes when the cells are not in their normal cellular context. However, our data also suggest that Sens must play another role that is required for the development of adult sensory organs, as overexpression of Scute in the absence of Sens is unable to promote es organ formation. This may be due to the inability of the progeny of the SOPs to survive or the inability to repress E(spl) expression (Figure 8).

The expression pattern of *sens* can be almost fully reconstructed in transgenic flies (data not shown) that carry the 3.4 kb and 5.9 kb genomic fragments (Figure 2A) upstream of *lacZ* in the *pP{CaSpeR-lacZ}* vector. Both fragments contain numerous E boxes, including a GCAGGTG E box, shown to be highly preferred by Scute/Daughterless heterodimers (Singson et al., 1994). Hence, combined with the temporal and spatial expression data (see above), these data suggest that *sens* expression may be directly controlled by proneural genes.

Role of Sens in the Notch Signaling Pathway

The ability of ectopic Sens to induce external sensory organ formation is most likely due to its ability to cause expression of many key players that are normally expressed in the proneural cluster. As shown in the model in Figure 8, proneural genes activate the transcription of the *E(spl)* genes (Kramatschek and Campos-Ortega, 1994; Singson et al., 1994) and *sens*. The Sens protein may then act via two pathways in the SOP. First, it may directly activate proneural gene expression participating in the initiation and/or maintenance of an autoregulatory loop (Culi and Modolell, 1998). This mode of action is supported by the observation that ectopic Sens can induce proneural gene expression in the absence of endogenous proneural proteins or E(spl) proteins (Figure 7G). In addition, the proneural genes contain consensus binding sites for the Sens protein, suggesting that the interaction may be direct. Second, Sens may first enhance and subsequently inhibit transcription of *E(spl)* genes. Expression of the genes of the *E(spl)* complex is clearly reduced in the SOPs to permit their specification (Jennings et al., 1994, 1995). We propose that Sens also plays a role in this process by inhibiting transcription of the *E(spl)* genes in the SOPs. This in turn may allow further upregulation of proneural gene expresssion, followed by ectopic expression of E(spl) in neighboring cells that do not express Sens, suggesting that they receive a signal from the proneural proteins expressing cells. This signal is most likely Delta, as Delta expression is clearly upregulated in the cells that express Sens (Hinz et al., 1994). Cells that do not express or express very low levels of Sens then accumulate more E(spl) than those that do express higher levels of Sens. Hence, the reduction of Notch signaling in the SOP may be strongly enhanced by the presence of Sens to help specify SOPs. Indeed, ectopic coexpression of E(spl)m8 and Sens dramatically reduces the action of Sens and in some areas of the notum creates a phenotype that is typically associated with overexpression of E(spl)m8 alone, i.e., loss of bristles (Ligoxygakis et al., 1999). This suggests that E(spl)m8 acts downstream of Sens (Figure 8). In ectodermal cells, Sens normally does not play a role because none of these cells acquire enough proneural gene expression to activate Sens at a level that is sufficient to activate proneural gene expression above a required threshold. The latter statement is supported by the expression pattern of Sens, which is restricted to those cells that express the highest levels of proneural proteins and by the observation that robust levels of ectopic proneural gene expression must be attained to induce ectopic Sens expression. In summary, we propose that the function of Sens is to integrate proneural gene expression into the Notch signaling pathway to promote proper SOP development in the *Drosophila* PNS.

Experimental Procedures

Fly Strains

The following fly strains were used in this study: Canton S (Bloomington Stock Center); *yw; P[lacZ, w^+]64A $sens^{M256}$ th st cu sr e^s ca/TM6, Hu P[w^+, abdA-lacZ] e Tb ca;* *yw; P[lacZ, w^+]64A $sens^{I235}$ th st cu sr e^s ca/TM6, Hu P[w^+, abdA-lacZ] e Tb ca ; yw; Df(3L)1228/4 P[lacZ, w^+]/TM3, Ser Sb* (Salzberg et al., 1997); *Ly^1/TM3, Sb* (Abbott, 1986); *P{$ry^{(+)}$=lArB}A77.1M3 /TM3, Sb ry* (Bellen et al., 1989); *$delta^{130}$P{$ry^{(+)}$= lArB}A77.1M3/TM3, Sb ry* (this work); *$sens^{E1}$ red e/TM3, Sb, e; $sens^{E2}$ red e/TM6B, Tb; $sens^{E53}$ red e/TM6B, Tb; $sens^{E54}$ red e/TM6b, Tb; $sens^{E58}$red e/TM6B, Tb; $sens^{E69}$ red e/TM6B, Tb* (H. Irick, Bloomington, Indiana); *$Lyra^{SX67}$/TM3, Sb* (P. Heitzler, Strasbourg, France); *y; d da^2 cn^1/Cyo, cn^2* (Caudy et al., 1988); *Df(2L)J27/SM1* (Lindsley and Zimm, 1992); *ato^1* (Jarman et al., 1994); *FM6-12/ Dp(1;Y) ct, y & FM6-12/Df(1) sc-B57, sn^3* (Gonzalez et al., 1989); *P{hsneo}l(3)neo19* (Spradling et al., 1999); *P[lArB]A101.1F3 ry[503]/ TM3, ry* (Bellen et al., 1989); *UAS-ato/TM3, Sb* #8 (Y.-N. Jan, San Francisco); *UAS-sc* (C. Doe, Eugene); *dpp-GAL4/TM6B* (G. Mardon, Houston); *hsFLP122; armlacZ, 70C FRT80b* (Vincent et al., 1994); *w; $sens^{E2}$, FRT802/TM6B* (G. Mardon, Houston); *UAS-sens(C1, C8, C5, C6)* (this work); *In(1) y^{3PL} sc^{8R}* (J. Skeath, St. Louis); *Df(1)sc10-1, sc^{10-1}/y^1 ac^{HW-1}* (Bloomington Stock Center); *UAS-m8* (S. Bray, Cambridge, UK); *w^{1118} ; P[m8-2.61]* (Kramatschek and Campos-Ortega, 1994); *w^{1118} ; P[m4-lacZ]-96A* (Bailey and Posakony, 1995); *E(spl)^{R1}/ TM6B, Tb^1* (Bloomington Stock Center); *f^{36a}; M(3) P{f+}/TM3, Ser* (P. Fernandez-Funez and P. Martin, Houston); *yw hs-FLP; UAS-scute/ apterous-GAL4; $sense^{E2}$ FRT80b/arm-lacZ, FRT80b* (this work).

Cloning, Plasmid Constructs, and Antibody Production

Standard molecular biology protocols were used. Based on the STS of *P{hsneo}l(3)neo19* (Spradling et al., 1999; Berkeley *Drosophila* Genome Project [BDGP]), we initiated a genomic walk (EMBL3) partially shown in Figure 2. *sens* cDNAs were isolated from an LD embryonic cDNA library (L. Hong and G. Rubin, BDGP). The *sens* cDNA was cloned into *pP{UAST}* and four transgenic *UAS-sens* lines (*C1*, *C8*, *C5*, and *C6*) were used in overexpression experiments. When driven by *dpp-GAL4*, *C1* causes the mildest phenotypes, whereas *C5* causes a very severe phenotype. *C8* and *C6* cause intermediate severe phenotypes. An 11 kb genomic SalI fragment containing the entire *sens* ORF was cloned into *pCaSpeR4* for rescue experiments.

For antibody production, two different constructs were cloned into the pET21 expression vector. The longer construct contains the entire open reading frame of *sens* and the shorter construct lacks the 4 zinc finger domains (amino acids 402–541). Purified inclusion bodies were used to immunize guinea pigs.

Immunological Stainings, Confocal Microscopy, and Scanning Electron Microscopy

The following primary antibodies were used: guinea pig anti-full-length Sens (1:1000) (this work); mAb22C10 (1:100) (Fujita et al., 1982); mouse anti-Cut (1:30) (Bodmer et al., 1987); rabbit anti-Asense

(1:1000) (Brand et al., 1993); rabbit anti-Scute (1:100) (a gift from G. Panganiban); rabbit anti–Couch Potato (1:1000) (Bellen et al., 1992); mouse anti-Prospero (1:4) (Vaessin et al., 1991); mouse anti-Delta (1:100) (a gift from M. Muskavitch); mouse anti-E(spl) mAb 323 (1:2) (a gift from S. Bray); mouse anti-β-galactosidase (1:1000) (Promega); rabbit anti-β-galactosidase (1:1000) (Cappel). Biotinylated secondary antibodies were from Vector Laboratories (anti-guinea pig/rabbit/mouse) and conjugated anti-rabbit/mouse/guinea pig antibodies fluorescent secondaries were from Molecular Probes (ALEXA) or Jackson Immunochemicals (Cy3). Confocal images were captured using a Bio-Rad MRC 1024 microscope, and scanning electron microscopy was performed using standard methods. All figures were processed with Adobe Photoshop software.

Clonal Analysis

f^{36a}; M(3) P{f+}/sens^{E2} 60 ± 24 hr old larvae were irradiated with 1000 rads. *sens* mutant bristles in clones should exhibit an extreme forked phenotype. To induce *sens^{E2}* clones in imaginal discs, we used FLP-mediated mitotic recombination (Xu and Rubin, 1993) by subjecting 36 ± 12 hr old larvae from a cross between *hsFLP122; arm-lacZ, 70C FRT80b* and *w; sens^{E2} FRT80b/TM6B* to a 1–2 hr heat shock at 38°C. Mutant clones are marked by the loss of the *arm-lacZ* reporter in imaginal discs (Vincent et al., 1994).

To remove *sens* in cells that overexpress Scute, we used *yw hs-FLP; UAS-scute/apterous-GAL4; sense^{E2} FRT80b/arm-lacZ, FRT80b* flies that were heat shocked as first or second instar larvae.

Gel Mobility Shift Assay

The *sens* constructs used to create antibodies were cloned into Bluescript and used in an in vitro transcription/translation Coupled Wheat Germ Extract System (Promega). The lysate was used in DNA binding assays. Probes (annealed oligonucleotides or DNA fragments) were ^{32}P-labeled with Klenow. Oligonucleotides used were as follows: CCGAAGTACCGTGATTTCAGGCATGCACGGG and GTGCATGCCTGAAATCACGGTACTTCGGGGG. DNA binding assays were done at RT for 30 min according to Murphy et al. (1996), and the reactions were separated on 5% native polyacrylamide gels.

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