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Suppressor of Hairless is required for signal reception during lateral inhibition in the *Drosophila* pupal notum

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SUMMARY

***Suppressor of Hairless* (S*u(H)*) activity is zygotically required in larval imaginal discs for the singling out of adult sense organ precursor (SOP) cells: loss of *Su(H)* function results in too many proneural cluster cells adopting the SOP fate, while overexpression of the Su(H) protein prevents SOP specification. *Su(H)* null mutant alleles are recessive lethal at the late larval and early pupal stages. The development of *Su(H)* mutant cells in pupae was therefore studied in somatic clones. Clonal analysis first showed that *Su(H)* is required for the regular spacing of microchaete precursor cells, as clusters of mutant SOPs were detected at positions where singled out sense organ cells are normally found. Second, *Su(H)* mutant SOPs produced neuron-like cells, consistent with a late defect in *Notch* (*N*) signalling. Third, a careful cell-by-cell analysis of clone borders showed that *Su(H)* mutant cells may adopt the SOP fate even when directly adjacent to wild-type cells. Finally, quantitative clone border analysis indicates that the relative level of *Su(H)* gene dosage appears to bias the selection of the future SOP: cells with a higher level of *Su(H)* activity are more likely to adopt the epidermal fate. These results show that notum cells strictly require *Su(H)* activity for receiving the lateral inhibitory signal. Thus, the DNA-binding protein encoded by the *Su(H)* gene may act downstream of the N receptor to implement the epidermal, non-SOP fate.**

Key words: *Suppressor of Hairless*, *Notch*, lateral inhibition, neurogenesis, peripheral nervous system, cell-cell interaction

INTRODUCTION

Intercellular signals play major roles in specifying cell fates during animal development. In *Drosophila*, the transmembrane Notch protein (N) acts as a receptor for intercellular signals in various cell fate decisions (Lehmann et al., 1983; Cagan and Ready, 1989; Hartenstein and Posakony, 1990; Corbin et al., 1991; Ruohola et al., 1991; Hartenstein et al., 1992). This function of *N* in cell-cell signalling appears conserved from worms to vertebrates [see Greenwald (1994) and Sternberg (1993) for reviews]. The role of N in lateral signalling between cells of equivalent developmental potential has been analysed in great detail for the formation of the sense organs of the adult fly.

A mechanosensory bristle is composed of four cells: two outer support cells, the trichogen and tormogen cells, which produce the stimulus-receiving cuticular structures (the shaft and the socket that surrounds the base of the shaft, respectively); the neuron and the thecogen cell, which are subepidermal and do not produce specialised cuticular structures. These four cells are generated from a single sensory organ precursor (SOP) cell that follows a stereotyped pattern of cell division (Hartenstein and Posakony, 1989). *N* activity is first required in late larvae and early pupae for singling out SOPs from groups (macrochaete SOPs) and rows (microchaete SOPs) of equipotent *achaete* and *scute*-expressing cells, called proneural clusters (Ghysen and Dambly-Chaudière, 1989; Romani et al., 1989; Cubas et al., 1991). *N* activity is also later required for specifying the distinct cell fates adopted by the four SOP progeny cells: a late reduction in *N* activity may thus result in all four sense organ cells adopting the neuronal fate (Hartenstein and Posakony, 1990).

The Delta (Dl) transmembrane protein may act as the ligand for the N receptor during SOP determination (Heitzler and Simpson, 1991). *Dl* also acts later to specify the non-neuronal fates of the SOP progeny cells: a late reduction in *Dl* activity yields a phenotype similar to *N*, i.e. sense organs composed of four neurons (Parks and Muskavitch, 1993).

The products of the genes *Hairless* (*H*: Bang and Posakony, 1992; Maier et al., 1992) and *Suppressor of Hairless* (*Su(H)*: Furukawa et al., 1992; Schweisguth and Posakony, 1992) may also be part of the lateral signalling pathway mediated by *Dl* and *N*. First, both *H* and *Su(H)* genetically interact with *N* (de la Concha et al., 1988; Dietrich and Campos-Ortega, 1984; Fortini and Artavanis-Tsakonas, 1994; Vässin et al., 1985), in addition to interacting with one another (Ashburner, 1982; Schweisguth and Posakony, 1992; F. S. and J. W. Posakony, unpublished results). Second, *Su(H)* null and *H* gain-of-function phenotypes appear similar to the neurogenic *N* loss-of-function phenotype: too many SOPs are specified from groups of competent cells (Schweisguth and Posakony, 1992; Bang and Posakony, 1992). Conversely, *Su(H)* gain-of-

function and *H* null mutant bristle phenotypes appear similar to the *Abruptex* phenotype associated with a gain of N function (Palka et al., 1990; de Celis et al., 1991): SOPs fail to develop resulting in bristle loss. *H* and *Su(H)* activities are also required later for specifying, again in an opposite manner, the trichogen (shaft) and tormogen (socket) fates (Bang et al., 1991; Bang and Posakony, 1992; Schweisguth and Posakony, 1994). Finally, direct protein-protein interactions have been identified both between Su(H) and the intracellular domain of N (Fortini and Artavanis-Tsakonas, 1994), and between Su(H) and H (Brou et al., 1994). Interestingly, Su(H) binds to the cdc10 repeats of N that were previously shown to be both necessary and sufficient for *N* signalling activity (Lieber et al., 1993; Rebay et al., 1993; Fortini et al., 1993; Struhl et al., 1993). In *Drosophila* S2 cells, transfected Su(H) can be found in the cytoplasm where it interacts with N. In an in vitro cell aggregation assay, binding of Dl-expressing cells to N-expressing cells relieves the N-Su(H) interaction, hence allowing translocation of Su(H) into the nucleus (Fortini and Artavanis-Tsakonas, 1994). These results suggest that Su(H) may act in transducing the N signal from the membrane into the nucleus.

Su(H) encodes a sequence-specific DNA-binding protein that has been highly conserved between *Drosophila* and man (Matsunami et al., 1989; Furukawa et al., 1991, 1992; Schweisguth and Posakony, 1992; Brou et al., 1994). *H* encodes a basic protein that acts as a negative regulator of the DNA-binding activity of Su(H) via direct protein-protein interactions (Brou et al., 1994). The human homologue of *Su(H)*, named Jκ-RBP/CBF1/KBF2, has been implicated in regulating the transcription of the adenovirus gene pIX (Dou et al., 1994) and from various cellular promoters expressed in B-cells (Grossman et al., 1994; Henkel et al., 1994; Zimber-Strobl et al., 1994). It is now clear that this protein is probably not involved in immunoglobulin VDJ recombination (Henkel et al., 1994; Schweisguth et al., 1994), as initially suggested (Matsunami et al., 1989). Although there is yet no evidence that Jκ-RBP/CBF1/KBF2 participates in *N* signalling in vertebrates, the importance of the developmental role of the mouse homologue of *Su(H)* is suggested by the embryonic lethality of homozygous knock-out mice (cited in Chung et al., 1994).

In this paper, the role of *Su(H)* during lateral inhibition signalling is investigated in vivo by clonal analysis. This methodology has been instrumental to first suggest that several cells are competent to produce a bristle at a given macrochaete position, and that negative signalling from bristle precursor cells may prevent these neighbouring competent cells from forming additional bristles (Stern, 1954). Also, the notion that equally competent cells compete to become SOPs and that this competition mechanism depends upon the relative levels of expression of Dl and N arose from studies of genetic mosaics (Heitzler and Simpson, 1991). Finally, the cell-autonomous behaviour of *N* mutant cells, and the contrasting non-autonomous behaviour of *Dl* mutant cells showed that Dl and N may act as the ligand and receptor, respectively, during lateral inhibition (Heitzler and Simpson, 1991, 1993). Here *Su(H)* mutant cells are shown to adopt autonomously the SOP fate when located in presumptive proneural regions. I further show that, at the border separating territories with different *Su(H)* gene dosage, cells with a higher level of *Su(H)* activity are more likely to become epidermal. These results constitute the first in vivo evidence that *Su(H)* acts, as *N*, in cells receiving the lateral inhibitory signal. Following SOP determination, clusters of *Su(H)* mutant SOPs delaminate from the epithelial cell monolayer and develop neuronal properties, consistent with a late defect in *N* signalling.

MATERIALS AND METHODS

Drosophila stocks

Flies were cultured on standard corn meal-sugar-agar medium at 25°C. The recombinant *Su(H)^{SF8} A 1-2-29 P[ry$^+$; hs-neo; FRT] 40A* chromosome was described in (Schweisguth and Posakony, 1994). The SF8 allele of *Su(H)* is a null allele (Schweisguth and Posakony, 1992). A 1-2-29 is an enhancer-trap marker that specifically reveals the shaft- and socket-secreting cells in late pupae and adults (Hartenstein and Jan, 1992; Schweisguth and Posakony, 1992). A 1-2-29 staining is however not yet detectable in 20-24 hours after puparium formation (APF) pupae. A101 is a P[*lacZ, ry*$^+$] enhancer-trap allele of *neu* (Boulianne et al., 1991) that is specifically expressed in the SOP and its progeny cells (Huang et al., 1991; Usui and Kimura, 1993). However, A101 staining is essentially not detectable in emerging adults. The epitope tagged cell-autonomous marker hs-NM31E, the hsFLP1, the FRT40A and the FRT82B constructs are described in Xu and Rubin (1993). The hsFLP38 is described in Chou and Perrimon (1992). The *pr pwn hsFLP38* and the *FRT82B kar^2 ry^{506} bx^{34e} Dp(2;3)P32 pwn$^+$* chromosomes were constructed and generously provided by P. Heitzler and P. Simpson. The P[*Su(H)*$^+$]-8 et P[*Su(H)*$^+$]-10 constructs, which include a 6 kb genomic fragment encoding a fully active copy of the *Su(H)*$^+$ gene (Schweisguth and Posakony, 1992), were recombined onto the *FRT82B P[w$^+$]87E Sb^{63c}* chromosome (Xu and Rubin, 1993).

Clones of cells homozygous mutant for *Su(H)* were produced in female pupae of the following genotype: *w^{1118}/y w hsFLP1 ;Su(H)^{SF8} A 1-2-29 P[ry$^+$; hs-neo; FRT] 40A/P[w$^+$, hs-NM]31E P[ry$^+$; hs-neo; FRT]40A*; A101/+. These were F_1 progeny from crossing *w^{1118}/y w hs-FLP1; Su(H)^{SF8} A 1-2-29 P[ry$^+$; hs-neo; FRT] 40A/In(2LR)Gla, Gla Bc Elp* females to *w^{1118}/Y; P[w$^+$, hs-NM]31E P[ry$^+$; hs-neo; FRT]40A*; A101/TM3, *Sb* males. Note that only 50% of the female progeny carry the *hsFLP1* construct and may thus be mosaic, and of these, only 50% may also contain the A101 *lacZ* marker. The male progeny does not bear the hsFLP1 construct, and are thus discarded. Thus, at most, only 25% of the female progeny is of potential interest. In practice, less than 10% of the dissected female pupae showed mosaicism after immunostaining and were A101 positive.

Clones of cells differing in their *Su(H)* gene dosage were produced in flies of the following genotypes:

(a) *pr pwn hsFLP38/ pr pwn; P[ry$^+$; hs-neo; FRT]82B P[w$^+$]87E Sb^{63c} P[w$^+$; Su(H)$^+$]-8 / P[ry$^+$; hs-neo; FRT]82B kar^2 ry^{506} bx^{34e} Tp(2;3)P32, pwn$^+$*.

(b) *pr pwn hsFLP38/ pr pwn; P[ry$^+$; hs-neo; FRT]82B P[w$^+$]87E Sb^{63c} P[w$^+$; Su(H)$^+$]-10 / P[ry$^+$; hs-neo; FRT]82B kar^2 ry^{506} bx^{34e} Tp(2;3)P32, pwn$^+$*.

(c) control: *pr pwn hsFLP38/ pr pwn; P[ry$^+$; hs-neo; FRT]82B P[w$^+$]87E Sb^{63c} / P[ry$^+$; hs-neo; FRT]82B kar^2 ry^{506} bx^{34e} Tp(2;3)P32, pwn$^+$*.

Chromosomes and mutations not described herein are described in Lindsley and Zimm (1992).

Heat-shock treatment

(a) Clonal analysis of cells homozygous mutant for *Su(H)*

A first heat-shock was applied between 24 and 48 hours after egg laying to induce FLP recombinase expression. Vials containing the larval progeny from the cross given above were placed in a 37°C water bath for 2× 1 hour, separated by a 1 hour interval at 25°C. A few days later, female prepupae were selected at puparium formation, placed in a humid chamber, aged for 19 hours at 25°C, and then heat-

shocked for 1 hour in a 37°C water bath to induce the expression of the NM molecular marker.

(b) Clonal analysis of cells differing in their *Su(H)* gene dosage

A 2× 1 hour heat-shock regimen, separated by a 1-day interval at 25°C, was applied to 24-72 hours old larvae, as described above.

Antibody and β-galactosidase staining

Heat-shocked 20 hours APF female pupae were dissected in PBS, fixed in 4% paraformaldehyde for 15 minutes, rinsed in PBS containing 0.05% Triton X-100 (PBS-T), blocked in PBS-T containing 1% BSA (PBT), and incubated overnight with the following primary antibodies: mouse monoclonal antibody (mAb) 1-9E10.2 (anti-MYC, obtained from the ATCC) from supernatant diluted 1:5 to 1:10; rabbit polyclonal anti-Horse Radish Peroxidase (HRP) serum (Cappel) diluted 1:1000. Tissues were then washed in PBT, incubated either with the biotinylated anti-mouse/rabbit secondary antibody (Vector) diluted 1:1000 in PBT for peroxidase activity (brown) staining using the Elite kit (Vectastain), or with an alkaline phosphatase (AP) anti-mouse antibody (Biosys) diluted 1:1000 for AP (blue) staining. All blocking, incubation and wash steps were at 4°C. Following immunostaining, tissues were incubated overnight for X-gal staining as described in Romani et al. (1989).

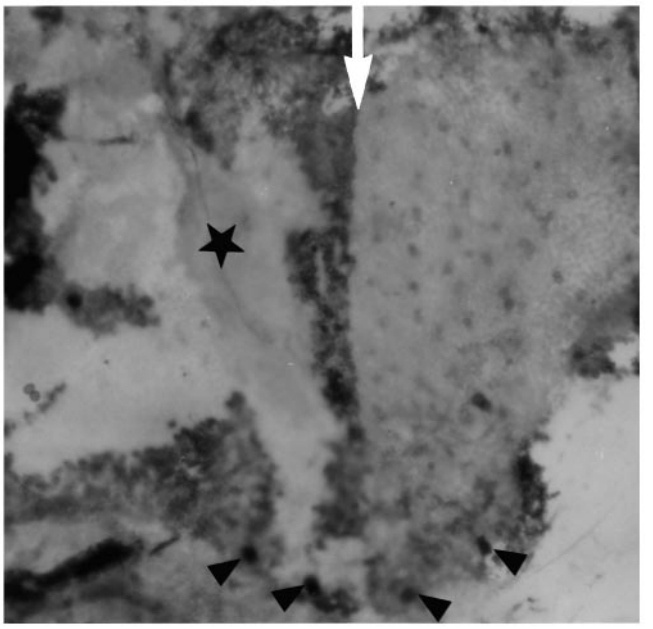


Fig. 1. Expression of the NM marker in a 20 hours APF mosaic pupae.The unstained mutant clone is indicated by a star in the left heminotum, and is surrounded by darkly stained wild-type cells carrying two copies of the NM marker. The right heminotum shows an intermediate staining level corresponding to expression from a single copy of the NM marker by cells heterozygous for *Su(H)*. Anterior is at the top. The midline is indicated by a white arrow, and scutellar bristles are shown by arrowheads. Note that NM expression is elevated in bristle cells upon heat-shock (see also Fig. 3E).

RESULTS

Generating clones of marked homozygous *Su(H)* mutant cells in the pupal notum

Genetic mosaics for *Su(H)* were generated using the FLP/FRT method (Xu and Rubin, 1993). Expression of the yeast FLP recombinase was induced upon heat-shock in first instar larvae, between 24 and 48 hours after egg laying. The FLP recombinase catalyses site-specific recombination between two FRT sites inserted at the base of chromosome arm 2L (Xu and Rubin, 1993). One of the two FRT-bearing chromosomes is mutant for a null allele of *Su(H)* (SF8: Schweisguth and Posakony, 1992). The other homologue carries the MYC-tagged NM cell-autonomous marker (Xu and Rubin, 1993). Clones mutant for *Su(H)*, induced in first and second instar larvae, could therefore be identified in dissected pupae, around 20 hours after puparium formation (APF) as groups of cells that do not express the NM protein. Fig. 1 shows an example of a large *Su(H)* mutant clone that covers one half of an heminotum. This clone is identified as a group of unstained cells following immunochemical detection of the NM marker using the anti-MYC 1-9E10.2 mAb. The twin clone can also be identified as a group of more darkly stained cells as it expresses two copies of the NM marker. Thus complete loss of *Su(H)* activity does not cause cell lethality or prevent cell proliferation in the pupal notum. These properties, together with the high frequency of mitotic recombination (see Materials and methods), allowed a detailed investigation of the development of these *Su(H)* mutant clones.

Su(H) mutant cells located at wild-type sensory organ positions adopt the SOP fate

The role of *Su(H)* in controlling the fates of sense organ cells was investigated in a cell-by-cell analysis of the mutant clones in dissected pupae around 20 hours APF. Eighteen clones, from thirteen notum, were analysed. Five are shown in Fig. 2. The fate of the *Su(H)* mutant cells was followed in the developing head and notum using the enhancer-trap line A101 where bacterial *lacZ* is expressed in the SOPs and their progeny (Ghysen and O'Kane, 1989; Huang et al., 1991; Usui and Kimura, 1993). The epidermis of the dorsal and posterior parts of the head and of the notum derive from specific regions of the eye-antennal and wing imaginal discs, respectively. In these tissues, A101 expression is first detected around 30 hours before puparium formation in the earliest macrochaete precursor cells and, around puparium formation, A101 is expressed in every macrochaete precursor cells of the notum (Huang et al., 1991). Microchaete precursor cells appear later, again as revealed by A101 expression, between 8 and 14 hours APF (Usui and Kimura, 1993). They divide twice soon after, between 15 and 20 hours APF (Hartenstein and Posakony, 1989; Usui and Kimura, 1993).

Around 20 hours APF, large clusters of *lacZ*-expressing cells are observed within *Su(H)* mutant clones, at positions where individual sensory organ cells would normally be present (Fig. 2). For example, in the posterior head clone shown in Fig. 2B, a group composed of more than 100 cells express the A101 marker. These mutant SOPs are found at the position expected for the proneural cluster that gives rise to the 15-20 occipital bristles (see Fig. 2A). This suggests that an excess number of clustered SOPs arise at positions where individual SOPs normally emerge, as first observed in imaginal discs from *Su(H)* mutant larvae (Schweisguth and Posakony, 1992). Indeed, in none of the eighteen clones studied here were *lacZ*-expressing cells detected at ectopic positions, i.e. in regions where no sense organ is known to develop. That mutant

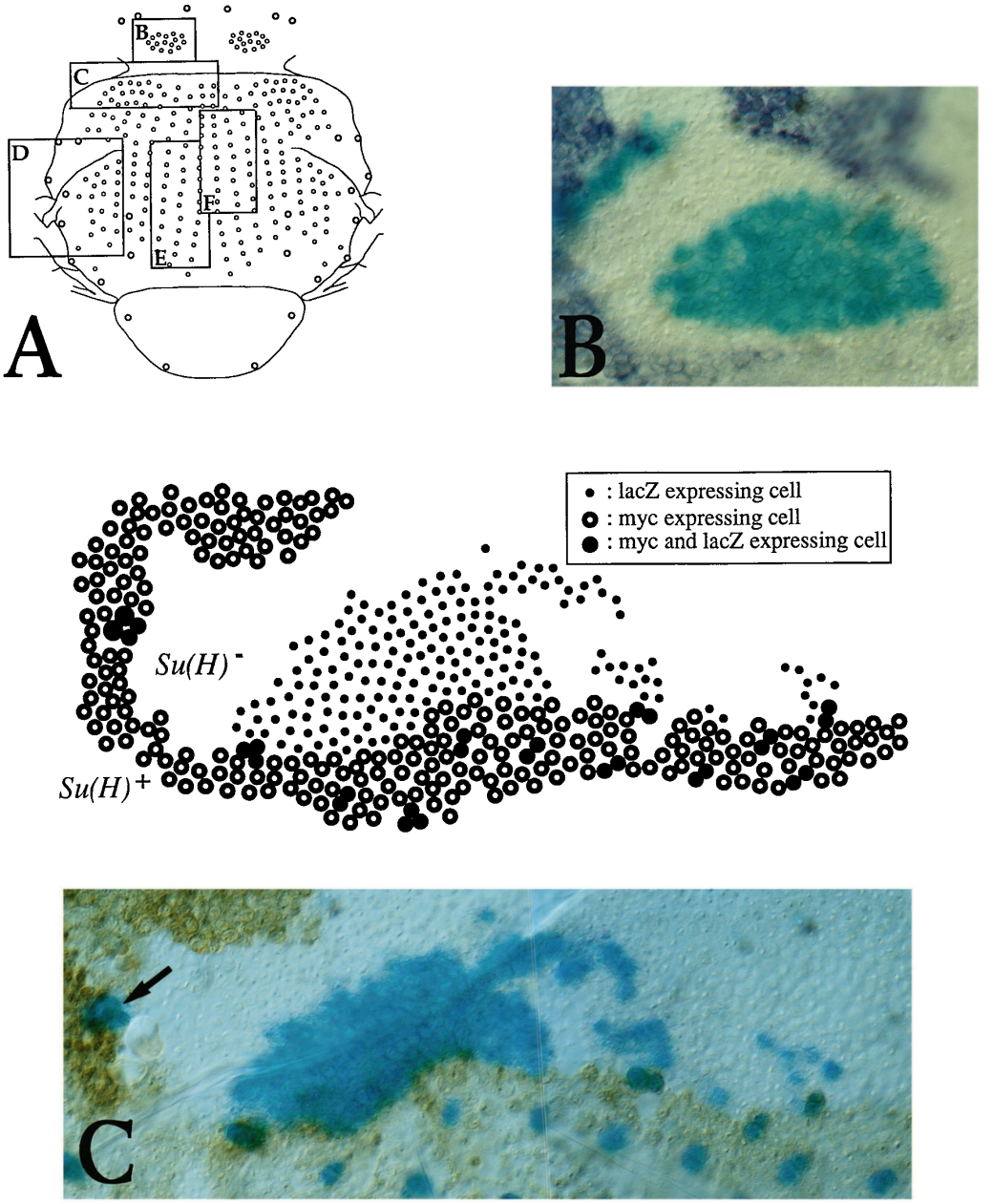
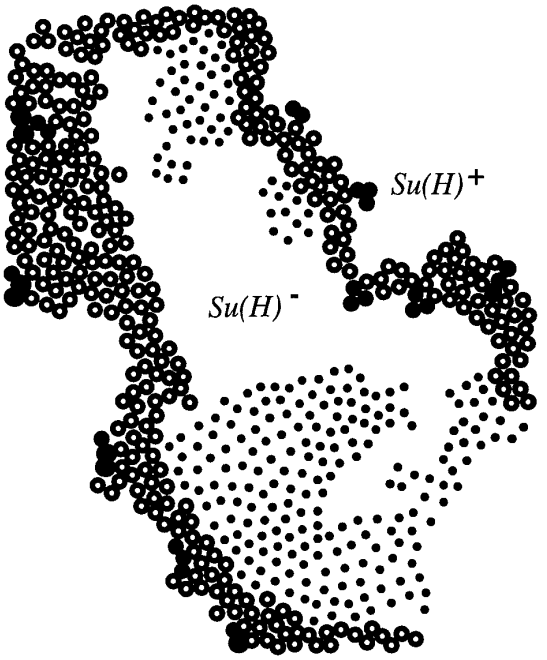
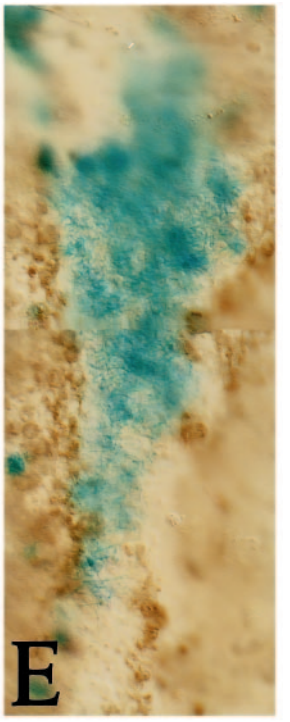


Fig. 2. A101 expression in mosaic pupae around 20 hours APF. (A) Diagram of a wild-type notum. Positions of B-F are indicated. Clones in B and D are from the same pupae. Macrochaetes and microchaetes are represented by large and small circles, respectively (see Huang et al., 1991; Lindsley and Zimm, 1992; Usui and Kimura, 1993; for notal structure and bristle nomenclatures). (B) Photograph of a mutant clone encompassing the occipital bristle precursor cells. NM-expressing (wild-type) cells are stained in dark blue (cytoplasmic AP staining). Mutant cells do not express the NM marker, and may or may not express the A101 marker (*lacZ*-positive cells appear blue-green). Note the A101-expressing cells in the upper left corner of the picture, at the position of the antero orbital bristle. (C-F) Diagrammatic representations of the regions of mosaic nota containing a mutant clone on top (C), or on right (D-F), and photographs of the corresponding clone below (C) or on left (D-F). The symbols used in the diagrams are given in the corner box of the C diagram. Note that mutant cells that do not express *lacZ* are not represented for clarity. Thus, in these diagrams, the clone borders are outlined by the NM-expressing cells only. NM-expressing cells are stained in brown (peroxydase staining) in C and F, or in dark blue (AP staining) in D-E. All clones are shown anterior side at the top. (C) Antero-lateral clone, bordered on its posterior-left margin by one humeral bristle (indicated with an arrow). Mutant cells located anteriormost in the notum, where no sense organs normally develop, do not express *lacZ*. (D) Lateral clone showing that mutant SOPs in the central microchaete field (indicated with a white arrow) have aggregated and lost cell contact anteriorly with the rest of the epithelium. Note in particular the epithelium disruptions shown with black stars. Anterior and posterior notopleural, and anterior and posterior supraalar bristles are seen on the left-hand side, and indicated with black arrows (from top to bottom). Here too, mutant cells that do not express *lacZ* are found at the edge of the microchaete field (see A, and Fig. 1 in Heitzler and Simpson (1991)]. (E-F) Central clones encompassing microchaete rows 1 to 3. Again, note that cells found close to the midline may not express *lacZ*. The notum shown in F appears younger: SOPs in row 2 (indicated with a short open arrow) have not yet divided to produce the two secondary precursor cells (compare with rows 1 and 3, that are shown with short black arrows, where SOPs divide earlier: see Usui and Kimura (1993) for details). Midline position is given by a thin black arrow.


Su(H)+
Su(H)-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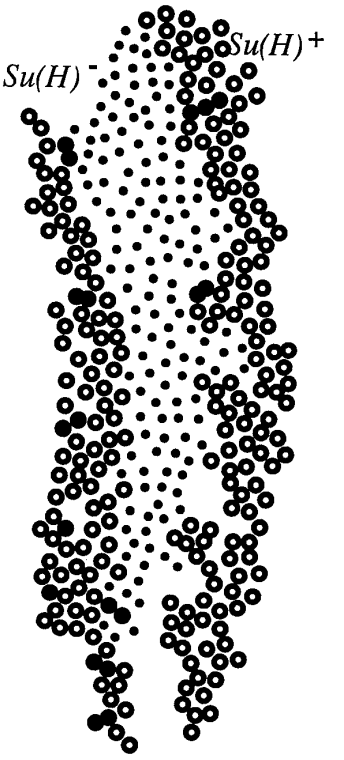
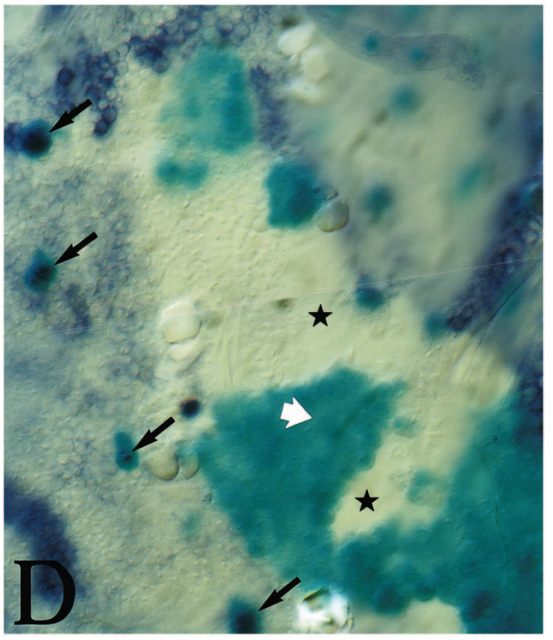

Su(H)+
Su(H)-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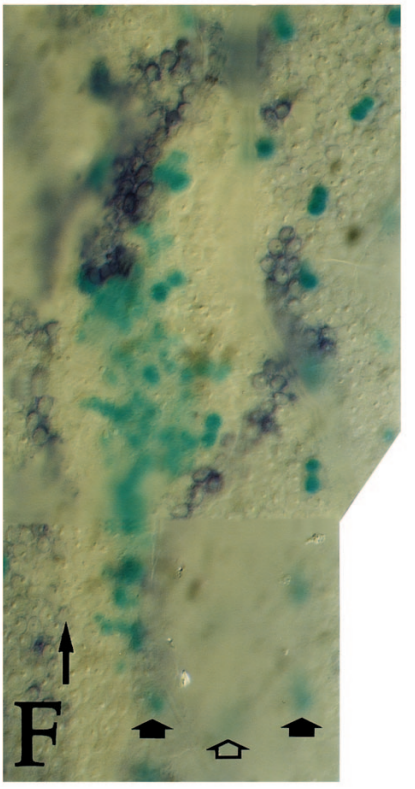
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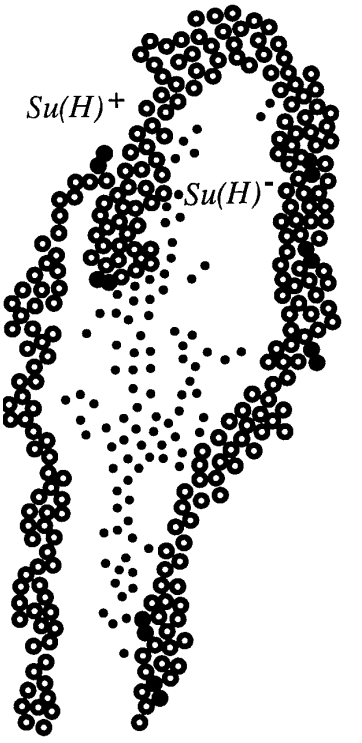
Su(H)+
Su(H)-

SOPs are only observed at normal SOP positions is fully consistent with the near normal positioning of the proneural clusters in *Su(H)* mutant wing discs (Schweisguth and Posakony, 1994).

This clone (as well as nearly all others: see below) also includes many *Su(H)* mutant cells that do not express *lacZ* (Fig. 2B). One possible interpretation is that mutants cells lying too close to wild-type cells are inhibited by the latter to adopt the SOP fate. However, mutant cells that do express *lacZ* can be found in direct contact with wild-type cells. For example in Fig. 2B, cells located near the anterior orbital bristles (that were likely part of the orbital proneural cluster) express the A101 marker. Alternatively, these *Su(H)* mutant cells do not adopt the SOP fate because they are not part of the proneural cluster and thus are not competent to adopt the SOP fate. Indeed, in the notum, mutant cells that do not express the A101 marker are usually found in peripheral regions of the notum (Fig. 2C,D), at the intrascutal suture (Fig. 2D), or along the midline (Fig. 2E,F). These regions do not give rise to sense organs in wild-type flies (see Fig. 2A), or even in the absence of *N* signalling (Heitzler and Simpson, 1991). Therefore, *Su(H)* mutant cells adopt the SOP fate only when located within presumptive proneural clusters, in pupae as in larvae (Schweisguth and Posakony, 1992, 1994).

Su(H) mutant cells may adopt the SOP fate even when directly juxtaposed to wild-type cells

The neurogenic phenotype described above for *Su(H)* could equally result from a defect in sending and/or receiving the lateral inhibitory signal (Heitzler and Simpson, 1991, 1993). To distinguish between these two possibilities, the fates adopted by mutant and wild-type cells that are found at the clone border were analysed. The two following questions were specifically addressed: first, can the fate of *Su(H)* mutant cells be influenced by neighbouring wild-type cells? Second, can the *Su(H)* mutant cells influence the behaviour of wild-type cells at the clone border?

First, when clone borders cross the central region of the heminotum, where stereotyped rows of microchaetes develop, most *Su(H)* mutant cells express the A101 marker, even when directly juxtaposed to wild-type cells (Fig. 2C-E). Thus, these *Su(H)* mutant cells do not respond to the lateral inhibitory signal sent by neighbouring wild-type cells (that should be capable of both sending and receiving the signal). This indicates that *Su(H)* acts in a cell-autonomous manner for the adoption of the SOP versus epidermal fate, and that *Su(H)* activity is strictly required in receiving the lateral inhibition signal. Not only do mutant SOPs express the A101 marker but they also aggregate and lose cell contact with the neighbouring non-SOP cells, leading to a disruption of the monolayer of epithelial cell (see Fig. 2D). Similar changes in cell adhesion were also observed in *Su(H)* mutant wing discs (unpublished results).

The fates adopted by wild-type cells at the clone border were also examined. In many cases, wild-type sensory organ cells can be found directly juxtaposed to *Su(H)* mutant cells (22 wild-type sense organs were identified in 10 out of 18 clones). This suggests that mutant cells do not necessarily prevent neighbouring wild-type cells from adopting the SOP fate (see discussion).

Finally, I also observed one mutant clone that contained many non-SOP cells, even though these cells are found in the notum region that normally gives rise to several microchaetes from row 2 (Fig. 2F): only a few mutant SOPs are detectable around row 2 position, while many mutant SOPs are found in the alignment of row 1 microchaetes. Interestingly, microchaete precursor cells appear sequentially in wild type, first along row 1 (and 5), and last along row 2 (and 4) (Usui and Kimura, 1993). That non-SOP mutant cells are found at late emerging SOP position may indeed be significant since this notum appears younger than most others (staging is based here upon the number of SOP progeny cells: only two A101-expressing cells, instead of the full complement of four post-mitotic cells, are detected at microchaete positions along rows 1 and 3).

A higher level of *Su(H)* activity favors the adoption of the epidermal fate at the clone border

I next investigated whether different levels of *Su(H)* activity within cells directly juxtaposed at the clone border could possibly influence their fate. Although it was possible, at least in some favorable cases, to visualize the twin clone border separating $Su(H)^+$ homozygous and heterozygous cells (see Fig. 1), the high level of expression of the NM marker in bristle cells upon heat-shock (see Fig. 1 and 3E) did not allow me to unambiguously identify the genotype of these sensory cells. Thus, a quantitative analysis of cell fate choices at the border could not be done in these clones. Alternatively, clones of cells expressing four wild-type copies of the *Su(H)* gene were generated in flies trisomic for *Su(H)* (see Materials and methods for details). These clones were marked using the *pawn* (*pwn*) cuticular marker that labels both sensory bristles and epidermal hairs (Heitzler and Simpson, 1991). Clones homozygous for two different P-element generated $Su(H)^+$ transpositions on the third chromosomes were analysed in adult cuticular preparations and compared to control clones. The frequencies with which marked bristles, produced by cells expressing four copies of *Su(H)*, are found directly adjacent to unmarked epidermal cells, expressing two (twin clone) or three (genetic background) copies of *Su(H)* are shown in Table 1. Along the clone border, bristles are more frequently produced by unmarked cells than by *pwn* cells. This bias indicates that cells with more *Su(H)* gene product are more likely to adopt the epidermal fate. This result provides further evidence that *Su(H)* acts in cells receiving the lateral inhibitory signal. It further indicates that *Su(H)* acts in the initial singling out of the SOP precursor when proneural cells compete for the adoption of the SOP fate.

Su(H) mutant SOPs produce neuron-like cells

The adult phenotypes associated with these mutant clones were also analysed. Previous studies showed that *Su(H)* activity is also strictly required for the adoption of the tormogen (socket) versus trichogen (shaft) fate (Schweisguth and Posakony, 1994). Therefore, mutant clones might be expected to produce clusters of shaft cells at the cuticular surface, resulting from the successive failures of epidermal and socket cell determination. This phenotype is indeed observed, but only at a very specific position on the head corresponding to the vibrissae bristles (Fig. 3A-A'). However, on the notum, patches of naked cuticle are detected (Fig. 3B). When *yellow* (*y*) is used as a bristle marker for mutant cells

Table 1. Influence of the relative *Su(H)* gene dosage upon SOP selection along mosaic borders

$Su(H)^+$ duplications	*Su*(*H*) gene copy number in pwn^-/pwn^+ cells	Frequency of pwn^- bristles adjacent to unmarked hairs
–	2/2	35/92 (38%)
P[$Su(H)^+$]-8	4/2 or 3	35/157 (20%)
P[$Su(H)^+$]-10	4/2 or 3	53/193 (27%)

The genotypes used in these experiments are described in Materials and methods. The frequencies shown in the last column are expressed as the ratios of pwn^- bristle to the total number of bristles observed at the mosaic clone border. In the control experiment [first line: no $Su(H)^+$ duplication], a weak bias is observed at the clone border as pwn^- bristles are less frequently observed than pwn^+ ones. Since both marked and unmarked cells have the exact same *Su(H)* wild-type genotype, an equal distribution could have been expected. This bias does not result from the *pwn* marker itself (see Table 3 in Heitzler and Simpson (1991)). It may in part be due the small size of the clones analysed here (many clones were 10-20 cells large). This would indeed result in an overall larger number of pwn^+ cells (outer border) relative to pwn^- cells (inner border). For example, at the border of a compact clone composed of 12 pwn^- cells at the time of bristle precursors determination, 10 pwn^- cells would contact 16 pwn^+ cells (assuming that each hexagonal cell contact 6 neighbouring cells): the frequency of pwn^- bristles at the clone border would thus be 38% (10/26). For both P[$Su(H)^+$] duplications studied here, the frequency bias is significantly greater than the one observed in control clones.

[see Schweisguth and Posakony (1994) for experimental details], y^- sense organ never develop at close proximity of the bald areas (not shown). Also, epidermal hairs appear severely disorganised and cuticle can even be featureless (see open arrows in Fig. 3B). Thus, mutant SOPs do not give rise to differentiated shaft or socket cells, but may either degenerate, remain undifferentiated, revert to an epidermal fate or generate only neuronal and/or thecogen cells. This cuticular phenotype is identical to the one associated with *N* mutant clones: cells mutant for a null allele of *N* do not differentiate epidermal hairs or sensory organ bristles in the central area of each heminotum: instead, a bald patch of wild-type epidermal cells is observed (Heitzler and Simpson, 1991). *N* mutant cells are thought to all differentiate as neurons (Hartenstein and Posakony, 1990). Emerging *Su(H)* mosaic adults were therefore examined for the presence of cells of neural origin underneath these patches of naked cuticle. Clusters of *lacZ*-stained cells, extending long processes that resemble axon bundles, are observed (Fig. 3C-C'). This suggests that some of the mutant SOPs may have adopted the neuronal fate. This hypothesis was tested in doubly stained pupae, at 44 hours APF, using a neuron-specific anti-HRP serum (brown staining in Fig. 3D) and the anti-MYC mAb to identify wild-type cells (in blue). The *Su(H)* mutant cells that are detected underneath the epidermis are stained with the anti-HRP serum (Fig. 3D). Furthermore, the processes extending out of these clusters connect to nearby axons produced by wild-type neurons. These data indicate that most *Su(H)* mutant SOPs, if not all, express a neuronal fate. Still, it is possible that mutant thecogen cells could also be part of these clusters together with anti-HRP positive neuron-like cells. Finally, mutant cells are also detected in the developing epidermis along the thoracic midline, consistent with the observation that *Su(H)* mutant cells located along the thoracic midline do not express the A101 marker (see Fig. 2E-F).

DISCUSSION

Successive requirements for *Su(H)* activity during sensory organ development in the notum

The analysis of *Su(H)* function in SOP determination had so far been mostly limited to a few sense organ precursor cells that appear early within the wing imaginal disc (Schweisguth and Posakony, 1992, 1994). This limitation inherent to the late larval lethality associated with *Su(H)* null alleles can be overcome by studying genetic mosaics. Clones of mutant cells are shown here to produce too many macrochaete and microchaete precursor cells on the head and notum: clusters of mutant SOPs develop at the presumed positions of bristle proneural clusters. It is believed that expression of the A101 *lacZ* marker is not transient but reflects stable commitment to the SOP fate since mutant SOPs aggregate, delaminate from the epithelial cell layer and produce neuron-like cells, resulting in naked cuticular patches. Similar cuticular phenotypes have been reported for *N* and *Dl* (Heitzler and Simpson, 1991). Therefore *Su(H)* activity appears strictly required for the singling out of most adult SOPs. It may be of interest to note that *Su(H)* mutant clones ultimately produce large clusters of mutant SOPs, as if nearly all proneural cluster cells become SOPs. This raises the possibility that both the initial refinement of proneural competence to a limited number of proneural cluster cells (Ghysen et al., 1993) that express *achaete* and *scute* at a higher level (Cubas et al., 1991), and the final singling out of SOPs from these refined clusters, could be impaired in these *Su(H)* mutant clones.

At a later stage, *Su(H)* was known to control the socket cell fate (Schweisguth and Posakony, 1994). Here, the complete loss of *Su(H)* function is shown to result in the loss of both shaft and socket cells and in the production of neuron-like cells. This phenotype is entirely consistent with a role of *Su(H)* in *N* signalling, since similar phenotypes were observed for *N* and *Dl* late loss of function (Hartenstein and Posakony, 1990; Parks and Muskavitch, 1993). This observation supports a recent hypothesis proposing that *Su(H)* participates in each alternative cell fate decision of the lineage (Posakony, 1994): first in the secondary precursor cell fate decision, followed by the shaft versus socket cell and neuron versus thecogen cell decisions. One exception is however noted here: the secondary precursor cells of the vibrissae bristles that would normally generate shaft and socket cells do not require *Su(H)* function for its determination.

Cell-autonomous requirement for *Su(H)* activity in signal reception

Whether *Su(H)* mutant cells behave cell-autonomously or not during SOP specification was analysed directly in the developing notum, soon after SOP determination. *Su(H)* mutant cells were shown here to adopt the SOP fate even when directly adjacent to wild-type cells. Thus mutant cells adopt the SOP fate cell autonomously when located within proneural clusters. This cell-autonomous requirement for *Su(H)* activity in selecting individual SOPs thus indicates that *Su(H)* activity is strictly required in cells receiving lateral inhibition. This conclusion fully supports the notion that *Su(H)* acts to transduce the signal directly from N at the membrane into the nucleus (Fortini and Artavanis-Tsakonas, 1994).

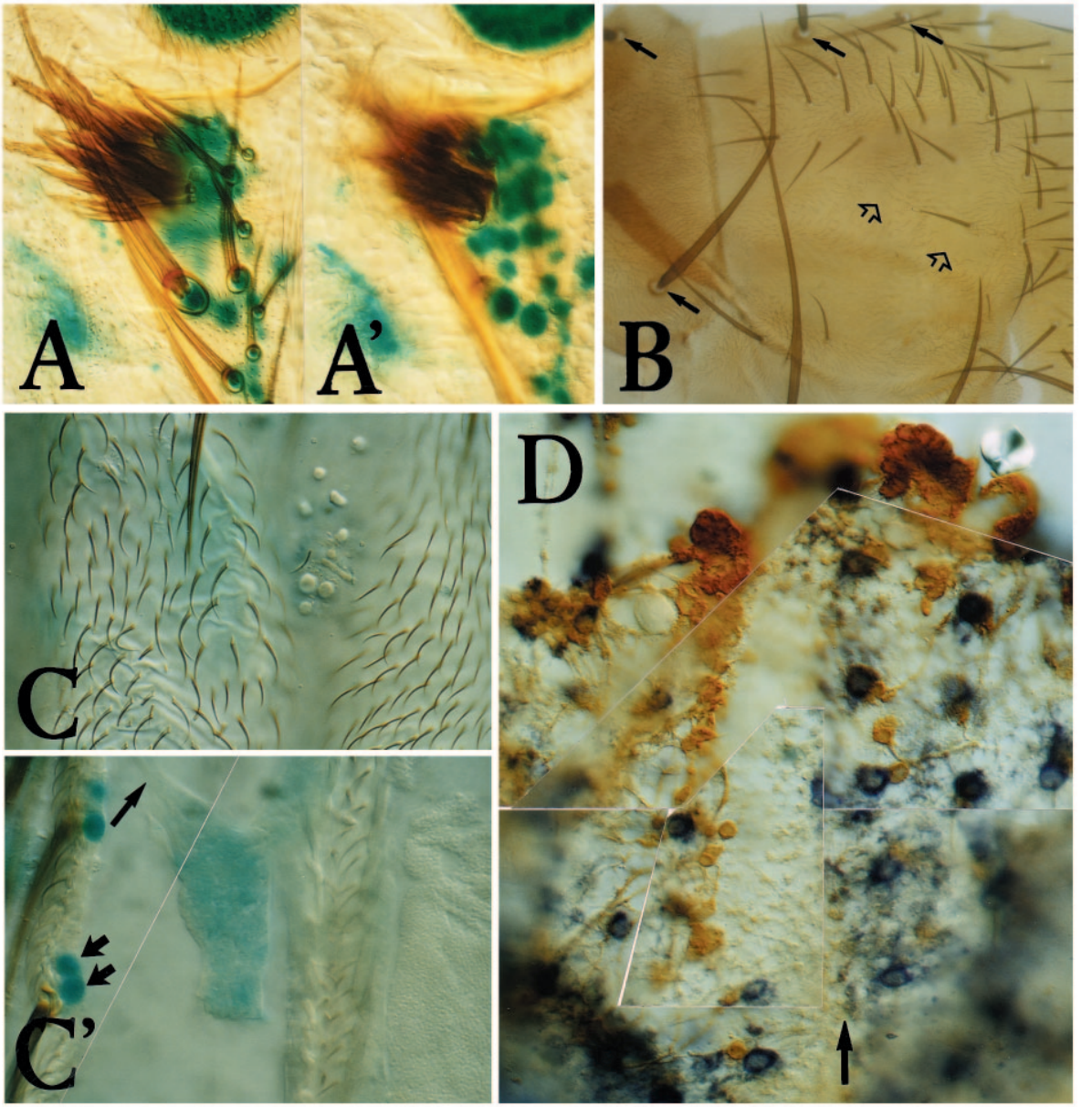

Fig. 3. Mutant SOPs may produce neuron-like cells. (A, A') Photographs of a mutant clone carrying vibrissae bristles from a young adult fly. The usual two large upper vibrissae bristles are replaced by a tuft of bristle shafts. These mutant bristles appear *yellow* (*y*) when *y* is used as a bristle marker for mutant cells (not shown). I never observed a bristle-loss phenotype at this particular position. Althought these flies carry both the A101 and A-1-2-29 enhancer trap *lacZ* markers, the staining of large nuclei likely results predominantly from the A-1-2-29 marker that confers specific *lacZ* expression in the shaft and socket cells (see C' and Hartenstein and Jan (1992), and Schweisguth and Posakony (1994)), since A101 expression is not detectable at this stage in wild-type flies. The number of large support cell nuclei is increased correspondingly to the number of shafts. These two focal planes further highlight the absence of socket cells at the base of the tufted shafts. Dorsal side is at the top. (B) Photograph of a cuticular preparation showing a large patch of naked cuticle at the normal position of the dorsocental bristles (absent here) on the right heminotum. In its center, epidermal hairs are disorganised or even absent, as pointed out by the open arrows. The two anterior scutellar and the dorsocentral bristles of the left heminotum are indicated with arrows. Anterior is at right. (C,C') Clusters of *lacZ*-expressing cells are found underneath the patches of naked cuticle, here in a young adult fly. *lacZ* expression likely results from abnormally persisting A101 expression (rather than from the A-1-2-29 enhancer-trap marker that specifically reveals the nuclei of both shaft and socket cells, shown here with thick arrows) since these clusters are not revealed by the A-1-2-29 marker alone (not shown). Note the processes extending out of this cell cluster (as indicated with a thin arrow). (D) Photograph of a 44 hours APF mosaic pupae, stained with the anti-MYC (blue AP staining) to detect the *Su(H)* mutant cells, and with an anti-HRP (brown staining) to reveal neuronal cells. The midline runs vertically at the center of the photograph (anterior is up). Part of the right heminotum is mutant for *Su(H)*, as judged from the lack of blue staining. A number of clustered neuron-like cells are detected that do not appear to innervate any mechanosensory organs. Nevertheless, these neuron-like cells project axonal processes that meet with those sent by wild-type neurons innervating normal bristles. The number of these mutant neuron-like cells is probably underestimated since it is likely that some of these cell clusters may have been lost during the incubation and washes steps. Note also that many cells found in the epidermal cell layer do not express the NM antigen, raising the possibility that some of the *Su(H)* mutant cells found along the midline may also develop as epidermal cells. Midline position is indicated with a thin arrow. Anterior is at the top.

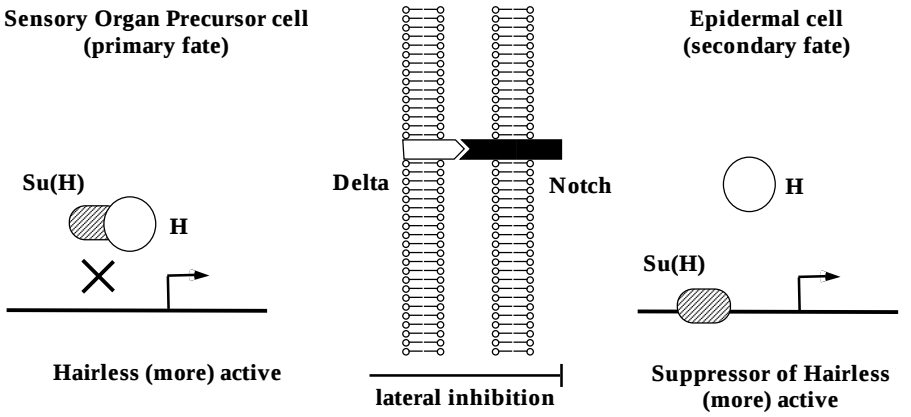

Fig. 4. Predicted roles for *Su(H)* and *H*. *Su(H)* activity is strictly required in the future epidermal cells that adopt the secondary fate in response to lateral inhibition. At the cell membranes, binding of Dl to the N receptor mediates negative signalling. In the inhibited cell, shown on the right side, Su(H) would bind DNA and regulate the transcription from yet unidentified downstream target promoters. The SOP cell, which expresses the primary fate, is shown on the left. In this cell, *H* is predicted to antagonize the effects of lateral inhibition by down-regulating Su(H) DNA-binding activity (Brou et al., 1994).

Is *Su(H)* activity also required in sending the lateral inhibitory signal? That wild-type cells directly juxtaposed to mutant SOPs may also adopt the SOP fate, i.e. express the A101 *lacZ* marker and divide to generate four daughter cells, may suggest that *Su(H)* activity is required for both receiving and sending inhibitory signals. This interpretation is however not supported by the observation that *Su(H)* mutant SOPs express the Dl protein at a high level (Schweisguth and Posakony, 1994), implying that *Su(H)* mutant SOPs still have at least part of the signalling properties of the wild-type SOPs. The clustering of mutant SOPs raises the alternative possibility that these cells lose contact with neighbouring wild-type cells soon after being committed to the SOP fate. Negative signalling by mutant SOPs would then be rendered ineffective if cell-contact dependent (as this appears to be the case for *glp-1* signalling in nematodes (Mello et al., 1994)), thereby allowing wild-type cells to later adopt the SOP fate. Whether *Su(H)* activity is required for sending the signal thus remains an open question.

Finally, the relative level of *Su(H)* gene dosage appears to bias the selection of the future SOP in favor of cells with a lower level of *Su(H)* activity. This result confirms that *Su(H)* acts in signal reception. It also indicates that it does so when notum cells are still competing for the adoption of the SOP fate, thereby implying that *Su(H)* may act in the initial selection of the future SOP. *Su(H)* could thus be one component of the feedback mechanism whereby cells that are less efficient in signal reception and/or transduction will have an increased ability to inhibit their immediate neighbours (Heitzler and Simpson, 1991). For example, *Su(H)* could up-regulate *N* transcription and/or down-regulate *Dl* expression. Indeed transcriptional control is a critical component of an analogous feedback mechanism involving the lin-12 receptor in *C. elegans* (Wilkinson et al., 1994). Interestingly, *lin-12*-positive autoregulation was shown to require a 67 bp long regulatory element (Wilkinson et al., 1994) that contains a CATGGGAAC motif that can be bound in vitro by the mouse homolog of *Su(H)* (Tun et al., 1994). Whether *lag-1* (Lambie and Kimble, 1991), which encodes the *C. elegans* homolog of *Su(H)* (S. Christensen, V. Kodoyianni and J. Kimble, personal communication), mediates *lin-12*-positive autoregulation through this regulatory element remains to be investigated.

That *Su(H)* is required in inhibited cells for the stable adoption of the epidermal fate has important implications for the *H-Su(H)* interaction. In the future epidermal cells, the Su(H) protein may be activated in response to *N* signalling and binds the promoter sequences of its direct downstream target genes (Fig. 4), which remain to be identified. Nuclear translocation of Su(H) may participate in the signal-dependent activation of Su(H) (Fortini and Artavanis-Tsakonas, 1994). This view implies that the Su(H) protein does probably not interact with H in inhibited cells. Otherwise, the H protein might prevent Su(H) to bind DNA (Brou et al., 1994). *H* activity may be more effective in the future SOP cells, thereby protecting them from residual *Su(H)* (epidermalizing) activity, as initially suggested by Bang and Posakony (1992). Thus, the H-Su(H) protein complexes are predicted to form in the future SOP cells (Fig. 4).

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