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Antagonistic activities of *Suppressor of Hairless* and *Hairless* control alternative cell fates in the *Drosophila* adult epidermis

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SUMMARY

Successive alternative cell fate choices in the imaginal disc epithelium lead to the differentiation of a relatively invariant pattern of multicellular adult sensory organs in *Drosophila*. We show here that the activity of *Suppressor of Hairless* is required for both the sensory organ precursor (SOP) versus epidermal cell fate decision, and for the trichogen (shaft) versus tormogen (socket) cell fate choice. Complete loss of *Suppressor of Hairless* function causes most proneural cluster cells to accumulate high levels of the achaete and Delta proteins and to adopt the SOP fate. Late or partial reduction in *Suppressor of Hairless* activity leads to the apparent transformation of the tormogen (socket) cell into a second trichogen (shaft) cell, producing a 'double shaft' phenotype. We find that overexpression of *Suppressor of Hairless* has the opposite phenotypic effects. SOP determination is prevented by an early excess of *Suppressor of Hairless* activity, while at a later stage, the trichogen (shaft) cell is transformed into a second tormogen (socket) cell, resulting in 'double socket' bristles. We conclude that, for two different cell fate decisions in adult sensory organ development, decreasing or increasing the level of *Suppressor of Hairless* function confers mutant phenotypes that closely resemble those associated with gain and loss of *Hairless* activity, respectively. These results, along with the intermediate SOP phenotype observed in *Suppressor of Hairless; Hairless* double mutant imaginal discs, suggest that the two genes act antagonistically to commit imaginal disc cells stably to alternative fates.

Key words: *Hairless*, *Suppressor of Hairless*, neurogenic genes, asymmetric cell division, *Drosophila*, peripheral nervous system, sensory organ development

INTRODUCTION

The generation of cell diversity among groups of equipotent cells is a fundamental aspect of the development of multicellular organisms. The *Drosophila* adult peripheral nervous system provides an excellent experimental system in which to address this problem genetically. Each external sense organ of the adult fly is composed of only a few cells that in most cases are the progeny of a single sensory organ precursor (SOP) cell (Hartenstein and Posakony, 1989). SOPs are determined during the late larval and early pupal stages within undifferentiated epithelial sheets, the imaginal discs, which will give rise to cuticular structures of the adult fly. In the first step of this determination process, the spatially restricted activities of the *achaete* (*ac*) and *scute* (*sc*) genes confer upon 'proneural clusters' of cells the competence to adopt the SOP fate (Cubas et al., 1991; Skeath and Carroll, 1991). Subsequently, one proneural cluster cell is singled out to become the future SOP, while the remaining cells of the cluster are inhibited from expressing this fate by local cell-cell interactions, referred to as lateral inhibition.

Proper singularization of the SOP requires the activity of multiple loci of the neurogenic group (Dietrich and Campos-Ortega, 1984; Hartenstein and Posakony, 1990; Heitzler and Simpson, 1991; Parks and Muskavitch, 1993; Simpson, 1990), as well as the functions of the genes *Hairless* (*H*) (Bang et al., 1991) and *Suppressor of Hairless* [*Su(H)*] (Schweisguth and Posakony, 1992). *H* activity is required for the stable determination of the SOP cell fate, and flies carrying null or strong hypomorphic alleles of *H* display an extensive 'bristle loss' phenotype (Bang et al., 1991). In addition, *H* acts as an antagonist of neurogenic gene function (Vässin et al., 1985; A. Bang and J. W. P., unpublished results), and an excess of *H* activity causes phenotypes that closely resemble neurogenic gene loss-of-function phenotypes (Bang and Posakony, 1992). Conversely, *Su(H)*, a dominant modifier of *H* (Ashburner, 1982), acts as a neurogenic gene, in that it is required to limit the expression of the SOP fate among proneural cluster cells (Schweisguth and Posakony, 1992).

Following its stable determination, the SOP follows a stereotyped pattern of division (Hartenstein and Posakony, 1989). The precursor cell for a typical mechanosensory bristle divides to yield two secondary precursor cells; one gives rise to the sensory neuron and thecogen cell, while the other gives rise to the trichogen and tormogen cells. The three non-neuronal cells form concentric sheaths around the dendrite of the neuron and produce the stimulus-receiving cuticular structures. In particular, the trichogen and tormogen cells produce the bristle shaft and the socket that surrounds the base of the shaft, respectively. The adoption of unique cell fates by the four sensory organ

cells requires the neurogenic genes *Notch* (*N*) (Hartenstein and Posakony, 1990) and *Delta* (*Dl*) (Parks and Muskavitch, 1993), as well as *H*. Partial loss of *H* function leads to a nearly complete transformation of the trichogen (shaft) cell into a second tormogen (socket) cell, yielding a 'double socket' phenotype (Bang et al., 1991; Lees and Waddington, 1942), while a gain of *H* function results in the converse transformation and produces a 'double shaft' phenotype (Bang and Posakony, 1992). A reduction of *Su(H)* dosage suppresses the *H* double socket effect (Schweisguth and Posakony, 1992); however, a strict requirement for *Su(H)* activity in this late cell fate choice has not yet been demonstrated.

The *H* gene encodes a novel basic protein of unknown function (Bang and Posakony, 1992; Maier et al., 1992). *Su(H)* is the *Drosophila* homologue of the mouse *J$_\kappa$–Recombination signal Binding Protein* gene (*J$_\kappa$–RBP*) (Furukawa et al., 1992; Schweisguth and Posakony, 1992). The J$_\kappa$–RBP and Su(H) proteins are 82% identical over most of their length, and are likely to share an as yet unidentified biochemical activity involving sequence-specific DNA binding.

MATERIALS AND METHODS

Drosophila stocks

Flies were cultured on standard yeast-cornmeal-molasses-agar medium at 25°C. Mutant alleles of *Suppressor of Hairless* [*Su(H)*; 2-50.5] and the *Su(H)* deficiency *Df(2L)TE35BC-24* are described in Schweisguth and Posakony (1992) and in Lindsley and Zimm (1992). The 8xSu(H) line was derived by recombination and conventional crosses using three independent P[w^+, *Su(H)*$^+$] transposon insertion lines (C1, C3 and C11; Schweisguth and Posakony, 1992). The number of P transposon insertions in the 8xSu(H) line was verified by genomic Southern blot analysis (not shown). Mutant alleles of *Hairless* (*H*) are described in Bang et al. (1991), except for H^{E31}. The E31 allele was isolated in an F$_1$ screen for *H* alleles induced by the imprecise excision of the D179 P[w^+, *lacZ*] enhancer trap transposon inserted in the 5′ untranslated region of the *H* coding sequence (Bang and Posakony, 1992). Genomic Southern blot analysis indicates that E31 corresponds to a small deficiency, from the w^+ minigene through about two-thirds of the *H* protein coding sequence (unpublished results). For the *Su(H)/H* epistasis experiment, *CyO* and *TM3* balancer chromosomes carrying a P[w^+, *actin-lacZ*] transposon (kindly provided by J. Thomas) were used to balance the *Su(H)* and *H* mutant chromosomes. Male flies of the following genotype were analyzed in the FLP recombination experiment (Golic and Lindquist, 1989; Xu and Rubin, 1993): *y w hsFLP1/Y; Su(H)^{SF8} A1-2-29 P[ry^+; hs-neo; FRT]40A/P[ry^+; y^+]25F P[ry^+; hs-neo; FRT]40A*. They were F$_1$ progeny of a cross between *y w hsFLP1; P[ry^+; y^+]25F P[ry^+; hs-neo; FRT]40A* females and *w^{1118}; Su(H)^{SF8} A1-2-29 P[ry^+; hs-neo; FRT]40A/CyO* males [the enhancer trap insertion A-1-2-29 (see below) is fully viable when homozygous, and was used in this experiment only as a w^+ recombination marker]. Flies of the genotype w^{1118} were used as wild-type controls. The enhancer trap transposon insertions P[ry^+, *lacZ*]A37 (kindly provided by Y. Hiromi and C. O'Kane), P[ry^+, *lacZ*]A101 (kindly provided by H. Bellen), and P[w^+, *lacZ*]A-1-2-29 (kindly provided by Y. N. Jan) were used as markers for sensory organ precursor cells and their progeny (Bang et al., 1991; Ghysen and O'Kane, 1989; Hartenstein and Jan, 1992; Huang et al., 1991). An *achaete-lacZ* fusion gene (Van Doren et al., 1992) and the *scabrous* P[ry^+, *lacZ*] enhancer trap transposon insertion A2-6 (Mlodzik et al., 1990) were used as proneural cell markers. Mutations and chromosomes not described herein are described in Lindsley and Zimm (1992).

Identification of mutant larvae

Trans-heterozygous *Su(H)* and *H* mutant larvae are identified unambiguously using the dominant *Black cells* (*Bc*) and *Tubby* (*Tb*) mutations (Bang et al., 1991; Schweisguth and Posakony, 1992). In some cases, mutant genotypes were also confirmed by observing the characteristic wing pouch phenotypes associated with *H* and *Su(H)* mutations (see Fig. 5). In the *Su(H)/H* epistasis experiment, imaginal discs from double mutant larvae were identified as those lacking the strong β-galactosidase activity staining that results from the expression of the *actin-lacZ* fusion gene carried by the balancer chromosomes.

Germline transformation

A 2.8 kb *Hin*dIII/*Xba*I full-length *Su(H)* cDNA fragment was isolated from plasmid pKS12 (Schweisguth and Posakony, 1992). The *Hin*dIII terminus was converted into an *Xba*I site by filling in using the Klenow polymerase, followed by the addition of a phosphorylated *Xba*I linker (NEB). The resulting *Xba*I/*Xba*I fragment was then cloned into the CaSpeR-*Hsp70* transformation vector (Bang and Posakony, 1992) at the unique *Xba*I site. The resulting P[w^+, *Hs-Su(H)*] transposable element was introduced into the germline of w^{1118} recipient embryos by coinjection with a Δ2-3 helper plasmid (Rubin and Spradling, 1982). Eleven stocks, designated P[*Hs-Su(H)*]–1 to –11, were established from independent w^+ G1 adults.

Heat-shock treatment

Staged pupae were placed in a humid chamber and subjected to heat shock using a precise temperature-controlled water bath. Heat-shocked animals were then returned to 25°C and allowed to develop. Pharate adults (manually removed from the pupal case) and newly eclosed adults were dissected and prepared for light microscopy as described (Bang and Posakony, 1992).

β-galactosidase activity staining

Histochemical staining for β-galactosidase activity was carried out as described by Romani et al. (1989).

Antibody staining

Immunodetection of the ac protein was carried out according to a protocol provided by J. Skeath. Imaginal discs were dissected in phosphate-buffered saline (PBS), fixed overnight in 2% formaldehyde in 'Brower fix' (100 mM Pipes, 2 mM $MgSO_4$, 1 mM EGTA, 0.1% NP40, pH 6.9), and then washed in PBS containing 0.3% Triton X-100 (PBS-T). The tissue was incubated overnight with the primary antibody (Skeath and Carroll, 1991) diluted at 1:50 in PBS-T, washed in PBS-T over a 1-hour period, incubated for 90 minutes with the biotinylated anti-mouse secondary antibody (Vector) diluted at 1:200 in PBS-T and then washed. All fixation, incubation and wash steps were at 4°C. Peroxidase activity staining was carried out using the Elite kit (Vector) at room temperature over a period of a few hours. Immunostaining using the mouse monoclonal antibodies 22C10 [a specific marker for SOP progeny cells (Hartenstein and Posakony, 1989)] and 202 (anti-Delta) was performed as follows. Dissected tissues were fixed for 20 minutes in 4% paraformaldehyde in PBS at room temperature. The monoclonal primary antibody was diluted at 1:100 and incubated overnight at 4°C or 2 hours at room temperature. Secondary antibody reaction and peroxidase staining were performed using the anti-mouse Elite kit (Vector). For the rabbit polyclonal anti-HRP serum (Cappel), a 1:1000 dilution was used. Anti-rabbit secondary antibody coupled to alkaline phosphatase (Biosys) was used at a 1:1000 dilution. β-galactosidase staining was carried out as described above immediately following the immunostaining reaction.

RESULTS

Su(H) function is strictly required for the determination of the socket-producing tormogen cell

Loss-of-function alleles of *Su(H)* are potent dominant suppressors of the *H* 'double socket' phenotype (Schweisguth and Posakony, 1992; unpublished results), suggesting that reduction of *Su(H)* activity favors the trichogen (shaft) cell fate. Consistent with this interpretation, 'double shaft' macrochaetes are occasionally found on flies *trans*-heterozygous for the *Su(H)* hypomorphic allele HG36 and a null allele (Fig. 1A). We have directly investigated the role of *Su(H)* in the trichogen/tormogen cell fate choice by generating somatic clones of homozygous *Su(H)* mutant cells in an otherwise heterozygous fly, using the FLP/FRT method developed by Golic and Lindquist (1989) and Xu and Rubin (1993). Briefly, the yeast FLP recombinase, expression of which is driven by the *Drosophila Hsp70* promoter, catalyzes site-specific recombination between two FRT sites inserted at the base of chromosome arm 2L, at cytological position 40A (Xu and Rubin, 1993). One of the two FRT-bearing chromosomes is also mutant for *Su(H)* (at 35B9-10); the null allele SF8 was used in this study. The other homologue carries a P element transposon that includes a wild-type copy of the cuticular marker gene *yellow* (*y*) (inserted at 25F; Xu and Rubin, 1993), and the experiment is carried out in a y^- genetic background. Upon heat induction of FLP-mediated recombination, y^- *Su(H)*$^-$ mutant clones are generated, and their phenotypes on the adult cuticle can be analyzed.

Two bristle phenotypes are observed in the mosaic flies. When recombination is induced in first and second instar larvae, patches of naked cuticle are found (the cellular defects associated with this phenotype will be presented elsewhere). Recombination events induced later in development (i.e., in third instar larvae) predominantly yield double-shaft bristles. All these double-shaft bristles are y^-, indicating that they are homozygous mutant for *Su(H)*. Double-shaft bristles may be found at every head and notum macrochaete position (Fig. 1B), and at microchaete positions on the notum and abdomen (Fig. 1C). They almost always appear individually, except on the abdomen, where clones containing several double-shaft microchaetes may be observed (Fig. 1C). Thus, it is likely that this 'double shaft' phenotype is primarily associated with late-arising somatic clones of *Su(H)* mutant cells. We conclude that a reduction in *Su(H)* activity may result in the transformation of the tormogen (socket) cell into a second trichogen (shaft) cell, just as is observed with an excess of *H* activity (Bang and Posakony, 1992).

High levels of achaete protein accumulation and proneural activity in *Su(H)* mutant proneural cluster cells

In the complete absence of *Su(H)* function, most or all cells in imaginal disc proneural clusters express the SOP-specific markers A37 and A101, indicating that, like certain genes of the neurogenic group, *Su(H)* is required early in adult sensory organ development to restrict the expression of the SOP fate (Schweisguth and Posakony, 1992). However, it has not been determined which other characteristics of wild-type SOPs are exhibited by these *Su(H)* mutant proneural cluster cells.

One distinguishing feature of normal SOPs is that they accumulate a higher level of proneural proteins than the other cells in the proneural cluster (Skeath and Carroll, 1991). Using an anti-ac monoclonal antibody (a generous gift of J. Skeath and S. Carroll; Skeath and Carroll, 1991), we have found that, in *Su(H)* mutant imaginal discs, most or all of the cells in the proneural clusters accumulate ac protein at a high level (Fig. 2A). These clusters of strongly ac-positive cells appear identical in size and location to the clusters shown previously to express the SOP-specific marker A101 (Schweisguth and Posakony, 1992).

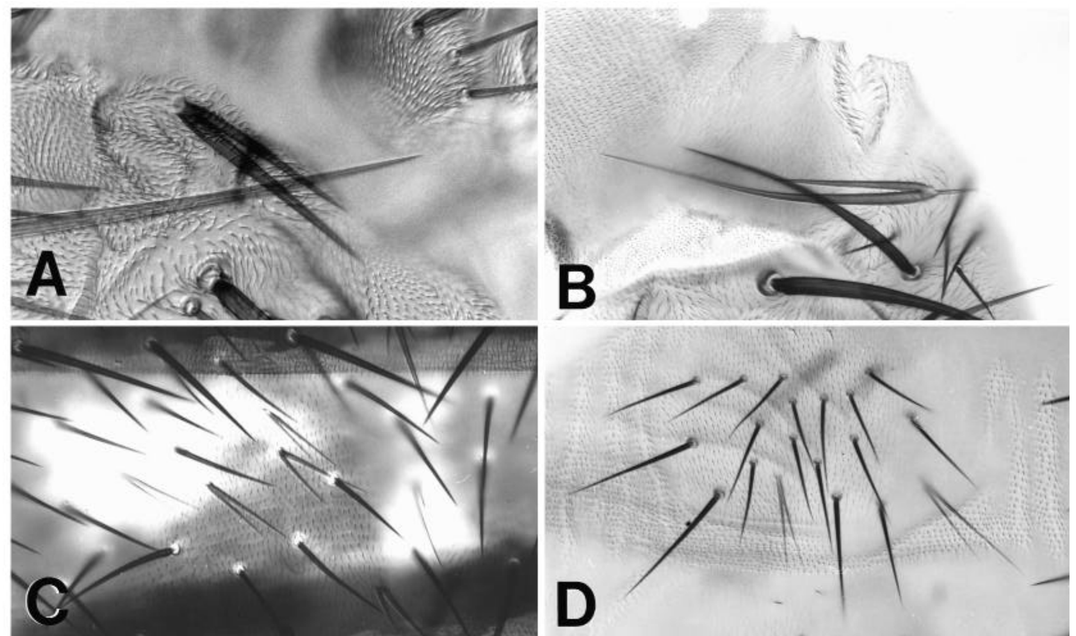

Fig. 1. Partial or late loss of *Su(H)* activity causes a 'double shaft' bristle phenotype. Light micrographs of adult cuticle preparations showing the notopleural region of a *Su(H)*^{SF8}/*Su(H)*^{HG36} pharate adult (A); a humeral macrochaete (B); and tergite (C) and sternite (D) abdominal macrochaetes from *y w hsFLP1/Y; Su(H)*^{SF8} *A1-2-29 P[ry*$^+$*; hs-neo; FRT]40A/P[ry*$^+$*; y*$^+$*]25F P[ry*$^+$*; hs-neo; FRT]40A* male flies, after FLP-mediated recombination is induced in early third instar larvae (2-hour heat shock at 37°C). In B-D, all double shaft bristles are phenotypically yellow, and thus develop from clones of *Su(H)* mutant cells. The clone borders in the epidermis cannot be reliably distinguished using the *P[ry*$^+$*; y*$^+$*]25F* marker, except in the strongly melanized tergites (C).

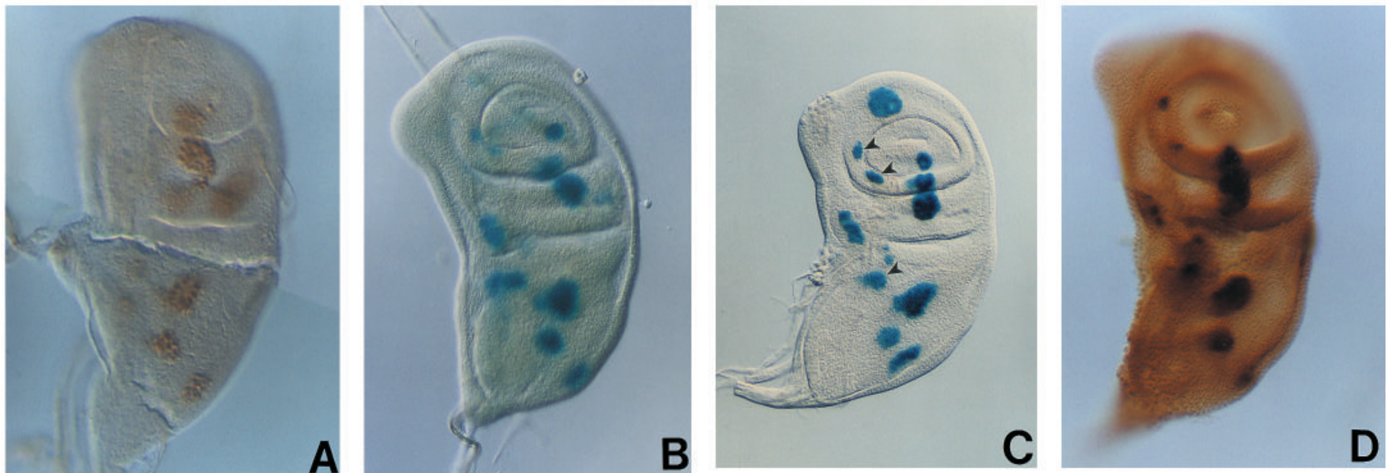

Fig. 2. Spatial patterns of achaete and Delta protein accumulation and of proneural gene activity in *Su(H)* mutant late third instar wing imaginal discs. Nomarski micrographs of *Su(H)* mutant discs after immunostaining (A,D) or β-galactosidase-staining (B,C) reactions. (A) Distribution of ac protein in a $Su(H)^{SF8}/Df(2L)TE35BC\text{-}24$ disc. A pattern similar to that shown is also observed in $Su(H)^{SF8}/Su(H)^{AR9}$ mutant wing discs. (B) *lacZ* activity in an $ac\text{-}lacZ\ Su(H)^{SF8}/ac\text{-}lacZ\ Su(H)^{AR9}$ imaginal disc. (C) *lacZ* activity in an $A2\text{-}6\ Su(H)^{SF8}/A2\text{-}6\ Su(H)^{AR9}$ imaginal disc. (D) Distribution of Dl protein in a $Su(H)^{SF8}/Su(H)^{AR9}$ imaginal disc. High levels of ac and Dl protein accumulation (A,D) and of proneural regulatory activity (B,C) are observed in clusters of cells in a pattern very similar to that described previously for the A101 and A37 SOP markers (Schweisguth and Posakony, 1992). Additional proneural clusters, indicated by arrowheads in (C), are detected using these 'early' markers, compared to A101 and A37 (Schweisguth and Posakony, 1992). These newly detected clusters normally give rise to SOPs that appear next in the wild-type sequence of SOP emergence described by Huang et al. (1991), consistent with our earlier interpretation that a general arrest in the development of the *Su(H)* mutant wing disc may prevent detection of late-arising SOP clusters (Schweisguth and Posakony, 1992).

The elevated accumulation of ac protein in $Su(H)^-$ proneural clusters is accompanied by a high level of proneural activity in these cells. An *ac-lacZ* fusion gene that is expressed under the direct positive control of *ac* and *sc* in wild-type discs (Van Doren et al., 1992) is activated at a high level in *Su(H)* mutant proneural clusters (Fig. 2B). The enhancer-trap marker A2-6, inserted at the *scabrous* (*sca*) gene, is expressed specifically in the proneural clusters of wild-type discs, and this expression is largely dependent on *ac* and *sc* (Mlodzik et al., 1990; data not shown). Moreover, the single SOPs that arise from wild-type proneural clusters exhibit an elevated level of A2-6 expression relative to the remaining cells of the cluster (Mlodzik et al., 1990). Fig. 2C shows that A2-6 is strongly expressed in most or all cells in $Su(H)^-$ proneural clusters, further indicating that proneural activity is high in these cells.

Finally, we examined the expression of the neurogenic gene *Dl*, using an anti-Dl antibody (mAb 202, generated in the laboratory of S. Artavanis-Tsakonis and kindly provided by M. Muskavitch). High levels of accumulation of the Dl protein are detected in most *Su(H)* mutant proneural cluster cells (Fig. 2D).

We conclude that high levels of ac protein accumulation and proneural gene activity are found together with high-level expression of the neurogenic protein Dl in all, or most, proneural cluster cells adopting the SOP fate in *Su(H)* mutant wing discs. Recent data from our laboratory indicate that loss of *H* function has the opposite effect: In H^- imaginal discs, the single presumptive SOP cell fails to maintain high levels of ac protein and proneural regulatory activity (A. Bang and J. W. P., unpublished data). These results support the conclusion that *H* and *Su(H)* have opposing functions in controlling the expression of the SOP fate (Bang et al., 1991; Bang and Posakony, 1992; Schweisguth and Posakony, 1992).

Gain of *Su(H)* function results in 'bristle loss' and 'double socket' phenotypes

The dominant modification of *H* mutant phenotypes by both gain and loss of *Su(H)* function led Ashburner (1982) to suggest that *Su(H)* acts as a negative regulator of *H* activity. The molecular cloning of the *Su(H)* gene (Schweisguth and Posakony, 1992) allows us to test whether an increase in *Su(H)* activity in an otherwise wild-type fly may lead to phenotypes similar to those associated with *H* loss-of-function mutations.

Previously, we described a P element transposon carrying a *Su(H)* genomic DNA fragment that rescues all aspects of the *Su(H)* null phenotype in transformed flies, and provides $Su(H)^+$ activity at a level quantitatively similar to the endogenous gene (Schweisguth and Posakony, 1992). We have established a transformant line that is homozygous for three copies of this transposon; this line thus carries the equivalent of 8 $Su(H)^+$ doses per diploid genome and is called 8xSu(H). As shown in Fig. 3A, many bristles on the head and thorax of 8xSu(H) adult flies exhibit a 'double socket' phenotype similar to that observed in *H* hypomorphic mutants. In addition, specific macrochaetes fail to appear; these correspond to those that are most sensitive to loss of *H* function (e.g., postvertical and humeral bristles; see Bang et al., 1991). The finding that *Su(H)* hyperactivity is more effective in interfering with trichogen cell differentiation than with expression of the SOP fate is consistent with the greater sensitivity of the trichogen/tormogen decision versus the SOP/epidermal decision to loss-of-function mutations in *H* (Bang et al., 1991).

We further investigated these cell fate changes using an inducible system for overexpression of *Su(H)*. A *Hs-Su(H)* fusion gene, consisting of a full-length *Su(H)* cDNA under the control of the *Hsp70* heat-shock promoter, was introduced into

the genome by P element-mediated germline transformation. The resulting *P[Hs-Su(H)]* transformant lines do not generally exhibit detectable bristle defects in the absence of heat-shock treatment (Fig. 3B). One interesting exception is line P[*Hs-Su(H)*]-8, which in heterozygous condition exhibits a fully penetrant 'double socket' phenotype at macrochaete positions in the posterior part of the notum (Fig. 3E). We applied a 6-hour heat-shock regimen (3×60 minutes at 37°C, separated by two 90-minute intervals at 25°C) to *P[Hs-Su(H)]* pupae at 16, 20 or 24 hours APF; i.e., during and following the division of microchaete precursor cells but well after the macrochaete cells have begun their differentiation (Hartenstein and Posakony, 1989). The resulting pharate adults exhibit a bristle phenotype very similar to the *H* double socket phenotype at most notum

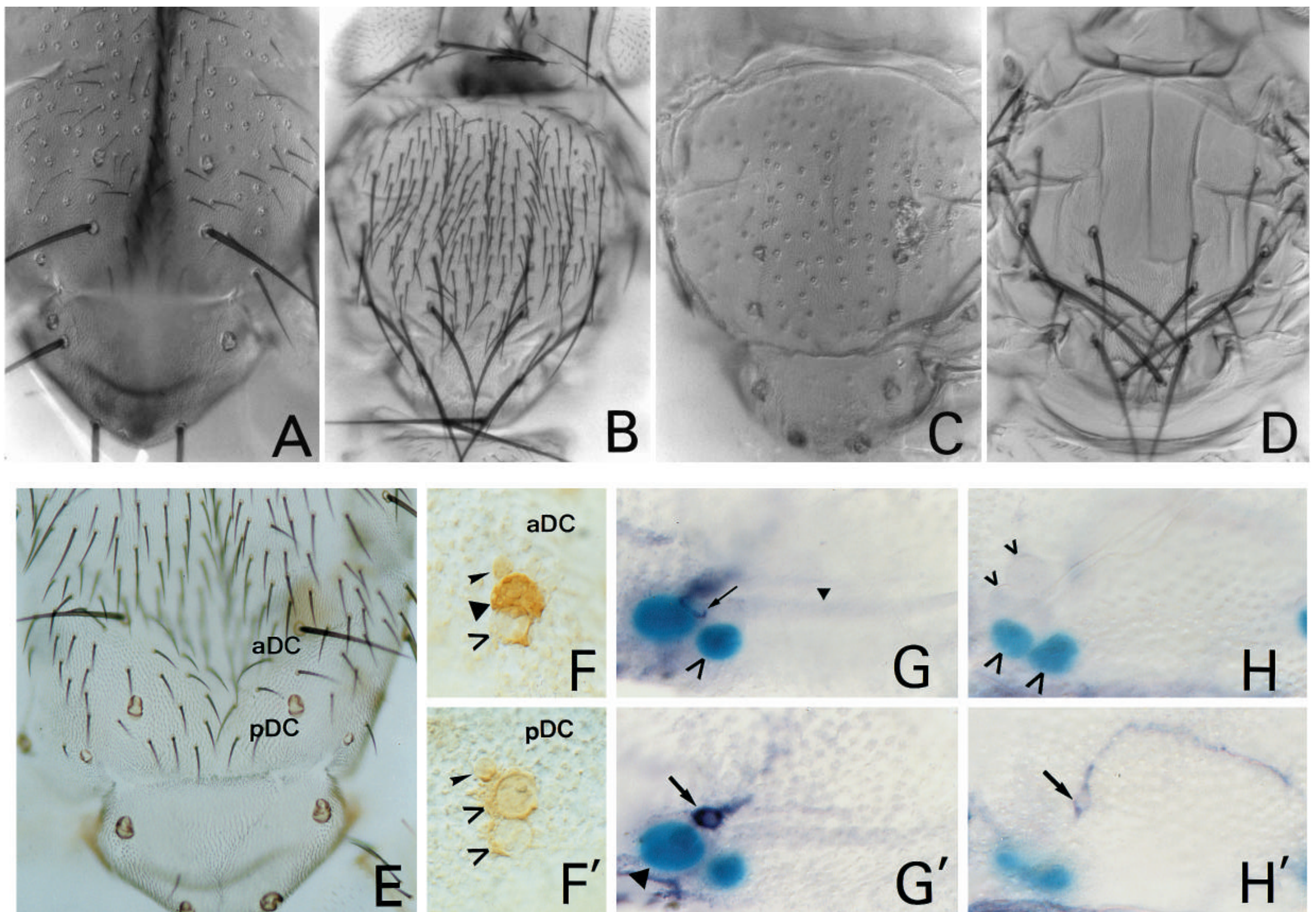

Fig. 3. Phenotypic consequences of overexpression of *Su(H)*. Nomarski micrographs of nota dissected from pharate or eclosed adults of the following genotypes: (A) 8xSu(H). (B-D) P[*Hs-Su(H)*]-1/P[*Hs-Su(H)*]-1. (E,F,F′) P[*Hs-Su(H)*]-8/*CyO*. (G,G′) P[w^+, *lacZ*]A-1-2-29/P[w^+, *lacZ*]A-1-2-29. (H,H′) P[*Hs-Su(H)*]-8 P[w^+, *lacZ*]A-1-2-29/*CyO*. G-G′ and H-H′ each show two different focal planes at the posterior scutellar (pSC) macrochaete position. An extensive 'double socket' phenotype is observed in 8xSu(H) flies (A), in P[*Hs-Su(H)*]-1 homozygotes subjected to a 6-hour heat-shock regimen starting at 20 or 24 hours APF (C), and in P[*Hs-Su(H)*]-8/*CyO* flies (E). This effect appears to result from the transformation of the trichogen (shaft) cell into a second tormogen (socket) cell. As shown in F, a normal aDC macrochaete stained at 24 hours APF with mAb 22C10 includes two polyploid cells (large nuclei): one is strongly stained, with a subepidermal cell body (the trichogen, indicated by a large arrowhead); the other is more lightly stained, with its cell body in the epidermal plane (the tormogen, indicated by an open arrowhead). By contrast, a double-socket pDC macrochaete from the same animal (F′) shows two lightly stained, polyploid cells within the epidermal plane (i.e., two tormogens, indicated by open arrowheads); this is characteristic of double-socket bristles in *H* mutants (Bang et al., 1991). At least one smaller 22C10-positive cell (neuron/thecogen, indicated by a small arrowhead) can be detected in both normal and double-socket bristles (F,F′). Similar results are obtained by comparing at 40 hours APF a normal pSC macrochaete from a P[w^+, *lacZ*]A-1-2-29/P[w^+, *lacZ*]A-1-2-29 pupa (G,G′) with a double-socket pSC bristle from a P[*Hs-Su(H)*]-8 P[w^+, *lacZ*]A-1-2-29/*CyO* pupa (H,H′). The shaft of the normal bristle is indicated by a small filled arrowhead (G); the two sockets of the mutant bristle are indicated by small open arrowheads (H). A single (G) and two (H) large epidermal nuclei (i.e., tormogens, indicated by large open arrowheads) are detected at normal and double-socket bristle positions, respectively, while a large subepidermal nucleus (i.e., trichogen, indicated by a large filled arrowhead) is observed only in the wild-type bristle (G′). A single neuron, indicated by a large arrow, is observed at both normal (G′) and double-socket (H′) pSC positions; note the dendrite at the base of the shaft in G (small arrow). The 'bristle loss' phenotype shown in D results from overexpression of *Su(H)* during the time of microchaete SOP determination (90-minute heat shock at 36.5°C, starting at 6 hours APF). Comparable microchaete loss effects were observed in eight of nine P[*Hs-Su(H)*] lines tested.

microchaete and macrochaete positions (Fig. 3C). This effect is still detectable when the heat shock is commenced at 26 hours APF, 10 hours after the division of the microchaete secondary precursors. Such late heat shocks cause many microchaete shafts to be shortened and thickened, with socket-like structures at their base (data not shown). No effect on either macrochaete or microchaete differentiation is detectable with heat shocks applied after 32 hours APF. Thus, the critical developmental period during which *Su(H)* overexpression is able to produce the double socket effect in microchaetes (16-32 hours APF) is in good agreement with the period of microchaete accessory cell differentiation (Hartenstein and Posakony, 1989).

The cellular basis of the double socket defect was examined in pupal nota of the P[*Hs-Su(H)*]-8 transformant line (Fig. 3E). At wild-type macrochaete positions, using mAb 22C10 as a marker in 24 hours APF pupae, the trichogen cell is detected as a strongly stained, subepidermal, polyploid cell, while the tormogen cell lying above it appears more faintly stained (Hartenstein and Posakony, 1989; Fig. 3F). At double socket macrochaete positions in the same pupae, by contrast, mAb 22C10 detects two and only two lightly stained, polyploid cells in the same epidermal plane (Fig. 3F′). This indicates that the trichogen cell is transformed into a second tormogen cell. We also used the enhancer trap insertion P[w^+, *lacZ*]A-1-2-29 as a specific marker for the trichogen and tormogen cells (Hartenstein and Jan, 1992). In wild-type pupae at 40 hours APF, two β-galactosidase-positive polyploid nuclei are detected at the pSC position; the tormogen cell nucleus appears in the epidermal plane (Fig. 3G), while the trichogen cell nucleus is located subepidermally (Fig. 3G′). In P[*Hs-Su(H)*]-8 pupae, again only two β-galactosidase-positive polyploid nuclei are observed at the 'double socket' pSC position; however, these two nuclei are in the same (epidermal) plane (Fig. 3H). Finally, a single neuron is revealed by an anti-HRP polyclonal antiserum at the pSC position in both wild-type and P[*Hs-Su(H)*]-8 pupae (Fig. 3G′,H′). These results indicate that the *P[Hs-Su(H)]* 'double socket' phenotype results from the specific transformation of the shaft-producing subepidermal trichogen cell into a second socket-producing epidermal cell (tormogen), while the sensory neuron/thecogen half of the lineage does not seem to be affected.

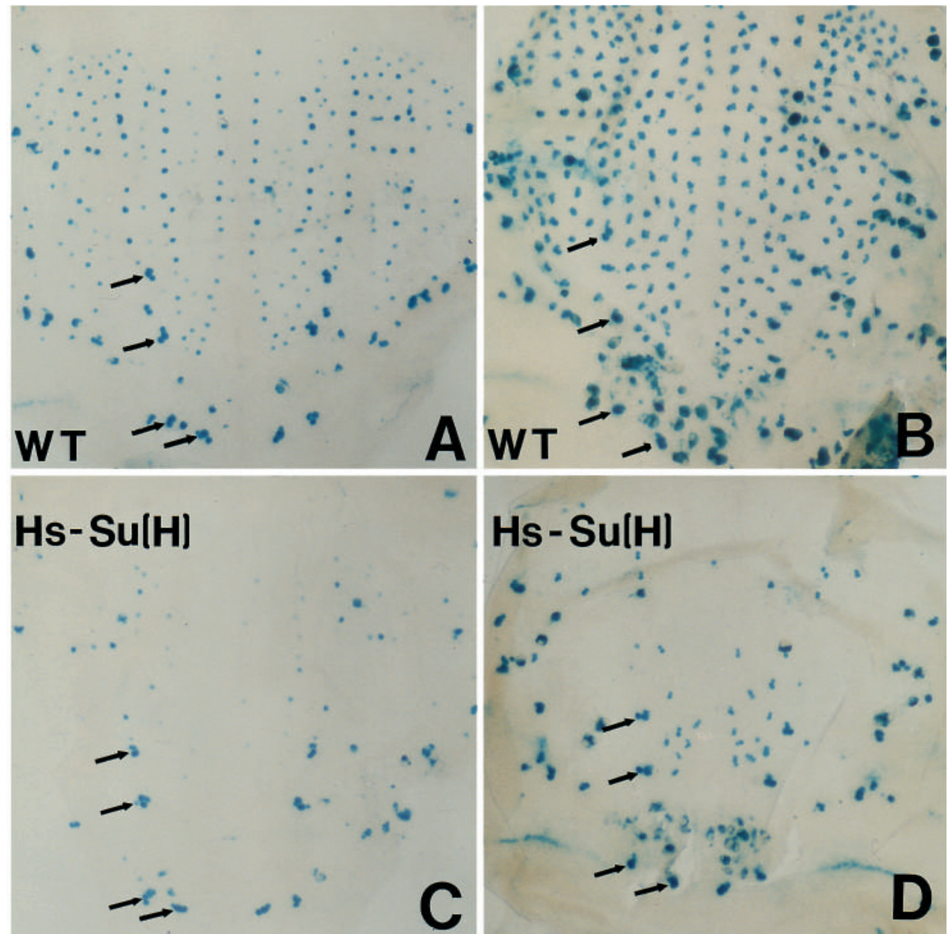

Fig. 4. Failure of SOP development is the cellular basis of the microchaete loss phenotype associated with overexpression of *Su(H)*. Non-Tb progeny of a cross between P[*Hs-Su(H)*]-1/*TM6* virgin females and A101/*TM6B, Tb* males were heat shocked at 6 hours APF (90 minutes at 36.5°C); see Fig. 3D. The nota of these animals were dissected and stained for β-galactosidase activity at 14 hours APF (A,C) or 24 hours APF (B,D). Two distinct *lacZ* activity patterns were observed in approximately equal numbers. These patterns correspond to A101/*TM6* (A,B) and P[*Hs-Su(H)*]-1/A101 (C,D) pupae. Unambiguous genotypic identification was obtained in a parallel experiment using a third chromosome balancer carrying a *lacZ* marker (*TM3, Sb* P[w^+, *act5C-lacZ*]) in trans to the P[*Hs-Su(H)*]-1 chromosome. Arrows indicate the dorsocentral and scutellar macrochaete positions. In the control pupae (A101/*TM6*), the notum contains a large number of single cells, arranged in a reproducible pattern of regular rows, that are β-galactosidase-positive at 14 hours APF (A); these cells are microchaete SOPs (Bang et al., 1991). Pupae of the same genotype not subjected to heat shock exhibit a very similar pattern of A101 expression (data not shown). At this stage, macrochaete SOPs have divided twice to produce the four daughter cells (all A101-positive) that comprise the bristle organ. At 24 hours APF (B), the microchaete SOPs have divided, and four cells are now detectable at microchaete positions. Overexpression of *Su(H)* dramatically affects SOP development (C,D). At 14 hours APF, many fewer cells in the microchaete field express A101, and those that do show a very low level of *lacZ* activity (C). By contrast, A101 expression is apparently unaffected in the progeny of the macrochaete SOPs. At 24 hours APF, A101 expression is observed at only a small number of notum microchaete positions (D); the level appears similar to that of control flies (B). Only two or three A101-positive cells are observed at many microchaete positions, instead of the usual four, suggesting that overexpression of *Su(H)* not only interferes with SOP determination but might also delay or disrupt the execution of the SOP fate.

An earlier, shorter heat shock (90 minutes at 36.5°C) applied at 6 hours APF, prior to the first division of the microchaete SOPs (Hartenstein and Posakony, 1989), results in the loss of nearly all notum microchaetes (Fig. 3D). Macrochaetes appear to be unaffected by this treatment, consistent with the earlier specification of macrochaete SOPs in late third instar larvae and early pupae (Hartenstein and Posakony, 1989; Huang et al., 1991). We determined the cellular basis of the microchaete loss phenotype by following the development of microchaete SOPs in *P[Hs-Su(H)]* flies using the A101 enhancer-trap marker (Fig. 4). A101/*P[Hs-Su(H)]*-1 and control A101/TM6 pupae were heat shocked at 6 hours APF (90 minutes at 36.5°C), and their dissected nota stained for β-galactosidase activity at 14 or 24 hours APF. At 14 hours APF, nearly all microchaete SOPs of A101/TM6 pupae are A101-positive, while only a few weakly stained cells are detected in A101/*P[Hs-Su(H)]*-1 pupae (Fig. 4A,C). At 24 hours APF, again only a reduced number of A101-expressing cells are observed at microchaete positions in P[*Hs-Su(H)*]-1/A101 pupae compared to the full pattern of bristle cells in A101/TM6 individuals (Fig. 4B,D). We conclude that, by the criterion of A101 expression, the microchaete loss phenotype observed in *P[Hs-Su(H)]* adults most likely results from a failure of SOP determination. Thus, both loss of *H* function (Bang et al., 1991) and hyperactivity of *Su(H)* lead to adult bristle loss by interfering with the expression of the SOP cell fate.

An intermediate SOP phenotype in *Su(H); H* double mutant imaginal discs

The finding that both loss- and gain-of-function mutations of *H* and *Su(H)* have opposite effects on the SOP/epidermal and trichogen/tormogen cell fate decisions raises the question of their epistatic relationship. To address this issue, we examined SOP determination in the imaginal discs of late third instar larvae carrying null mutations in both genes, using the A37 enhancer-trap as an SOP marker. In imaginal discs, A37 is specifically expressed in SOP cells and their progeny (Bang et al., 1991; Ghysen and O'Kane, 1989; Huang et al., 1991; see Fig. 5A). Single A37-expressing cells are occasionally detected in *H* mutant wing discs at the dorsal radius (dR) and posterior scutellar (pSC) positions, and at the prospective anterior wing margin (Fig. 5B; see also Bang et al., 1991). By contrast, five large clusters of A37-positive cells are consistently observed in *Su(H)* mutant wing discs at positions where single SOPs normally arise (Fig. 5C). These clusters appear to correspond to the ventral radius sensillum (vR), the second campaniform sensillum of vein L3 (L3-2), the giant sensillum of the radius (GSR), the dorsal radius sensillum

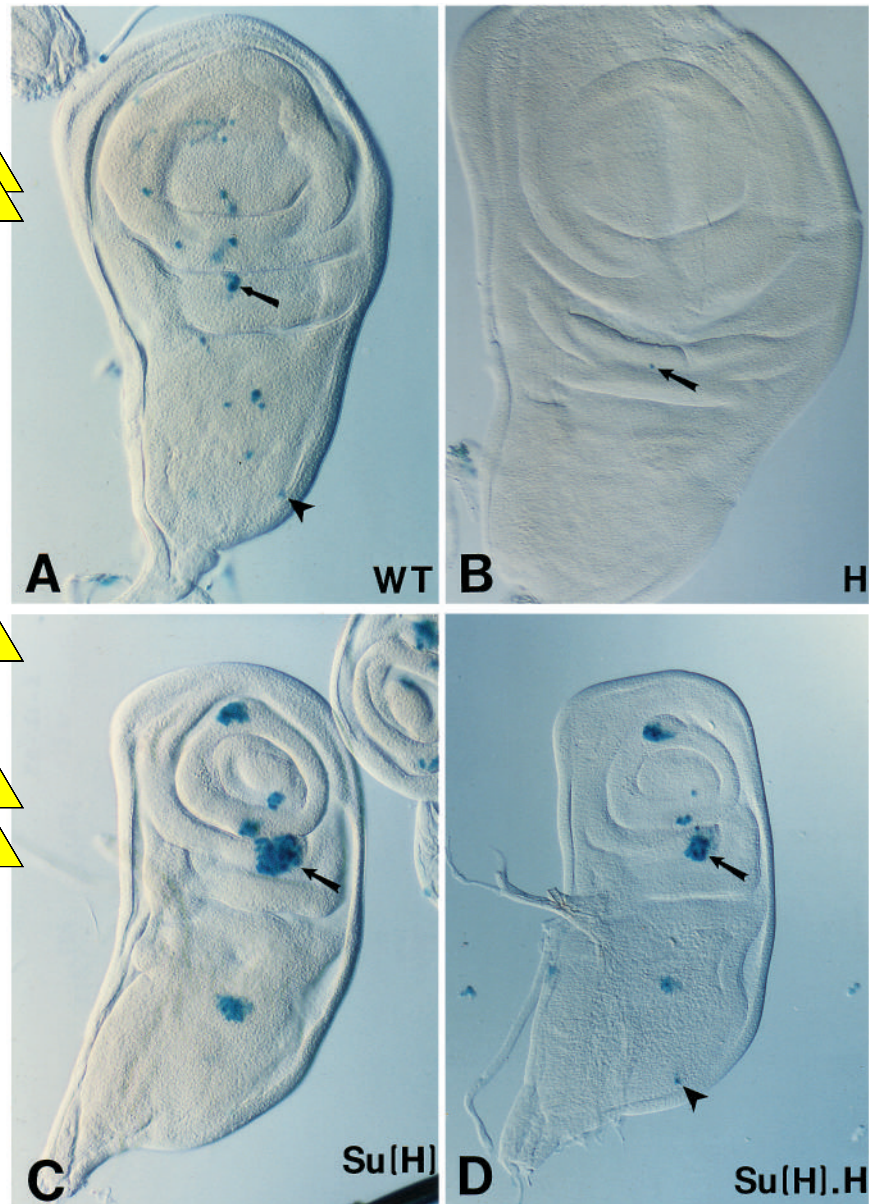

Fig. 5. Epistatic relationship between *H* and *Su(H)* in SOP determination. Nomarski micrographs of wing imaginal discs from late third instar larvae of the following genotypes: (A) A37/A37. (B) A37 H^{E31}/A37 H^{2}. (C) $Su(H)^{SF8}/Su(H)^{AR9}$; A37/A37. (D) $Su(H)^{SF8}/Su(H)^{AR9}$; A37 H^{E31}/A37 H^{2}. Discs are shown at the same magnification. Arrow (A-D) indicates the position of the dorsal radius (dR) sensillum, while the arrowhead (A,D) indicates the posterior scutellar (pSC) bristle precursor cell. Detection of *lacZ* activity at the pSC position in D is an indication of the age of the disc [see Huang et al. (1991) for a description of the spatio-temporal pattern of SOP development]. Although by this criterion the double mutant disc (D) appears at least as old as the *Su(H)* mutant disc (C), the size of the SOP clusters in D appears reduced relative to those in C. As previously reported (Bang et al., 1991; Schweisguth and Posakony, 1992), *H* and *Su(H)* loss-of-function mutations have dramatic, and opposite, phenotypic effects on the size and morphology of the wing pouch region of the late third instar wing disc. The double mutant disc (D) appears most similar in size and morphology to the *Su(H)* mutant disc (C), suggesting that *Su(H)* may be more clearly epistatic to *H* for this aspect of their phenotype.

(dR), and the posterior postalar bristle (pPA). Faint β-galactosidase staining is sometimes detected at the pSC cluster position. In the *Su(H); H* double mutant, four to six clusters of A37-expressing cells are reproducibly observed (Fig. 5D). These include positions where *lacZ* activity can be detected in the *H* mutant disc (dR and pSC), as well as positions where *lacZ* expression is observed in *Su(H)* but not *H* mutant discs (vR, L3-2, GSR, pPA). In these latter positions, however, A37-positive cells appear significantly reduced in number in the double mutant. It appears, first, that cells that normally strictly require *H* function to express the A37 marker may adopt the SOP fate in the absence of both *H* and *Su(H)* activity; and second, that some of the cluster cells that would express the SOP fate in a *Su(H)* mutant disc apparently fail to do so when *H* function is absent as well. This interpretation of our results indicates that no strict epistatic relationship can be established between *H* and *Su(H)* for the specification of the SOP fate.

DISCUSSION

Antagonistic functions of *Su(H)* and *H* in the trichogen (shaft) versus tormogen (socket) alternative cell fate decision

Two novel effects of *Su(H)* mutant conditions on the differentiation of adult mechanosensory bristles are described in this study: A loss-of-function 'double shaft' phenotype, in which the bristle develops with two shafts and no socket, and a gain-of-function 'double socket' phenotype, in which two sockets appear at the expense of the shaft. These differentiative defects appear to result, respectively, from the transformation of the tormogen (socket) cell into a second trichogen (shaft) cell, and the converse.

The opposite phenotypic effects have been described for *H*: A partial loss of H^+ activity results in the development of many double-socket bristles, while *H* overexpression produces double-shaft bristles (Bang et al., 1991; Bang and Posakony, 1992). Taken together, these data indicate that a high level of *Su(H)* activity or a low level of *H* promote the socket fate, while, conversely, a low level of *Su(H)* or a high level of *H* favor the shaft fate. The finding from these and earlier studies (Bang and Posakony, 1992) that the trichogen/tormogen cell fate decision may be altered well after the birth of these cells indicates a continuing requirement for antagonistic *Su(H)* and *H* activities during their differentiation.

As bristle differentiation proceeds, the cell body of the trichogen comes to lie beneath that of the tormogen (Hartenstein and Posakony, 1989; Lees and Waddington, 1942). This rearrangement is similar in part to the delamination of embryonic neuroblasts; shaft-producing cells and neuroblasts both loosen their contact with the apical surface to move beneath the socket-producing and epidermal cells, respectively. We suggest that the choice between the trichogen and tormogen cell fates shares significant similarities with other neurogenic decisions, both in its genetic requirements (Posakony, 1994) and in its possible association with changes in cell-cell adhesion (Hartenstein et al., 1992). In this respect, it is interesting to note that 'double shaft' and 'double socket' phenotypes have both been observed in specific *N* loss-of-function (de Celis et al., 1991) and gain-of-function (see Fig. 5D of Rebay et al., 1993) conditions, respectively.

Control of the SOP versus epidermal alternative cell fate decision by *Su(H)*

We have shown here that in a *Su(H)* mutant wing disc, high levels of ac protein accumulation and of proneural regulatory activity are detected in the same cluster pattern that we described earlier for the expression of the SOP-specific marker A101 (Schweisguth and Posakony, 1992). We have further observed a high level of Dl protein accumulation in the identical cluster pattern in *Su(H)* mutant discs. This result is consistent with recent data indicating that wild-type SOPs exhibit elevated Dl expression (A. Bang, J. Kavaler, and J. W. P., unpublished observations; A. Parks and M. Muskavitch, unpublished observations). Thus, several important characteristics of normal SOPs are shared by clusters of cells in *Su(H)* null imaginal discs, strongly supporting our earlier conclusion that a complete loss of *Su(H)* function results in the commitment of all, or most, imaginal disc proneural cluster cells to the SOP fate (Schweisguth and Posakony, 1992).

Somatic mosaic studies in adult flies indicate that *Dl* acts non-cell-autonomously and is an essential component of the inhibitory signal that prevents the determination of more than one SOP cell within a proneural cluster (Heitzler and Simpson, 1991). Moreover, higher *Dl* activity in a cell appears to confer a higher capacity to inhibit neighboring cells (Heitzler and Simpson, 1991). Accordingly, the simultaneous presence of high levels of ac and Dl in multiple neighboring cells in *Su(H)* proneural clusters might be viewed as somewhat paradoxical. One simple interpretation is that *Su(H)* is normally required for proneural cluster cells to respond to the *Dl*-mediated inhibitory signal, so that, in the absence of *Su(H)* function, this signal becomes ineffective at antagonizing proneural gene expression and function.

Antagonistic functions of *Su(H)* and *H* in the SOP versus epidermal cell fate decision

Several lines of evidence firmly support our conclusion that *H* and *Su(H)* encode antagonistic activities that have opposite effects on the SOP versus epidermal cell fate decision in imaginal discs. First, *H* and *Su(H)* have opposite null phenotypes with respect to SOP development. Second, both loss of *H* function and overexpression of *Su(H)* cause adult bristle loss and, in both cases, the developmental basis for this phenotype is the failure to specify and/or execute the SOP cell fate. Third, bristle loss resulting from *Su(H)* overexpression is strongly enhanced by reduction of *H* function (unpublished results).

Our phenotypic analysis of *Su(H); H* double mutant larvae indicates that proneural cluster cells may adopt the SOP or epidermal fates in the absence of both functions. Thus, in *Su(H)* mutant wing discs, some cells require H^+ activity to express the SOP fate, while other cells do not. Similarly, in the absence of H^+ activity, $Su(H)^+$ function is required for some, but not all, cells to express the epidermal fate. These findings suggest that *Su(H)* and *H* act primarily to stably establish alternative cell fates, rather than to specify cell identities initially. The lack of a clear epistatic relationship between *H* and *Su(H)* for SOP determination further implies that these two genes do not act sequentially in a strictly linear genetic pathway controlling this process.

Considering that *H* and *Su(H)* transcripts appear ubiquitously distributed in the third instar wing imaginal disc (Bang

and Posakony, 1992; Schweisguth and Posakony, 1992), both proteins may be present in all proneural cluster cells. It is formally possible that H and Su(H) are constitutively active in all the cells of the cluster, and that alterations in their relative levels only secondarily affect an asymmetry established independently. Alternatively, the activities of the H and/or Su(H) proteins may be spatially regulated within the proneural cluster in response to a lateral inhibitory signal from the presumptive SOP cell. We have previously suggested that H acts within the presumptive SOP to make it resistant to inhibitory signaling by its neighbors in the proneural cluster (Bang and Posakony, 1992). It is possible that Su(H) is activated in the non-SOP cells of the cluster; this could lead to the specific inhibition of H activity in these cells, which would thus be prevented from stably adopting the SOP fate.

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