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Sensory Organ Precursor Cell Fate by Negatively Regulating the Activity of the *Notch* Signaling Pathway

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In *Drosophila* imaginal discs, the function of the *Hairless* (*H*) gene is required at multiple steps during the development of adult sensory organs. Here we report the results of a series of experiments designed to investigate the *in vivo* role of *H* in sensory organ precursor (SOP) cell specification. We show that the proneural cluster pattern of proneural gene expression and of transcriptional activation by proneural proteins is established normally in the absence of *H* activity. By contrast, single cells with the high levels of *achaete, scabrous,* and *neuralized* expression characteristic of SOPs almost always fail to appear in *H* mutant proneural clusters. These results indicate that *H* is required for a relatively late step in the development of the proneural cluster, namely, the stable commitment of a single cell to the SOP cell fate. We also show that expression of an activated form of the Notch receptor leads to bristle loss with the same cellular basis—failure of SOP determination—as loss of *H* function and that simultaneous overexpression of *H* suppresses this effect. Finally, we demonstrate by epistasis experiments that the failure of stable commitment to the SOP fate in *H* null mutants requires the activity of the genes of the *Enhancer of split* complex, including *groucho.* Our results indicate that *H* promotes SOP determination by antagonizing the activity of the *Notch* pathway in this cell, thereby protecting it from inhibitory signaling by its neighbors in the proneural cluster. We propose a simple threshold model in which the principal role of *H* in SOP specification is to translate a quantitative difference in the activity of the *Notch* pathway (in the SOP versus the non-SOP cells) into a stable binary cell fate decision.

INTRODUCTION

The *Notch* (*N*) cell–cell signaling pathway, named for the gene that encodes its transmembrane receptor, plays a prominent role in conditional cell fate specification during *Drosophila* development (Fortini and Artavanis-Tsakonas, 1993). It acts in a variety of stages and processes, including neurogenesis, myogenesis, and oogenesis, and is typically responsible for restricting the expression of a particular cell fate among two or more cells that have the potential to adopt that fate.

The structure and operation of the *N* pathway has been most fully elucidated in the development of the external sensory organs of the adult peripheral nervous system (PNS). The most numerous of these organs are the mechanosensory bristles, which cover much of the body surface in a relatively invariant pattern and are each composed of four cells—a neuron and three nonneuronal accessory cells—that derive via a fixed lineage from a single sensory organ precursor cell, or SOP (Hartenstein and Posakony, 1989). Adult mechanoreceptors develop during the late larval and early pupal stages within undifferentiated epithelial sheets, the imaginal discs and histoblast nests, that ultimately give rise to the cuticular structures of the fly. First, the spatially restricted expression and activity of the achaete (ac) and scute (sc) proneural proteins, which are basic helix–loop–helix (bHLH) transcriptional activators, confer on small groups of ectodermal cells the competence to adopt the SOP cell fate (Cubas *et al.*, 1991; Skeath and Carroll, 1991). Then, within each of these proneural clusters (PNCs), local inhibitory cell–cell signaling mediated by the *N* pathway confines the expression of the SOP fate to a single cell; the remaining cells of the cluster become ordinary epidermal cells (Dietrich and Campos-Ortega, 1984; Hartenstein and Posakony,

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1990; Heitzler and Simpson, 1991; Parks and Muskavitch, 1993; Schweisguth and Posakony, 1992; Schweisguth and Posakony, 1994). The *N* pathway again plays a critical role in the lineage by which the SOP cell generates the four component cells of the bristle (Hartenstein and Posakony, 1990; Parks and Muskavitch, 1993; Schweisguth and Posakony, 1994). This lineage, which consists of three asymmetric cell divisions, requires *N*-mediated signaling to ensure that at each division the daughter cells adopt alternative fates (Posakony, 1994).

The known protein constituents of the *N* pathway are encoded by genes of the neurogenic group, including *N* itself, *Delta* (*Dl*), *Suppressor of Hairless* [*Su(H)*], and the *Enhancer* of split complex [E(spl)-C]. Genetic (Heitzler and Simpson, 1991) and biochemical (Fehon *et al.,* 1990; Rebay *et al.,* 1991) experiments indicate that the Dl protein is a transmembrane ligand for the N receptor; moreover, it appears to be the major component of the inhibitory signal in the cell fate decisions referred to above (Heitzler and Simpson, 1991; Parks and Muskavitch, 1993). The Su(H) protein has been implicated, again by both genetic (Schweisguth and Posakony, 1992, 1994; Schweisguth, 1995) and biochemical (Fortini and Artavanis-Tsakonas, 1994) evidence, as a transducer of the inhibitory signal within the receiving cell. Su(H) binds to the ankyrin repeats of the intracellular domain of the N receptor and is thus retained in the cytoplasm; cell culture experiments have shown that upon interaction of N with the Dl ligand (presented by the sending cell), Su(H) is released and translocated to the nucleus (Fortini and Artavanis-Tsakonas, 1994). Here, it appears to function as a sequence-specific DNA-binding protein and transcription factor (Brou *et al.,* 1994; A.M.B. and J.W.P., submitted for publication). The E(spl)-C includes seven transcription units that encode bHLH repressor proteins (Delidakis and Artavanis-Tsakonas, 1992; Knust *et al.,* 1992); these are thought to be the ultimate nuclear effectors of the *N*-mediated inhibitory signal. The E(spl)-C also contains several other genes, including *groucho* (*gro*), which encodes a nuclear protein of the WD-40 family (Delidakis *et al.,* 1991; Hartley *et al.,* 1988) that interacts directly with the E(spl)-C bHLH proteins and appears to function as a corepressor (Paroush *et al.,* 1994), and *E(spl)m4,* which encodes a small protein of unknown function (Klämbt *et al.,* 1989). Recently, we have demonstrated that both bHLH and non-bHLH genes of the E(spl)-C are directly activated by Su(H) in response to N receptor activity (A.M.B. and J.W.P., submitted for publication). The function of the E(spl)-C is required in the same way as *N, Dl,* and *Su(H)* activity for multiple alternative cell fate decisions during adult PNS development (Tata and Hartley, 1995).

Like the genes of the *N* pathway, *Hairless* (*H*), which encodes a highly basic 109-kDa protein (Bang and Posakony, 1992; Maier *et al.,* 1992), acts both in the development of the SOP cell and in the specification of cell fates in the bristle lineage (Bang *et al.,* 1991; Bang and Posakony, 1992). However, *H* functions antagonistically to these genes (Dietrich and Campos-Ortega, 1984; Lindsley and Zimm, 1992; Vässin *et al.,* 1985), so that its loss- and gain-of-function phenotypes are the opposite of those of the neurogenic genes. Specifically, loss of *N* (Hartenstein and Posakony, 1990; Shellenbarger and Mohler, 1978), *Dl* (Parks and Muskavitch, 1993), or *Su(H)* (Schweisguth and Posakony, 1992, 1994) function leads to the commitment of too many cells in the PNC to the SOP fate and hence to the appearance of supernumerary sensory organs, while *H* null mutants fail to establish functional SOP cells and consequently lack bristles on the adult cuticle (Bang *et al.,* 1991). Conversely, hyperactivity of *Su(H)* leads to a failure of SOP determination and bristle loss (Schweisguth and Posakony, 1994), while hyperactivity of *H* leads to the commitment of additional cells in each PNC to the SOP fate and thus to bristle multiplication (Bang and Posakony, 1992). Brou *et al.* (1994) have recently reported *in vitro* evidence that H inhibits the DNA-binding activity of Su(H) by direct protein–protein interaction.

Here we report the results of a series of experiments designed to investigate the *in vivo* role of *H* in SOP specification. We show that the PNC pattern of proneural gene expression and transcriptional activation by proneural proteins is established normally in the absence of *H* activity. By contrast, single cells with the high levels of *ac, scabrous* (*sca*), and *neuralized* (*neu*) expression characteristic of SOPs almost always fail to appear in *H* mutant PNCs. These results indicate that *H* is required for a relatively late step in the development of the PNC; namely, the stable commitment of a single cell to the SOP cell fate. We also show that expression of an activated form of the N receptor leads to bristle loss with the same cellular basis—failure of SOP determination—as loss of *H* function, and that simultaneous overexpression of *H* suppresses this effect. Finally, we demonstrate by epistasis experiments that the failure of stable commitment to the SOP fate in *H* null mutants requires the activity of the bHLH genes of the E(spl)-C, as well as *gro.* Our results indicate that *H* promotes commitment to the SOP cell fate by antagonizing the activity of the *N* pathway in this cell, thereby protecting it from inhibitory signaling by its neighbors in the PNC. We propose a simple threshold model in which the principal role of *H* in SOP specification is to translate a quantitative difference in the activity of the *N* pathway (in the SOP versus the non-SOP cells) into a stable binary cell fate decision.

MATERIALS AND METHODS

Drosophila Stocks

Flies were cultured on standard yeast–cornmeal–molasses–agar medium at 25°C. Chromosomes and marker mutations not described herein are described in Lindsley and Zimm (1992).

***H* mutant alleles.** As a standard for a *H* null genotype we used H^{20}/H^{E31}. The H^{E31} allele is a small deficiency which deletes two-thirds of the *H* ORF sequence (F. Schweisguth, unpublished observations), while H^{20} is a ~2-kb inversion with both breakpoints within the *H* ORF sequence (Bang *et* *al.*, 1991; Bang and Posakony, 1992); it is very likely that these alleles are protein null. All other mutant alleles of *H* used in this study are described in Lindsley and Zimm (1992), Bang *et al.* (1991), and Bang and Posakony (1992).

E(spl)-C mutations. For the sake of clarity in presenting and discussing our results, we have used the following nomenclature convention for mutations in the E(spl)-C. All mutations that affect multiple gene functions within the complex (in some cases, including *gro*) are indicated by the symbol *E(spl)*, accompanied by a superscript lesion or allele designation. Apparent point mutations in *gro* are indicated by the symbol *gro*, accompanied by a superscript lesion or allele designation. Accordingly, we have redesignated the following mutations of the E(spl)-C from their original names (designations used here appear first): $E(spl)^{r8.1}$ = $gro^{r8.1}$, $E(spl)^{r72.1}$ = $gro^{r72.1}$, $E(spl)^{b32.2}$ = $gro^{b32.2}$, gro^{E48} = $E(spl)^{E48}$, gro^{E73} = $E(spl)^{E73}$. All other mutations are referred to by their original names. Mutations listed below, in Table 1, and in Fig. 5 are described in the following references under their original designations: $E(spl)^{RA7.1}$ (Knust *et al.*, 1987a,b); gro^{B88} (Ziemer *et al.*, 1988); $E(spl)^{r8.1}$, $E(spl)^{r72.1}$, $E(spl)^{b32.2}$, $Df(3R)boss^{16}$ (Schrons *et al.*, 1992); gro^{E48}, gro^{E73}, $Df(3R)E(spl)^{BX36}$ (Preiss *et al.*, 1988).

Transgenic fly stocks. These are described in the cited publications: *P*[w^+, gro^+] (Schrons *et al.*, 1992); *P*[ry^+, *Hs-N(intra)*]*#2*; ry^{506} (Struhl *et al.*, 1993); *P*[w^+, *Hs-H*]*-3* (Bang and Posakony, 1992); *P*[ry^+, *lacZ*]*A101* (Bellen *et al.*, 1989); *P*[w^+, *ac-lacZ*]*A1-1* (Van Doren *et al.*, 1992).

In Situ Hybridization

In situ hybridization was performed essentially as described by Tautz and Pfeifle (1989) with modifications by Jiang *et al.* (1991). Fixation of imaginal discs was performed as in Schweisguth and Posakony (1992). Antisense RNA probes, labeled with digoxygenin-UTP, were prepared exactly as described by the manufacturer (Boehringer-Mannheim) using full-length *ac*, *sc*, and *sca* cDNA clones as templates (Baker *et al.*, 1990; Mlodzik *et al.*, 1990; Van Doren *et al.*, 1991).

Antibody Labeling

Labeling of imaginal discs with the anti-ac monoclonal antibody was performed as described previously (Skeath and Carroll, 1991).

Histochemical Staining for β-galactosidase Activity

Histochemical demonstration of β-galactosidase activity was carried out as described by Romani *et al.* (1989).

Heat Shock Treatment

Staged pupae were placed in a humid chamber and subjected to heat shock at 37°C. Development was then allowed to proceed at 25°C.

Preparation of Adult Cuticles for Light Microscopy

Adult cuticles were prepared for light microscopy as described by Bang and Posakony (1992).

FLP/FRT-Mediated Production of Mitotic Clones

The FLP/FRT method for inducing site-specific recombination to generate mitotic clones is described by Golic (1991), as are the *P*[$>w^{hs}>$]*75A* and *hsFLP2B* chromosomes. The *P*[$>w^{hs}>$]*75A* FRT insertion site is at cytological position 85B (K. Golic, personal communication). Progeny of appropriate crosses were heat shocked at 37°C for 2 hr at 48–72 hr of development and subsequently grown at 25°C. Somatic clones were identified by virtue of their phenotype. Recovery of clones depended strictly on the presence of the mutation(s), the *hsFLP* gene, and two FRT sites, as well as heat shock induction; sibling genotypes did not exhibit clones. Approximately 10 clones per animal were recovered.

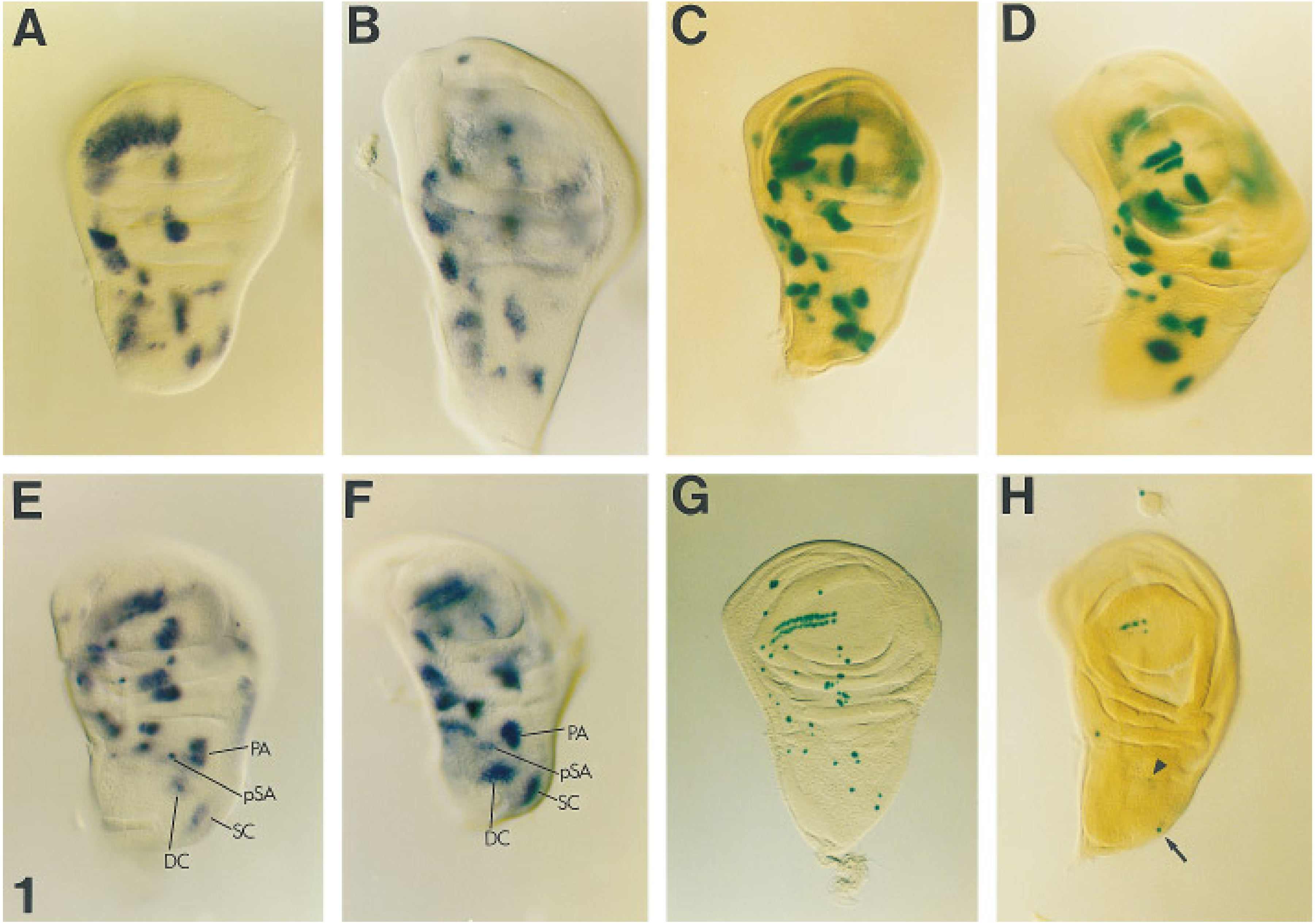
RESULTS

Proneural Gene Expression and Function in H Mutant Proneural Clusters

We have shown previously that the *H* bristle loss phenotype reflects an early defect in sensory organ development, namely the failure to specify and/or execute the SOP cell fate (Bang *et al.*, 1991). This conclusion was based on our finding that, in regions of the notum exhibiting bristle loss in adult *H* mutants, we were unable at the appropriate stages of development to detect sensory organ-specific cell types, the precursor cell divisions that generate them, or the SOP cells themselves. In these experiments the enhancer-trap transposon insertion A37 (Ghysen and O'Kane, 1989) was used as a specific marker for SOP cells. Later studies have shown that A37 is expressed late in the development of an SOP cell, just prior to its division (Blair *et al.*, 1992). Here we have used a variety of earlier PNC and SOP markers in order to determine the specific step of SOP development at which H^+ activity is first required.

We first investigated whether the normal PNC pattern of proneural gene expression can be established in the absence of *H* function. *In situ* hybridization was used to examine the distribution of *ac* and *sc* transcripts in wing imaginal discs from late third instar larvae. As shown in Figs. 1A and 1B, *sc* transcripts accumulate in an apparently normal PNC pattern in *H* mutant wing discs. Similar results were obtained for *ac* (data not shown). Though the resolution of this assay does not allow us to discern small differences in level of expression or in details of pattern within the PNC, it is clear that *H* activity is not required for the overall spatial pattern of proneural gene expression in the wing disc.

The bHLH proteins encoded by *ac* and *sc* function as transcriptional activators in the PNCs of the wing disc (Van Doren *et al.*, 1992; Martinez *et al.*, 1993; Singson *et al.*, 1994). We tested whether *H* function is required for this activity by examining the expression of a reporter gene con-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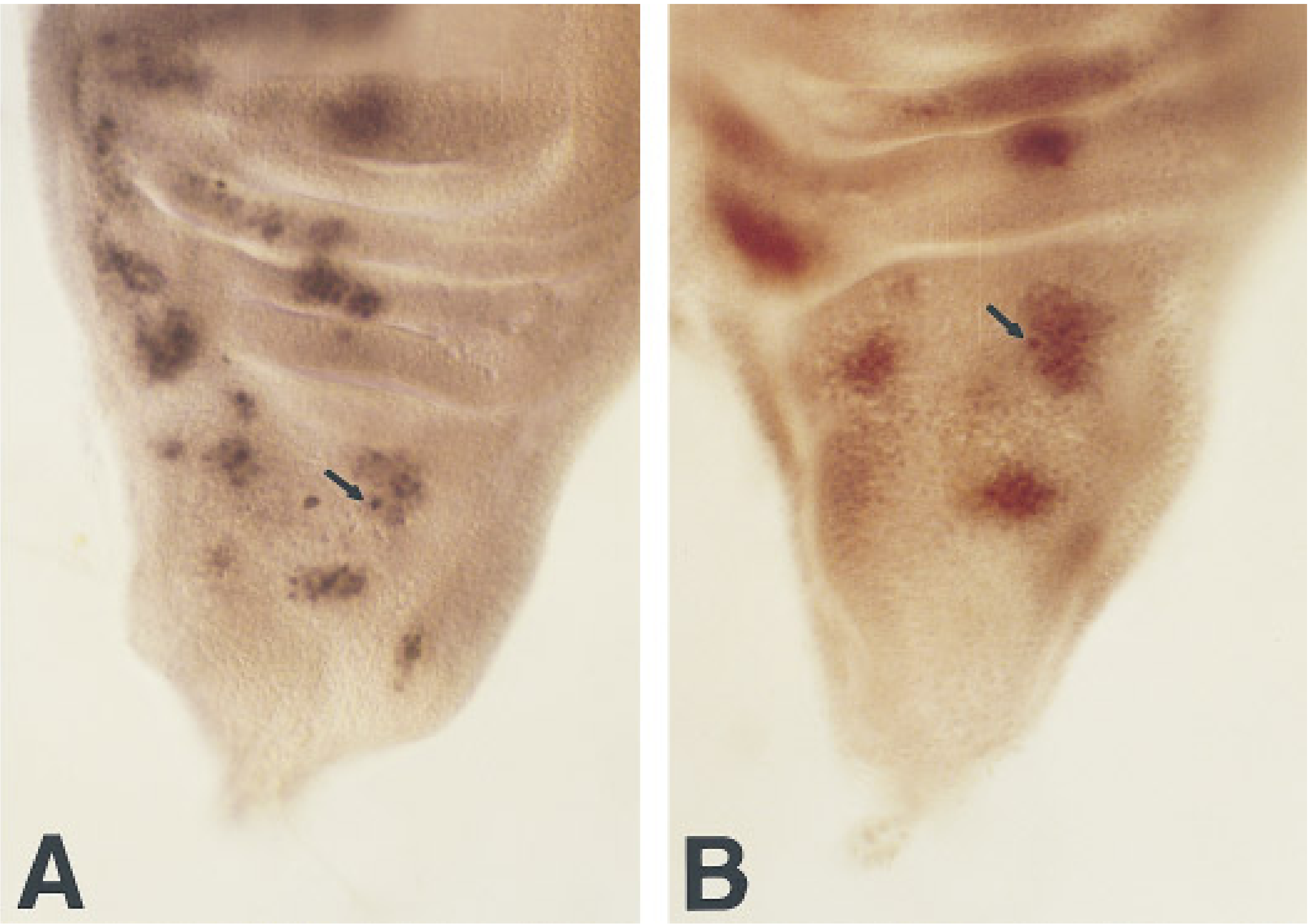


FIG. 2. Comparison of ac protein accumulation in wild-type and *H* null PNCs and SOPs. Anti-ac monoclonal antibody labeling of wing discs from wild-type (A) or H^{20}/H^{E31} (B) late third instar larvae. PNCs in *H* mutant wing discs generally fail to exhibit a singled-out cell with the high level of ac accumulation characteristic of wild-type SOPs (arrow in A). Occasionally an individual cell is observed in a *H* mutant PNC with a detectably higher level of ac (arrow in B); these occur preferentially in certain PNCs, such as those corresponding to the pSC, aPA, and pDC macrochaetes.

struct in which a 0.9-kb fragment of the *ac* promoter is fused to the *Escherichia coli lacZ* gene. In a wild-type background (Fig. 1C), this transgene is directly activated in a PNC pattern by the endogenous *ac* and *sc* genes (Van Doren *et al.,* 1992; Martinez *et al.,* 1993). Figure 1D shows that this construct displays an apparently normal PNC expression pattern in *H* mutant wing discs, indicating that the transcriptional activation function of the proneural proteins in the PNCs is not dependent on *H*.

SOP Cell Fate Determination in H Mutant Imaginal Discs

A very useful marker for the establishment and development of PNCs and the selection of SOPs is *sca* (Baker *et*

FIG. 1. Expression of PNC and SOP markers in wing imaginal discs from wild-type and *H* null late third instar larvae. (A,B,E,F) Wild-type (A,E) and H^3/H^3 (B,F) wing discs hybridized *in situ* with a digoxygenin-labeled RNA probe for *sc* (A,B) or *sca* (E,F). (C,D,G,H) Wild-type (C,G) and *H* null [H^2/H^2 (D); H^{20}/H^{E31} (H)] wing discs, stained for β-galactosidase activity to detect expression of an *ac–lacZ* reporter gene (C,D) or the A101 enhancer-trap marker (G,H). *H* mutant disc shown in (D) is younger than the wild-type disc shown in (C) and thus exhibits somewhat less extensive *ac–lacZ* expression in some areas. Proneural clusters giving rise to certain thoracic macrochaetes are labeled in E and F for comparison: postalar (PA), posterior supraalar (pSA), dorsocentral (DC), and scutellar (SC). Two macrochaete positions which consistently express A101 in *H* mutant wing discs, the pSC and the aPA, are indicated, respectively, by an arrow and an arrowhead in (H). Note, especially in (B), (D), and (H), the wing-pouch overgrowth phenotype observed in strong *H* loss-of-function mutants.

al., 1990; Mlodzik *et al.*, 1990). Expression of *sca* in the PNCs of the wing disc (with the exception of those that give rise to chordotonal organs) requires *ac* and *sc* function and appears to be directly activated by proneural protein complexes (Singson *et al.*, 1994). In wild-type discs (Fig. 1E), the initially high level of *sca* transcript accumulation throughout the PNC gives way to very reduced levels in non-SOP cells, accompanied by sustained strong expression in the presumptive SOP (Mlodzik *et al.*, 1990). We find that, as with *ac*, *sc*, and the *ac–lacZ* transgene, the initial establishment of *sca* expression in the PNC pattern is unimpaired in *H* mutant wing discs (Fig. 1F), confirming our conclusion that transcriptional activation by proneural proteins does not require *H*. However, single cells with the stable, high level of *sca* transcript characteristic of normal SOPs nearly always fail to appear in *H* mutant PNCs (Figs. 1E and 1F). For example, in a wild-type disc, the PNC for the posterior supra-alar (pSA) macrochaete characteristically includes one cell with high *sca* expression surrounded by other cells with much lower expression (Fig. 1E), while the same PNC in a *H* mutant disc exhibits only a low level of *sca* transcript (Fig. 1F). This observation suggests that *H* activity is required for the proper selection of the SOP from surrounding non-SOP cells in the PNC.

As with *sca*, the ac and sc proteins are initially expressed in all cells of the PNC, but their expression is progressively refined by the action of the neurogenic genes (Cabrera, 1990; Cubas *et al.*, 1991; Skeath and Carroll, 1991, 1992). The refinement process eventually results in the accumulation of high levels of ac and sc in the SOP and a reduction or loss of their expression in the remaining cells of the cluster. Using an anti-ac monoclonal antibody (Skeath and Carroll, 1991), we compared ac protein expression in wing imaginal discs from wild-type and *H* mutant late third instar larvae. Although the overall PNC pattern of ac protein accumulation appeared normal in *H* discs, single cells with the high levels of ac characteristic of SOPs were generally not observed (Figs. 2A and 2B). Within some of the PNCs that give rise to dorsal thorax macrochaetes, we did occasionally detect higher ac expression in an individual cell (Fig. 2B), more frequently for some clusters than for others (see below and Fig. 1 legend). One possible explanation for this inconsistency is that high-level ac accumulation is unstable in *H* mutant SOP cells. In any case, our results concerning ac protein expression are in accord with those for *sca* transcript accumulation in indicating that *H* is required for the stable singularization of the SOP cell.

Expression of the neurogenic gene *neuralized* (*neu*) is one of the earliest known indicators of SOP specification (Boulianne *et al.*, 1991). The A101 enhancer-trap transposon insertion in the 5′ promoter region of the *neu* gene expresses β-galactosidase in all SOP cells and their progeny (see Fig. 1G), faithfully reflecting the wild-type expression pattern of *neu* (Bellen *et al.*, 1989; Boulianne *et al.*, 1991; Huang *et al.*, 1991). In *H* mutant wing discs, A101 generally fails to be expressed in the SOPs that give rise to the macrochaetes of the dorsal thorax, with two exceptions: the posterior scutellar (pSC), which exhibits strong staining similar to wild-type levels, and the anterior postalar (aPA), which exhibits only weak staining relative to wild-type levels (Figs. 1G and 1H). Other macrochaete SOPs are occasionally detectable, but they exhibit extremely weak β-galactosidase activity (data not shown). By staining *H* mutant wing discs dissected at puparium formation, we investigated the later development of the A101-positive cells and found that they do not go on to divide (data not shown). Thus, despite their expression of the A101 marker, the presumptive pSC and aPA SOPs fail to express the SOP cell fate in *H* mutant wing discs.

Since A101 is a recessive embryonic lethal allele of *neu* (Boulianne *et al.*, 1991), we investigated the possibility that it is not an unbiased reporter of the effects of loss of *H* function by making use of a duplication, $Dp(3;3)Antp^{+R8}$, which includes neu^{+}. The patterns of lacZ-expressing cells in late third instar wing discs from animals of the genotypes $A101\ H^{20}/H^{E31}\ Dp(3;3)Antp^{+R8}$ and $A101\ H^{20}/H^{E21}$ are identical (data not shown), indicating that the expression of A101 in specific *H* mutant SOPs does not reflect a suppression of the *H* phenotype by reduction of *neu* function.

Taken together, the inconsistent, low, or undetectable expression of the SOP markers described above strongly suggest that *H* is required for a relatively late step in the development of the PNC; namely, the stable commitment of a single cell within the PNC to the SOP cell fate.

Deregulated Activity of the N Receptor Prevents Commitment to the SOP Cell Fate

Loss of mechanosensory bristles from the adult notum is one phenotypic consequence of the expression of activated derivatives of the N receptor protein (Rebay *et al.*, 1993; Struhl *et al.*, 1993). For example, Struhl and colleagues have reported that constitutive expression of a transgene encoding only the intracellular domain of *N*, [*Notch(intra)*] can lead to balding of the dorsal thoracic cuticle (Struhl *et al.*, 1993). We have investigated the cellular basis for this cuticular phenotype, because of its superficial similarity to the *H* null phenotype.

We made use of a transgenic fly line in which *Notch(intra)* expression is under the control of the inducible *Hsp70* promoter (Struhl *et al.*, 1993) to define a restricted period of time during which constitutive N receptor activity results in a bristle loss phenotype. The application of a 90-min heat shock (37°C) to $P[ry^{+}, Hsp70\text{-}Notch(intra)]$ transformants at 7 hr after puparium formation (APF) leads to a highly penetrant loss of microchaetes (Fig. 3B). If the heat shock treatment is applied earlier (at 0 hr APF) or later (at 16 hr APF), the microchaete pattern is not significantly affected (data not shown). Thus, the time at which expression of *Notch(intra)* causes a microchaete loss phenotype accords well with the time of emergence of microchaete SOPs from the pupal notum epithelium at 8–12 hr APF (Usui and Kimura, 1993).

To investigate the cellular basis of the *Notch*(*intra*) bristle loss phenotype, we used the A101 enhancer trap insertion as a specific marker for SOPs and their progeny (Bellen *et* *al.*, 1989; Huang *et al.*, 1991; Usui and Kimura, 1993). Pupae of the genotype *P*[*ry*$^+$, *Hsp70-Notch(intra)*]*#2/+; A101/+* were subjected to heat shock (90 min at 37°C) at 7 hr APF and then incubated at 25°C until either 14 or 24 hr APF. At these times, nota were dissected, fixed, and stained for β-galactosidase activity (Figs. 3D and 3F). In wild-type animals at 14 hr APF, A101 is expressed in the complete array of microchaete SOPs (Figs. 3A and 3C), while β-galactosidase activity is almost never detected at microchaete positions in *Notch(intra)* animals (Fig. 3D). By 24 hr APF, microchaete SOPs are still absent from *Notch(intra)* nota (Fig. 3F), whereas in wild-type pupae, the lineal descendants of the SOP cells continue to express the A101 marker (Fig. 3E). By the criterion of A101 expression, then, the loss of adult sensory organs resulting from expression of *Notch(intra)* is due to a failure of SOP cell fate determination. We conclude that the deregulated activity of the N receptor prevents the establishment of the SOP cell fate, just as loss of *H*$^+$ function does (Bang *et al.*, 1991; this paper).

Simultaneous Overexpression of H Counteracts the Phenotypic Effects of Activated N

The observation that both loss of *H* activity and expression of *Notch(intra)* interfere with the determination of the SOP cell suggests that hyperactive signaling by the *N* pathway overwhelms the capacity of endogenous *H*$^+$ function to promote stable commitment to the SOP cell fate (Posakony, 1994). If so, then it might be expected that elevated *H*$^+$ activity could alleviate the effect of *Notch(intra)* expression on SOP specification. We tested this hypothesis by examining the phenotypic consequences of the simultaneous overexpression of *Notch(intra)* and *H* during the determination of microchaete precursor cells. Pupae of the genotypes *P*[*ry*$^+$, *Hsp70-Notch(intra)*]*#2/+*, *P*[*w*$^+$, *Hs-H*]-*3/+* (Bang and Posakony, 1992), and *P*[*ry*$^+$, *Hsp70-Notch(intra)*]*#2/+; P*[*w*$^+$, *Hs-H*]-*3/+* were subjected to heat shock (90 min at 37°C) at 7 hr APF. SOP development was evaluated at the cuticular level as the animals reached adulthood (Fig. 4). Under such a regimen, the expression of *Notch(intra)* alone leads to extensive loss of microchaetes from the notum (Figs. 3B and 4B), and the overexpression of *H* alone causes a mild increase in the density of sensory organs (see Bang and Posakony, 1992), while the phenotype that results from the concomitant overexpression of *Notch(intra)* and *H* is intermediate (Fig. 4C). Within large territories of the nota of *Hsp70-Notch(intra)/+; Hs-H/+* adults that have undergone heat shock treatment, the pattern of bristles closely resembles the wild type (compare Figs. 4C and 4A). Thus, hyperactivity of *H*$^+$ counteracts the phenotypic effects of *Notch(intra)* expression and restores the capacity for appropriate execution of the SOP versus epidermal cell fate decision (see Discussion).

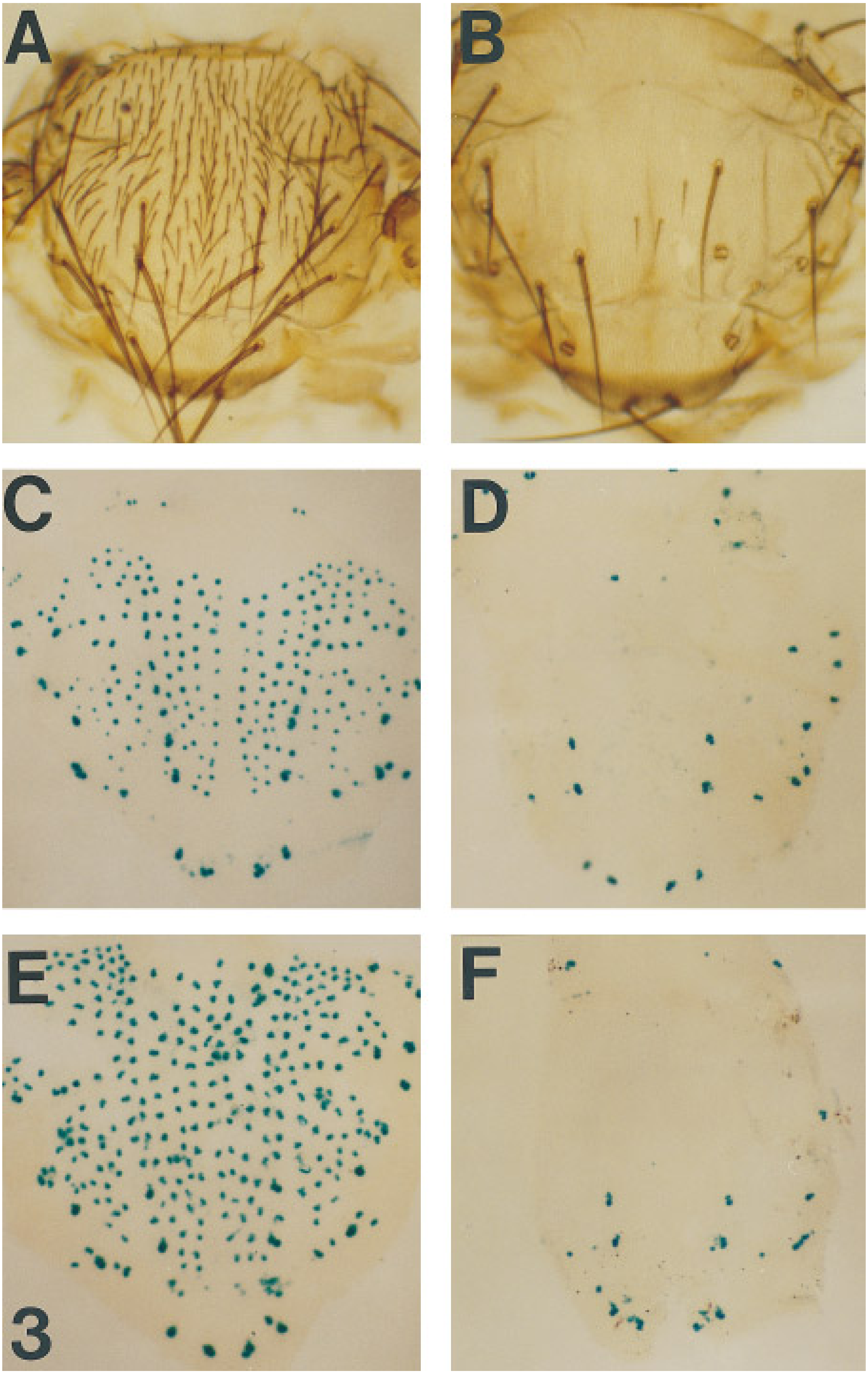
Loss-of-Function Mutations in E(spl)-C bHLH Genes and gro Suppress SOP Loss in H Null Mutants

We have suggested previously (Bang and Posakony, 1992; Posakony, 1994) that *H* acts to protect the SOP from inhibitory signaling in the PNC by antagonizing the activity of *N* pathway genes in this cell. If, as this hypothesis suggests, the failure of SOP determination in *H* null mutants results from the inappropriate activity of at least some *N* pathway components, then removal of these functions should suppress the *H* null phenotype. We examined the epistatic relationship between *H* and the E(spl)-C bHLH genes and between *H* and *gro* in adult SOP development in order to test this prediction.

The effect of incrementally reducing E(spl)-C activity on the *H* bristle loss phenotype was assessed by introducing into *H* null backgrounds combinations of E(spl)-C deletions which remove varying numbers of bHLH transcription units (of 14 possible). Since the E(spl)-C deletion chromosomes we used (Fig. 5) have lesions that reduce or eliminate *gro* activity, a *gro*$^+$ P element transposon was included in the genotype so that the observed phenotypic effects could be attributed specifically to loss of E(spl)-C bHLH gene function. This resulted in two genotypic series, one in which the *gro*$^+$ dosage is approximately 2 and another with a *gro*$^+$ dosage of >2, both differing in the number of E(spl)-C bHLH genes (Table 1).

Considering the first genotypic series, we find that deletion of only two of the E(spl)-C bHLH genes [both copies of *m8;* genotype *P*[*gro*$^+$]*;* H^{E31} $E(spl)^{r8.1}/H^{E31}$ $E(spl)^{r8.1}$] is not suppressive (Table 1). In animals of the genotype *P*[*gro*$^+$]*;* H^{20} $E(spl)^{r72.1}/H^{E31}$ $E(spl)^{r72.1}$, in which four bHLH genes are removed (both copies of *m7* and *m8*), we detect a mild suppression of *H* bristle loss in which two dorsal thorax macrochaetes, the aPA and the pSC, are consistently rescued, along with a subset of the microchaete pattern (Fig. 6B and Table 1). The strongest viable combination of E(spl)-C deficiencies (Schrons *et al.*, 1992), which removes 9 of 14 bHLH genes, displays maximal suppression of the *H* bristle loss phenotype, as observed in pharate adults of the genotype *P*[*gro*$^+$]*;* H^{20} $E(spl)^{b32.2}/H^{E31}$ $E(spl)^{r72.1}$ (Fig. 6C and Table 1).

FIG. 3. Temporally restricted expression of *Notch(intra)* causes a failure of SOP cell determination. (B,D,F) Pupae of the genotype *P*[*ry*$^+$, *Hsp70-Notch(intra)*]*#2/+; A101/+* were subjected to a 90-min heat shock (37°C) at 7 hr APF and compared to *A101/TM2* control animals (A,C,E) with respect to development of adult sensory organ structures (A,B) and expression of the A101 enhancer trap insertion in the developing pupal notum (C–F). β-Galactosidase activity was assayed at 14 hr APF (C,D), when in wild-type nota (C) the complete array of microchaete SOPs expresses A101, and at 24 hr APF (E,F), when A101 is expressed in all four progeny of the SOP in wild-type nota (E). Loss of adult microchaete bristles caused by expression of *Notch(intra)* (B) correlates with persistent loss of A101-positive microchaete SOPs (D,F); expression of A101 at macrochaete positions remains.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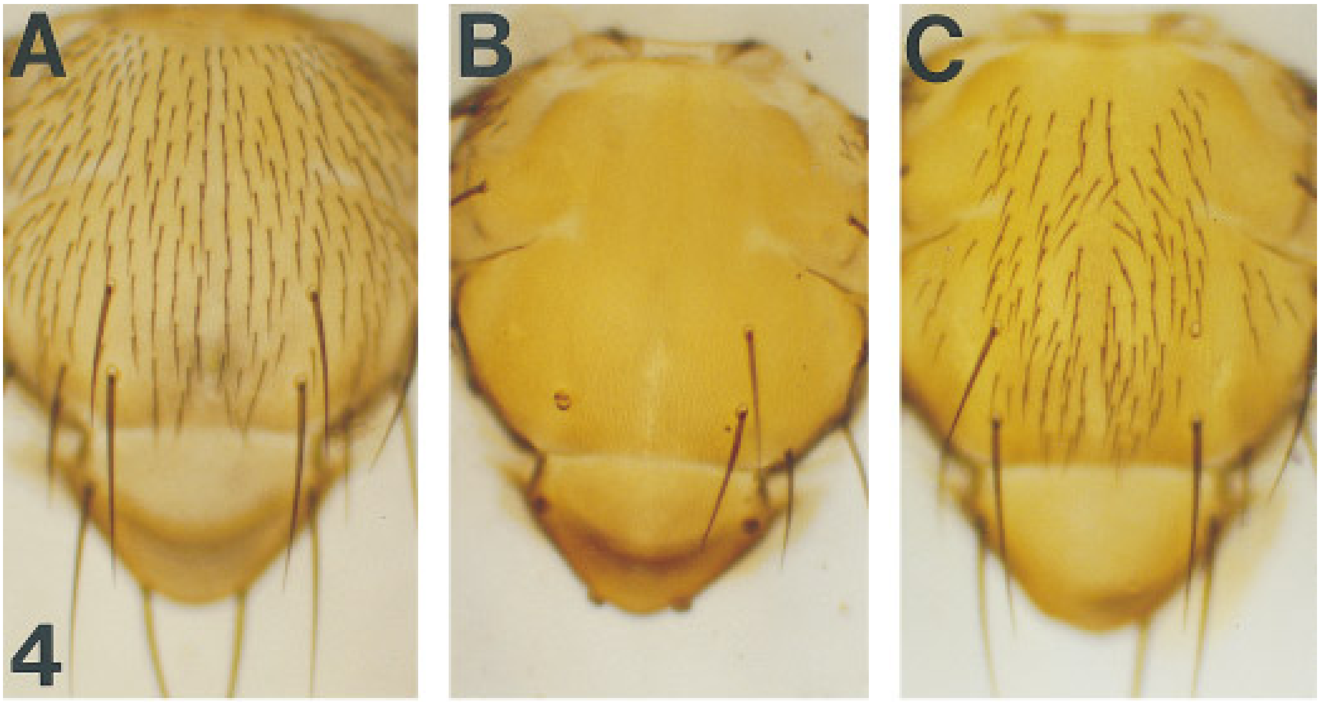

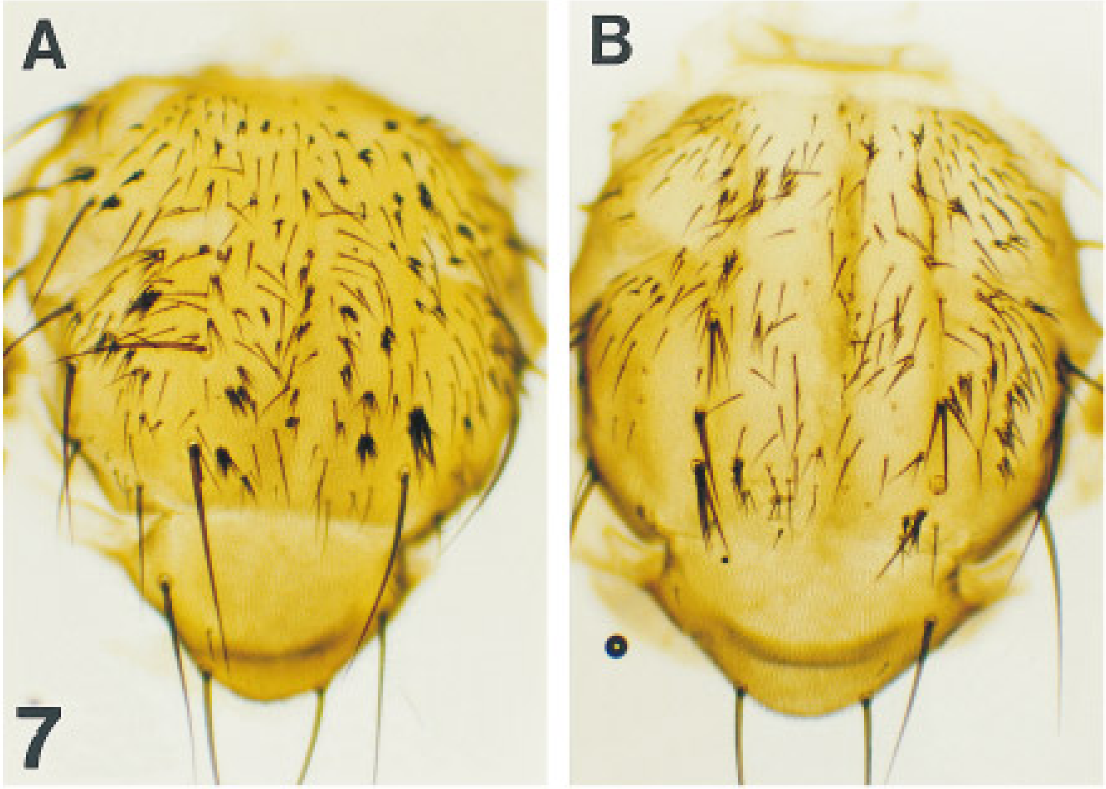

FIG. 4. Phenotypic consequences of *Notch(intra)* expression are overcome by simultaneous overexpression of *Hairless*. Cuticle preparations of adult animals developing from (A) w^{1118} control pupa not subjected to heat shock; (B) Pupa of the genotype *P*[ry^+, *Hsp70-Notch(intra)*]*#2/+* subjected to heat shock (37°C) for 90 min at 7 hr APF; (C) Pupa of the genotype *P*[ry^+, *Hsp70-Notch(intra)*]*#2/+; P*[w^+, *Hs-H*]*-3/+* subjected to heat shock as above.

FIG. 7. The *gro* null bristle multiplication (''tufting'') phenotype is epistatic to the *H* null bristle loss phenotype. Cuticle preparations of nota dissected from adults (which had been heat shocked as second instar larvae to induce FLP/FRT-mediated mitotic recombination)

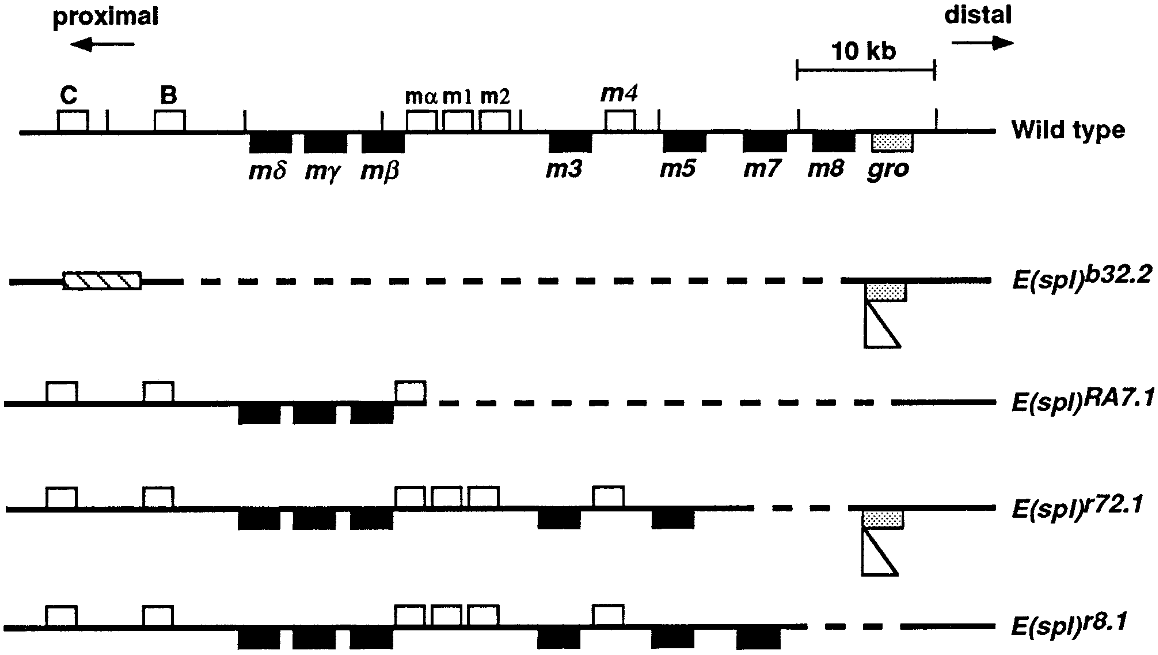

FIG. 5. Map of the E(spl)-C mutant chromosomes used for the epistasis experiments of Fig. 6 and Table 1; adapted from Schrons *et al.* (1992). Black boxes represent bHLH transcription units; white boxes represent additional transcription units of unknown function; gray boxes represent *gro*. Triangles indicate P element insertions: $E(spl)^{r8.1}$, $E(spl)^{r72.1}$, and $E(spl)^{b32.2}$ were all induced by P element excision. Dashed lines indicate deleted regions; the hatched box shows limits of uncertainty for the proximal breakpoint of $E(spl)^{b32.2}$.

Almost all thoracic macrochaetes, as well as the entire microchaete array, are restored. We observe a similar progression in the suppression of the *H* null phenotype in the second genotypic series (Table 1).

Interestingly, removal of the E(spl)-C bHLH genes *m3* and *m5* does not appear to contribute to suppression of the *H* bristle loss phenotype, as shown by the similar degree of suppression observed in pharate adults of the genotypes $P[gro^+]$; H^{20} $E(spl)^{RA7.1}/H^{E31}$ and $P[gro^+]$; H^{20} $E(spl)^{r72.1}/H^{E31}$ $E(spl)^{RA7.1}$ compared to pharate adults of the genotypes $P[gro^+]$; H^{20} $E(spl)^{r72.1}/H^{E31}$ and P[gro$^+$]; H^{20} $E(spl)^{r72.1}/H^{E31}$ $E(spl)^{r72.1}$, respectively (Table 1). It is possible that reduction of *m4* gene dosage counteracts the suppressive effect of removing *m3* and *m5*, since this transcription unit is also deleted by the $E(spl)^{RA7.1}$ mutation. In any case, it appears that the degree of suppression of the *H* null phenotype caused by reduction of E(spl)-C activity depends on both the number and the identity of E(spl)-C transcription units removed.

Though it is clear that SOP determination and development has been rescued in these experiments, the rescued bristles are not normal, but exhibit a "double socket" phenotype (Figs. 6B and 6C). This is consistent with the fact that the trichogen/tormogen (shaft/socket) cell fate decision in the SOP lineage is especially sensitive to reduction of *H* function (Bang *et al.*, 1991). We have found that in a *H* hypomorphic background (H^{22}/H^{22}), comparable reductions of E(spl)-C activity do suppress the double socket phenotype (data not shown).

We conclude from these genetic epistasis experiments that the failure of SOP determination in *H* null mutants requires the activity of the bHLH genes of the E(spl)-C.

Since null mutations in *gro* do not allow the survival of homozygotes to the pupal stage, we made use of a somatic

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of the following genotypes: (A) w^{1118}; *hsFLP2B/+*; $P[>w^{hs}>]75A\ gro^{E48}/P[>w^{hs}>]75A+$ and (B) w^{1118}; *hsFLP2B/+*; $P[>w^{hs}>]75A\ H^{20}\ gro^{E48}/$ $P[>w^{hs}>]75A{+}{+}$. Multiple mosaic patches exhibiting bristle multiplication phenotypes are present in both genotypes, but the clones are otherwise unmarked. A large fraction of both gro^- and H^- gro^- mosaic clones contain bristle structures with multiple shafts and no sockets. Control H^- clones exhibited only the characteristic *H* null bristle loss phenotype (data not shown). The additional bristle loss apparent in B is probably not within the H^- gro^- clonal territories, since we observe it in animals not subjected to the heat shock treatment that generates the clones. Instead, it is due to the *H gro/++* heterozygous background of the animals used in the experiment (as opposed to the *gro/+* background for A), which causes both bristle loss and some "double socket" effects (the latter can be seen at some positions on the thorax shown in B).

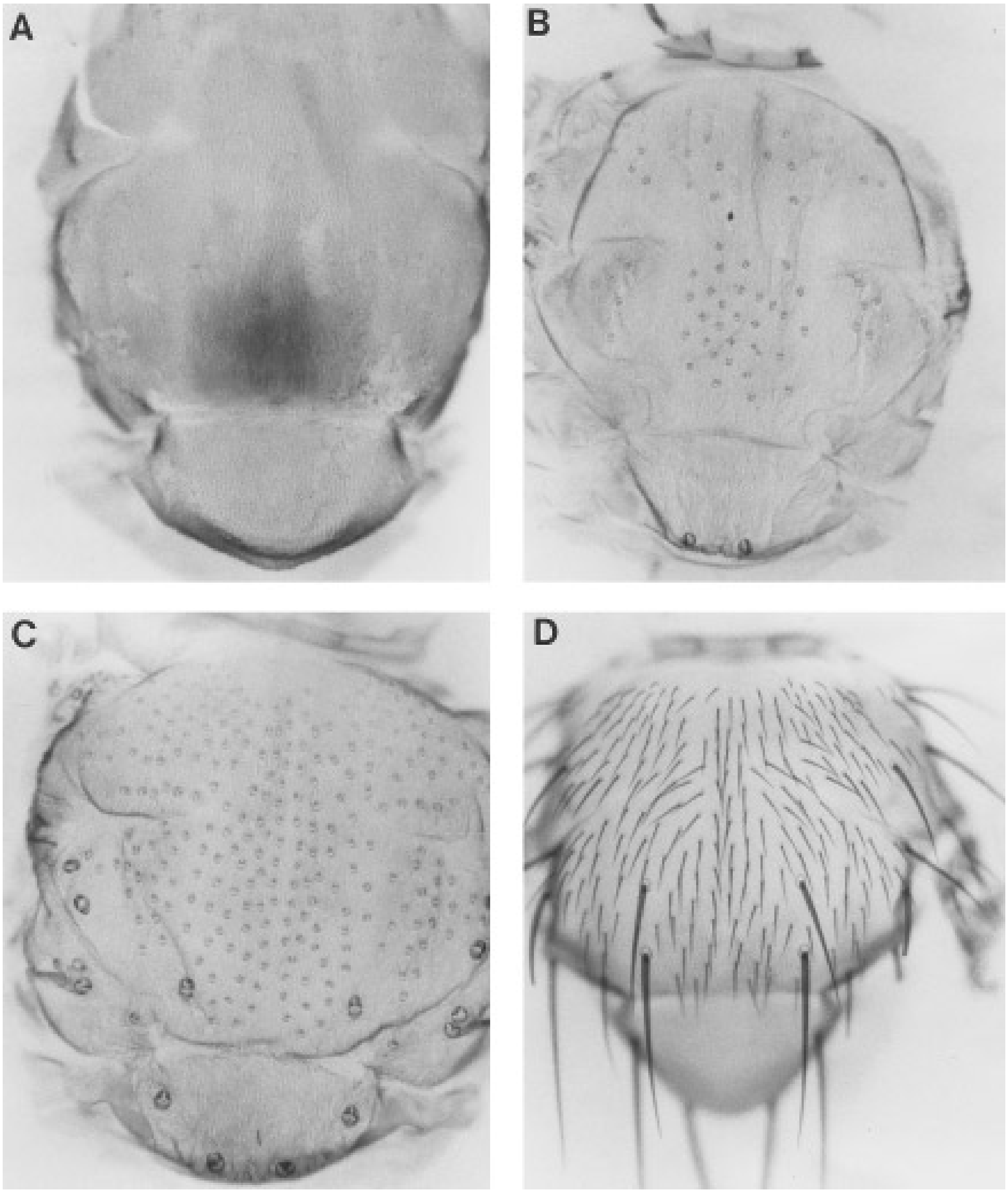

FIG. 6. Reduction of E(spl)-C bHLH gene function suppresses the *H* null bristle loss phenotype. Cuticle preparations of nota dissected from pharate adults of the following genotypes: (A) H^{20}/H^{E31}; (B) $P[gro^{+}]$; H^{20} $E(spl)^{r72.1}/H^{E31}$ $E(spl)^{r72.1}$; (C) $P[gro^{+}]$; H^{20} $E(spl)^{b32.2}/H^{E31}$ $E(spl)^{r72.1}$; (D) wild type. The anterior dorsocentral (aDC) macrochaete, which is very sensitive to loss of *H* function (Bang *et al.*, 1991), consistently fails to be rescued even by strong reduction of E(spl)-C activity (C). The results shown are not allele- or chromosome-specific, as we have also observed suppression of *H* bristle loss with the E(spl)-C deficiencies $Df(3R)E(spl)^{BX36}$ and $Df(3R)boss^{16}$ in H^{20}, H^{E31}, H^{2}, H^{3}, and H^{22} backgrounds (data not shown).

mosaic analysis to investigate the epistatic relationship between *H* and *gro*. gro^{-} clones generated by radiation-induced mitotic recombination have been reported to display two mutant phenotypes, bristle tufting and bristle loss (de Celis *et al.*, 1991; Schrons *et al.*, 1992), similar to the phenotypes of strong loss-of-function mutations in other *N* pathway genes. At the cuticular level, we cannot assay the epistatic relationship between the gro^{-} and H^{-} bristle loss pheno-

TABLE 1

Reduction of E(spl)-C bHLH Gene Dosage Suppresses the Bristle Loss Phenotype of *H* Null Mutants

Genotype	H^+ dose	gro^+ Dose	No. of bHLHs removed	Effect on *H* bristle loss phenotype
$P[gro^+]; H^{E31}\ E(spl)^{r8.1}/H^{E31}E(spl)^{r8.1}$	0	2	2	No effect
$P[gro^+]; H^{20}\ E(spl)^{r72.1}/H^{E31}E(spl)^{r72.1}$	0	2	4	Suppressed (+)
$P[gro^+]; H^{20}\ E(spl)^{r72.1}/H^{E31}E(spl)^{RA7.1}$	0	2	6	Suppressed (+)
$P[gro^+]; H^{20}\ E(spl)^{b32.2}/H^{E31}E(spl)^{r72.1}$	0	2	9	Suppressed (+++)
$P[gro^+]; H^{20}\ E(spl)^{r72.1}/H^{E31}$	0	>2	2	No effect
$P[gro^+]; H^{20}\ E(spl)^{r72.1}/H^{E31}\ E(spl)^{r8.1}$	0	>2	3	Suppressed (+)
$P[gro^+]; H^{20}\ E(spl)^{RA7.1}/H^{E31}$	0	>2	4	No effect
$P[gro^+]; H^{20}\ E(spl)^{b32.2}/H^{E31}$	0	>2	7	Suppressed (++)

Note. The effects of incrementally reducing E(spl)-C bHLH gene function on the *H* null bristle loss phenotype was assayed by examining cuticle preparations of pharate adults of the genotypes indicated. Because the E(spl)-C deletion chromosomes used for these experiments (see Fig. 5) have lesions that reduce or eliminate *gro* activity, a gro^+ P element transposon was included in the background, so that the observed phenotypic effects could be attributed specifically to loss of E(spl)-C bHLH function. This resulted in two genotypic series, one in which the gro^+ dosage is approximately 2 (upper set) and another with a gro^+ dosage of >2 (lower set), both with varying numbers of E(spl)-C bHLH genes. The relative degree of suppression observed is indicated on a qualitative scale: (+), mild; (++), intermediate; (+++), strong. In the crosses used to generate these progeny, the chromosome with the less severe E(spl)-C mutation always came from the female parent, in order to minimize maternal effects from these mutations (see Bang, 1993 for additional details).

types, since they are indistinguishable, but we can readily establish an epistatic relationship between gro^- bristle tufting and *H* null bristle loss. The cellular basis of the gro^- bristle tufting phenotype has not been investigated, but since adult bristle structures are produced, it clearly involves the commitment of (probably multiple) cells in the PNC to the SOP fate—the opposite of *H* SOP loss.

High frequencies (∼10 per animal) of mosaic patches homozygous for gro^{E48} or doubly homozygous for H^{20} and gro^{E48} were generated using the FLP–FRT site-specific recombination system (Golic and Lindquist, 1989; Golic, 1991). Both gro^{E48} and $H^{20}\ gro^{E48}$ mosaic patches exhibited bristle tufting phenotypes (Figs. 7A and 7B). Similar results were obtained for gro^{E73}, $H^{E31}\ gro^{E48}$, $H^{20}\ gro^{E73}$, and $H^{E31}\ gro^{E73}$ homozygous clones (data not shown). We conclude that the gro^- bristle tufting phenotype is epistatic to the *H* null bristle loss phenotype. This in turn indicates that, as for the E(spl)-C bHLH genes, the failure of stable commitment to the SOP fate in *H* null mutants depends upon gro^+ function.

DISCUSSION

H Promotes Stable Commitment to the SOP Cell Fate

Our previous studies have established that *H* plays an essential role in the specification and/or execution of the SOP cell fate in the wing imaginal disc (Bang *et al.*, 1991; Bang and Posakony, 1992). Here, by analyzing the expression of various PNC and SOP markers in a *H* mutant background, we have obtained strong evidence that *H* is required for a relatively late step in the determination of the SOP; namely, the stable commitment of a single PNC cell to this fate. We have found, first, that the initial expression of the proneural genes *ac* and *sc* appears to be established normally in *H* mutant PNCs. Second, the activity of the proneural proteins as transcriptional regulators in the PNCs appears to be similarly unimpaired, as assayed by the patterns of expression of an *ac–lacZ* transgene and the endogenous *sca* gene, both of which are subject to direct activation by protein complexes that include ac and sc. By these criteria, *H* activity is not required for the establishment or early development of the PNCs. Rather, the first detectable defect in *H* mutant PNCs is at a later step, in the emergence of a single cell that in the wild-type disc expresses a higher level of the proneural protein ac, a higher level of *sca* transcript, and the early SOP-specific marker A101. All three of these indicators reveal a very severe defect in SOP singularization from the PNC in *H* mutant discs: Nearly all PNCs in the notum region of the wing disc fail to develop a single cell with elevated ac and *sca* expression, and nearly all lack an A101-expressing cell. We take the fact that we do occasionally observe *H* mutant PNCs containing an individual cell with a higher level of ac and the fact that certain *H* mutant SOPs express A101 (though they do not go on to divide) as additional evidence of the relative lateness of the requirement for H^+ function in SOP determination.

H Is a Negative Regulator of the N Signaling Pathway

If, as we have concluded, the principal role of *H* in imaginal disc PNCs is to ensure the stable commitment of one cell to the SOP fate, there remains the question of its specific regulatory function. We have proposed previously (Bang and Posakony, 1992; Posakony, 1994) that *H* acts to protect the presumptive SOP from lateral inhibitory signaling within the PNC. We believe that the results presented in this paper and in Schweisguth and Posakony (1994) strongly support this hypothesis and indicate specifically that *H* functions as a negative regulator of the *N* cell–cell signaling pathway.

It is now clear that *H* acts *in vivo* as an antagonist of the activity of three key members of the *N* pathway: the N receptor itself (this paper; our unpublished observations), the putative transducer and transcription factor Su(H) (Brou *et al.*, 1994; Schweisguth and Posakony, 1994), and the family of proteins encoded by the E(spl)-C, including gro (this paper). First, we have shown that overexpressing an activated form of N causes bristle loss with the same cellular basis (failure of normal SOP commitment) as loss of *H* function. Our finding that simultaneous overexpression of *H* overcomes this phenotypic effect of activated N indicates that H antagonizes the activity of the receptor *in vivo*. Second, earlier genetic studies have shown that *H* and *Su(H)* encode antagonistic activities that have opposite effects on the SOP versus epidermal cell fate decision (Bang *et al.*, 1991; Bang and Posakony, 1992; Schweisguth and Posakony, 1992, 1994). Loss of *Su(H)* function was found to be epistatic to loss of *H* function in most PNC cells (Schweisguth and Posakony, 1994), consistent with the conclusion that *H* antagonizes *Su(H)* activity in normal SOP determination. Brou *et al.* (1994) have recently provided *in vitro* evidence that the H–Su(H) interaction is direct and that H inhibits the DNA-binding and transcriptional activation functions of Su(H). Finally, we have shown that loss of E(spl)-C bHLH gene function and loss of *gro* function are largely or fully epistatic to loss of *H* function in SOP determination, indicating that *H* inhibits the activity of these genes in this process.

We believe that the best interpretation of the body of results just summarized is that loss of *H* function leads to the inappropriate activity of *Su(H)* in the presumptive SOP; this in turn results in inappropriate E(spl)-C activity in this cell, resulting in the inhibition of the SOP cell fate via the same mechanism that normally acts in non-SOP cells of the PNC.

It is striking that reduction of E(spl)-C function in a *H* null background leads to the restoration of an almost normal spatial pattern of macrochaetes and microchaetes (Fig. 6C). This observation has a number of important implications. First, it indicates that although *H* is required for normal SOP specification in adult PNS development, it does not provide a primary ''proneural'' function; instead, its positive role in SOP cell fate determination derives from its action as a negative regulator of a pathway that antagonizes this fate. In support of this conclusion, we have found that overexpression of *H* via a *Hs–H* transgene fails to rescue any bristles in an $ac^- sc^-$ double mutant (Bang, 1993), though by other criteria such transgenes have potent biological activity (Bang and Posakony, 1992; this paper). Second, the experiment of Fig. 6 suggests that spatial variation in *H* activity is not responsible for establishing the initial asymmetry between the SOP and the non-SOP cells, since a single presumptive SOP cell can emerge from within the PNC in the absence of *H* function. Finally, our results further illustrate the importance of a delicate balance in the relative levels of activity of *H* and the genes of the *N* pathway, which we have noted previously (Bang and Posakony, 1992; Schweisguth and Posakony, 1994).

As described above, we believe that neighboring cells in the PNC are the source of the inhibitory signaling that must be antagonized in the SOP by *H* (Bang and Posakony, 1992; Posakony, 1994). A number of lines of experimental evidence support the idea of ''mutual inhibition'' within the PNC (Goriely *et al.*, 1991; Parks and Muskavitch, 1993). In addition, the patterns of expression of the genes that encode components of the *N* pathway suggest that all cells in imaginal disc PNCs are capable of sending, receiving, and transducing inhibitory signals. Thus, in the late third instar wing disc, *N* (Fehon *et al.*, 1991), *Su(H)* (Schweisguth and Posakony, 1992), and *gro* (A.G.B. and J.W.P., unpublished observations) are expressed ubiquitously; *Dl* is expressed in broad territories that appear to fully overlap the PNCs (Kooh *et al.*, 1993); and genes of the E(spl)-C other than *gro* are expressed specifically throughout the PNCs (Hinz *et al.*, 1994; Singson *et al.*, 1994). Our finding that the failure of SOP specification in *H* null imaginal discs depends on the activity of *N* pathway components downstream of the receptor [*Su(H)* (Schweisguth and Posakony, 1994); the E(spl)-C bHLH genes and *gro* (this paper)] provides, we believe, strong evidence that the SOP itself is subject to *N* pathway-mediated inhibitory signaling from neighboring cells in the PNC.

Cell Specificity of H Action in SOP Determination

The foregoing discussion raises the critical question of how the cell specificity of *H* action in SOP determination is achieved. That is, why does *H* successfully antagonize the activity of the *N* pathway in the SOP but not in the remaining cells of the PNC? We believe that our present understanding of *H* function in SOP determination in the imaginal disc (summarized above) is compatible with a relatively simple model that addresses this question, which we will refer to as the *H* threshold model. The principal elements of this hypothesis are that the activity of *H* in the cells of the PNC is relatively uniform and constitutive and that a substantial difference in the activity of the *N* pathway in the SOP versus the non-SOP cells is principally responsible for their different responses to *H* regulation. In this view, *H* does not itself establish the initial asymmetry between the SOP and non-SOP cells in the PNC; instead, it ensures that a previously established asymmetry leads to the stable commitment of a single cell to the SOP fate.

The model (Fig. 8) suggests that, although all cells in the PNC are subject to inhibitory signaling via the N receptor, the total quantity of signal received by the non-SOP cells is substantially greater than that received by the SOP. For example, it is likely that this signal is mediated principally by the Dl protein, though it might also involve other possible ligands for the N receptor (e.g., sca); up-regulation of Dl (Kunisch *et al.*, 1994) (or sca; Mlodzik *et al.*, 1990) expres-

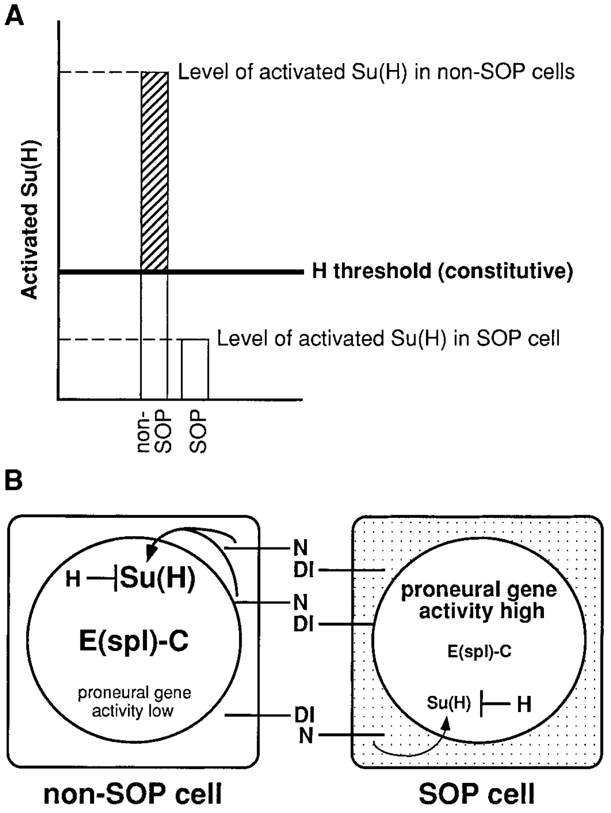
A
Activated Su(H)
Level of activated Su(H) in non-SOP cells
H threshold (constitutive)
Level of activated Su(H) in SOP cell
non-SOP
SOP
B
H —| Su(H)
E(spl)-C
proneural gene activity low
N
Dl
N
Dl
Dl
N
proneural gene activity high
E(spl)-C
Su(H) |— H
non-SOP cell
SOP cell

FIG. 8. A threshold model of H function in SOP specification. (A) The constitutive level of H activity in the PNC is sufficient to inhibit the relatively low level of Su(H) activity in the SOP cell but not the higher levels in non-SOP cells, leaving uninhibited Su(H) (hatching) free to function in these cells. (B) The H threshold translates a quantitative difference in the activity of the *N* pathway in SOP versus non-SOP cells into a stable binary cell fate decision. Up-regulation of Dl expression in the presumptive SOP (Kunisch *et al.*, 1994) would contribute to higher levels of activated Su(H) in the non-SOP cells. In these cells, activated Su(H) protein not inhibited by H elevates the level of E(spl)-C gene expression; we have recently shown that this occurs by direct transcriptional activation (A.M.B. and J.W.P., submitted for publication). This in turn leads to the down-regulation of proneural gene activity and a consequent loss of SOP potential. By contrast, the same level of H activity in the presumptive SOP is sufficient to inhibit the lower level of activated Su(H) in this cell. E(spl)-C expression thus remains low, and proneural gene activity high, leading to stable commitment to the SOP cell fate. This model appears to account satisfactorily for events in most PNC cells (presumably including the normal SOP), in which Schweisguth and Posakony (1994) found that *Su(H)* is epistatic to *H* for SOP determination; it does not by itself account for the remaining cells which failed to show commitment to the SOP fate in the absence of both gene functions.

sion in the SOP would certainly be expected to contribute to the proposed differential in signal received. The result of this and other regulatory differences would be a higher level of activated Su(H) protein in non-SOP cells than in the SOP. The model further postulates that the constitutive level of H activity in the PNC establishes a threshold of inhibition of Su(H) activity. If the level of activated Su(H) in a cell is at or below this threshold, then there is no net Su(H) activity, and the cell fails to respond to the inhibitory signal. However, in cells in which the level of activated Su(H) is above the H threshold, the "excess" activated Su(H) (that which is not inhibited by H) is then free to elevate the expression of E(spl)-C genes, and this in turn inhibits the cell from adopting the SOP fate. In the model, the level of activated Su(H) in the SOP is below the H threshold, while that in the non-SOP cells is substantially above it (Fig. 8).

According to the model, a *H* null imaginal disc has a very low or zero threshold, so that there is excess (uninhibited) Su(H) activity even in the cell that would normally become the SOP—with the result that it has inappropriate E(spl)-C activity and is inhibited from committing to the SOP fate. Overexpression of activated N or of Su(H) would mimic this effect by elevating the level of active Su(H) in the SOP so that it exceeds the existing H threshold. Conversely, overexpression of *H* raises the threshold, so that even the higher level of activated Su(H) characteristic of non-SOP cells is fully inhibited in at least some of these cells, leading to their stable commitment to the SOP fate.

This relatively simple version of the *H* threshold model is of course not a comprehensive hypothesis for the regulatory events involved in the SOP/non-SOP cell fate decision. Moreover, the basic premises of the model (that H activity is constitutive within the PNC and that non-SOP cells have more *N* pathway activity than the SOP) clearly require experimental support and could be invalidated by future studies. In particular, it is entirely possible that H activity is itself subject to modulation within the PNC, either by the *N* pathway or by some other regulatory system. For example, an activity dependent on proneural gene function could elevate H activity preferentially in the SOP.

At present, however, we favor a model in which the principal role of H in SOP specification is to translate a quantitative difference in the activity of the *N* pathway in the SOP versus the non-SOP cells (a difference which itself results from preexisting biases in proneural potential within the PNC) into a stable binary cell fate decision.

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Note added in proof. The paper described herein as "A.M.B. and J.W.P., submitted for publication" has now been accepted: Bailey, A. M., and Posakony, J. W. (1995). Suppressor of Hairless directly activates transcription of Enhancer of split Complex genes in response to Notch receptor activity. *Genes Dev.,* in press.

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