Drosophila *Notch* Receptor Activity Suppresses *Hairless* Function During Adult External Sensory Organ Development

Daniel F. Lyman and Barry Yedvobnick

Department of Biology, Emory University, Atlanta, Georgia 30322

Manuscript received March 15, 1995
Accepted for publication September 12, 1995

ABSTRACT

The neurogenic *Notch* locus of Drosophila encodes a receptor necessary for cell fate decisions within equivalence groups, such as proneural clusters. Specification of alternate fates within clusters results from inhibitory communication among cells having comparable neural fate potential. Genetically, *Hairless* (*H*) acts as an antagonist of most neurogenic genes and may insulate neural precursor cells from inhibition. *H* function is required for commitment to the bristle sensory organ precursor (SOP) cell fate and for daughter cell fates. Using *Notch* gain-of-function alleles and conditional expression of an activated *Notch* transgene, we show that enhanced signaling produces *H*-like loss-of-function phenotypes by suppressing bristle SOP cell specification or by causing an *H*-like transformation of sensillum daughter cell fates. Furthermore, adults carrying *Notch* gain of function and *H* alleles exhibit synergistic enhancement of mutant phenotypes. Over-expression of an H^+ transgene product suppressed virtually all phenotypes generated by *Notch* gain-of-function genotypes. Phenotypes resulting from over-expression of the H^+ transgene were blocked by the *Notch* gain-of-function products, indicating a balance between *Notch* and *H* activity. The results suggest that *H* insulates SOP cells from inhibition and indicate that *H* activity is suppressed by *Notch* signaling.

CELL communication, employing ligand-receptor interactions coupled to intracellular elements, is a component of many cell fate decisions. In Drosophila, the segregation of neural and epidermal primordia in the embryonic and imaginal neuroectoderm involves communication within groups of cells having comparable developmental potential (proneural clusters). These equivalence groups arise from restricted expression of proneural genes, such as the *achaete-scute complex* (*AS-C*), *atonal* and *daughterless* (CUBAS *et al.* 1991; CAMPUZANO and MODOLLEL 1992; SKEATH and CARROL 1992; GHYSEN *et al.* 1993; JARMAN *et al.* 1994), which encode basic helix-loop-helix (bHLH) transcriptional regulatory proteins that apparently regulate genes required for neural development. Commitment of cells within these equivalence groups to neural development is associated with maintenance/enhancement of proneural gene expression; neighboring cells cease expressing these genes in response to inhibitory communication and adopt the epidermal fate.

Neurogenic genes encode elements of this signaling pathway and are functionally linked, based upon genetic and molecular criteria (LEHMANN *et al.* 1983; VASSIN *et al.* 1985; DE LA CONCHA *et al.* 1988; FEHON *et al.* 1990; XU *et al.* 1990; FORTINI and ARTAVANIS-TSAKONAS 1994; JENNINGS *et al.* 1994). The neurogenic pathway has been studied in both embryonic neurogenesis and adult external sensory organ development. Null mutations of neurogenic genes cause excess production of neural precursor cells and a corresponding loss of epidermis, resulting in recessive embryonic lethality or surplus adult external sensory organs (neurogenic phenotypes). In adult sensory organ development both specification of sensory organ precursor (SOP) cells within proneural clusters (HARTENSTEIN and POSAKONY 1989; CUBAS *et al.* 1991; SKEATH and CARROL 1992) and daughter cell fates (HARTENSTEIN and POSAKONY 1990; PARKS and MUSKAVITCH 1993; POSAKONY 1994) are regulated by the neurogenic pathway.

The neurogenic *Notch* (*N*) and *Delta* (*Dl*) loci encode interacting membrane proteins; *Delta* protein (Delta) bound to *Notch* protein (Notch) on adjacent cell surfaces has been demonstrated (FEHON *et al.* 1990), supporting the proposed role of Delta as a ligand for the Notch receptor (HEITZLER and SIMPSON 1991). The complex intracellular domain of Notch includes ankyrin repeats, necessary for cell communication (LIEBER *et al.* 1993; LYMAN and YOUNG 1993; REBAY *et al.* 1993) and interaction with other proteins during Notch signaling (DIEDERICH *et al.* 1994; FORTINI and ARTAVANIS-TSAKONAS 1994). Several neurogenic genes encode nuclear proteins including *mastermind* (SMOLLER *et al.* 1990), *neuralized* (PRICE *et al.* 1993) and the *Enhancer of split Complex* [*E(spl)C*]. The *E(spl)C* region encodes multiple bHLH factors (KLAMBT *et al.* 1989; KNUST *et al.* 1992; DELIDAKIS and ARTAVANIS-TSAKONAS 1992) and

Corresponding author: D. F. Lyman, Department of Biology, 1510 Clifton Rd., Emory University, Atlanta, GA 30322.
E-mail: dlyman@biology.emory.edu

groucho (*gro*), which appears to participate in a transcriptional repression complex with other *E(spl)C* factors (HARTLEY *et al.* 1988; PAROUSH *et al.* 1994). The *E(spl)C* appears to be epistatic to other neurogenic genes and to be the target of the cell signaling pathway (VASSIN *et al.* 1985; DE LA CONCHA *et al.* 1988; LIEBER *et al.* 1993). The neurogenic gene *Suppressor of Hairless* [*Su(H)*] encodes a homologue of CBF1 (a mammalian transcriptional regulatory protein, HENKEL *et al.* 1994) that has recently been shown to bind to the Notch ankyrin repeats (FORTINI and ARTAVANIS-TSAKONAS 1994). Moreover, in cell culture, Delta-Notch interaction results in nuclear translocation of the *Su(H)* product and possible activation of the *E(spl)C* (BROU *et al.* 1994; FORTINI and ARTAVANIS-TSAKONAS 1994; JENNINGS *et al.* 1994). Although not shown to produce a neurogenic phenotype, the *deltex* (*dx*) locus interacts genetically with neurogenic genes and encodes a cytoplasmic protein which also binds to the Notch ankyrin repeats (XU and ARTAVANIS-TSAKONAS 1990; GORMAN and GIRTON 1992; DIEDERICH *et al.* 1994). The *SuH* and *dx* products may compete for binding to Notch. Thus, neurogenic gene products constitute elements of a cell signaling pathway from cell surface to nucleus.

Hairless (*H*), a potent antagonist of neurogenic genes, is also required for neural fate (*e.g.*, bristle) development (BANG *et al.* 1991; BANG and POSAKONY 1992). *Hairless* loss-of-function mutations (H^-) produce a dominant alteration of bristle daughter cell fates or suppress SOP cell determination. Over-expression of H^+ in transgenic flies results in either excess bristle production or a transformation of daughter cell fates opposite to *H* loss of function. In addition, H^- enhances *N* gain-of-function (*i.e., split* and *Abruptex*) phenotypes (see LINDSLEY and ZIMM 1992) and antagonizes embryonic and imaginal neurogenic phenotypes, except those of the *E(spl)C* (VASSIN *et al.* 1985; DE LA CONCHA *et al.* 1988). Originally, *Su(H)* was identified by its genetic interaction with *H* (ASHBURNER 1982); *Su(H)* loss of function restores bristle development in an H^- genetic background, whereas over-expression of a transgenic *Su(H)* product mimics adult H^- mutant phenotypes (SCHWEISGUTH and POSAKONY 1992). Recently, *H* and *Su(H)* proteins have been shown to interact *in vitro* and in cell culture (BROU *et al.* 1994). This interaction inhibits DNA binding and transcriptional activation by *Su(H)* of a reporter gene containing a potential *E(spl)C* promoter binding site. *H* has been proposed to encode a nuclear protein and to insulate SOP cells from inhibition by antagonizing the function of neurogenic genes (BANG and POSAKONY 1992; MAIER *et al.* 1992). Thus, *H* protein may insulate SOP cells from inhibition by counteracting Notch regulation of *Su(H)* protein activity.

Here, we examine the cellular basis by which certain *N* mutations suppress mechanosensory bristle production on the notum and we examine their interaction with *H*. Gain-of-function *Notch* genes, or expression of an activated *Notch* transgene ($Notch^{int}$), suppress SOP cell fate specification in a fashion indistinguishable from H^- mutations. These *N* gain-of-function phenotypes are also enhanced by H^- mutations. Expression of $Notch^{int}$ during third instar larval and pupal stages also produces many other H^--like phenotypes. Virtually all *Notch* gain-of-function phenotypes are blocked by expression of an $H^{\pm}$ transgene. Conversely, these *N* products suppress *H* gain-of-function phenotypes. Our results support the view that *H* insulates SOP and daughter cells from inhibition and suggest an intimate balance between *H* and *N* function in cell signaling events which suppress/promote one of two alternate cell fates during adult sensory bristle development.

MATERIALS AND METHODS

Drosophila stocks: Fly stocks were maintained at 25° on standard cornmeal, sucrose media. Stocks of *Hairless* alleles (H^1 *Pr/In(3R)C e*, $H^2/T(2;3)$ *ap*^{Xa}, $H^3/In(3R)C$ *Sb e l(3)e*, *p*^{p} $H^{C20}/TM6B$ and *p*^{p} H^{RP1} *e/TM6B*) were obtained from the Bloomington Stock Center (University of Indiana, Bloomington) or the laboratory of J. POSAKONY (UCSD, La Jolla, CA). The *Hairless* (*H3*) transgenic strain (w^{1118}; *P*[w^+; *hs-H*$^+$]*H3*) was generously provided by the laboratory of J. POSAKONY (BANG and POSAKONY 1992). The activated cytoplasmic Notch ($N^{Intracellular\ domain}$; herein referred to as $Notch^{int}$) transgenic stock (*y w P*[w^+; *hs-Notch*^{int}]*/FM7c*) was kindly provided by the laboratory of M. YOUNG (The Rockefeller University, New York, NY) (LIEBER *et al.* 1993). Expression of both transgene products is driven by a heat shock promoter. The *A37* and *A101* "enhancer trap" lines (*ru h P*[*lacZ; ry*$^+$]*A37 sr e*^{s} *ca* and *P*[*lArB*]*A101.1F3 ry*^{503}*/TM3, ry*) were kindly provided by H. BELLEN (Baylor College of Medicine, Houston, TX). Descriptions of mutant alleles, balancer chromosomes and transgenic stocks may be obtained from LINDSLEY and ZIMM (1992) or indicated references. Adult cuticle preparations (mounted in Permount or 90% glycerol) and vital stains of pupal tissue (toluidine blue and neutral red, mounted in 90% glycerol) were performed as described (ASHBURNER 1989).

Immunohistochemistry: White prepupae (0 hr after puparium formation, apf) were collected from vials and aged at 25° until dissection in 1× PBS on ice. Specimens were fixed in 4% paraformaldehyde/PBS (with gentle agitation) for 20–30 minutes at 25°, washed three times in PBS and stained as described (KIDD *et al.* 1989). Antisera (diluted to 50% of final titre) were preabsorbed (twice for 2 hr each at 25°) with fixed and permeablized wild-type embryos. Primary antibodies/antisera were used at the following concentrations: Monoclonal antibody (MAb) 22C10 (1:50), anti-cut (1:1000), anti-asense (1:5000) and anti-β-galactosidase (1:1500) – and visualized using a standard or elite peroxidase Vectastain ABC kit (Vector, Burlingame, CA). Pupal tissues were then mounted in Poly Bed 812 (Polysciences, Warrington, PA) or 90% glycerol. MAb 22C10 was kindly provided by S. BENZER (CIT, Pasadena). Antisera to *cut* and *asense* proteins were generously provided by the laboratory of Y. N. JAN (UCSF, San Francisco, CA). Rabbit antibody to β-galactosidase was obtained from Cappel (Durham, NC).

Detection of β-galactosidase activity: White prepupae were collected, aged at 25° and dissected. Fixation and histochemical detection of β-galactosidase activity was performed as de-

scribed (ROMANI *et al.* 1989), and tissues were mounted in 90% glycerol. β-galactosidase expressed by the *A37 P*-element transposon insertion labels the primary precursor cells of adult sensilla and their daughter cells (BANG *et al.* 1991).

Analysis of *asense* immunolabeling of microchaete SOP cells: Expression of *ase* protein in microchaete primary and secondary precursor cells (not previously reported; BRAND *et al.* 1993) was confirmed. Ax^{9B2} flies (controls) exhibit a wild-type distribution of microchaetes on the head and notum. Nota were prepared and stained with *ase* antiserum at 15 hr apf, during formation of secondary precursor cells (HARTENSTEIN and POSAKONY 1989; USUI and KIMURA 1993). Stained nota exhibited a wild-type pattern of microchaete sensilla (data not shown), which consisted of one or two labeled nuclei lying in the epidermal plane, reflecting the developmental stage (*i.e.*, before or after SOP cell division) at which nota were examined. No macrochaete precursor cells were detected with the antiserum since *ase* expression is extinguished in the terminal daughter cells of the sensilla. The scutellar region which lacks microchaetes was devoid of labeled nuclei.

Establishment of labeled "heteroallelic" pupal tissue genotype: The $l(1)N^B$ and N^{60g11} mutations were maintained over a balancer chromosome (*i.e.*, $y\ w^a\ l(1)N^B/FM7c$ and $w^a\ N^{60g11}/FM7c$). Crosses to $wa\ Ax^{9B2}$ produce balancer males and two classes of female progeny. $Ax^{9B2}/FM7c$ females exhibit the *Bar* phenotype (discernible at 72 hr apf) and normal bristle pattern, while $l(1)N^B/Ax^{9B2}$ (or N^{60g11}/Ax^{9B2}) sisters ("heteroallelic *N* mutants") exhibit a "T"-shaped pattern of mechanosensory bristles on the notum (Figure 1, B and C), due to bristle loss (LYMAN and YOUNG 1993).

Histochemical stains of such pupae with antibodies that label sensory organ cells exhibit a mixture of wild-type distribution of sensilla and a T pattern corresponding to the adult bristle loss pattern. Labeled pupae with the T pattern of sensilla were confirmed as heteroallelic female progeny. White prepupae (>40) from the cross were collected (0 hr apf) and aged 72 hr at 25°. *Bar* eye pupae were separated from non-*Bar* siblings and each class was stained with MAb 22C10. *Bar* eye pupae exhibited only a wild-type distribution of sensilla; non-*Bar* pupae demonstrated only the T pattern of labeled sensory organs.

Generation of adult mutant phenotypes by larval/pupal expression of $Notch^{int}$ and an H^+ transgene: Only transgenic females ($Notch^{int}/FM7c$) exhibited the reported mutant cuticle phenotypes in response to heat shock induction. No mutant effects were observed after heat shock treatment of the transgene host or balancer strains. Expression of $Notch^{int}$ and the H^+ transgene were induced in late (wandering) third instar larvae or in pupae at 0, 8, 16, 24 or 32 hr apf. Larvae or white prepupae were collected and aged at 25°, heat shocked at 37° for 30 minutes and then allowed to eclose at 25°. All heat shock developmental times noted include a ±1 hr variance. Preserved wings were dehydrated in ethanol and mounted in Canada Balsam (Sigma, St. Louis, MO). Other cuticle structures were prepared for dissecting microscope observation as described (ASHBURNER 1989).

Establishment of labeled $Notch^{int}$ pupal tissue genotype: Immunohistochemical stains were done to examine the cytoarchitecture of bristle sensory organs resulting from $Notch^{int}$ expression. To confirm "mutant" labeled pupae as transgenic females, only female third instar larvae were collected, heat shocked at 8 hr (n = 12) or 20 hr (n = 13) apf, aged to 36 hr apf at 25°, dissected and stained with MAb 22C10. The stained female pupae, following expression of $Notch^{int}$, exhibited only a "mutant" composition of sensilla (see RESULTS). Examination of noninduced female pupae (n = 20) demonstrated only the wild-type composition of sensilla (data not shown).

RESULTS

***Notch* gain-of-function alleles [$l(1)N^B$ and N^{60g11}] suppress SOP cell specification:** Distributed in a characteristic pattern over the adult integument is an array of external sensory organs. Two types of mechanosensory bristles, large (macrochaetes) and small (microchaetes), are located on the head and notum (Figure 1A). The macrochaetes, and to a lesser extent microchaetes, are distributed at characteristic positions. Each sensillum is composed of four cells derived from a primary sensory organ precursor (SOP) cell by two rounds of division (HARTENSTEIN and POSAKONY 1989). The tricogen (shaft) and tormogen (socket) arise from one secondary precursor; the other produces a bipolar sensory neuron and thecogen (sheath) cell. Macrochaete SOP cells are specified in third instar imaginal wing disc proneural clusters and divide around puparium formation. Microchaete SOP cells are specified approximately 10 hr after puparium formation (apf) and divide around 14 hr apf (HARTENSTEIN and POSAKONY 1989; USUI and KIMURA 1993). Following the secondary precursor cell divisions, daughter cell fate decisions ensue within a few hours. Terminal cell differentiation then forms the mature sensory organ. Except for the epidermally located socket cell, all daughter cells assume a subepidermal position during early bristle morphogenesis.

Previous analysis of two atypical, recessive lethal *Notch* (*N*) alleles [$l(1)N^B$ and N^{60g11}] demonstrated several gain-of-function phenotypes; notably bristle loss on the notum (LYMAN and YOUNG 1993). N^{60g11} is a frameshift truncation of the Notch intracellular domain immediately carboxy-terminal to the ankyrin repeats, while $l(1)N^B$ encodes a conservative substitution in an extracellular *lin-12/Notch* repeat (LYMAN and YOUNG 1993). Typically, $l(1)N^B/+$ females exhibit a mild loss of microchaetes on the mesonotum (Figure 2A), as do temperature-sensitive $N^{60g11}/+$ flies. A dramatic enhancement of bristle loss on the head and notum occurs when either allele is combined *in trans* (heteroallelic) with a gain-of-function *Abruptex* allele (Ax^{9B2}) of *Notch* (Figure 1, B and C). The loss of microchaetes in particular results in a characteristic pattern; one or two rows of bristles remain along each side of the dorsal midline as well as a small cluster along the anterior border of the notum. This distinctive T-shaped pattern of sensory organs was useful in evaluating the cellular basis of the mutant phenotype.

The involvement of *Notch* in the selection of cell fates suggested that these bristle phenotypes reflect suppression of precursors by overactive Notch receptors. However, *N* loss of function during external sensory organ development can also cause bristle loss, due to produc-

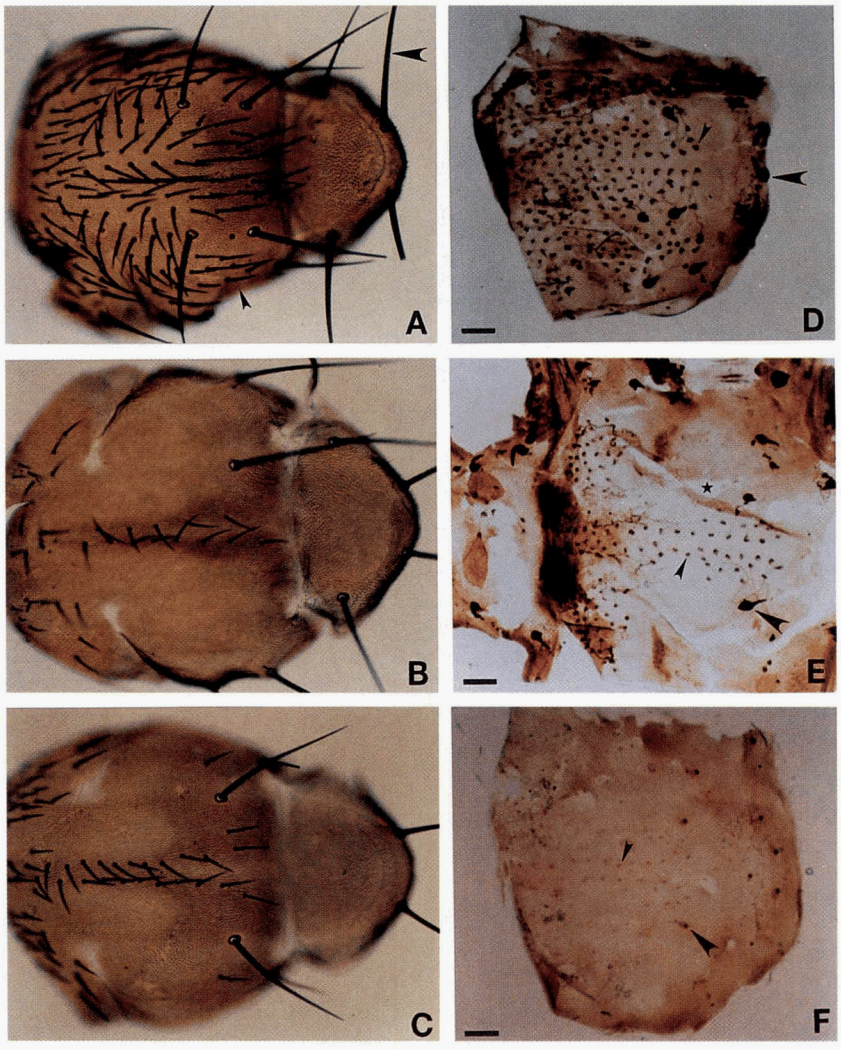

FIGURE 1.—*Notch* gain-of-function alleles suppress SOP fate specification. Heteroallelic combinations of specific *N* gain-of-function alleles result in significant loss of mechanosensory bristles on the notum (and head; not shown) due to lack of SOP cell specification. (A–F) Anterior is left. (A–C) Cuticle preparations illustrate the distribution, and loss, of macrochaete (large arrowhead) and microchaete (small arrowhead) sensilla on adult nota; (A) *Canton S*, (B) $l(1)N^{B}/Ax^{9B2}$ and (C) N^{60g11}/Ax^{9B2}. (A) Mechanosensory bristles on wild-type nota (particularly macrochaetes) exhibit a characteristic, reproducible distribution (see LINDSLEY and ZIMM 1992 for nomenclature). (B and C) Heteroallelic combinations of *N* gain-of-function alleles result in loss of both macrochaetes and microchaetes; a characteristic T pattern of microchaetes results leaving a large area of the notum devoid of bristles. Many macrochaetes are also lost. Despite some variability, specific macrochaetes lost correlate highly with those sensitive to *H* loss-of-function mutations (BANG *et al.* 1991; D. F. LYMAN, unpublished observations). (D–F) Immunohistochemical stains of pupal nota demonstrate that this mechanosensory bristle loss results from suppression of SOP fate specification. (D) Wild-type notum (36 hr apf) illustrating normal distribution of stained (MAb 22C10) macrochaete (large arrowhead) and microchaete (small arrowhead) sensilla. (E) $l(1)N^{B}/Ax^{9B2}$ notum stained (MAb 22C10, 36 hr apf) for bristle sensilla shows loss of specific macrochaetes (large arrowhead) and most microchaetes (small arrowhead). Comparable to the pattern seen for the bristle cuticle phenotype, large areas of the notum (star) are devoid of stained microchaete sensilla. Equivalent results are obtained in stained N^{60g11}/Ax^{9B2} nota (data not shown). (F) $l(1)N^{B}/Ax^{9B2}$; *A37*/+ notum stained (anti-β-galactosidase, 24 hr apf) for SOP cells lacks precursors for many macrochaetes (large arrowhead) and most microchaetes (small arrowhead). Microchaete SOP cells present on the notum are arranged in the characteristic T pattern. An equivalent effect is seen in stained N^{60g11}/Ax^{9B2}; *A37*/+ nota (data not shown). (D–F) Bar, 200 μm.

tion of excess sensory neurons at the expense of other cells (HARTENSTEIN and POSAKONY 1990; POSAKONY 1994). We used reagents that specifically label SOP cells or their progeny, to determine which stage of bristle formation was affected by the atypical *N* mutations.

Using MAb 22C10 and an antiserum to *cut* protein (cut), we determined that this defect results from loss of all four cells of the sensillum. In wild-type flies, both reagents label all four cells of the bristle organ (HARTENSTEIN and POSAKONY 1989; BLOCHLINGER *et al.* 1993). By 36 hr apf, all macrochaetes and microchaetes have differentiated on the wild-type head and notum and all four daughter cells have become MAb 22C10-positive (Figure 1D), though tormogen cell labeling is

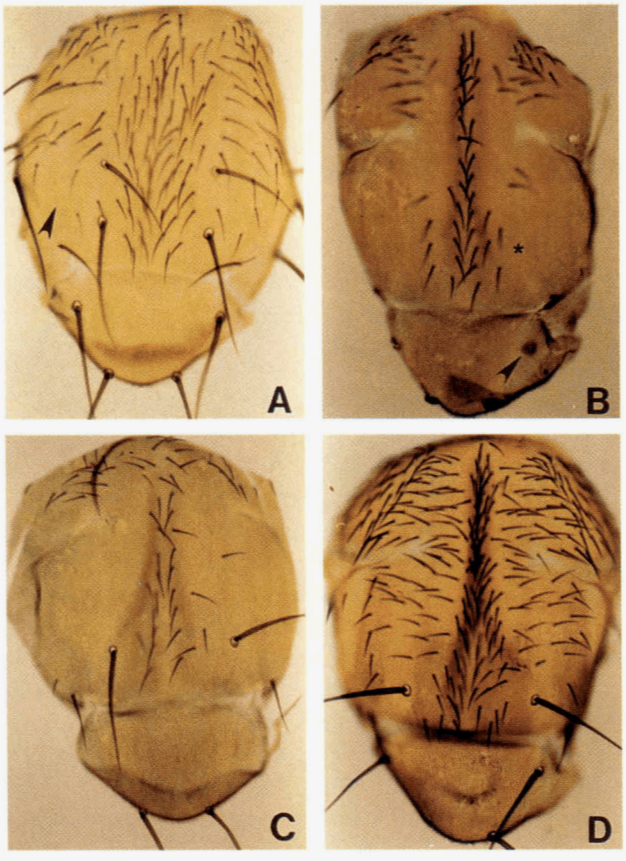

FIGURE 2.—*Hairless* gene dosage modifies *Notch* gain-of-function bristle phenotypes. (A) Adult cuticle showing the mild loss of microchaetes on the notum of an $l(1)N^B/+$ fly. In addition to a typical general reduction in the number of microchaetes on the notum (*Cf.* Figure 1A; LINDSLEY and ZIMM 1992; LYMAN and YOUNG 1993), patches of microchaete loss are also frequently evident (arrowhead). Loss of macrochaetes (*e.g.,* anterior dorsocentrals) is rarely observed in this genotype. (B) Adult cuticle showing the synergistic loss of mechanosensory bristles on the notum of a $l(1)N^B/+; H^1/+$ fly, characterized by the same T pattern of microchaetes seen in the heteroallelic *N* mutant flies (*cf.* Figure 1, B and C). Numerous macrochaetes are also either lost (*e.g.,* dorsocentrals; *) or exhibit a double socket phenotype (*e.g.,* scutellars; arrowhead, out of focus). $FM7c/+; H^1/+$ siblings (data not shown) exhibit only the typical H^- macrochaete phenotype. $l(1)N^B/+; +/+$ siblings exhibit only mild loss of microchaetes (comparable to 2A) and no effect on macrochaetes (data not shown). Other H^- alleles (data not shown) produce similar effects when combined with $l(1)N^B$. H^- alleles do not exhibit dominant microchaete loss (BANG *et al.* 1991; LINDSLEY and ZIMM 1992; D. F. LYMAN, unpublished observations). (C) Notum of a $l(1)N^B/Ax^{9B2}; P[hs\text{-}H^+]H3/+$ fly (development at 25°) in which the H^+ transgene was not heat shock activated. Lack of H^+ cDNA expression in this genotype results in mechanosensory bristle loss on the notum which is indistiguishable from that of $l(1)N^B/Ax^{9B2}; +/+$ flies (*cf.* Figure 1B). (D) Expression of an H^+ cDNA during microchaete SOP specification (8 hr apf) in heteroallelic *N* mutant flies ($l(1)N^B/Ax^{9B2}; P[hs\text{-}H^+]H3/+$) counteracts the suppression of microchaete bristle formation due to the *N* gain-of-function genotype (*cf.* Figures 2C and 1B). Restoration of macrochaetes also occurs in this genotype when the H^+ transgene is expressed during the appropriate stage of macrochaete SOP specification (data not shown). In addition, gain-of-function phenotypes resulting from expression of the H^+ cDNA alone (see text; BANG and POSAKONY 1992) were not observed in the presence of the *N* heteroallelic alleles. The apparent increased density of microchaetes along the midline of this specimen is an artifact due to buckling of the cuticle preparation. (A–D) Anterior is up.

variable. Cut is expressed in macrochaete and microchaete sensilla on the head and notum at 24 hr apf.

Examination of $l(1)N^B/Ax^{9B2}$ pupae at 36 hr apf, stained with MAb 22C10, revealed a T pattern of labeled sensilla on the notum in register with that of bristles on heteroallelic adult flies (Figure 1E). Each stained sensillum contained three or four cells having normal morphology and position. Lateral to the dorsal midline, however, a large area lacked stained sensilla. In 24 hr apf pupae labeled with cut antiserum identical results were obtained (data not shown). Equivalent results were obtained with N^{60g11}/Ax^{9B2} pupae (data not shown). These observations indicate that bristles on the head and notum of these *N* mutants do not form because either SOP cells are not properly specified or they do not correctly produce daughter cells. Since all four daughter cell types could be observed in the remaining labeled sensilla, these observations rule out the possibility that daughter cell fates were transformed from one sensory organ cell type to another, as seen with the conditional N^- allele, $l(1)N^{ts1}$ (HARTENSTEIN and POSAKONY 1990).

Since *Notch* also functions during SOP fate specification, we investigated whether this bristle loss resulted from a lack of SOP cells. We used two markers to visualize SOP cells: a *P*-element transposon insertion (*A37*) that expresses β-galactosidase in sensillum precursor cells and an antiserum to *asense* protein (data not shown), which is expressed specifically in determined SOP cells (BANG *et al.* 1991; BRAND *et al.* 1993). For both markers, among the labeled nota of both $l(1)N^B/Ax^{9B2}$ and N^{60g11}/Ax^{9B2} pupae, we found SOP cells only flanking the dorsal midline and bordering the anterior limit of the mesonotum; *i.e.,* in the characteristic T pattern (Figure 1F). In addition, examination of these nota with vital stains (at 16 and 20 hr apf) failed to produce evidence of cell death (data not shown). Therefore, the absence of stained SOP cells in regions of the head and notum corresponding to bristle loss indicated that these *N* heteroallelic combinations block initial selection or maintenance of bristle SOP cell fates. This interpretation is supported by analyses of flies carrying an activated *Notch* transgene (below).

Notch signaling activity suppresses *Hairless* function during SOP cell fate specification: Neurogenic mutations often cause overproduction of external sensory organs, analogous to over production of embryonic neuroblasts in homozygous mutants. In contrast, gain-of-function phenotypes of *Notch* reflect suppression of

neural fate determination due to enhancement of cell signaling (this work; LIEBER *et al.* 1993; LYMAN and YOUNG 1993; REBAY *et al.* 1993; STRUHL *et al.* 1993). In addition to proneural gene function, production of adult external sensory organs requires *Hairless* (*H*) activity (BANG *et al.* 1991; BANG and POSAKONY 1992). We were interested in the phenotypic similarities between *H* loss-of-function and *N* gain-of-function mutations, which suggested a link between *N* and *H*. Evidence presented here supports the idea that *H* activity is inhibited by Notch receptor signaling during mechanosensory bristle development. Thus, *H* function appears to be an integral negative regulatory element of the *Notch* signaling pathway.

Most macrochaetes are present in adults haploinsufficient for *H* (*i.e.*, $H^-/+$), but typically exhibit a "double socket" phenotype; *i.e.*, the tricogen cell forms a second socket rather than bristle shaft (Figure 3C; BANG *et al.* 1991; D. F. LYMAN, unpublished observations). Thus, the tricogen cell fails to shift from an epidermal to subepidermal position and two large sensory organ (tormogen) cells are detected in the epidermal plane. Further reduction of *H* function leads to bristle loss (due to suppression of SOP fate specification) or lethality.

We examined the phenotype of $l(1)N^B$ adults when combined with *H* loss-of-function genotypes. The mild loss of microchaetes on the notum of $l(1)N^B/+$ flies (Figure 2A) is dominantly enhanced by H^- alleles (Figure 2B), producing a bristle pattern similar to $l(1)N^B/Ax^{9B2}$ flies (Figure 1B). Thus, reduction of *H* enhances the mutant effect of elevated Notch activity, or enhanced Notch activity may reduce *H* function during external sensory organ development. Therefore, we tested whether expression of a heat shock-driven H^+ transgene could offset the bristle loss exhibited by $l(1)N^B/Ax^{9B2}$ flies. Expression of the H^+ transgene in a wild-type background during bristle SOP cell fate determination (*e.g.*, 8 hr apf) results in supernumerary bristles (BANG and POSAKONY 1992). In the $l(1)N^B/Ax^{9B2}$ genotype, expression of the H^+ transgene at 8 hr apf largely restored microchaete specification to a wild-type pattern (Figure 2D). Restoration of bristle development was heat shock dependent (Figure 2, C and D) and heat shocked $l(1)N^B/Ax^{9B2}$ flies lacking the H^+ transgene did not show bristle restoration (data not shown). This result demonstrates that extra H^+ protein activity compensates for hyperactive Notch activity. The resulting complement of microchaetes also indicates that H^+-induced production of excess bristle sensilla was blocked by the Notch gain-of-function products (Figure 2D), supporting the view that H^+ protein activity insulates SOP cells from inhibition and that *H* may be a target of Notch receptor activity.

Larval/pupal expression of an activated $Notch^{int}$ transgene produces H^--like phenotypes: The data presented above strongly suggest a functional link between *N* and *H*. We examined this possibility further by altering Notch activity at different stages of external sensory organ development using a heat shock-inducible, activated *N* transgene to produce H^--like phenotypes (double socket or bristle loss). This transgene ($Notch^{int}$) encodes only the protein intracellular domain and embryonic expression results in an anti-neurogenic phenotype due to inhibition of neuroblast specification (LIEBER *et al.* 1993; STRUHL *et al.* 1993); such effects are produced in the presence or absence of endogenous N^+ function.

Macrochaete SOP cells on the wing disc are determined during the third larval instar. Expression of $Notch^{int}$ at this stage caused selective loss of macrochaetes on the notum, and head (Table 1, Figure 3A). Specification of macrochaete SOP cells on the head and notum is completed by puparium formation after which the daughter cells are generated (HARTENSTEIN and POSAKONY 1989). Expression of $Notch^{int}$ during this phase (*i.e.*, 0–24 hr apf) resulted in double socket sensilla at all or most macrochaete sites on the head and notum of adult flies (Table 1, Figure 3, B, D, and E), which closely resemble the mutant sensilla resulting from H^- haploinsufficiency (FIGURE 3C; BANG *et al.* 1991).

Expression of $Notch^{int}$ during microchaete development produced equivalent phenotypes (Table 1). Microchaete SOP cell specification occurs at 8–12 hr apf (HARTENSTEIN and POSAKONY 1989; USUI and KIMURA 1993) and $Notch^{int}$ expression at this time inhibited microchaete formation (Figure 3D); macrochaete sensilla exhibited the double socket phenotype. Transgene induction at 16–24 hr apf (during morphogenesis of small and large bristles) resulted in double socket microchaete and macrochaete sensory organs (Figure 3E). Thus, enhancement of Notch activity during different phases of mechanosensory bristle development on the head and notum resulted in H^--like phenotypes which affect the corresponding phases of sensillum formation (Table 1).

These H^--like phenotypes suggest that Notch signaling inhibits H^+ activity at different stages of bristle formation. This was tested by co-expressing an H^+ transgene with $Notch^{int}$. As shown in Figure 3, F and G, expression of the H^+ transgene blocked the phenotypes caused by $Notch^{int}$ expression. Induction of the H^+ product with $Notch^{int}$ at 8 hr apf completely counteracted the microchaete bristle loss and macrochaete double socket phenotypes (Figure 3F). Co-expression during sensory organ morphogenesis (*e.g.*, 24 hr apf) fully blocked the double socket phenotypes for large and small bristles (Figure 3G). Thus, extra H^+ protein compensates for enhanced Notch signaling during bristle daughter cell fate determination, as well as during SOP cell fate specification.

Over-expression of the H^+ transgene during bristle

TABLE 1

***Hairless*-like macrochaete and microchaete phenotypes produced by *Notch^{int}* expression**

Structure	*Notch^{int}* time[a]	*Notch^{int}* phenotype[b]	H^- phenotype	Developmental time[c]	References[d]
Mch[e]					
	L3	SOP[f]	SOP	L3	1, 2
h/n	0–24	ds[g]	ds	>0	
	16–24	SOP	SOP	>15	2, 3
ab.	24–32	ds	ds	>15	
	0–8	SOP	SOP	L3–18	4
leg	16–24	ds	ds	L3–18	
mch[h]					
	0–8	SOP	SOP	3–12	1, 2, 5
h/n	16–24	ds	ds	>16	
	16–24	SOP	SOP	>15	2, 3
ab.	24–32	ds	ds	>15	

[a] Time of larval/pupal development at which expression of *Notch^{int}* produces the corresponding phenotype; L3, third instar (wandering) larvae; 0 (*etc.*), hours after puparium formation.

[b] All H^--like effects generated by *Notch^{int}* expression were suppressed by co-expression of the H^+ transgene.

[c] Time of larval/pupal development at which event normally occurs.

[d] References: (1) HARTENSTEIN and POSAKONY (1989); (2) BANG *et al.* (1991); (3) MADHAVAN and MADHAVAN (1980); (4) GRAVES and SCHUBIGER (1981); (5) USUI and KIMURA (1993).

[e] Mch, macrochaetes; h/n, head and notum; ab, abdomen.

[f] SOP, suppression of SOP cell specification.

[g] ds, double socket effect due to daughter cell fate transformation.

[h] mch, microchaetes.

SOP cell fate specification results in excess production of external sensory organs; expression during bristle morphogenesis produces sensilla with "double bristle" shafts, due to a tormogen to tricogen transformation (BANG and POSAKONY 1992). We have seen evidence of these effects with expression of one copy of the H^+ product (D. F. LYMAN, unpublished observations). However, these phenotypes are not observed with co-expression of *Notch^{int}* (data not shown), suggesting that H^+ protein function is suppressed by Notch signaling and further highlighting the balance between Notch receptor signaling and H^+ function.

***Notch^{int}* suppresses SOP cell fate specification and alters sensillum daughter cell fates in a manner similar to H^- mutations:** The bristle loss phenotypes generated by *Notch^{int}* and their responses to over-expression of an H^+ transgene suggested an effect on SOP cell specification. Similarly, the double socket phenotype produced by *Notch^{int}* suggested a tricogen to tormogen transformation. This was confirmed by examination of labeled sensilla in flies expressing the activated *N* transgene. We examined the cellular basis of the bristle effects following transgene expression at 8 hr apf (for bristle loss) or at 20 hr apf (for double socket sensilla).

Expression of *Notch^{int}* at 8 hr apf eliminates microchaete sensilla and produces double socket macrochaetes on the head and notum (Figure 3D). MAb 22C10 staining at 32 hr apf, following heat shock at 8 hr apf, revealed mutant *Notch^{int}* nota with few microchaete sensilla but many double socket macrochaete sensilla on the head and notum (Figure 4A). Close examination of the macrochaete sensilla (Figure 4A, inset) showed that each contained two large epidermally positioned cells (tormogens) associated with one or two small subepidermal cells (neuron and thecogen). A similar evaluation with cut antiserum yielded comparable results (Figure 4B). These observations suggest that *Notch* transgene expression during macrochaete bristle morphogenesis results in a tricogen to tormogen transformation. Expression of *Notch^{int}* at 16–24 hr apf results in both microchaete and macrochaete double socket sensilla (Figure 3E). Figure 4C shows that such nota exhibit both microchaete and macrochaete labeled sensilla characterized by two large cells (tormogens) in the epidermal plane associated with one or two small subepidermal cells. Thus, the double socket phenotypes of both large and small mechanosensory bristles following *Notch^{int}* activation represent a daughter cell fate transformation comparable to that of H^- mutations.

The effect of *Notch^{int}* on microchaete SOP cell fate specification was evaluated by examination of β-galactosidase labeled (*A37*) sensilla. Labeled *Notch^{int}* nota (24 hr apf) which received no heat shock treatment exhibited exclusively a wild-type composition of SOP cells (data not shown). *Notch^{int}* nota heat shocked at 8 hr apf exhibited β-galactosidase activity only in macrochaete sensilla and a striking lack of microchaete SOP cells (Figure 4D). The labeled macrochaete sensilla typically featured two large stained nuclei within the epidermal plane and a small subepidermal nucleus. Similar results were obtained on 16 hr preparations, heat shocked at 8 hr apf (data not shown).

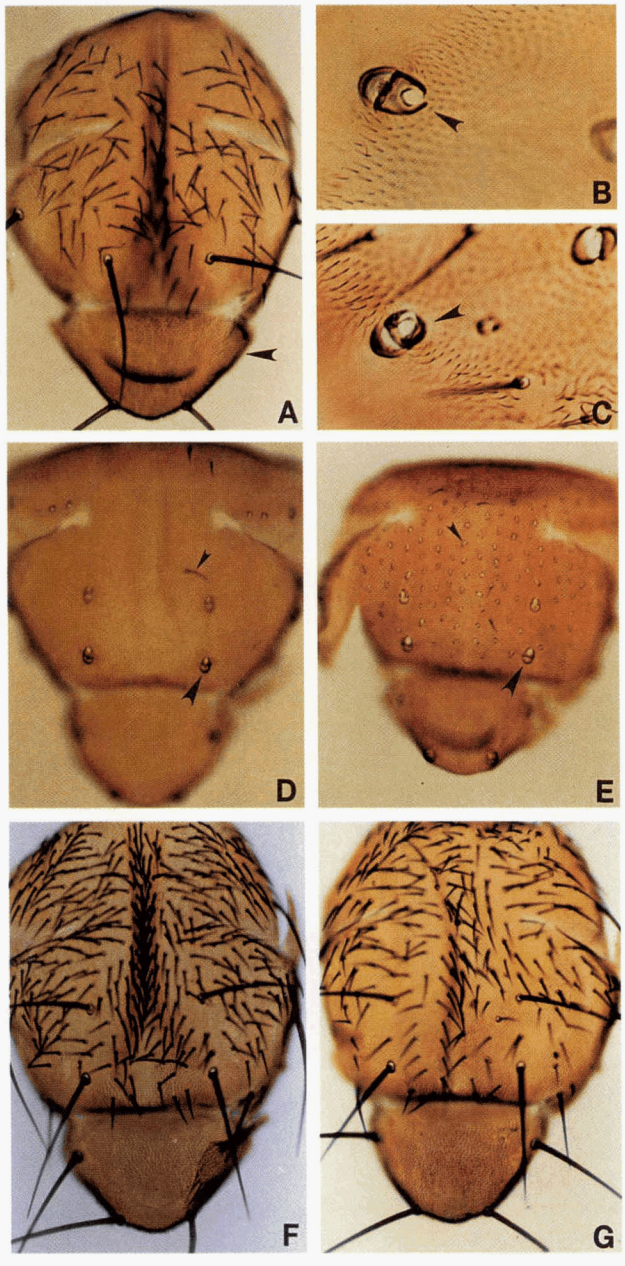

FIGURE 3.—*Hairless*-like bristle phenotypes are produced by an activated *Notch* transgene (*Notch^{int}*) and suppressed by co-expression of an H^+ cDNA. (A, B, D, and E) Expression of *Notch^{int}* (*i.e.*, *P*[*hs-Notch^{int}*]/*FM7c*) during specific stages of mechanosensory bristle development produces H^--like bristle phenotypes. (A) Larval (third instar) expression of *Notch^{int}* results primarily in selective loss of macrochaete bristles (*e.g.*, anterior scutellars; arrowhead). Specific macrochaetes that are lost appear to be related to when *Notch^{int}* is activated during the third instar larval period. (B) Pupal expression (*i.e.*, 0–24 hr apf) of *Notch^{int}*, during macrochaete bristle daughter cell morphogenesis, results in a characteristic double socket H^--like phenotype (arrowhead) for large bristles. (C) Double socket macrochaete phenotype (arrowhead) of $H^1/+$. Similarity to bristle phenotype in B is evident. (D) Expression of *Notch^{int}* at 8 hr apf (during microchaete SOP specification) produces a striking loss of microchaetes (small arrowhead) on the notum but double socket macrochaetes (large arrowhead). (E) Double socket macrochaetes (large arrowhead) and microchaetes (small arrowhead) on the notum result from expression of *Notch^{int}* at 16–24 hr apf (during macrochaete and microchaete daughter cell morphogenesis). (F and G) Co-expression of an H^+ cDNA with *Notch^{int}* (*P*[*hs-Notch^{int}*]/+; *P*[*hs-H$^+$*]*H3*/+) at 8 hr apf (F) or 16–24 hr apf (G) blocks the corresponding bristle loss or double socket phenotypes of the macrochaetes and microchaetes produced by expression of *Notch^{int}* alone (D and E). Modification of these bristle phenotypes does not occur when *Notch^{int}* is outcrossed to the H^+ transgene host strain (w^{1118}). H^+ gain-of-function phenotypes resulting from expression of the H^+ cDNA alone (see text) are not produced when *Notch^{int}* is co-expressed with the H^+ cDNA (F and G). (A, D–G) Anterior is up.

A selective depletion of β-galactosidase labeled macrochaete SOP cells was also observed in 24 hr apf transgenic nota heat shocked as third instar larvae (data not shown). In addition, examination of *Notch^{int}* nota with vital stains (at 16 and 20 hr apf) following heat shock treatment at 8 hr apf failed to exhibit evidence of cell death on the head or notum (data not shown). These observations indicate that expression of the activated *N* transgene during SOP fate specification of macrochaetes and microchaetes suppresses the bristle precursor fate in a manner similar to the heteroallelic *N* mutants and H^- mutations.

***Notch^{int}* produces additional H^--like phenotypes that are suppressed by co-expression of the H^+ transgene:** H^- mutations also produce shortening of wing veins, loss of campaniform sensilla on wing vein LIII, loss of bristles on the anterior wing margin (and nonsensory hairs along the posterior margin) and loss of interommatidial bristles (Tables 1 and 2; BANG *et al.* 1991). Some of these phenotypes are also observed with *N* gain-of-function alleles (*e.g.*, *Ax*, *l(1)N^B* and *N^{60g11}*; LINDSLEY and ZIMM 1992; D. F. LYMAN, unpublished observations). As summarized in Tables 1 and 2, expression of *Notch^{int}* at appropriate stages of development also produced similar, H^--like phenotypes. In each case the phenotype was suppressed by co-expression of the H^+ transgene (data not shown).

Formation of campaniform sensilla on wing vein LIII and the anterior cross vein was inhibited by expression of *Notch^{int}* in third instar larvae (Table 2) as was development of chemosensory bristles on the anterior margin of the wing (Figure 5B, Table 2). Expression of *Notch^{int}* in progressively older pupae (*i.e.*, from 0–24 hr apf; during daughter cell fate determination) caused an increase in mutant chemosensory organs on the wing margin consisting of a socket and lacking a shaft; comparable to a previously reported H^- phenotype (BANG *et al.* 1991). Development of stout mechanosensory bristles on the anterior wing margin was also affected (Table 2). Early *Notch^{int}* expression (8 hr apf) suppressed bristle formation while later activation (16–24 hr apf) produced a double socket phenotype (Figure 5, C and D), comparable to an effect in trans-heterozygotes of

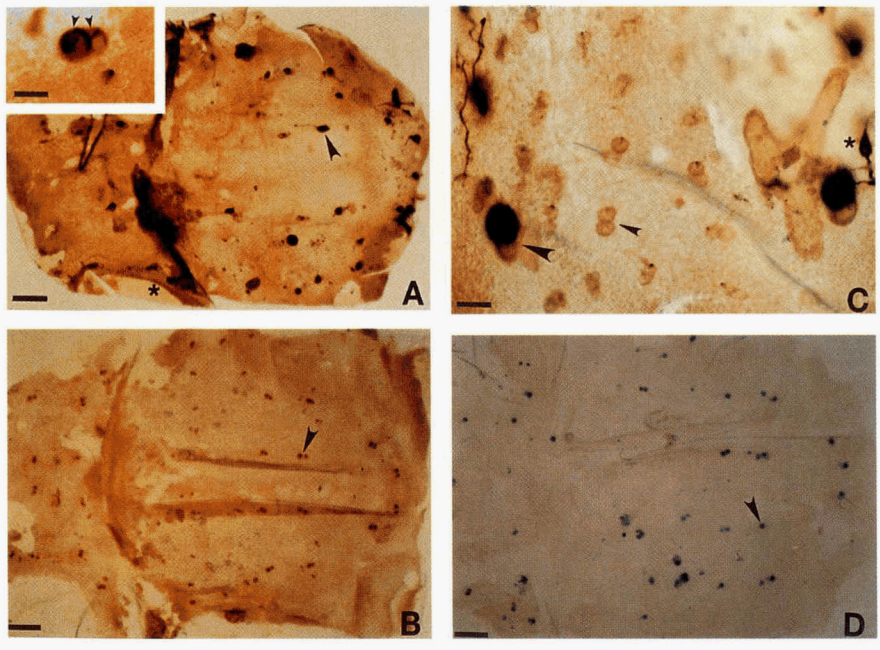

FIGURE 4.—The cellular bases of $Notch^{int}$-generated bristle phenotypes on the head and notum are indistinguishable from those of H^- mutations. Microchaete bristle loss produced by expression of $Notch^{int}$ results from loss of SOP fate specification whereas double socket macrochaete and microchaete sensilla produced by $Notch^{int}$ expression results from a tricogen to tormogen cell fate transformation. (A) 32 hr apf *P[hs-Notch^{int}]/FM7c* notum, heat shocked at 8 hr apf and stained (MAb 22C10) to visualize bristle sensory organs. Virtually the entire head (anterior to *) and notum is devoid of labeled microchaete sensilla. Only macrochaete sensilla (arrowhead) are detected. Closer examination of the macrochaete sensory organs (inset) reveals a double socket cytoarchitecture (*i.e.,* two large stained tormogen cells (arrowheads) in the epidermal plane accompanied by a subepidermal sensory neuron, out of the plane of focus). (B) *P[hs-Notch^{int}]/FM7c* notum, heat shocked at 8 hr apf, stained (24 hr apf) with cut antiserum. Results confirm the extensive lack of detectable microchaete sensilla on the notum and demonstrate two large, stained epidermal cells (nuclei) in each macrochaete sensillum (arrowhead). (C) Double socket cytoarchitecture of large and small mechanosensory bristles on the notum of *P[hs-Notch^{int}]/FM7c,* heat shocked at 16–24 hr apf and stained (MAb 22C10) at 36 hr apf, was confirmed. Throughout the notum (and head) both macrochaete (large arrowhead) and microchaete (small arrowhead) sensilla display two large, stained epidermal cells associated with a subepidermal sensory neuron. The bipolar sensory neuron (*) of one double socket macrochaete sensillum is shown. (D) β-galactosidase labeling (24 hr apf) of large and small bristle SOP cells on the head and notum of *P[hs-Notch^{int}]/+; A37/+* shows very few microchaete precursors. Thus, expression of $Notch^{int}$ at 8 hr apf suppresses microchaete SOP cell formation on the head and notum. Macrochaete SOP (or secondary precursor) cells (arrowhead) are readily observed. Equivalent results (data not shown) are obtained with the *A101* transposon. (A, B, and D) Anterior is left. Bar, 200 μm. (inset, C) Bar, 50 μm.

strong H^- alleles (BANG *et al.* 1991). Expression in pupae also inhibited production of nonsensory hairs on the posterior wing margin (Table 2, data not shown) and at 16–24 hr apf suppressed wing vein formation (Figure 5E, Table 2).

Bristle loss or double socket sensilla were also observed on the legs after $Notch^{int}$ expression at early (0–8 hr apf) and later (16–24 hr apf) pupal stages, respectively (Figure 6, A–C, Table 1). Similar effects were evident for abdominal bristles (Table 1). Primarily, loss of abdominal macrochaetes and microchaetes resulted from $Notch^{int}$ expression at 16–24 hr apf (Figure 6E) whereas expression at 24–32 hr apf produced double socket phenotypes (Figure 6F). As summarized in Tables 1 and 2, the various H^--like phenotypes produced by $Notch^{int}$ expression for each tissue correlated closely with the developmental status of each structure. These results strongly imply that H^+ activity is part of the Notch signaling pathway in many contexts.

Due presumably to basal expression of the transgene, mild bristle phenotypes (*e.g.,* loss or double socket) occur on the abdomen of $Notch^{int}$ females without heat shock treatment. A synergistic enhancement of these bristle defects resulted from a combination of *H* haploinsufficiency with the $Notch^{int}$ genotype (data not shown); bristle loss on the head and notum also occurred. Basal expression of the H^+ transgene has been previously reported (BANG and POSAKONY 1992). Introduction of the H^+ transgene into the $Notch^{int}$ stock suppressed (without heat shock) the abdominal bristle phenotypes. Other outcrosses of the $Notch^{int}$ stock did not produce this effect.

We also observed an unusual effect on interommatidial bristle development, which normally initiates after puparium formation at the eye disc center and radiates outward (CAGAN and READY 1989). $Notch^{int}$ expression in third instar larvae produced a narrow dorsoventral band of bristles (Figure 6, G and H, Table 2) that shifted

TABLE 2

Additional *Hairless*-like phenotypes produced by larval/pupal expression of *Notch^{int}*

Structure[a]	*Notch^{int}* time[b]	*Notch^{int}* phenotype[c]	H^- phenotype	Developmental time[d]	References[e]
C.S.	L3	SOP[f]	SOP	L3	1, 2
Ch.B.	L3	SOP	SOP		
	8–24	ds	ds	L3>0	1, 2
S.M.B.	8	SOP	SOP	6–8	
	16–24	ds	ds	>12	1, 2
N.S.H	8–16	Loss	Loss	ND[g]	2
I.O.B.	0–16	Loss	Loss	0–20	2, 3
W.V.	16–24	Shortened (LII, IV, V pcv)[h]	Shortened (LII, IV, V, pcv)	>9	2, 4

Pupal expression of *Notch^{int}* also suppressed production of bristles of the female terminalia, a H^--like phenotype (BANG *et al.* 1991). Dorsal pedicellar sensilla of the haltere (reduced in strong H^- trans-heterozygotes; BANG *et al.* 1991) was not examined in *Notch^{int}* flies. Short-term expression of *Notch^{int}* during larval or pupal development is not expected to result in an H^--like enlargement of the entire eye due to spatio-temporal eye development. The basis of the H^- enlargement of the eye is unreported (2). An unusual phenotype affecting interommatidial bristles due to short term expression of *Notch^{int}* during the third larval instar was observed (see text). The relationship of this phenotype to *H* is unknown. We also observed *spl*-like (twinned) bristles for many classes of sensilla when *Notch^{int}* was expressed around the time of secondary precursor cell division, as well as the *Notch^{int}* stock presumably due to basal expression of the transgene. Occasional bristles (usually macrochaetes) exhibiting this phenotype are observed in approximately 20% of $l(1)N^B/FM7c$ and $N^{60}g^{11}/FM7c$ flies (D. F. LYMAN, unpublished observations). Introduction of the H^+ transgene into the *Notch^{int}* stock suppressed the production of twinned bristles without heat shock induction, presumably due to basal expression of the H^+ cDNA (see text, BANG and POSAKONY 1992). Variable effects on wing and leg morphology also occurred due to expression of *Notch^{int}* during larval and pupal development. Wings typically failed to completely unfold, appearing crumpled to varying degrees. Proximal leg segments were often irregularly thickened, appearing bloated, while distal portions were typically thinner than normal. These wing and leg effects were suppressed by co-expression of the H^+ transgene.

[a] C.S., Campaniform sensilla; Ch.B., chemosensory bristles of the anterior wing margin. S.M.B., stout mechanosensory bristles of the anterior wing margin; N.S.H., nonsensory hairs of the posterior wing margin; I.O.B., interommatidial bristles; W.V., wing veins.

[b] Time of larval/pupal development at which expression of *Notch^{int}* produces the corresponding phenotype; L3, third instar (wandering) larvae; 0 (*etc.*), hours after puparium formation.

[c] All H^--like effects generated by *Notch^{int}* expression were suppressed by co-expression of the H^+ transgene.

[d] Time of larval/pupal development at which event normally occurs.

[e] References: (1) HARTENSTEIN and POSAKONY (1989); (2) BANG *et al.* (1991); (3) CAGAN and READY (1989); (4) SCHUBIGER and PALKA (1987).

[f] SOP, absence of sensilla suggest suppression of SOP cells in *Notch^{int}* flies, and presumably in H^- flies (data not shown; see text).

[g] ND, not determined (see 2).

[h] LII, IV, V, longitudinal wing veins II, IV, V; pcv, posterior cross vein.

anteriorly along the eye as progressively older larvae were heat shocked. Progress of the band reached the anterior border several hours prior to the morphogenetic furrow, suggesting an effect of activated Notch on eye development ahead of the furrow. A reduction and disorganization of bristles occurred ahead of the band (Figure 6, G and H) while bristles behind the band appeared normal by light microscopy. Production of this band was only partially suppressed by co-expression of the H^+ transgene (data not shown). Expression of activated Notch after puparium formation (*i.e.*, 0–16 hr apf) did not disrupt ommatidial organization, but produced eyes lacking interommatidial bristles (Table 2, data not shown).

DISCUSSION

We have examined the relationship between *Hairless* (*H*) activity and Notch receptor signaling in Drosophila. A potent antagonist of neurogenic genes, *H* has been proposed to insulate imaginal SOP cells from cell-cell inhibition and perhaps regulate *E(spl)C* function (VASSIN *et al.* 1985; DE LA CONCHA *et al.* 1988; BANG and POSAKONY 1992). Using mutant and engineered *Notch* (*N*) gain-of-function products, we provide evidence of a functional interaction between Notch signaling and *H* activity. The data suggest that *H* function is integrated in the *Notch* signaling cascade as a negative regulator and that effective intercellular communication mediated by Notch must surmount *H* activity. The data are consistent with emerging models of the *Notch* signaling pathway based on molecular analyses (BROU *et al.* 1994; FORTINI and ARTAVANIS-TSAKONAS 1994).

Our principal evidence for an antagonistic relationship between *N* and *H* is that elevation of Notch activity mimics *H* loss-of-function mutations. Dominant, gain-of-function *N* mutations result in loss of bristles and SOP cells in a manner similar to H^- mutations. Also,

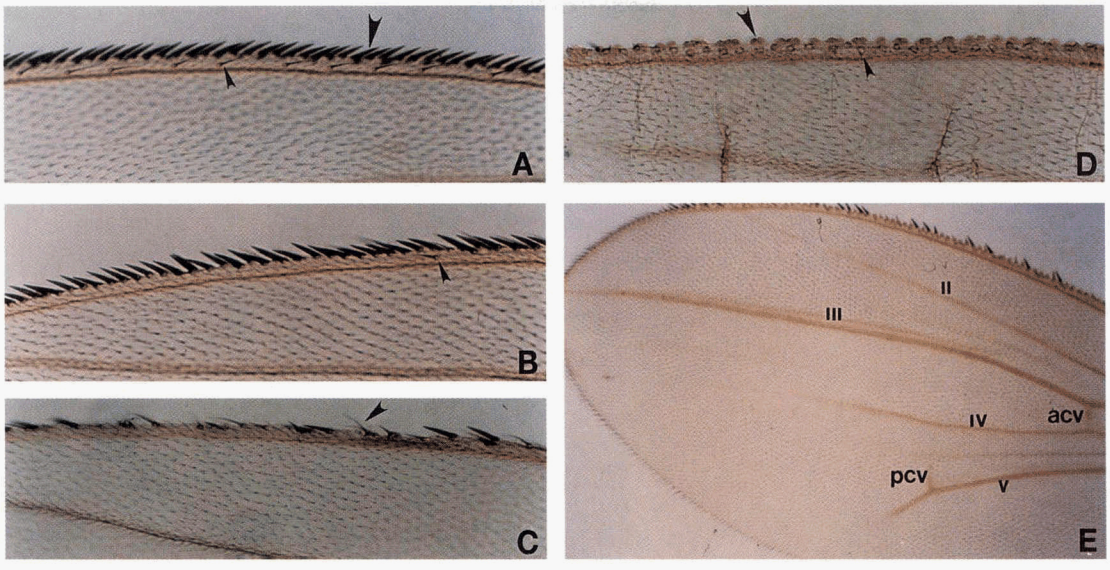

FIGURE 5.—*Hairless*-like wing phenotypes are caused by expression of $Notch^{int}$ during larval/pupal development. (A) Anterior wing margin of $P[hs\text{-}Notch^{int}]/FM7c$ (not heat shocked) showing the evenly spaced recurved (chemosensory) bristles (small arrowhead) and stout (mechanosensory) bristles (large arrowhead). (B) Expression of $Notch^{int}$ during third instar larval development results in partial loss of chemosensory bristles (arrowhead) along the anterior wing margin. Extent of bristle loss appears to correlate with when $Notch^{int}$ is expressed during the larval (third instar) or pupal periods. (C) Expression of $Notch^{int}$ at 8 hr apf typically causes both chemosensory and stout bristles to be lost. Some reduction of slender mechanosensory bristles (arrowhead), not visible in A and B, may also occur. (D) Expression of $Notch^{int}$ at 16–24 hr apf, results in "double socket" phenotypes of mechanosensory (large arrowhead) and chemosensory bristles (small arrowhead) on the anterior margin of the wing blade. Slender bristles are also absent. (B–D) Oriented opposite to A. (E) Incomplete formation of longitudinal wing veins II, IV and V and the posterior cross vein (pcv) is caused by expression of $Notch^{int}$ at 16–24 hr apf. acv, anterior cross vein.

expression of the gain-of-function $Notch^{int}$ transgene at appropriate developmental stages produced double socket, bristle loss and numerous other phenotypes that are exhibited by loss-of-function mutations of *H*. In all cases examined, the cellular basis for these phenotypes was consistent with those reported for H^- mutations (BANG *et al.* 1991). Additionally, H^- mutations were observed to enhance *N* gain-of-function phenotypes, whereas over-expression of an H^+ transgene suppressed these *N* phenotypes. Finally, both the *N* gain-of-function mutations and expression of the $Notch^{int}$ transgene blocked phenotypes produced by over-expression of the H^+ transgene.

Analysis of *Notch* gain-of-function mutations suggested a functional interaction with *Hairless* during cell signaling: Analysis of two atypical *N* alleles [$l(1)N^B$ and N^{60g11}] demonstrated several gain-of-function properties (LYMAN and YOUNG 1993 and this work). A synergistic loss of mechanosensory bristles occurs on the head and notum by combining these *N* alleles *in trans* with *Ax* mutations; trans-heterozygous combinations of either $l(1)N^B$ or N^{60g11} with Ax^{9B2} result in a dramatic and characteristic pattern of microchaete loss on the notum combined with extensive loss of macrochaetes.

Immunohistochemical analysis demonstrated that this bristle loss resulted from the absence of SOP cells that give rise to the four daughter cells, which comprise the mature bristle organ. The remaining stained sensilla were distributed in the characteristic (T) pattern of bristles seen on adults of this genotype and exhibited the normal cytoarchitecture of four mature bristle daughter cells. We saw no evidence of altered cell fates that could account for the absence of the external bristles in regions of the notum lacking sensory bristle organs. Additionally, there was no evidence of cell death during early or late phases of bristle development. These results indicate that the anti-neurogenic effects of these *N* gain-of-function mutations are exerted during the initial process of SOP cell fate commitment. Since *N* regulates the process of cell fate selection in proneural equivalence groups, we conclude that uncommitted proneural cells fail to specify, execute or maintain the SOP cell fate due to enhanced cell signaling by these overactive *N* products. We observed that this effect on mechanosensory bristle development is similar to, and enhanced by, *H* loss-of-function mutations. Expression of an H^+ transgene, however, blocked this bristle loss in the mutant heteroallelic *N* flies, indicating that *H* protein is capable of insulating SOP cells from Notch receptor signaling. These results also suggest that *H* is a target of Notch activity during bristle development.

Expression of an activated *Notch* transgene confirmed the antagonistic interaction between *N* and *H*: *N* and *H* are involved in the development of many imaginal structures. Phenocritical periods in larval and pupal de-

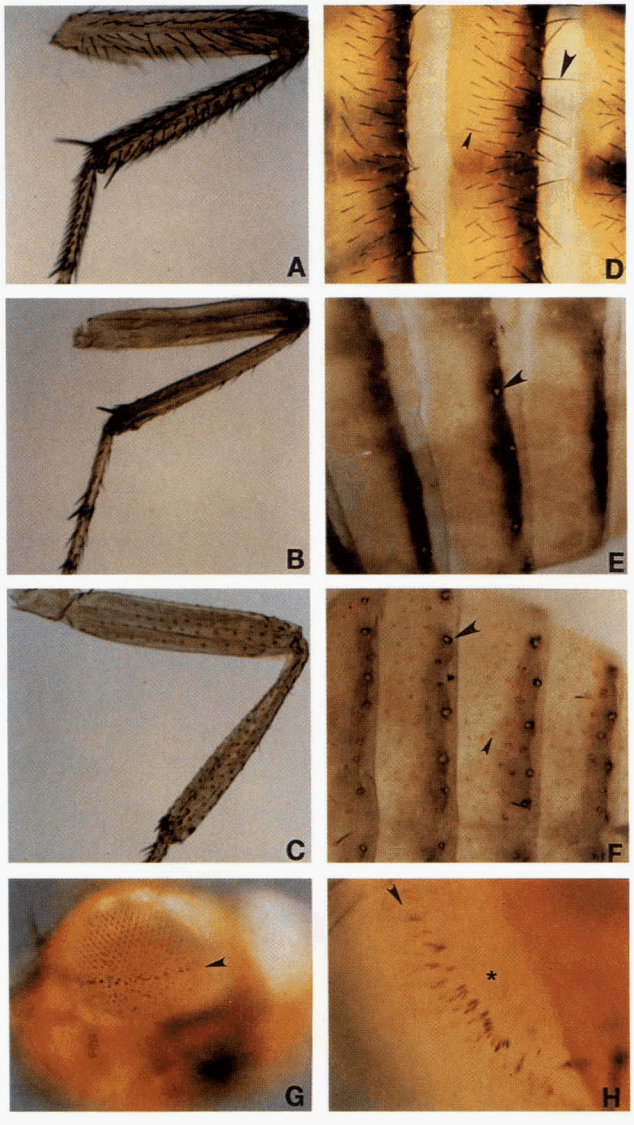

FIGURE 6.—Larval/pupal expression of *Notch*^{int} causes defects in many bristle types. (A–C) Effect of *Notch*^{int} expression on leg bristle development. (A) Wild-type leg showing normal distribution of mechanosensory bristles. (B) Expression of *Notch*^{int} (*i.e.*, *P*[*hs-Notch*^{int}]/*FM7c*) at 8 hr apf results in a striking loss of bristles on the leg, similar to the bristle loss on the head and notum (*cf.* Figure 3D). (C) Double socket bristle sensilla result on the leg after *Notch*^{int} expression at 16–24 hr apf, similar to the double socket bristle effect on the head and notum (*cf.* Figure 3E). (D–F) Activated *Notch*^{int} expression affects abdominal bristle development. Anterior is left. (D) Wild-type abdomen showing large (large arrowhead) and small (small arrowhead) bristle pattern. (E) Activation of *Notch*^{int} expression at 16–24 hr apf results in abdominal macrochaete and microchaete bristle loss, similar to the bristle loss on the head and notum (*cf.* Figure 3D). Some double socket macrochaetes (arrowhead) are produced as well. (F) Expression of *Notch*^{int} at 24–32 hr apf results in a double socket phenotype for both macrochaete (large arrowhead) and microchaete (small arrowhead) abdominal bristles, similar to the double socket bristle effect on the head and notum (*cf.* Figure 3E). (G and H) Larval (third instar) expression of *Notch*^{int} produces a dorsoventral band of interommatidial bristles across the eye (arrowhead). (G) Distribution of bristles posterior to this band is normal, but disrupted anteriorly with some loss of bristles. Anterior is down; posterior, up. (H) Higher magnification view of *Notch*^{int}-generated dorsoventral bristle band (arrowhead). Anterior is right *; posterior, left.

velopment have been demonstrated for sensory organ production with *l(1)N*^{ts1}; the type and sensitivity of effects correlate with the developmental status of the affected structure (SHELLENBARGER and MOHLER 1975; CAGAN and READY 1989; HARTENSTEIN and POSAKONY 1989). Similarly, larval and pupal expression of *Notch*^{int} resulted in numerous defects which closely correlated with the developmental status of the affected structures (Tables 1 and 2). Thus, although adult external sensory organs develop in largely similar ways, each initiates SOP cell specification, morphogenesis and differentiation according to its own developmental clock. Consistent with this, expression of *Notch*^{int} produced early and late phenotypes in each class of sensillum according to its developmental phase at the time of activated Notch expression.

We have shown that the early and late bristle defects caused by *Notch*^{int} expression are indistinguishable from H^- phenotypes. Like H^- mutations, enhancement of Notch signaling activity during early development of sensilla resulted in a bristle loss phenotype due to suppression of SOP cell fate specification. Enhancement of *N* function later, during sensillum morphogenesis, resulted in an H^--like double socket phenotype. We found that these later *Notch*^{int} phenotypes were also associated with an H^--like tricogen-to-tormogen transformation. Both the early and late bristle defects produced by *Notch*^{int} were suppressed by co-expression of the H^+ transgene. Furthermore, co-expression of *Notch*^{int} with the H^+ transgene suppressed both the early production of excess bristles and the late tormogen to tricogen fate transformation produced by ectopic H^+ expression (BANG and POSAKONY 1992), reflecting the sensitivity of *H* function to Notch signaling activity.

Limited expression of *Notch*^{int}, at selected times from late third instar larvae to 32 hr apf allowed us to evaluate the effects of Notch signaling during the development of many imaginal disc derivatives. We observed numerous mutant effects which correspond precisely to H^- phenotypes (Tables 1 and 2). Most phenotypes caused by H^- mutations (BANG *et al.* 1991) could be generated by *Notch*^{int} expression (Tables 1 and 2). Concerning bristle sensory organs on the abdomen, legs and wings, we observed bristle loss (early) and double socket (later) H^--like phenotypes (Tables 1 and 2), similar to the bristle effects on the notum. Co-expression of the H^+ transgene blocked each of these mutant phenotypes. Thus, Notch signaling appears to inhibit H activity in many developmental contexts.

Both *N* and *H* are required during eye development; however, the role of *H* has not been defined. Larval/

pupal *N* loss of function produces rough eyes due to alterations of cell fates (CAGAN and READY 1989; LINDSLEY and ZIMM 1992). Specification of cell types and ommatidial assembly commences as the morphogenetic furrow progresses anteriorly across the eye disc. All differentiating cell types behind the furrow are affected by disruption of *N* function. Rough eye effects due to cell fate alteration have also been reported for activated *Notch* transgenes under *sevenless* or *Actin 5C* promoter regulation (FORTINI *et al.* 1993; STRUHL *et al.* 1993). However, we have not seen a rough eye effect with $Notch^{int}$ under *hsp 70* promoter regulation, perhaps due to a comparatively brief duration of $Notch^{int}$ activity in our studies. Nevertheless, the enhanced signaling activity provided by $Notch^{int}$ during third instar larval development did produce an unusual eye bristle effect that suggests a possible involvement of *N* in cell-cell interactions anterior to the morphogenetic furrow. Further work, however, will be required to determine the cellular basis of this effect as well as that of the *split*-like bristle phenotype generated by expression of the activated *Notch* transgene at certain times during bristle development (Table 2, legend). Pupal expression of $Notch^{int}$, however, produced a loss of interommatidial bristles, similar to H^- mutations.

The role of Notch signaling in *Hairless* suppression: Molecular and genetic analyses have confirmed the interaction of many neurogenic gene products as components of a cell communication pathway, presumably initiated by physical interaction between Delta ligand and the Notch receptor and ending with *E(spl)C* activity. Genetic modification of neurogenic phenotypes by H^- alleles suggests an involvement of *H* in this pathway. Reduction of *H* activity decreases the severity of neurogenic phenotypes while an increase in H^+ enhances neurogenic effects (VASSIN *et al.* 1985; DE LA CONCHA *et al.* 1988; LINDSLEY and ZIMM 1992). Since the phenotype of *E(spl)C* loss of function is not affected by *H* dosage, the *E(spl)C* has been proposed to be the most direct target of *H* activity (VASSIN *et al.* 1985; DE LA CONCHA *et al.* 1988). The genetic interactions between *H* and *N*, the generation of H^- phenotypes by *N* gain-of-function products and their comparable cellular basis shown in this work, further support an involvement of *H* in this pathway as a target of Notch receptor signaling.

H has been suggested to be a nuclear protein due to its overall basic charge and similarities to some transcription factors (BANG and POSAKONY 1992; MAIER *et al.* 1992). Transcription of *H* is ubiquitous in the larval wing disc, suggesting that spatial restriction of expression is not a means of *H* function regulation (BANG and POSAKONY 1992). Rather, post-transcriptional modification may serve this purpose. Our observations that enhanced Notch activity produces H^--like phenotypes and is capable of suppressing the effects of ectopic H^+ protein expression support the view that the *Notch* signaling pathway modifies *H* protein, rather than gene, function. However, we can not rule out that Notch signaling inhibits *H* gene expression.

Evidence from diverse sources suggest a molecular basis for the interaction of *N* and *H*. Mutations of *Su(H)* exhibit strong antagonism of adult H^- bristle phenotypes as well as interactions with *N* and other neurogenic genes (LINDSLEY and ZIMM 1992; FORTINI and ARTAVANIS-TSAKONAS 1994). We have also observed genetic interactions between *N* and *Su(H)* (D. F. LYMAN, unpublished observations). As in the case of $Notch^{int}$, over-expression of a transgenic $Su(H)^+$ product results in H^--like bristle loss or double socket phenotypes (SCHWEISGUTH and POSAKONY 1992). *Su(H)* protein binds to the Notch ankyrin repeats (FORTINI and ARTAVANIS-TSAKONAS 1994), which are necessary for cell signaling (LIEBER *et al.* 1993; LYMAN and YOUNG 1993; REBAY *et al.* 1993), and the interaction of Delta with Notch is associated with nuclear translocation of *Su(H)* product in cell culture (FORTINI and ARTAVANIS-TSAKONAS 1994). Also, *Su(H)* protein (a Drosophila homologue of the mammalian transcription factor, CBF1, HENKEL *et al.* 1994) binds a mouse *E(spl) m8* gene promoter and activates transcription in S2 cells (BROU *et al.* 1994). This binding is inhibited by interaction with *H* protein. Thus, Notch signaling may drive redistribution of *Su(H)* protein to overcome inhibition by *H* protein, perhaps by titration (BROU *et al.* 1994). Notch activation may therefore promote nuclear translocation and regulation of *E(spl)C* bHLH gene expression by *Su(H)* protein in various contexts, such as early and late stages of mechanosensory bristle development.

Consistent with this, Notch signaling has been shown to activate expression of *E(spl)C* bHLH genes and promote adoption of the epidermal cell fate during embryonic neurogenesis (JENNINGS *et al.* 1994). One might anticipate a similar effect of Notch signaling on expression of *E(spl)C* genes during adult SOP fate specification. However, as shown in both embryonic neurogenesis and imaginal sensory organ development (KRAMATSCHEK and CAMPOS-ORTEGA 1994; SINGSON *et al.* 1994), expression of genes in the *E(spl)C* also appear to be regulated by products of the *AS-C* and by cross- or auto-regulation, emphasizing the molecular complexity of neural *vs.* epidermal cell fate selection. Similarly, the asymmetric promotion of alternate cell fates by *H* during adult external sensory organ development may result from asymmetries in Notch receptor signaling or from *Su(H)* protein activity. These might in turn result from inequities in ligand activity or perhaps additional signal transducing factors. In any case, it appears that *H* activity is intimately involved in negatively regulating the *Notch* signaling pathway during adult sensory organ development and possibly during embryonic neurogenesis.

Special appreciation is expressed to Dr. H. ELLIS for helpful discussions, help in pupal dissections and a critical review of this manuscript. Reviewers comments were also most appreciated. We also thank the laboratories that provided a number of strains and antisera. This work was supported by grants to B.Y. from the National Science Foundation and the Emory University Research Committee.

LITERATURE CITED

ASHBURNER, M., 1982 The genetics of a small autosomal region of *Drosophila melanogaster* containing the structural gene for alcohol dehydrogenase. III. Hypomorphic and hypermorphic mutations affecting the expression of *Hairless*. Genetics **101:** 447–459.

ASHBURNER, M., 1989 *Drosophila. A Laboratory Handbook*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

BANG, A. G., and J. W. POSAKONY, 1992 The *Drosophila* gene *Hairless* encodes a novel basic protein that controls alternative cell fates in adult sensory organ development. Genes Dev. **6:** 1752–1769.

BANG, A. G., V. HARTENSTEIN and J. W. POSAKONY, 1991 *Hairless* is required for the development of adult sensory organ precursor cells in *Drosophila*. Development **111:** 89–104.

BLOCHLINGER, K., L. Y. JAN and Y. N. JAN, 1993 Postembryonic patterns of expression of *cut*, a locus regulating sensory organ identity in *Drosophila*. Development **117:** 441–450.

BRAND, M., A. P. JARMAN, L. Y. JAN and Y. N. JAN, 1993 *asense* is a *Drosophila* neural precursor gene and is capable of initiating sense organ formation. Development **119:** 1–17.

BROU, C., F. LOGEAT, M. LECOURTOIS, J. VANDEKERCKHOVE, P. KOURILSKY *et al.*, 1994 Inhibition of the DNA-binding activity of *Drosophila Suppressor of Hairless* and of its human homologue, KBF2/RBP-J, by direct protein-protein interaction with *Drosophila Hairless*. Genes Dev. **8:** 2491–2503.

CAGAN, R. L., and D. F. READY, 1989 *Notch* is required for successive cell fate decisions in the developing *Drosophila* retina. Genes Dev. **3:** 1099–1112.

CAMPUZANO, S., and J. MODOLLEL, 1992 Patterning of the *Drosophila* nervous system: the *achaete-scute* gene complex. Trends Genet. **8:** 202–208.

CUBAS, P., J. DE CELIS, S. CAMPUZANO and J. MODOLLEL, 1991 Proneural clusters of *achaete-scute* expression and the generation of sensory organs in the *Drosophila* imaginal wing disc. Genes Dev. **5:** 996–1008.

DE LA CONCHA, A., U. DIETRICH, D. WEIGEL and J. A. CAMPOS-ORTEGA, 1988 Functional interactions of neurogenic genes of *Drosophila melanogaster*. Genetics **118:** 499–508.

DELIDAKIS, C., and S. ARTAVANIS-TSAKONAS, 1992 The *Enhancer of split* [*E(spl)*] locus of *Drosophila* encodes seven independent helix-loop-helix proteins. Proc. Natl. Acad. Sci. USA **89:** 8731–8735.

DIEDERICH, R. J., K. MATSUNO, H. HING and S. ARTAVANIS-TSAKONAS, 1994 Cytosolic interaction between *deltex* and *Notch* ankyrin repeats implicates *deltex* in the *Notch* signaling pathway. Development **120:** 473–481.

FEHON, R. G., P. J. KOOH, I. REBAY, C. L. REGAN, T. XU *et al.*, 1990 Molecular interactions between the protein products of the neurogenic loci *Notch* and *Delta*, two EGF-homologous genes in *Drosophila*. Cell **61:** 523–534.

FORTINI, M. E., and S. ARTAVANIS-TSAKONAS, 1994 The *Suppressor of Hairless* protein participates in *Notch* receptor signaling. Cell **79:** 273–282.

FORTINI, M. E., I. REBAY, L. A. CARON and S. ARTAVANIS-TSAKONAS, 1993 An activated *Notch* receptor blocks cell-fate commitment in the developing *Drosophila* eye. Nature **365:** 555–557.

GHYSEN, A., C. DAMBLY-CHAUDIERE, L. Y. JAN and Y. N. JAN, 1993 Cell interactions and gene interactions in peripheral neurogenesis. Genes Dev. **7:** 723–733.

GORMAN, M. J., and J. R. GIRTON, 1992 A genetic analysis of *deltex* and its interaction with the *Notch* locus in *Drosophila melanogaster*. Genetics **131:** 99–112.

GRAVES, B., and G. SCHUBIGER, 1981 Regional differences in the developing foreleg of *Drosophila melanogaster*. Dev. Biol. **85:** 334–343.

HARTENSTEIN, V., and J. W. POSAKONY, 1989 Development of adult sensilla on the wing and notum of *Drosophila melanogaster*. Development **107:** 389–405.

HARTENSTEIN, V. and J. W. POSAKONY, 1990 A dual function of the *Notch* gene in *Drosophila* sensillum development. Dev. Biol. **142:** 13–30.

HARTLEY, D. A., A. PREISS and S. ARTAVANIS-TSAKONAS, 1988 A deduced gene product from the *Drosophila* neurogenic locus *Enhancer of split* shows homology to mammalian G-protein subunit. Cell **55:** 785–795.

HEITZLER, P., and P. SIMPSON, 1991 The choice of cell fate in the epidermis of *Drosophila*. Cell **64:** 1083–1092.

HENKEL, T., P. D. LING, S. D. HAYWARD and M. G. PETERSON, 1994 Mediation of Epstein-Barr virus EBNA2 transactivation by recombination signal-binding protein Jκ. Science 265: 92–95.

JARMAN, A. R., E. H. GRELL, L. ACKERMAN, L. Y. JAN and Y. N. JAN, 1994 *atonal* is the proneural gene for *Drosophila* photoreceptors. Nature 369: 398–400.

JENNINGS, B., A. PREISS, C. DELIDAKIS and S. BRAY, 1994 The Notch signaling pathway is required for *Enhancer of split* bHLH protein expression during neurogenesis in the *Drosophila* embryo. Development **120:** 3537–3548.

KIDD, S., M. K. BAYLIES, G. P. GASIC and M. W. YOUNG, 1989 Structure and distribution of the *Notch* protein in developing *Drosophila*. Genes Dev. **3:** 1113–1129.

KLAMBT, C., E. KNUST, K. TIETZE and J. A. CAMPOS-ORTEGA, 1989 Closely related transcripts encoded by the neurogenic gene complex *Enhancer of split* of *Drosophila melanogaster*. EMBO J. **8:** 203–210.

KNUST, E., H. SCHRONS, F. GRAWE and J. A. CAMPOS-ORTEGA, 1992 Seven genes of the *Enhancer of split* complex of *Drosophila melanogaster* encode helix-loop- helix proteins. Genetics **132:** 505–518.

KRAMATSCHEK, B., and J. A. CAMPOS-ORTEGA, 1994 Neuroectodermal transcription of the *Drosophila* neurogenic genes *E(spl)* and *HLH-m5* is regulated by proneural genes. Development **120:** 815–826.

LEHMANN, R., F. JIMENEZ, U. DIETRICH and J. A. CAMPOS-ORTEGA, 1983 On the phenotype and development of mutants of early neurogenesis in *Drosophila melanogaster*. Roux's Arch. Dev. Biol. **192:** 62–74.

LIEBER, T., S. KIDD, E. ALCAMO, V. CORBIN and M. W. YOUNG, 1993 Anti-neurogenic phenotypes induced by truncated *Notch* proteins indicate a role in signal transduction and may point to a novel function for *Notch* in nuclei. Genes Dev. **7:** 1949–1965.

LINDSLEY, D., and G. G. ZIMM, 1992 *The Genome of Drosophila melanogaster*. Academic Press, San Diego.

LYMAN, D., and M. W. YOUNG, 1993 Further evidence for function of the *Drosophila Notch* protein as a transmembrane receptor. Proc. Natl. Acad. Sci. USA **90:** 10395–10399.

MADHAVAN, M. M., and K. MADHAVAN, 1980 Morphogenesis of the epidermis of adult abdomen of *Drosophila*. J. Embryol. Exp. Morphol. **60:** 1–31.

MAIER, D., G. STRUMM, K. KUHN and A. PREISS, 1992 *Hairless*, a *Drosophila* gene involved in neural development, encodes a novel serine rich protein. Mech. Dev. **38:** 143–156.

PARKS, A. L., and M. A. MUSKAVITCH, 1993 *Delta* function is required for bristle organ determination and morphogenesis in *Drosophila*. Dev. Biol. **157:** 484–496.

PAROUSH, Z., R. L. FINLEY, T. KIDD, S. M. WAINWRIGHT, P. W. INGHAM *et al.*, 1994 *Groucho* is required for *Drosophila* neurogenesis, segmentation and sex determination and interacts with *hairy*-related bHLH proteins. Cell *79:* 805–815.

POSAKONY, J. W., 1994 Nature *vs.* nurture: asymmetric cell divisions in *Drosophila* bristle development. Cell **76:** 415–418.

PRICE, B. D., Z. CHANG, R. SMITH, S. BOCKHEIM and A. LAUGHON, 1993 The *Drosophila neuralized* gene encodes a C_5HC_4 zinc finger. EMBO J. **12:** 2411–2418.

REBAY, I., R. G. FEHON and S. ARTAVANIS-TSAKONAS, 1993 Specific truncations of *Drosophila Notch* define dominant activated and dominant negative forms of the receptor. Cell **74:** 319–330.

ROMANI, S., S. CAMPUZANO, E. R. MACAGNO and J. MODOLLEL, 1989 Expression of *achaete* and *scute* genes in *Drosophila* imaginal discs and their function in sensory organ development. Gene Dev. **3:** 997–1007.

SCHUBIGER, M., and J. PALKA, 1987 Changing spatial patterns of

DNA replication in the developing wing of *Drosophila*. Dev. Biol. **123:** 145–153.

SCHWEISGUTH, F., and J. W. POSAKONY, 1992 Antagonistic activities of *Suppressor of Hairless* and *Hairless* control alternative cell fates in the *Drosophila* adult epidermis. Development **120:** 1433–1441.

SHELLENBARGER, D. L., and J. D. MOHLER, 1975 Temperature sensitive periods and autonomy of pleiotropic effects of $l(1)N^{ts1}$, a conditional *Notch* lethal in *Drosophila*. Dev. Biol. **62:** 432–446.

SINGSON, A., M. W. LEVITEN, A. G. BANG, X. H. HUA and J. W. POSAKONY, 1994 Direct downstream targets of proneural activators in the imaginal disc include genes involved in lateral inhibitory signaling. Genes Dev. **8:** 2058–2071.

SKEATH, J. B., and S. B. CARROL, 1992 Regulation of proneural gene expression and cell fate during neuroblast segregation in the *Drosophila* embryo. Development **114:** 939–946.

SMOLLER, D., C. A. S. FRIEDEL, D. BETTLER, L. LAM and B. YEDVOBNICK, 1990 The *Drosophila* neurogenic locus *mastermind* encodes a nuclear protein unusually rich in amino acid homopolymers. Genes Dev. **4:** 1688–1700.

STRUHL, G., K. FITZGERALD and I. GREENWALD, 1993 Intrinsic activity of the *lin-12* and *Notch* intracellular domains *in vivo*. Cell **74:** 331–346.

USUI, K., and K. KIMURA, 1993 Sequential emergence of the evenly spaced microchaetes on the notum of *Drosophila*. Roux's Arch. Dev. Biol. **203:** 151–158.

VASSIN, H., J. VIELMETTER and J. A. CAMPOS-ORTEGA, 1985 Genetic interactions in early neurogenesis of *Drosophila melanogaster*. J. Neurogenet. **2:** 291–308.

XU, T., I. REBAY, R. J. FLEMMING, T. N. SCOTTGALE and S. ARTAVANIS-TSAKONAS, 1990 The *Notch* locus and the genetic circuitry involved in early *Drosophila* neurogenesis. Genes Dev. **4:** 464–475.

Communicating editor: R.E. DENELL