Only a subset of the binary cell fate decisions mediated by Numb/Notch signaling in *Drosophila* sensory organ lineage requires *Suppressor of Hairless*

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SUMMARY

In *Drosophila*, an adult external sensory organ (bristle) consists of four distinct cells which arise from a sensory organ precursor cell via two rounds of asymmetric divisions. The sensory organ precursor cell first divides to generate two secondary precursor cells, IIa and IIb. The IIa cell then divides to produce the hair cell and the socket cell. Shortly after, the IIb cell divides to generate the neuron and the sheath cell. The membrane-associated protein Numb has been shown to be required for the first two asymmetric divisions. We now report that a new hypomorphic numb mutant not only displays a double-socket phenotype, due to a hair cell to socket cell transformation, but also a double-sheath phenotype, due to a neuron to sheath cell transformation. This provides direct evidence that numb functions in the neuron/sheath cell lineage as well. Those results, together with our observation from immunofluorescence analysis that Numb forms a crescent in the dividing IIa and IIb cells suggest that asymmetric localization of Numb is important for the cell fate determination in all three asymmetric cell divisions in the sensory organ lineage. Interestingly, we found that in the hair/socket cell lineage but not the neuron/sheath cell lineage, a *Suppressor of Hairless* mutation acts as a dominant suppressor of numb mutations whereas *Hairless* mutations act as enhancers of numb. Moreover, epistasis analysis indicates that *Suppressor of Hairless* acts downstream of numb, and results from in vitro binding analysis suggest that the genetic interaction between numb and Hairless may occur through direct protein-protein interaction. These studies reveal that *Suppressor of Hairless* is required for only a subset of the asymmetric divisions that depend on the function of numb and Notch.

Key words: numb, *Suppressor of Hairless*, cell fate, neuron, sheath, sensory organ, *Drosophila*

INTRODUCTION

Asymmetric cell division is a fundamental process for the generation of daughter cells with different fates. Two types of mechanisms may be used in generating asymmetric cell divisions, an extrinsic mechanism and an intrinsic mechanism. The intrinsic mechanism involves the segregation of an inherited determinant preferentially into one of the daughter cells. The extrinsic mechanism may be executed through cell-cell communication (reviewed by Horvitz and Herskowitz, 1992). In *Drosophila*, both mechanisms are used to generate the distinct fates of the cells within an adult sensory organ which, like the external sensory organ in the embryonic peripheral nervous system (PNS), is derived from a single sensory organ precursor (SOP) cell (Hartenstein and Posakony, 1989).

A simple sensory organ comprises four cells with distinct fates: a neuron, a sheath cell, a socket cell and a hair cell (Fig. 1A). To generate these different cells of a sensory organ, the SOP cell follows a stereotyped pattern of asymmetric cell divisions (Hartenstein and Posakony, 1989). The SOP cell first divides to generate two secondary precursor cells, IIa and IIb. The IIa cell then divides to produce two outer support cells, the hair cell and the socket cell. Shortly after the IIa cell division, the IIb cell divides to produce two inner cells, the neuron and the sheath cell (Fig. 5A).

Previous studies of the *Drosophila* embryonic and adult PNS suggest that the gene numb plays a key role in generating asymmetry in the SOP lineage (Uemura et al., 1989; Rhyu et al., 1994). Immunocytochemical studies suggest that Numb is a membrane-associated protein and is asymmetrically localized in the dividing SOP cell. Upon SOP division, Numb is preferentially segregated into the IIb cell (Rhyu et al., 1994). In numb loss-of-function mutants, the SOP cell divides symmetrically to produce two IIa cells, which produce four outer support cells (Uemura et al., 1989). Ectopic expression of Numb causes the opposite cell fate transformation in embryos (Rhyu et al., 1994). The generation of the four different cells of a sensory bristle in adult flies also requires the function of numb. Mosaic analysis reveals that the most extreme numb loss-of-function phenotype is the formation of four socket cells. This four-sOCKET phenotype arises from the abnormal symmetric division of SOP to produce two IIa cells and the abnormal symmetric
division of each Ia cell to produce two socket cells (Rhyu et al., 1994). The transformation of Ib to Ia has therefore precluded the examination of the role of numb in the asymmetric division of the Ib cell in numb null mutants.

Besides the cell-intrinsic mechanism mediated by numb, a cell-extrinsic mechanism mediated by Notch and Delta is also used for asymmetric divisions in the SOP lineage (Hartenstein and Posakony, 1990; Parks and Muskavitch, 1993). Analyses of temperature-sensitive mutants of Notch and Delta reveal that reduction of Notch or Delta function during the first SOP division causes both daughter cells to assume the Ib cell fate, which then divide symmetrically to produce four neurons (Hartenstein and Posakony, 1990; Parks and Muskavitch, 1993). In addition, Notch is also involved in both the Ia cell division and the Ib cell division, affecting the cell fates of both the socket/hair lineage and the neuron/sheath lineage (Guo et al., 1996). Notch and Delta probably mediate cell-cell communication through a receptor-ligand interaction in which Notch acts as the receptor and Delta as a ligand (Heitzler and Simpson, 1991; Rebay et al., 1991), and Numb acts by suppressing Notch activity during embryonic sensory organ development, probably via direct protein-protein interactions between Numb and Notch (Guo et al., 1996).

In addition to their function in the lateral inhibition step during the SOP formation, the genes Suppressor of Hairless (Su(H)) and Hairless (H) help control the fates of adult sensory organ cells (Lees and Waddington, 1942; Bang et al., 1991; Bang and Posakony, 1992; Furukawa et al., 1992; Schweisguth and Posakony, 1992; Schweisguth, 1995). Su(H) is a potent dominant suppressor of H. Loss of function or overexpression of Su(H) during SOP divisions causes the Ia cell to divide symmetrically into two hair cells or two socket cells, respectively (Schweisguth and Posakony, 1992). Conversely, loss of function or overexpression of H exhibits a two-socket or two-hair phenotype, respectively (Bang et al., 1991; Bang and Posakony, 1992). Shortly after the division of the Ia cell, the Su(H) protein specifically accumulates in the nucleus of the socket cell (Gho et al., 1996). These results suggest that Su(H) is required for socket cell formation and H is required for hair cell formation.

There is growing evidence suggesting that Su(H) functions in the Notch signaling pathway. Both Su(H) and H show allele-specific genetic interactions with Notch and Delta during SOP formation (Vaessen et al., 1985; de la Concha et al., 1988; Fortini and Artavanis-Tsakonas, 1994). The Su(H) gene encodes a protein that is highly homologous to the mammalian RBP-Jκ DNA-binding protein (Furukawa et al., 1992; Schweisguth and Posakony, 1992), and by binding specifically to sites in the promoter of the genes of the Enhancer of Split (E(spl)) complex, acts as a transcriptional activator of these genes (Bailey and Posakony, 1995; Lecourtis and Schweisguth, 1995). Activated Notch causes transcriptional activation of these E(spl) genes in transgenic flies, and this activation requires Su(H), providing further evidence for the participation of Su(H) in Notch signaling (Bailey and Posakony, 1995; Lecourtis and Schweisguth, 1995). A functional link between Su(H) and Notch has also been established in cultured Drosophila cells. When expressed in the S2 cells, the Su(H) protein is localized to the nucleus. Coexpression of Su(H) and Notch causes Su(H) protein to be retained in the cytoplasm, probably due to direct binding of Su(H) to Notch. Upon activation of Notch by its ligand Delta, the Su(H) protein translocates to the nucleus (Fortini and Artavanis-Tsakonas, 1994). This ability of activated Notch to cause translocation of Su(H) protein is inhibited by Numb (Frise et al., 1996).

It has been proposed that Su(H) is involved in all three binary decisions in the sense organ lineage (Posakony, 1994; Schweisguth, 1995), implying that Notch signaling is transduced by Su(H) in all three binary decisions. However, Schweisguth and Posakony (1994) did not observe a sheath-to-neuron transformation in flies carrying extra copies of Su(H). This result by itself does not prove but does raise the possibility that Su(H) is not required for the neuron/sheath cell fate decisions. It is therefore important to resolve whether Su(H) is indeed involved in all binary cell fate decisions mediated by Notch and numb.

In this report, we first present the characterization of a hypomorphic mutant of numb, which exhibits both a hair-to-socket transformation and a neuron-to-sheath cell transformation. We then show that Su(H) is epistatic to numb and that a Su(H)
interaction as a dominant suppressor of the *numb* hypomorph while a *H* mutation acts as an enhancer of the *numb* hypomorph. Moreover, the interactions of *Su(H)* and *H* with *numb* only occur in the hair/sheath cell lineage, indicating that *Su(H)* and *H* are not required in determining the neuron/sheath cell lineage. Further, in vitro binding analysis suggests that the genetic interaction between *numb* and *H* probably occurs through physical association of their protein products.

**MATERIALS AND METHODS**

**Drosophila strains and genetics**

*Drosophila* strains were raised on standard cornmeal-yeast agar medium at 25°C or at room temperature. The three mutant alleles of *numb* (*numb*\(^1\), *numb*\(^2\), and *numb*\(^3\)) and the deficiency that uncovers the *numb* locus (Df(2L)A-C) are described by Uemura et al. (1989). The null mutation of *Su(H)* (Su(H)\(^{FS8}\)) was as described by Schweisguth and Posakony (1992). The four haploinsufficient alleles of *H* (H\(^{BS5}\), H\(^{DEBP1}\), H\(^{P521}\) and H\(^{BIO6}\)) were isolated and characterized by E. Grell (E. Grell, personal communication).

The *numb*\(^{SW}\) allele was isolated through an F\(_1\) revertant screen of the P-element-induced *numb*\(^{1}\) allele. Dysgenic males of the genotype *numb*\(^1\) ICyO; Dr \(\Delta\) 2-3/+ were crossed to *numb*\(^2\) pr cn Elp/CyO females. The F\(_1\) Cy\(^{+}\) progeny were collected and examined for abnormal sensory bristle phenotypes on the fly notum. Each candidate with abnormal bristles was retested for reproducible bristle defects by crossing to the other *numb* alleles. One mutation (*numb*\(^{SW}\) allele) was isolated from screening 3600 F\(_1\) Cy\(^{+}\) progeny. This *numb*\(^{SW}\) allele displayed a double-sOCKET bristle phenotype when trans-heterozygous to other *numb* mutations.

All four alleles of *numb* were balanced over the second chromosome balancer CyO. To examine the bristle phenotypes, *numb*\(^{SW}\) ICyO flies were mated to *numb*\(^2\) ICyO flies and the Cy\(^{+}\) flies were scored for sensory bristle defects (X represents 1, 2, 3, or the other 2L). To study the inner cell fates of the *numb*\(^{SW}\) mutant notata, we crossed homozygous *numb*\(^{SW}\) flies to *numb*\(^2\) pr cn Bc/CyO flies. Third instar larvae that displayed the marker Black cell (Bc) were selected and maintained at room temperature or 25°C till the formation of white prepupa. After staging, the prepupae were dissected and stained with antibodies used in this study have been described previously: the rabbit anti-Prospero antibody (V aessin et al., 1996), the rabbit anti-Numb antibody (Rhyu et al., 1994), the rat anti-

sensory organ lineage. For adult notum *Drosophila* strains and genetics yellow (y) and crinkled (ck) marked clones, we mated yw UAS-FLP; p[y\(^+\), y\(^{+}\)\(^{V\_C}\) ck FRT40A Gal4\(^{109-68}\)/CyO females to yw; *numb*\(^2\) Su(H)\(^{FS8}\)/CyO males. To examine the Numb protein localization, prepupae were maintained at 25°C till the formation of white prepupa. After staining, the prepupae were dissected and stained with antibodies that recognize the inner cells of each sensory organ.

**Antibody staining and confocal microscopy**

The fixed pupal nota were washed in PBT (PBS containing 0.3% Triton X-100). The fly nota were incubated with primary antibodies for 2 hours at room temperature. After washing several times with PBS, the nota were incubated with the secondary antibodies conjugated with either fluorescein or rhodamine. The nota were washed first with PBT and then with PBS and subsequently mounted in 90% glycerol with 2% n-propyl-gallate. The antibodies used in this study have been described previously: the rabbit anti-Prospero antibody (Vaessin et al., 1999), the monoclonal anti-Elav antibody (mAb44C11) (Bier et al., 1988), the rabbit anti-Numb antibody (Rhyu et al., 1994), the rat anti-Cut antibody (Blochlinger et al., 1990), and the rat anti-Su(H) antibody (Gho et al., 1996), which were used at 1:1000, 1:5, 1:1000, 1:5000, and 1:1000 dilutions. The anti-Su(H) antibody was kindly provided by F. Schweisguth. A Zeiss microscope and a Bio-Rad MRC-600 confocal microscope were used to analyze the images.

**Generation of UAS-numb transhormans**

The full length *numb* cDNA was derived from the KpnI fragment of hs-numb\(^2\) (Rhyu et al., 1994) and subcloned into the KpnI site of the pUAST vector. The pUAST-*numb* DNA was introduced into w\(^{-}\) embryos to generate transgenic flies by standard injection methods (Rubin and Spradling, 1982). The transgenic flies were crossed to sca-

Transformation analysis. To examine the Numb protein localization, prepupae were maintained at 25°C to 24-30 hours after puparium formation (APF) for cell fate trans-formation analysis. To examine the Numb protein localization, prepupae were maintained at 25°C for 14-16 hours APF, 16-18 hours APF, and 18-20 hours APF. Staged pupae were then dissected in PBS and fixed for 10 minutes in 5% formaldehyde in PEM solution (100 mM PIPES pH6.9, 1 mM MgCl\(_2\), 1 mM EGTA).

The notch intracellular domain and various Numb fragments were selected and staged to prepupae for pupal notum antibody staining of the inner cells of each sensory organ.

For studying the bristle phenotypes caused by overexpression of *numb*, the homozygous UAS-*numb*\(^{34}\) flies were crossed to the GAL4 enhancer-trap line Gal4\(^{109-68}\)/CyO, and Cy\(^{+}\) flies were analyzed. Prepupae were collected from the same cross for pupal notch antibody staining of the inner cells of each sensory organ.

The genetic interaction analysis between *numb*\(^{SW}\) and *Su(H)*\(^{FS8}\) was carried out as follows. The *numb*\(^2\) allele and *Su(H)*\(^{FS8}\) allele were first recombined onto the same chromosome, generating flies of the genotype *yw; numb*\(^2\) Su(H)\(^{FS8}\) ICyO *y\(^{+}\), y\(^{+}\)*. Homozygous *yw; numb*\(^{SW}\) flies were crossed to *yw; numb*\(^2\) Su(H)\(^{FS8}\) ICyO *y\(^{+}\), y\(^{+}\)* flies and Cy\(^{+}\) flies were examined for phenotypes. For inner sense organ cell analysis, third instar larvae with yellow mouth hooks (y\(^{-}\)) were selected and staged to prepupae for pupal notum staining.

For each of the four alleles of *H* was double balanced to *numb*\(^{SW}\) using CyO and TM6 *p[y\(^{+}\), y\(^{+}\)*, y\(^{+}\)*] generating flies of the genotype *yw; numb*\(^{SW}\) ICyO; H/ITM6 *p[y\(^{+}\), y\(^{+}\)*]. Females homozygous for *yw; numb*\(^{SW}\) ICyO; H/ITM6 *p[y\(^{+}\), y\(^{+}\)*] males. Bristle phenotypes were scored in *Cy\(^{+}\)* and y flies (yw; *numb*\(^{SW}\) ; *H*\(^{+}\)) as compared to *Cy\(^{+}\)* and y flies (yw; *numb*\(^{SW}\) ; +/TM6 *p[y\(^{+}\), y\(^{+}\)*]).

**Double mutant mosaic analysis**

For the loss-of-function mitotic recombinant clones in adults and pupae, different GAL4 enhancer trap lines, Gal4\(^{109-68}\) (Frisch et al., 1996; Guo et al., 1996) and sca-Gal4 (Nakao and Campos-Ortega, 1996) were used to drive UAS-FLP recombinase expression in the

**In vitro binding assays**

The Notch intracellular domain and various Numb fragments were
incubated in 150 ml labeled in vitro translated protein. The protein mixture was phenotypic generated in some of the clones that have lost generated in numb SW mutation is indeed a hsp70 promoter (data not shown), suggesting that this new genotype (Table 1). The homozygous severe of the double-socket bristle phenotype varies with the numb alleles (Fig. 1C,F). Double-socket bristles are produced at the expense of a hair in these partial loss of numb function mutants. The three previously isolated numb function mutants. The hair to neuron to sheath transformations (Fig. 1). The dissection and doubly stained with the neuronal marker mAb44C11 and one Prospero-expressing sheath cell (Fig. 2A-C).

RESULTS

Reduction of numb function causes both hair to socket and neuron to sheath transformations

The three previously isolated numb alleles (numb1, numb2 and numb3) cause recessive embryonic lethality, rendering these mutants unsuitable for the study of interactions of numb with other genes during adult sensory organ formation. We have therefore carried out a P-element revertant screen in order to isolate more numb mutants that are viable and show visible phenotypes in adult sensory bristles. We started with the allele numb1, which was generated for insertion of the transposon pUCHsneo (see Materials and Methods for details), and isolated a viable hypomorphic numb allele (numbSW) which exhibits a double-socket phenotype (Fig. 1).

The sensory organs on the fly notum (dorsal thorax) include both macrochaetae and microchaetae, large and small bristles, positioned in a fixed pattern. Each bristle has an external sensory structure that is composed of a hair and a socket (Fig. 1). In the numbSW mutant flies, both macrochaetae and microchaetae exhibit abnormal external sensory structures (Fig. 1C,F). Double-socket bristles are produced at the expense of a hair in these partial loss of numb function mutants. The severity of the double-socket bristle phenotype varies with the genotype (Table 1). The homozygous numbSW flies show a small number of double-socket bristles (3 out of 26 macrochaetae on the notum), while transheterozygous numbSW/numb2 flies display a very strong double-socket phenotype (12 out of 26 macrochaetae). These abnormal bristles with double sockets are observed on almost every part of the fly, including notum, head, wing (Fig. 1E), abdomen, legs and sex combs. This double-socket phenotype can be rescued by overexpression of numb driven by the heat-shock hsp70 promoter (data not shown), suggesting that this new mutation is indeed a numb allele. The double-socket phenotype generated in numbSW flies is similar to the double-socket phenotype generated in some of the clones that have lost numb function (Rhyu et al., 1994) and confirms the function of numb in the hair/socket lineage.

Besides a hair and a socket, a normal sensory bristle also contains two inner cells, a neuron and a sheath cell (Fig. 1A). To examine the inner cells of sensory bristles in the numbSW mutant we have used a neuronal nuclear marker, the monoclonal antibody mAb44C11 (an anti-Elav antibody), and a sheath cell nuclear marker, the anti-Prospero antibody, and doubly stained numbSW mutant nota at 24-30 hours APF when all four cells of a sensory organ are fully differentiated. In wild-type nota, each sensory bristle has one mAb44C11-positive neuron and one Prospero-expressing sheath cell (Fig. 2A-C).

Table 1. Numbers of double-socket macrochaetae in flies that are trans-heterozygous for numbSW and various numb alleles, as compared to those of the wild-type (+/+) flies

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Notum</th>
<th>Head</th>
</tr>
</thead>
<tbody>
<tr>
<td>numbSW/numb1</td>
<td>11.2</td>
<td>7.9</td>
</tr>
<tr>
<td>numbSW/numb2</td>
<td>12.4</td>
<td>10.2</td>
</tr>
<tr>
<td>numbSW/numb3</td>
<td>11.8</td>
<td>9.2</td>
</tr>
<tr>
<td>numbSW/numbSW</td>
<td>3.0</td>
<td>1.9</td>
</tr>
<tr>
<td>numbSW/Df(2L)A-C</td>
<td>15.4</td>
<td>11.3</td>
</tr>
</tbody>
</table>

Wild-type macrochaetae number: notum, 26; head, 14.
In contrast, in the numbSW mutant nota, some sensory bristles contain two Prospero-positive sheath cells but no neurons (Fig. 2D-F), indicating that the neuron is transformed into a sheath cell. The total number of cells within each sensory bristle does not seem to change, as revealed by staining with an anti-Cut antibody which recognizes all four cells of the sensory organ (data not shown). About 50%-60% of the sensory bristles have a neuron to sheath cell transformation (Table 2A), showing that numb also functions in the IIb cell lineage for the formation of the neuron and the sheath cell. Therefore, partial loss of numb activity in the numbSW allele causes both IIa and IIb cells to divide symmetrically, resulting in the formation of two socket cells and two sheath cells (Fig. 5B).

Previously it was shown that the Numb protein is asymmetrically localized in the dividing SOP cell, forming a crescent in the cell cortex of SOP (Rhyu et al., 1994). We were therefore interested in knowing whether Numb protein localization is affected in the numbSW mutants. Since the Numb protein localization in later stages (i.e. the IIa and IIB divisions) during the SOP development has not been studied, we have first examined the Numb protein localization on the pupal nota of wild type during each of the divisions (SOP, IIa, and IIb) of a microchaete bristle (Fig. 3). In this experiment, we used the anti-Cut antibody which stains the nuclei of all the cells within each sensory organ, and the anti-Numb antibody, and doubly stained pupal nota at 14-16 hours APF (Fig. 3A-C), 16-18 hours APF (Fig. 3D-F), and 18-20 hours APF (Fig. 3G-I). On a notum at 14-16 hours APF when dividing SOPs are frequently present as revealed by the non-nuclear staining of the anti-Cut antibody, a clear crescent staining of Numb is associated with each dividing SOP cell. At 16-18 hours APF, each adult sensory organ is at the two-cell stage, with the IIa cell being ready to divide. We observed a Numb crescent in each dividing IIa cell (Fig. 3D-F). A couple of hours after the division of the IIa cell, the IIb cell starts to divide (18-20 hours APF). Numb crescent also forms in the IIb cell before it divides during a three-cell stage. The observation of asymmetric localization of Numb protein in the dividing SOP, IIa cell and IIb cell suggests that Numb is preferentially segregated into one daughter cell during each of the three divisions in the SOP lineage, thus generating daughter cells with distinct fates. Similar crescent localization of Numb was also observed during the SOP development on the pupal nota of numbSW mutants (data not shown). Therefore, the numbSW mutation most likely affects the Numb signaling efficiency rather than Numb localization.

**Overexpression of numb can cause cell fate transformations opposite to those of the numb hypomorphic allele**

Previously, it has been shown that overexpression of numb using the heat-shock hsp70 promoter (hs-numb) results in two major phenotypes, balding (loss of external sensory organ structures) and twinned hairs with no socket. These two phenotypes presumably are due to the transformation of the IIa cell into the IIb cell and the socket cell into the hair cell in the IIa

<table>
<thead>
<tr>
<th>Pupa no.</th>
<th>1 Pros+ cell</th>
<th>2 Pros+ cells</th>
<th>Total macrochaetae counted</th>
</tr>
</thead>
<tbody>
<tr>
<td>A numbSW/numb2</td>
<td>1</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>10</td>
<td>18</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>11</td>
<td>18</td>
</tr>
<tr>
<td>B numbSW+/numb2Su(H)SF8</td>
<td>1</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>13</td>
<td>21</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>10</td>
<td>20</td>
</tr>
</tbody>
</table>

Average neuron to sheath transformation: numbSW/numb2, 58%; numbSW+/numb2Su(H)SF8, 55%.

Fig. 3. Numb is asymmetrically localized in all three divisions during SOP development. Pupal nota at 14-16 hours APF, 16-18 hours APF, and 18-20 hours APF were dissected and doubly stained with the anti-Cut (red) and the anti-Numb (green) antibodies. Similar results were obtained with both wild-type and numbSW mutant pupal nota. (A-C) A region of pupal notum at 14-16 hours APF. Arrowhead indicates the Numb crescent, which is associated with the dividing cell, the IIa cell, whereas the neighboring cell with nuclear staining of Cut is presumably the IIb cell. The arrowhead shows a two-cell cluster positive for nuclear staining of Cut, corresponding to a IIa cell and a IIb cell resulted from a SOP division. (D-F) A region of pupal notum at 16-18 hours APF. Arrow indicates the Numb crescent. Note that the Numb crescent is associated with the dividing cell, the IIa cell, whereas the neighboring cell with nuclear staining of Cut is presumably the IIb cell. The arrowhead shows a two-cell cluster positive for nuclear staining of Cut, corresponding to a stage before the IIa cell enters mitosis. (G-I) A region of pupal notum at 18-20 hours APF. Arrow indicates the Numb crescent, which is associated with the IIb cell during mitosis. The arrowhead shows the two cells next to the IIb cell which are positive for nuclear staining of Cut. These two neighboring cells are presumably the hair cell and the socket cell. Brackets show two clusters of three cells with nuclear staining of Cut, suggesting that the IIb cells in these three-cell clusters are at a stage before entering mitosis.
cell lineage, respectively (Rhyu et al., 1994). We examined the effects of *numb* overexpression on the cell fate of the inner cells, the neuron and sheath cell (daughters of the IIb cell).

The UAS-GAL4 system (Brand and Perrimon, 1993) was used to study the effect of *numb* overexpression. Transgenic flies carrying one copy of UAS-*numb* (#34A) were crossed to Gal4109-68 and the Gal4109-68/+; UAS-numb/+ flies were examined. (A,C,D) Twinned hairs caused by the socket to hair transformation. (B,D) Balding phenotype due to the transformation from IIa into IIb. (E-G) A pupal notum of Gal4109-68/+; UAS-*numb*/+ mutant at 24-30 hour APF. The UAS-GAL4 system (Brand and Perrimon, 1993) was used to study the effect of *numb* overexpression. Transgenic flies carrying one copy of UAS-*numb* (#34A) were crossed to Gal4109-68 and the Gal4109-68/+; UAS-numb/+ flies were examined. (A,C,D) Twinned hairs caused by the socket to hair transformation. (B,D) Balding phenotype due to the transformation from IIa into IIb. (E-G) A pupal notum of Gal4109-68/+; UAS-numb/+ mutant at 24-30 hour APF.

**Fig. 4.** Overexpression of *numb* can generate phenotypes that are opposite to those from reduction of *numb* function. The homozygous UAS-*numb* (#34A) flies were crossed to Gal4109-68 and the Gal4109-68/+; UAS-numb/+ flies were examined. (A) Twinned hairs caused by the socket to hair transformation. (B) Balding phenotype due to the transformation from IIa into IIb. (E-G) A pupal notum of Gal4109-68/+; UAS-numb/+ mutant at 24-30 hour APF. The three neurons and one sheath cell phenotype is marked by arrowheads, two neurons and two sheath cells, and two neurons with no sheath cells by *s*, and two neuron with no sheath cells by arrows.

The strong genetic interaction between *numb SW* and *Su(H)*SF8 made us wonder how *numb* and *Su(H)* act antagonistically. Shortly after division of the IIa cell, the *Su(H)* protein is strongly expressed in the SOP cell and its progeny. Two phenotypes resulted from such overexpression of *numb*, twinned hairs (Fig. 4A,C) and balding (Fig. 4B,D), the same as the phenotypes caused by hs-*numb* (Rhyu et al., 1994). Sensory bristles in the dissected pupal notae of flies carrying both UAS-*numb* and Gal4109-68 were doubly stained using the neuronal marker mAb44C11 and the sheath cell marker anti-Prospero antibody. Four types of phenotypes were observed for the inner cells: four neurons, three neurons and one sheath cell, two neurons and two sheath cells, and two neurons with no sheath cells (Fig. 4E-G). Most likely, transformation of both the IIa cell to a IIb cell and the sheath to a neuron caused these phenotypes.

Therefore, overexpression of *numb* can cause phenotypes opposite to the loss or reduction of *numb* function phenotypes. These overexpression experiments confirm the finding that *numb* functions in all three divisions of the SOP lineage. Fig. 5 summarizes the cell fate transformations in the IIa and IIb lineage within an adult sensory organ in *numb SW* and UAS-*numb*, as compared to the wild type.

**A *Su(H)* mutation acts as a dominant suppressor of the *numb* hypomorph in determining the hair/socket lineage but not the neuron/sheath lineage**

Partial reduction of *Su(H)* activity produces a twinned hair phenotype, resulting from transformation of the socket cell into a hair cell (Schweisguth and Posakony, 1994), opposite to the phenotype caused by decreased *numb* function. Conversely, overexpression of *Su(H)* causes a double-socket phenotype, presumably resulting from transformation of the hair cell into a socket cell (Schweisguth and Posakony, 1994). To test whether these genes act in the same pathway, we assessed the influence of the *Su(H)* null allele, *Su(H)*SF8, on the hypomorphic allele *numb SW*. As described above, *numb SW*/*numb 2* flies showed the double-socket phenotype in about 50% of the adult sensory bristles (Table 1) and a neuron to sheath transformation in 50-60% of pupal sensory bristles (Table 2A). Removing one copy of the *Su(H)* gene in *numb SW*/*numb 2* flies (i.e. *numb SW*/*numb 2* Su(H)SF8) strongly suppressed the double-socket phenotype (Fig. 6A-C). In contrast, reduction of *Su(H)* function had no effect on the *numb SW* double-sheath phenotype. As shown in Fig. 6D-F and summarized in Table 2B, double-labeling of the sensory bristle cells with mAb44C11 and anti-Prospero clearly showed the double-sheath cell phenotype characteristic of *numb SW*/*numb 2* mutant. These results establish that *Su(H)*SF8 behaves as a dominant suppressor of the hair cell to socket cell transformation but not of the neuron to sheath cell transformation in the *numb SW*/*numb 2* mutant.

**Numb determines the hair cell identity by negatively regulating *Su(H)* protein expression**

The strong genetic interaction between *numb SW* and *Su(H)*SF8 made us wonder how *numb* and *Su(H)* act antagonistically. Shortly after division of the IIa cell, the *Su(H)* protein is strongly expressed in the SOP cell and its progeny. Two phenotypes resulted from such overexpression of *numb*, twinned hairs (Fig. 4A,C) and balding (Fig. 4B,D), the same as the phenotypes caused by hs-*numb* (Rhyu et al., 1994). Sensory bristles in the dissected pupal notae of flies carrying both UAS-*numb* and Gal4109-68 were doubly stained using the neuronal marker mAb44C11 and the sheath cell marker anti-Prospero antibody. Four types of phenotypes were observed for the inner cells: four neurons, three neurons and one sheath cell, two neurons and two sheath cells, and two neurons with no sheath cells (Fig. 4E-G). Most likely, transformation of both the IIa cell to a IIb cell and the sheath to a neuron caused these phenotypes.

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Interaction of Su(H) with numb

Epistatic relationship between Su(H) and numb

The finding that loss-of-function mutations of numb and Su(H) have opposite effects on the hair/socket cell fate specification and the strong genetic interaction between these two genes raise the question of their epistatic relationship. To investigate this issue, we used UAS-FLP and specific GAL4 enhancer trap lines to target FRT mediated clones of both numb and Su(H) to the SOP lineage (Frise et al., 1996) (Zeng et al., personal communication). We took advantage of two GAL4 enhancer-trap lines, Gal4109-68 and sca-Gal4, which express GAL4 specifically in the SOP and its daughter cells. Both Gal4109-68 and sca-Gal4 show similar expression of a reporter gene during SOP divisions, although the expression in sca-Gal4 appears to be stronger. The reporter gene expression in sca-Gal4 also starts earlier in proneural clusters before the SOP is singled out. We expected that the FLP recombinase would be expressed in a similar pattern from the UAS-FLP transgene. The SOP lineage specific FLP expression induced mitotic recombination between a FRT chromosome carrying mutations of numb, Su(H), or numb Su(H) and a ‘wild-type’ FRT chromosome, producing one daughter cell homozygous for the null mutations and the other daughter cell homozygous for the wild-type genes.

Loss of numb function in the IIa cell due to UAS-FLP and Gal4109-68 caused a double-socket phenotype (Fig. 8A-C), which is indistinguishable from the double sockets observed in the numbSW allele (Fig. 1C,F) or in heat-shock FLP-numb FRT mutant clones generated during third instar larval and pupal stage (Rhyu et al., 1994). Interestingly, sensory organs with three sockets and one hair (Fig. 8D) or four sockets (data not shown) were observed with a moderate frequency when clones for the null mutation of numb were generated using the GAL4 line sca-Gal4. These phenotypes are most likely due to an early mitotic recombination event induced by the early expression of sca-Gal4 in the proneural cluster before the division that gives rise to the SOP cells. In this case, the SOP itself would be homozygous for the numb null mutation, causing transformation of the IIb cell into the IIa cell. Subsequently, loss of numb function in the two IIa cells may cause one IIa cell to divide symmetrically to form two socket cells, whereas the other IIa cell may either give rise to a hair cell and a socket cell due to an incomplete transformation or two socket cells due to a complete transformation.

Twinned hairs as well as several other bristle phenotypes were observed when either Gal4109-68 or sca-Gal4 was used to remove Su(H) function in the SOP cell and its daughter cells (Fig. 8E-I). The twinned hair phenotype suggests that Su(H) functions in the IIa division to determine the socket cell fate (Schweisguth and Posakony, 1994). Loss of Su(H) function, like that of numb, also yields a broad spectrum of transformations. The phenotypes are always in the direction

activity. Numb may normally be present primarily in the hair cell but not in the socket cell, thus resulting in low Su(H) protein level in the hair cell and high Su(H) protein level in the socket cell. When numb function is reduced, the level of Su(H) protein is increased in the hair cell, leading to the transformation of the hair cell into a socket cell. Conversely, when numb function is increased, the level of Su(H) protein is suppressed in the socket cell, resulting in the transformation of the socket cell into a hair cell.

Fig. 6. Reducing one copy of Su(H) suppresses the double-socket phenotype but not the double-sheath phenotype of numbSW/numb2 mutants. (A) An adult notum from a numbSW/+ Su(H)SF8 fly showing the normal looking macrochaete and microchaete bristles. This indicates that the double-sheath phenotype generated in the numb hypomorph is suppressed by removing one copy of the wild-type Su(H) gene. (B) The dorsal central region of the notum in (A) at higher magnification. (C) The wing margin bristles from a numbSW/+ Su(H)SF8 fly showing a similar suppression of the double socket bristle phenotype. (D-F) Staining of a pupal notum from numbSW/+ Su(H)SF8 mutants with the anti-Prospero antibody and a single socket bristle of the wild-type pupal notum, a single sheath cell is restricted to the socket/hair lineage.
of forming two hair cells and no socket cell, and they range from a remnant of a hair cell associated with an abnormal socket to the formation of twinned hairs at the expense of the socket cell. These phenotypes (Fig. 8E,F) may be explained by partial transformation of the socket cell to a hair cell and are therefore intermediate phenotypes toward the formation of twinned hairs. We then examined the phenotype in the double mutants where both numb and Su(H) function were removed from the SOP and/or its daughter cells. We found that a range of phenotypes was produced in the numb<sup>2</sup> Su(H)<sup>SF8</sup> mosaic flies when either Gal4<sup>109-68</sup> or sca-Gal4 was used (Fig. 8J-N). Phenotypes of the double mutants were indistinguishable from those due to loss of function of Su(H) alone (Fig. 8E-I). Heat-shock FLP induced mitotic recombination, generated in second instar larvae, also produced double null mutant clones with phenotypes
indistinguishable from those of Su(H) null mutant clones (data not shown). Therefore, the Su(H) gene appears to act downstream of numb in the same genetic pathway in determining the fates of the IIa daughter cells, the hair cell and the socket cell.

We have further examined the inner cells on the pupal nota of these mutants at 24-30 hours APF by double staining with the anti-Prospero antibody and the monoclonal antibody mAb44C11 which specifically stain the sheath cells and the neurons, respectively (Fig. 9). As expected, the loss-of-function mutation of numb caused a double-sheath phenotype (Fig. 9A-C). In loss-of-function clones of Su(H), the sheath/neuron cell lineage is completely normal (Fig. 9D-F).

In the double mutants where null mutations of both numb and Su(H) were generated in the SOP lineage, the double-sheath phenotype due to loss of numb function did not seem to be affected by the removal of the Su(H) function (Fig. 9G-I). In the double mutants where null mutations of both numb and Su(H) were generated in the SOP lineage, the double-sheath phenotype due to loss of numb function did not seem to be affected by the removal of the Su(H) function (Fig. 9G-I).

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Similar phenotypes of the inner cells in these double mutants were generated when either Gal4109-68 or sca-Gal4 was used. These results provide further evidence for Su(H) acting as a downstream target of numb only in determining the cell fates in the IIa lineage for the formation of the hair cell and the socket cell. Consistent with this scenario, overexpression of Su(H) via the heat-shock promoter (hs-Su(H)) did not affect the neuron/sheath cell lineage, as revealed by double staining with the anti-Prospero antibody and the mAb44C11 antibody (data not shown). Our results strongly indicate that Su(H) and numb function in the same genetic pathway, with Su(H) acting downstream of numb and negatively regulated by numb. However, this interaction is restricted to the hair/socket lineage and there is no such epistatic relationship in the neuron/sheath cell lineage where apparently Su(H) is not required.

**H acts as a dominant enhancer of numb in determining the hair/socket lineage**

Previously, it has been shown that Su(H) and H act antagonistically to control the cell fates in the adult sensory organs (Schweisguth and Posakony, 1994). The Su(H) mutation acts as a dominant suppressor of the various haplo-insufficient alleles of H to suppress the double-socket phenotype of H mutants. The double-socket phenotype exhibited by H loss-of-function mutants is similar to that of the numb loss-of-function phenotype. Given that numb and Su(H) act in the same genetic pathway, we have further examined the genetic interaction of H with numb. Homozygous numbSW flies display a very weak double-socket phenotype (middle column in Table 3; Fig. 10A,D). Removing one copy of H greatly enhances the double-socket phenotype of the numbSW flies (Fig. 10C,F). Table 3 summarizes the effect of four different H alleles on the numbSW flies. We found that the

**Table 3. Numbers of the double-socket cells counted in numbSW nota compared to those in numbSW; H/+ nota**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>numbSW/numbSW; H/+</th>
<th>numbSW/numbSW; +/+</th>
<th>numbSW/+; H/+</th>
</tr>
</thead>
<tbody>
<tr>
<td>H&lt;sup&gt;B55&lt;/sup&gt;</td>
<td>12.1</td>
<td>2.0</td>
<td>4.2</td>
</tr>
<tr>
<td>H&lt;sup&gt;DEB1&lt;/sup&gt;</td>
<td>13.0</td>
<td>2.8</td>
<td>2.1</td>
</tr>
<tr>
<td>H&lt;sup&gt;B21&lt;/sup&gt;</td>
<td>11.7</td>
<td>2.5</td>
<td>2.7</td>
</tr>
<tr>
<td>H&lt;sup&gt;D9&lt;/sup&gt;</td>
<td>7.1</td>
<td>2.7</td>
<td>0.7</td>
</tr>
</tbody>
</table>

The wild-type nota contains 26 macrochaetae.
number of double-sockets in numbSW mutants carrying one copy of the H mutation is two- to three-fold greater than the sum of those in H and numbSW single mutants (Table 3). Thus the hair/socket cell lineage is very sensitive to the dosage of both H and numb. H mutations function as dominant enhancers of numbSW in the specification of cell fates of the hair/socket cell lineage of adult sensory bristles.

In a genetic screen for modifiers of numb using the numbSW allele, we isolated a dominant enhancer of numbSW (data not shown). This enhancer mutant was subsequently found to be allelic to H, further substantiating the genetic interaction we observed between numb and H (Fig. 10 and Table 3).

**H and Numb physically interact with each other**

Given that numb, Notch, Su(H), and H all participate in the same genetic pathway in determining the IIa cell lineage and that Su(H) binds to H (Bailey and Posakony, 1995), Notch binds to Su(H) (Fortini and Artavanis-Tsakonas, 1994), and Numb binds to Notch (Guo et al., 1996), we wondered whether the interactions of numb with Su(H) and H are due to physical associations of their gene products. We performed an in vitro binding assay to determine whether Numb binds to Su(H) or H. As shown in Fig. 11, a bacterial fusion protein, GST-Numb, and in vitro translated H protein were coprecipitated by glutathione agarose beads (Fig. 11, lane 2). Similar coprecipitation of GST-Notch and the in vitro translated H protein was also observed (Fig. 11, lane 1). The in vitro translated H protein binds the Numb-N fragment which encompasses the PTB domain of Numb (Fig. 11, lane 3), whereas the GST-Numb-C (Fig. 11, lane 4) or GST alone (Fig. 11, lane 5) failed to bind to H. Similar binding analysis with the in vitro translated Su(H) protein showed no detectable binding of Su(H) to Numb (data not shown).

**DISCUSSION**

By isolating and characterizing a hypomorphic numb mutant, numbSW, we have confirmed and extended previous studies to show that numb functions in all three asymmetric divisions that give rise to a simple external sensory organ. Moreover, we found that the IIa lineage which gives rise to the hair and the socket, but not the IIb lineage which gives rise to the neuron and the sheath cell, requires the functions of Su(H) and presumably H. These findings, and the genetic interactions among these genes, are discussed below.

**numb functions in all three asymmetric cell divisions during the formation of adult sensory organs**

Studies of numb in embryos indicate that numb functions during the SOP division to produce the two distinct secondary precursors, the IIa cell and the IIb cell (Uemura et al., 1989; Rhyu et al., 1994). Moreover, results from overexpression and mosaic analysis in adult flies suggest that numb functions during both SOP division and the IIa division in the development of adult sensory organs (Rhyu et al., 1994). Therefore, numb plays an important role in specifying the IIb cell fate and the hair cell fate. Although it was proposed previously that numb also plays a role in specifying the progeny cell fates of IIb, it has not been demonstrated directly owing to the transformation of the IIb cell into the IIa cell in numb null mutants. The characterization of the newly isolated hypomorphic allele of numb (numbSW) clearly indicates that numb is also involved in the sheath/neuron lineage (the IIb cell lineage), as revealed by the double-sheath cell phenotype on fly pupal nota as revealed by staining with anti-Prospero antibody (Fig. 2). Similar double-sheath phenotypes were also observed in the external sensory organs in fly embryos (data not shown). This involvement of numb in the choice between the neuron and the sheath cell fates was also confirmed by mosaic analysis using the UAS-FLP/FRT with GAL4 and a numb null allele (numb0) (Fig. 9). In addition, we have performed immunofluorescence analysis and found that the Numb protein is asymmetrically localized in the dividing SOP, the dividing Ila cell, and the dividing IIb cell (Fig. 3). Thus, Numb functions to promote the IIb cell fate in the SOP division, the hair cell fate in the Ila lineage, as well as the neuron fate in the IIb lineage presumably by being preferentially segregated into the IIb cell, the hair cell, and the neuron, respectively. The normal presence of asymmetric Numb localization in numbSW mutants suggests that the hypomorphic mutation of numb may affect the Numb signaling efficiency.
**Su(H) and H are required for determining the hair/socket cell lineage but not the neuron/sheath cell lineage**

It has previously been shown that Su(H) and H are required for the determination of the socket cell and the hair cell within an adult sensory organ, respectively (Schweisguth and Posakony, 1994). Loss of function alleles of Su(H) are potent dominant suppressors of the double-socket phenotype in H mutants (Schweisguth and Posakony, 1992), suggesting that reduction of Su(H) activity favors the hair cell fate. Consistent with this hypothesis, a twinned hair phenotype without a socket was produced by either partial loss of function or the null mutant of Su(H) in mosaic analysis (Schweisguth and Posakony, 1994). Overexpression of Su(H) produces the opposite transformation. When Su(H) activity is increased during the Ila cell division, the hair cell is transformed into a socket cell, resulting in a double-socket phenotype (Schweisguth and Posakony, 1994). In contrast, reduction of function or overexpression of H during the later stage of the SOP lineage yields a double-socket or twinned hair phenotype, respectively (Bang et al., 1991; Bang and Posakony, 1992). Therefore, unlike Su(H), which is required for socket cell formation, H activity seems to be required for hair cell formation.

Although it was proposed that the Su(H) and H activities are required for all three binary cell fate decisions in sense organ development (Posakony, 1994), Schweisguth and Posakony (1994) observed that flies which carry extra copies of Su(H) contain a single neuron within each sensory organ, as revealed by staining with the anti-HRP antibody. This observation raised the possibility but did not prove that Su(H) is not involved in the neuron/sheath cell fate decision because it was not known whether the extra copies of Su(H) caused overexpression of Su(H) at the right place and right time to produce sheath/neuron transformation. Importantly, whether neuron/sheath decision is affected by Su(H) loss-of-function mutant was not tested. Here, we have used anti-Prospero antibody, which labels the nuclei of sheath cells, and the monoclonal antibody mAb44C11, which labels the nuclei of neurons, to analyze sensory bristle cells of pupal nota of both the null mutant (Fig. 9A-C) and mis-expression (hs-Su(H), data not shown) of Su(H). These experiments show that the neuron/sheath cell lineage is not affected by the loss or increase of Su(H) activity.

**numb interacts with Su(H) and H in determining hair/socket lineage**

As described above, numb is involved in all three asymmetric divisions during the formation of a sensory organ in both embryos and adults. The twinned hair phenotype due to loss of function of Su(H) is opposite to the double-socket phenotype due to loss of function of H or numb during the Ila cell division. It therefore raises the possibility that numb, Su(H), and H may determine the Ila cell lineage by acting in the same genetic pathway. Consistent with this, we found that loss of activity of one copy of Su(H) almost completely prevented the double-socket formation in numb mutants, indicating that Su(H)SF8 behaves as a dominant suppressor of numb mutants. Conversely, we showed that various haplo-insufficient alleles of H act as dominant enhancers of the numbSW allele, since partial reduction of H function greatly increased the double-socket phenotype in flies homozygous for numbSW.

In contrast to the suppression of the double-socket phenotype of numbSW/numb2 mutants (Fig. 6; Table 2), we failed to detect any effect on the double-sheath phenotype of the numbSW/numb2 mutants when one copy of the wild-type Su(H) gene was removed, suggesting that this genetic interaction between numb and Su(H) is restricted to the Ila lineage.

**Su(H) acts downstream of numb in the hair/socket lineage**

Having found that partially reduced Su(H) activity prevents the double-socket phenotype in the partial loss of numb function mutant, we asked whether Su(H) acts downstream of numb by examining mitotic recombination induced clones that lack the functions of both Su(H) and numb in the SOP lineage.

Adult flies carrying clones of null mutations of both numb and Su(H) showed a twinned hair phenotype, resembling the loss of Su(H) function phenotype but opposite to the loss of numb function phenotype (Fig. 8). This result indicates that Su(H) is epistatic to numb in the same genetic pathway that leads to the determination of daughter cell fates of Ila. Given that complete removal of Su(H) functions had no effect on the neuron/sheath cell lineage nor did it alter the double-sheath cell phenotype generated by null mutations of numb (Fig. 9), it appears that Su(H) has no essential role in the neuron/sheath cell specification.

How might numb, Su(H), and H regulate one another to specify the hair or the socket cell fate? Our immunostaining results indicate that the Numb protein is asymmetrically localized in the dividing Ila cell (Fig. 3D-F). Therefore, this intrinsic factor Numb is probably preferentially segregated into the future hair cell upon the asymmetric division of the Ila cell. High level of Numb leads to down regulation of Su(H) and the hair fate. Conversely, low level of Numb leads to high level of Su(H) and the socket fate. How does Numb (and possibly H) level affect Su(H) level is unknown. An intriguing possibility is that the regulation is at the level of protein stability as has been shown in the case of Tramtrack in photoreceptor specification (Li et al., 1997; Tang et al., 1997).

**Numb/Notch signaling mediated by Su(H)-dependent versus Su(H)-independent pathways**

Notch and numb are involved in an antagonistic manner in all three asymmetric divisions of the SOP lineage during embryogenesis and adult sensory organ formation (Hartenstein and Posakony, 1990; Rhyu et al., 1994; Guo et al., 1996). It has been proposed that Notch is a downstream target gene that is negatively regulated by numb in the determination of daughter cell fate during asymmetric division of the SOP and its daughter cells in the PNS (Guo et al., 1996) as well as the asymmetric division of MP2 in the CNS (Spana et al., 1995; Spana and Doe, 1996) during embryogenesis. Most likely, this epistatic relationship of Notch and numb also applies to adult sensory organ development.

Su(H) is known to function in the Notch signaling pathway. Drosophila sense organ development provides a striking example for Su(H) action in only a subset of the binary cell fate decisions controlled by Numb/Notch signaling. Existence of Su(H)-independent Notch signaling is not limited to Drosophila sense organs. Lecourtois and Schweisguth (1995) found that Su(H)-independent Notch signaling is involved in mesectodermal cell fate specification. In a vertebrate muscle
cell culture system, Shawber et al. (1996) have demonstrated that Notch can inhibit muscle cell differentiation by a mechanism that is independent of CBF1 (a human homolog of Su(H)). An important task for the future is to identify the molecular components used in Su(H)-independent Notch signaling pathways. The Drosophila sense organ should prove to be a useful system for this purpose.

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