A Notch-Independent Activity of Suppressor of Hairless Is Required for Normal Mechanoreceptor Physiology

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Summary
Suppressor of Hairless [Su(H)]/Lag-1/RBP-Jk/CFB1 is the only known transducing transcription factor for Notch receptor signaling. Here, we show that Su(H) has three distinct functions in the development of external mechanosensory organs in Drosophila: Notch-dependent transcriptional activation and a novel auto-repression function, both of which direct cell fate decisions, and a novel auto-activation function required for normal socket cell differentiation. This third phase of activity, the first known Notch-independent activation function for Su(H) in development, depends on a cell type-specific autoregulatory enhancer that is active throughout adult life and is required for proper mechanoreception. These results establish a direct link between a broadly deployed cell signaling pathway and an essential physiological function of the nervous system.

Introduction
One of the most important contributions of molecular biology and genetics to the study of development is the discovery that a handful of evolutionarily conserved cell–cell signaling pathways direct a majority of developmental patterning and cell fate specification processes in metazoans. The Notch (N) signaling pathway is a prime example: Its major components are conserved across bilaterian phyla, and within a single organism it is employed repeatedly, in many different contexts, during development (reviewed by Artavanis-Tsakonas et al., 1999). N receptor signaling is used to specify cell fates in all three germ layers, in such disparate contexts as T cell precursor differentiation in mammals, notochord specification in the ascidian Ciona, vulval development in C. elegans, and selection of muscle, gut, and sensory organ precursors in the Drosophila embryo. Such versatility raises the simple but important question of how N signaling controls cell fate. Yet we remain largely ignorant of the regulatory linkages that connect a common cell fate specification system such as the N pathway to the particular differentiation program of an individual N-dependent cell type. By studying this “specification/differentiation interface” in detail, we hope to elucidate the mechanisms underlying specificity in cell–cell signaling, and to learn how a given signaling pathway is enlisted for the specification of novel structures during metazoan evolution.

Only one direct transducing transcription factor has been identified so far for the N receptor: Suppressor of Hairless [Su(H)] (Schweisguth and Posakony, 1992) and its orthologs, including mammalian CBF1/RBP-Jk (Hamaguchi et al., 1989). Upon association with ligand, the transmembrane N receptor is proteolytically cleaved, and a resulting intracellular domain fragment (NIC) acts as a transcriptional coactivator for Su(H) (Artavanis-Tsakonas et al., 1999). The role of Su(H) in N signaling has been extensively studied in both vertebrates and invertebrates. During neurogenesis in both insects and mammals, for example, basic helix-loop-helix (bHLH) repressor-encoding genes of the hairy/Enhancer of split class are directly activated by Su(H) in response to the N signal (Bailey and Posakony, 1995; Jarriault et al., 1995). Su(H)’s DNA binding specificity and its interaction with N are evolutionarily conserved, and its activity is required for almost all known N-mediated patterning and cell fate specification events (Artavanis-Tsakonas et al., 1999). Though Su(H)-independent N signaling events have been described (Shawber et al., 1996; Matsumo et al., 1997; Wang et al., 1997; Ordentlich et al., 1998), no other DNA binding partner or transducer for N has been identified. Thus, we might predict that a great deal of the cell-type specificity of the response to activated N is manifested in the regulation of Su(H) activity, or of genes downstream of Su(H).

The development of the adult mechanosensory bristle of Drosophila includes at least four distinct N-mediated cell fate decisions (Posakony, 1994). The sensory organ precursor (SOP) cell prevents its neighbors from adopting the SOP fate via N-mediated lateral inhibitory signaling (Heitzi and Simpson, 1991). The SOP then executes a characteristic lineage to generate the cells that make up the bristle organ: a bipolar sensory neuron, a sheath cell (thecogen), a shaft cell (trichogen), a socket cell (formogen), and, in some cases, one or more glial cells (Hartenstein and Posakony, 1989; Gho et al., 1999; Van De Bor et al., 2000). The bristle lineage consists of multiple asymmetric cell divisions, including pIIA/pIIB (the immediate progeny of the SOP), socket/shaft, and sheath cell/neuron. N signaling acts to specify one of the two sister cell fates (the first) in each of these divisions. Su(H), which is expressed ubiquitously at moderate levels in all developing tissues, is dramatically upregulated in only one cell type of the fly: the socket cell of external sensory organs (ESOs) (Schweisguth and Posakony, 1992; Gho et al., 1996). The mechanism and function of this activation have heretofore been unknown.

We have identified a discrete socket cell-specific tran-
Figure 1. Socket Cell-Specific Transcription of Su(H) Is Driven by a Discrete Enhancer Module

(A) Diagram of the Su(H) gene. RC-wt is a genomic DNA fragment that rescues the Su(H) null phenotype (Schweisguth and Posakony, 1992). Boxes represent Su(H) exons; black indicates protein-coding regions; white denotes UTRs. Ovals indicate the nine Su(H) protein binding sites (see Figure 2).

(B) Embryonic (stage 17) pattern of GFP expression driven by the Autoregulatory Socket Enhancer (ASE), a 1.9 kb fragment downstream of the Su(H) gene (see [A]). The ASE-GFP reporter gene is active specifically in socket cells of all ESOs.

(C) ASE-GFP expression in socket cells of ESOs (arrowheads) in a late third-instar larva.

(D) β-galactosidase activity in ovaries of flies carrying -403Su(H)-lacZ, in which lacZ is driven by the Su(H) promoter. Note reporter gene activity in all nurse cells, as well as in the oocyte nucleus (on, arrow) and posterior pole follicle cells.

(E and F) General β-galactosidase activity expressed from (E) -403Su(H)-lacZ and (F) ASE + -403Su(H)-lacZ in late third-instar wing imaginal discs.

(G) Socket cell-specific activity of ASE5-GFP in the thorax of a pupa at 36 hr APF.

(H) ASE5-lacZ activity in socket cells of the adult thorax.

(I) ASE-GFP expression in socket cells of all adult ESOs, including sensory bristles on the thorax, abdomen, and wing margin, as well as
scriptional enhancer of the Su(H) gene that is activated shortly after the birth of this cell, and remains active in socket cells of all ESOs throughout the adult life of the fly. This enhancer is directly activated in the socket cell by Su(H)’s own protein product and requires auto-activation for adult socket cell expression. We find that the maintenance of Su(H) auto-activation does not require continued N signaling; this is the first known instance of N-independent transcriptional activation by Su(H) in development.

We show here that flies lacking socket cell-specific Su(H) auto-activation properly specify all N-dependent cell fates during development, including the socket cell fate. However, they exhibit severe defects in mechanoreception. Thus, a core component of the N pathway has been recruited for an essential differentiative/physiological function specifically in one cell type. We also find that direct auto-repression by Su(H) in the shaft cell is necessary for the proper specification of the shaft cell fate. Taken together, these results lead us to propose a model of mechanosensory organ development in which Su(H) employs three distinct mechanisms of transcriptional regulation: N-dependent auto-activation in the early socket cell, auto-repression in the shaft cell, and a later phase of N-independent auto-activation that is required for the proper physiological activity of adult mechanoreceptors.

Results

The Su(H) ASE: A Cell Type-Specific Transcriptional Enhancer Active in Developing and Mature Sensory Organs

Transgenic ß-galactosidase reporter gene analysis of genomic DNA sequences flanking the Su(H) gene (Figure 1A) identified two separate cis-regulatory regions that together recapitulate the complete Su(H) expression pattern. The Su(H) promoter and proximal upstream region drives ß-galactosidase expression ubiquitously, at moderate levels, in the embryo (data not shown), in larval-stage imaginal discs (Figure 1E), and in the nurse cells and follicle cells of the ovary (Figure 1D). Su(H) promoter activity in the third-instar wing imaginal disc is uniform and does not appear to be regulated by N signaling in the proneural clusters or at the presumptive wing margin, both sites of active N signaling (Figure 1E).

A 1.9 kb transcriptional enhancer module located downstream of Su(H) (Figure 1A) drives robust reporter gene expression specifically in the socket cells of all ESOs of both the larval and adult PNSs (Figures 1B, 1C, and 1I). This genomic DNA fragment contains eight binding sites for the Su(H) protein and is transcriptionally auto-activated in socket cells (see Figure 2); we therefore refer to this element as the Su(H) Autoregulatory Socket Enhancer, or ASE. A 372 bp 5' subfragment of the enhancer containing five Su(H) binding sites, called ASE5 (Figure 1A), drives socket cell-specific expression that is qualitatively and quantitatively indistinguishable from that observed with the full ASE (Figures 1G and 1H); the 1.5 kb 3' subfragment, called ASE3, drives considerably weaker socket cell expression (not shown). The ASE is active at very high levels in socket cells of developing larval and adult ESOs (Figures 1B and 1G). It is noteworthy that the ASE is active in no other cell type of the fly, including cells of the wing imaginal disc (Figure 1F), where active N signaling results in the direct transcriptional activation of many Su(H) target genes, including members of the Enhancer of split Complex in proneural clusters (Bailey and Posakony, 1995; Nellesen et al., 1999) and genes such as vestigial at the presumptive wing margin (Halder et al., 1998). Strikingly, the Su(H) ASE is active in socket cells of larval ESOs throughout all three larval instars (Figure 1C), and in adult ESOs throughout adult life (Figure 1I).

In flies carrying a temperature-sensitive allele of N (Nts/Nnull), shifting to the restrictive temperature during the N-dependent socket–shaft cell fate decision causes many presumptive tormogen (socket) cells to express the trichogen (shaft) fate instead (Figure 1J; Bailey, 1996). In these cells, the Su(H) ASE is inactive (Figure 1J'; white arrowheads). Conversely, Hairless (H) hypomorphic flies (H<sup>HP</sup>/H<sup>HP</sup>), in which the trichogen is transformed to a socket cell by inappropriate N pathway activity (Figure 1K; Bang et al., 1991), show ASE activity in both the normal tormogen and the converted trichogen (Figure 1K'). Thus, transcriptional activity of the Su(H) ASE acts as a marker for the socket cell fate and, like the socket cell fate, is a downstream consequence of N signaling.

The Su(H) ASE Is Auto-Activated in Socket Cells of Adult Mechanoreceptors

Computer analysis of the sequence of the Su(H) ASE (Adams et al., 2000) indicated the presence of eight predicted high-affinity binding sites for the Su(H) protein (Figures 1A and 2A; consensus binding site YRTGDGAD derived from Tun et al., 1994; our unpublished data; Bailey and Posakony, 1995; Nellesen et al., 1999). Electrophoretic mobility shift assays (EMSAs) demonstrate that purified Su(H) protein binds in vitro to each of these predicted sites (Figure 2B). Mutating from G to C, the residue in the sixth position of a Su(H) binding site (indicated by arrowheads in Figure 2A) severely reduces or destroys its in vitro affinity for the protein (Bailey and...
Su(H) Binds to and Activates the Su(H) ASE

Figure 2. Su(H) Binds to and Activates the Su(H) ASE

(A) Conservation of Su(H) binding sites in the Su(H) ASE between D. melanogaster and D. virilis. Flanking sequences are shown in lower case; dots denote identical bases. Arrowheads indicate the single base changed (from G to C) in mutated sites; this point mutation abolishes Su(H) binding in vitro (see [B]).

(B) Labeled oligonucleotide probes containing predicted Su(H) binding sites in the ASE from D. melanogaster, as well as from D. virilis (where different), were tested for binding by purified Su(H) fusion proteins in vitro. Su(H) binds efficiently to all nine predicted sites in the Su(H) gene (lanes 2–10), but not to mutated sites (lane 11; our unpublished data). Sites in the D. virilis Su(H) gene that differ from those in D. melanogaster were also tested; all were bound strongly by Su(H) except DvS6 (lanes 12–15).

(C and D) Integrity of Su(H) binding sites is essential for ASE activity in adult socket cells. (C) ASE-GFP exhibits high levels of GFP expression in abdominal bristles. (D) ASEm-GFP, containing a single base-pair mutation in each Su(H) binding site, is inactive in adult socket cells.

(E) DvASE-GFP, containing a genomic DNA fragment downstream of the D. virilis Su(H) gene that includes seven Su(H) binding sites, is expressed specifically in socket cells of adult D. melanogaster.

(F and G) The Su(H) binding sites in ASE5 are not sufficient for socket cell expression. (F) ASE5-lacZ is strongly expressed in adult socket cells. (G) 5xSu(H)-lacZ, which contains the same five Su(H) sites as the ASE5, but lacks all other ASE sequences, fails to be expressed detectably in socket cells.

Posakony, 1995); this rule holds true for Su(H) binding sites in the ASE (Figure 2B and additional data not shown). When all eight Su(H) sites in the ASE have been mutated in this way, the enhancer (ASEm) is no longer active in adult socket cells (Figures 2C and 2D). Auto-activation of the Su(H) ASE is therefore essential for its activity in adult socket cells.

We cloned the Su(H) gene from Drosophila virilis, which diverged from D. melanogaster approximately 60 million years ago (Beverley and Wilson, 1984), and found that a downstream enhancer (DvASE) is able to drive socket cell-specific GFP expression in transgenic D. melanogaster (Figure 2E). Sequencing of DvASE reveals that several blocks of sequence are highly conserved between D. melanogaster and D. virilis, including the Su(H) binding sites (Figure 2A), of which four (DvS3,4,7,8) are perfectly conserved and three (DvS2,5,9) have diverged but still bind the protein efficiently in vitro (Figure 2B). Only DvS6 binds poorly, as predicted (Figures 2A and 2B). Thus, seven Su(H) binding sites in the Su(H) ASE have been functionally conserved between D. melanogaster and D. virilis, providing further evidence of biological significance for these sequence elements.

Since integrity of Su(H) binding sites is required for the adult activity of the ASE, we tested whether it is sufficient for transcriptional activation in the socket cell by constructing 5xSu(H)-lacZ, a reporter gene in which the five Su(H) binding sites from ASE5 were placed upstream of lacZ. Although ASE5-lacZ is strongly expressed in adult socket cells (Figure 2F), 5xSu(H)-lacZ is incapable of driving reporter gene expression in the socket cell, or in any other cell type (Figure 2G). We conclude that Su(H) binding sites, though necessary, are insufficient for ASE activation, and that additional DNA binding transcriptional activator(s) are required in conjunction with Su(H) for enhancer activity.

Adult Su(H) Auto-Activation Is Independent of N Signaling

The N receptor and its ligand Delta (Dl) are expressed throughout the epidermal epithelium during socket-shaft cell fate specification. By mid-pupal stages, however, Dl is no longer expressed in the epidermis and is detectable only in the trichogen and, more weakly, in the tormogen (Parks et al., 1997). The trichogen degenerates around the time of eclosion, raising the possibility that there is no Dl-expressing cell in contact with the adult tormogen. Indeed, in positively controlled experiments,
neither Dl nor N is detectable by antibody staining in adult bristles (data not shown). This suggests that ASE-mediated Su(H) auto-activation in adult bristles is a N signaling-independent process. We tested this hypothesis by assaying ASE activity in ASE5-GFP adult flies subjected to conditions that cause a loss of N signaling.

Flies carrying the temperature-sensitive allele Nts1 in trans to an N null mutation suffer socket-to-shaft cell fate conversions when subjected to the restrictive temperature during the socket–shaft fate decision, due to a loss of N pathway activity in the tormogen (Figures 3A and 3B; Bailey, 1996). As we have shown, these converted tormogen cells do not activate the ASE (see Figure 1J). However, when adult Nts1/Nnull flies are subjected to the restrictive temperature, the activity of the Su(H) ASE is not affected (Figures 3F and 3G). Likewise, a dominant-negative form of N lacking the intracellular transcriptional coactivator domain (UAS-ECN; Jacobsen et al., 1998) similarly affects the socket–shaft decision when overexpressed in early pupae (Figure 3C), but has no effect on ASE5-GFP expression when overexpressed in adults (Figure 3H). The same holds true for Dlts/Dlnull flies; an early pupal-stage shift to the nonpermissive temperature causes a failure of N-mediated cell fate specification in the socket cell (Figure 3D), but an identical temperature shift in adults has no observable effect on the activation of ASE5 (Figure 3I).

H encodes an important negative regulator of N signaling (Bang and Posakony, 1992) that can inhibit the in vitro DNA binding activity of Su(H) through direct protein–protein interactions (Brou et al., 1994). Overexpression of H causes socket-to-shaft cell fate conversions (Bang and Posakony, 1992), while loss of H function causes shaft-to-socket conversions and can enhance the effects of Su(H) overexpression (Lees and Waddington, 1942; Bang et al., 1991; Schweisguth and Posakony, 1994). We find that overexpressing H by subjecting hs-GAL4; UAS-NDN pupae (Go et al., 1998) to heat shock during the socket–shaft cell fate decision indeed causes socket-to-shaft conversions that resemble the effects of loss of N, Dl, or Su(H) function (Figure 3E). The same H overexpression regime, performed in adults, completely and irreversibly abolishes ASE5-GFP expression (Figure 3J). This experiment serves as an important positive control, by demonstrating that ASE activity in adults can be interrupted by a treatment that is expected to interfere with the transcriptional activation function of Su(H). Taken together, our results indicate that Su(H) auto-activation in the adult tormogen via the ASE is independent of N signaling.

**Su(H) Auto-Activation Is Not Required for Mechanoreceptor Cell Fate Specification or External Morphology**

A 6.5 kb genomic DNA fragment that includes the Su(H) gene (here called RC-wt, for wild-type rescue construct)
is capable of completely rescuing the Su(H) null phenotype in transgenic flies (Schweisguth and Posakony, 1992). By deleting the ASE from this rescue construct (RC-ΔASE) or by specifically mutating all nine of the Su(H) binding sites it contains (RC-9Xm; constructs are diagrammed in Figure 1A), we sought to assay the effects of loss of Su(H) autoregulation without affecting the ubiquitous or maternal expression of the gene, which are driven by upstream regulatory sequences, as described above. Indeed, we find that transgenic mutant flies of the genotypes Su(H)−/−; RC-ΔASE/+ or Su(H)−/−; RC-9Xm/+ are viable and fertile, but are defective in Su(H) transcriptional auto-activation, as assayed by in situ hybridization to Su(H) transcripts (Figures 4A–4C). In these Su(H) ASE mutant backgrounds, the ASE5-GFP reporter is not active in adult socket cells (Figures 4D–4F). We conclude that these mutants are indeed deficient in Su(H) auto-activation.

Because the tormogen and trichogen generate large, easily identifiable external cuticular structures (socket and shaft, respectively), these structures are commonly used as markers of cell fate. Altering the level of N pathway activity during the time of the socket–shaft cell fate decision can result in the development of sensory organs with two shaft structures (Figure 1J) or two socket structures (Figure 1K). Perhaps surprisingly, autoregulation-deficient Su(H) mutations do not affect the ability of the very great majority of tormogens to generate a normal socket structure. More than 99.5% of macrochaete bristles in adult flies of the genotypes Su(H)−/−; RC-ΔASE/− or Su(H)−/−; RC-9Xm/− exhibit a wild-type cuticular phenotype (Figures 4G–4I). We observe a low frequency of both double shafts and missing bristles (<0.5% combined), both of which are observed in Su(H) hypomorphic mutants (Schweisguth and Posakony, 1994). This result may suggest that the ASE makes a small contribution to the ubiquitous expression of Su(H), and is consistent with our observation that when the ASE is placed upstream of −403Su(H)-lacZ, a mild increase in β-galactosidase activity is observed in third-
instar wing imaginal discs (Figures 1E and 1F). The very weak effect on external cuticular phenotype of abrogating Su(H) auto-activation is fully suppressed by adding a second copy of the mutant rescue construct to the genotype (data not shown), indicating that it is a quantitative, not a qualitative, phenomenon.

In order to confirm that cell fate specification occurs normally in autoregulation-deficient Su(H) mutants, we stained developing mechanoreceptors for D-Pax2 protein. D-Pax2, the Drosophila ortholog of vertebrate Pax-2, is expressed in the shaft and sheath cells, but not the socket cell, of mechanosensory bristles in pupae at 32 hr after puparium formation (APF) (Fu and Noll, 1997; Kavaler et al., 1999). Moreover, this asymmetry in D-Pax2 expression (on in the trichogen and off in the tormogen) is a downstream consequence of N pathway activation in the tormogen; thus, tormogens that have been converted to the shaft fate express D-Pax2 (Kavaler et al., 1999). In autoregulation-deficient Su(H) mutants, D-Pax2 is expressed in the trichogen but not the tormogen (Figures 4J-4L), confirming that N- and Su(H)-dependent socket and shaft cell fate specification occurs normally in the absence of Su(H) auto-activation.

**Su(H) Autoregulation Is Required for Normal Mechanoreceptor Physiology**

The tormogen, a well-characterized component of insect ESOS, plays a major role in mechanoreception. Cell-specific ablation of all tormogens in flies results in severe uncoordination (S. B. and J. W. P., unpublished results), indicating that the presence of the socket cell is essential for proper function of the PNS. The cellular morphogenesis of the mature tormogen includes the following (reviewed by Hartenstein and Posakony (1989) and Keil (1997), and diagrammed in Figure 5A): generation of the cuticular socket structure; polyplody and dramatic cell growth; envelopment of the shaft cell, sheath cell, and neuron; formation of the socket septum, which physically links the sensory dendrite to the cuticular wall; and extensive infoldings of the apical membrane surrounding the mechanotransduction apparatus. These apical folds, which vastly increase the surface area of the tormogen, have been implicated by both ultrastructural and biochemical studies in the active pumping of potassium ions into the receptor lymph space surrounding the dendrite (Keil, 1997). The high K⁺ concentration of the receptor lymph is responsible for the transepithelial potential (TEP), or positive voltage of the receptor lymph with respect to the hemolymph (Thurm and Küppers, 1980). Deflection of the bristle shaft deforms the sensory dendrite, opening mechanosensitive channels and allowing a K⁺ flux into the neuron (Thurm and Küppers, 1980; Keil, 1997; Walker et al., 2000).

Our observations that the Su(H) ASE is highly active in the tormogen throughout adult life, and is not required for socket cell fate specification, led us to hypothesize that Su(H) autoregulation may contribute to mechanoreceptor function. To test this, electrophysiological recordings were made from adult mechanosensory bristles of wild-type and mutant flies. We measured each bristle’s TEP by means of a reference electrode placed in contact with the hemolymph and a recording/stimulating electrode placed over the cut end of the bristle shaft, which is hollow in adult flies and contains receptor lymph (Kernan et al., 1994; Figure 5A). In wild-type Drosophila bristles, resting TEPs in the range of 20–80 mV are typically observed (Kernan et al., 1994; Figure 5B). Mechanical stimulation of the bristle allows K⁺ flow into the neuron; the resultant decrease in the TEP is measured as the mechanoreceptor potential (MRP; Figure 5B). Wild-type [Su(H)+/+ ] and Su(H)-/--; RC-wt flies show comparable mean TEPs (44 ± 4 mV and 41 ± 4 mV, respectively; Figures 5C and 5E). In contrast, both autoregulation-deficient Su(H) mutants, Su(H)-/--; RC- ∆ASE and Su(H)-/--; RC-9Xm, have very significantly lower (p < 0.001) average TEPs (9 ± 1 mV and 13 ± 2 mV, respectively; Figures 5C and 5E). Given their much-reduced mean TEPs, it is not surprising that MRPs are also strongly reduced in mutant bristles (Figures 5B and 5C). However, it is noteworthy that several Su(H)-/--; RC-9Xm bristles that show TEPs in the normal range still have substantially reduced MRPs (Figure 5C), indicating a failure of mechanotransduction.

By recording from stimulated bristles under voltage-clamped conditions, we can determine the current flow across the dendritic membrane (called the transepithelial current or TEC), a measure of mechanotransduction (Walker et al., 2000). Wild-type voltage-clamped bristles normally show increased current upon mechanical stimulation as mechanosensitive ion channels open, reducing the resistance of the dendritic membrane (Thurm and Küppers, 1980; Walker et al., 2000). This increase in TEC is called the mechanoreceptor current, or MRC (Figure 5D). Bristles of both wild-type and Su(H)-/--; RC-wt flies show comparable mean MRCs (138 ± 24 pA and 102 ± 12 pA, respectively), while both Su(H)-/--; RC- ∆ASE and Su(H)-/--; RC-9Xm bristles show very significantly reduced (p < 0.001) average MRCs (37 ± 4 pA and 23 ± 2 pA, respectively; Figures 5D and 5E). This result suggests that autoregulation-deficient Su(H) mutant bristles not only fail to generate a proper TEP, but also may be partially defective in their ability to open mechanosensitive channels.

Ultrastructural analysis revealed that a clearly identifiable tormogen cell is evident in both Su(H)-/--; RC- ∆ASE and Su(H)-/--; RC-9Xm mutant bristles (Figures 5F and 5G). The sheath cell and neuron are also present in these mutant organs (Figures 5F and 5G). Thus, the severely reduced mechanosensory capacity of the mutants is not due to the loss of any bristle cell type, but instead is likely to be caused by defects in tormogen differentiation and/or maintenance of proper tormogen morphology and function. This may in turn have secondary effects on other cells in the mechanoreceptor organ.

**Su(H) Auto-Repression in the Shaft Cell Is Required for Proper Cell Fate Specification**

Transgenic fly lines carrying the RC-9Xm rescue construct display an unexpected gain-of-function phenotype: double-socket bristles (resulting from a shaft-to-sOCKET cell fate conversion) are observed when two copies of the mutant transgene are present in a Su(H)+/+ background (Figure 6C). This phenotype is similar to that of Su(H)+/+- flies carrying six copies of RC-wt (6x RC-wt; Schweisguth and Posakony, 1994; Figure 6B),
standard error of the mean. Su(H) +/- and RC-wt responses are not significantly different, while RC-ΔASE and RC-9Xm mutant bristles show very significantly reduced mechanotransduction capacity (p < 0.001, **).

(F and G) TEMs of cross sections through ASE mutant [Su(H) +/-; 1x RC-ΔASE and Su(H) +/-; 1x RC-9Xm] adult macrochaetae bristles. In both genotypes, a clearly identifiable socket cell (so) surrounds the sheath cell (sh) and the dendrite (den) of the neuron. Characteristic apical folds (af) of the socket cell are apparent in the RC-9Xm bristle (G).

Discussion

We have reported here that Su(H), a core component of the ubiquitous N signaling pathway, has been re-
Figure 6. Su(H) is a Transcriptional Auto-Repressor in the Shaft Cell

(A–D) SEMs of the dorsal aspect of adult heads of otherwise wild-type flies carrying wild-type or mutant Su(H) rescue transgenes. Arrowheads indicate the positions of postvertical macrochaetes.

(A) Flies with two copies of the wild-type Su(H) rescue transgene (2x RC-wt), as well as two wild-type endogenous Su(H) genes, display normal socket and shaft morphology.

(B) Su(H)1/1 flies carrying six copies of the wild-type transgene (6x RC-wt) exhibit shaft-to-socket transformations (Schweisguth and Posakony, 1994).

(C) Su(H)1/1 flies carrying two copies of the Su(H) binding site mutant transgene RC-9Xm show shaft-to-socket transformations. This image depicts one of the most strongly expressive RC-9Xm lines; most lines show a milder double-socket phenotype (see [E]).

(D) The double-socket phenotype is reduced in severity in Su(H)1/1 flies with only one copy of RC-9Xm (compare to [C]).

(E and F) Quantitation of Su(H) gain-of-function double-socket phenotype. (E) Penetration and expressivity of double-socket effect among lines carrying Su(H) rescue transgenes. X axis shows percentage of double-socket macrochaetes on head and dorsal thorax of adult flies: mild, 0%–10%; moderate, 10%–50%; severe, >50%. (F) Percentage of double-socket macrochaetes averaged across all transgenic lines of the indicated genotype.

(G and H) The double-socket phenotype conferred by RC-9Xm is partially suppressed by loss of endogenous Su(H) activity. (G) Anterior orbital macrochaete of a Su(H)1/1;2x RC-9Xm fly. The shaft cell (right) has undergone a complete phenotypic transformation to the socket fate. (H) Anterior orbital macrochaete of a Su(H)mum/Su(H)mum;2x RC-9Xm fly. In the absence of endogenous Su(H) function, RC-9Xm causes a less complete shaft-to-socket transformation.

(I and I9) A mutant ASE lacking Su(H) binding sites is active in both the tormogen and trichogen in early pupae. Shown are confocal fluorescence images of a thoracic microchaete in an ASEm-GFPnuc transgenic pupa at 24 hr APF. (I) In a more apical focal plane, GFP expression is evident in the socket cell nucleus (so), along with faint cytoplasmic accumulation. (I9) Less intense GFP fluorescence in the more basal shaft cell nucleus (sh) indicates ectopic activity of the mutant enhancer.

Two Separable Roles for Su(H) in the Tormogen: Cell Fate Specification and Differentiation/Physiology

In nearly all of the many developmental settings (both embryonic and post-embryonic) in which it acts as the key transducing transcription factor for the N pathway, Su(H) is expressed generally, at a similar low-to-moderate level in all cells of the tissue (Schweisguth and Posakony, 1992; Gho et al., 1996). The discovery that Su(H) transcript and protein levels are greatly elevated specifically in the socket cells of all ESOs of the larval and adult PNSs (Schweisguth and Posakony, 1992; Gho et al., 1996) posed two questions. First, what is the developmental function of this elevated expression, given that the much lower Su(H) protein levels in all other cell types of the fly are sufficient for transduction of the N signal? Second, how is this cell type-specific elevation of Su(H) transcript and protein accumulation achieved?

Our study of the transcriptional regulation of Su(H) has identified two distinct cis-regulatory modules: a promoter-proximal region that drives moderate general and maternal Su(H) expression, and a downstream enhancer, the ASE, which is responsible for strong socket cell-specific transcriptional activation. Because these two regulatory modules are physically separable, we were able to selectively eliminate the socket cell activation of Su(H) by deleting or mutating the ASE, while retaining the general and maternal Su(H) expression that is essential for viability and fertility. We found that the loss of socket cell-specific Su(H) activation has no sig-
significant effect on N-mediated specification of the socket cell fate, or on any other cell fate decision. Since Su(H) is genetically required for socket cell fate determination, we conclude that moderate levels of Su(H) protein are both necessary and sufficient for transduction of the N signal in the socket cell (and all other N-responsive cell types of the fly).

The characteristic high level of Su(H) in the socket cell is not only dispensable for the initial specification of the tormogen fate; several major aspects of tormogen differentiation, such as significant cell growth, envelopment of neighboring sensory organ cells, and generation of a cuticular socket structure, also proceed normally in its absence. However, in other respects, autoregulation-deficient tormogens appear to be defective, leading to a failure of mechanoreception. Taken together, our results support a relatively simple developmental model in which the moderate levels of Su(H) protein present in the newly born tormogen are sufficient to transduce the incoming N signal and implement the socket cell fate, while socket cell-specific upregulation of Su(H), initiated in response to N signaling, reflects a specific differentiative role for Su(H) in the socket cell that is required for normal mechanoreception.

**Su(H) Auto-Activation and N Signaling**

In this work, we demonstrate that socket cell-specific transcriptional elevation of Su(H) expression depends on direct auto-activation through eight Su(H) binding sites in the ASE. This is the first known autoregulatory activity for Su(H) in any species.

We suggest that the ASE-mediated Su(H) autoregulatory loop is initiated during the socket–shaft cell fate decision in the early pupa by the direct binding of Ncir/Su(H) activating complexes. We have shown, however, that maintenance of this loop does not require continued N signaling activity. When does Su(H) auto-activation become N signal-independent? Shifting Ncir/Nnull flies to the restrictive temperature after 72 hr of pupal development at 18°C has no detectable effect on either the cuticular morphology of the socket or ASE activity (S. B. and J. W. P., unpublished results); since temperature-shifting 36 hr (at 18°C) pupae can inhibit both the socket fate and ASE expression, we can very roughly estimate that Su(H) auto-activation becomes signal-independent within 36 hr (at 18°C) after the birth of the tormogen.

It is conceivable that Ncir generated during pupal-stage N signaling perdures in the tormogen and acts as a coactivator throughout adult life. While this remains a formal possibility, it would require that Ncir molecules in sufficient numbers to strongly activate Su(H) persist in the tormogen for several weeks. At present, we favor instead the notion that Su(H) utilizes a distinct coactivator in the adult tormogen.

**Su(H) Auto-Repression and Cell Fate**

CBF1, a mammalian Su(H) homolog, is well-documented as a transcriptional repressor. Two vertebrate corepressor proteins, Silencing Mediator for Retinoid and Thyroid receptor (SMRT) and CBF1 Interacting coRepressor (CIR), have been shown to act as bridges between CBF1 and a histone deacetylase (HDAC) complex, which effects transcriptional repression through chromatin remodeling (Kao et al., 1998; Hsieh et al., 1999). Studies of CBF1 function in vertebrate cells have led to a “transcriptional switch” model in which (1) CBF1 directly represses target genes by recruiting HDAC and (2) NIC (or the viral protein EBNA-2) binds to CBF1, displacing the corepressor complex and acting as a transcriptional coactivator (Hsieh and Hayward, 1995; Waltzer et al., 1995; Kao et al., 1998).

We found unexpectedly that mutating the autoregulatory Su(H) binding sites in the Su(H) gene causes a gain-of-function cell fate conversion phenotype in which shaft cells inappropriately assume the socket cell fate. This result, in which the loss of Su(H)-mediated repression leads to a cell fate transformation, lends support to the “transcriptional switch” model for Su(H)/CBF1 activity, and suggests that this model applies to *Drosophila* Su(H), not only in gene activation at cell boundaries (Morel and Schweisguth, 2000), but also in N-mediated asymmetric cell fate decisions.

One aspect of ASEm-GFP expression is potentially quite informative with respect to ASE regulation. ASEm is active in the tormogen in the early pupa, although its expression is substantially weaker than that of the wild-type enhancer, and unlike the wild-type ASE it is not active in adult tormogens. This early activity of the mu-
tant enhancer indicates that other transcriptional activator(s) besides Su(H) bind directly to the ASE. These activator(s) are evidently present or active only in the socket and shaft cells, since these are the only cells in which ASEM-GFP is expressed. For the sake of brevity, we will refer to the activity of this ASE binding factor or factors as “A” (see Figure 7), with the understanding that “A” may stand for either expression or activity modulation (e.g., by phosphorylation) of one or more transcriptional activators. The activity “A,” then, is sufficient to activate ASEM in the socket and shaft cells, but only during pupal stages. This provides an important clue to the necessity for Su(H) auto-repression. If “A” is sufficient to activate Su(H) in the developing shaft cell (and perhaps to initiate a positive autoregulatory loop), the ASE must somehow be repressed in that cell in order to prevent inappropriate Su(H) activation. However, this repression must not occur in the socket cell, where Su(H) auto-activation is necessary for proper differentiation. Hence Su(H), which acts as a repressor only in the absence of N signaling, is an ideal repressor for the Su(H) ASE.

Three Distinct Mechanisms of Su(H)-Mediated Transcriptional Regulation in the Mechanosensory Bristle

The results obtained in this study lead us to propose the following model for Su(H)-mediated transcriptional regulation during mechanosensory bristle development (Figure 7). Following the division of the precursor cell pIIA to give rise to the presumptive trichogen and tormogen, both daughter cells express the ligand Dl and the receptor N. However, the more anterior sister cell inherits from pIIA the N pathway inhibitor protein Numb, while the more posterior sister does not (Rhyu et al., 1994). In the absence of activated N (N0), Su(H) in the anterior cell acts as a auto-repressor via the ASE, preventing activation of Su(H) by the activity “A.” The posterior sister cell, however, contains nuclear N+, which acts as a coactivator for Su(H) and induces the expression of socket-specific Su(H) target genes, including Su(H) itself. While this Su(H) auto-activation loop is initiated by N signaling, it is a consequence, rather than a determinant, of the socket cell fate. In the absence of N-stimulated activation of Su(H) target genes (and in the absence of N-dependent activities that repress the trichogen differentiation program; Kavalera et al., 1999), the anterior cell adopts the shaft cell fate. In the socket cell, Su(H) auto-activation becomes N signaling-independent during the course of pupal development, perhaps through an interaction with an alternative coactivator. By the time of eclosion of the adult fly, both Su(H) and “A” are required to activate the ASE, but neither is sufficient. Su(H) auto-activation continues in the socket cell throughout pupal and adult life, where it is essential for specific aspects of late tormogen differentiation and for normal mechanoreception in the adult PNS.

Experimental Procedures

Cloning and Reporter Transgene Construction

All β-galactosidase, cytoplasmic eGFP, and nuclear eGFP reporter gene constructs were assembled in a gypsy-insulated P-element reporter vectors pPelican, pGreen Pelican, and pStinger, respec-

Drosophila Stocks, Crosses, and Temperature Shifts

N loss-of-function: N0+ females (Shellenbarger and Mohler, 1978) were crossed to wN+1/Y; P[ASE-GFP]/Cy Dp(N+, w+) males. N0+ is a deletion of the N locus (Grimmwade et al., 1985). Progeny were kept at 18°C either for 36 hr after pupariation formation (APF) or until the pharate adult stage, then shifted to 31°C in a thermal cycler for 18 hr and returned to 18°C. Female progeny were assayed for GFP expression. Curvy° progeny had not inherited the N° duplication on the second chromosome. DI loss-of-function: w1118; DlRF/TM6B males (Lehmann et al., 1983) were crossed to w1118; P[ASE-GFP]; DlRF/TM6B females (Parody and Muskavitch, 1993). Non-Tubby larvae resulting from this cross (genotype w1118; DlRF/TM6B) were kept at 18°C either for 36 hr APF or until the pharate adult stage, then shifted to 32°C in a thermal cycler for 18 hr and returned to 18°C (Parks and Muskavitch, 1993). H loss-of-function: H° is a hypomorphic allele of H (Bang et al., 1991). Overexpression of H and N°; w1118; P[ASE-GFP]; P[hs-GAL4]/TM6B (Brand et al., 1994) flies were crossed to w1118; P[UES-ECN] (Jacobson et al., 1998) flies. Non-Tubby progeny larvae were selected, kept at 18°C either for 36 hr APF or until the pharate adult stage, shifted to 37°C in a thermal cycler or water bath for 2 hr, and returned to 18°C for 16 hr.

Electrophysiological Recording

Voltage and current recordings from adult mechanosensory bristles were performed as described by Kerman et al. (1994) and by Walker et al. (2000). Macrochaete bristles at the following positions were used: anterior notopleural, anterior postalar, anterior and posterior scutellar, and posterior dorsocentral.

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