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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-Jκ/CBF1 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 autorepression 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-Jκ (Hamaguchi et al., 1989). Upon association with ligand, the transmembrane N receptor is proteolytically cleaved, and a resulting intracellular domain fragment (N^{IC}) 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; Matsuno 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 (Heitzler 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 (tormogen), 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-

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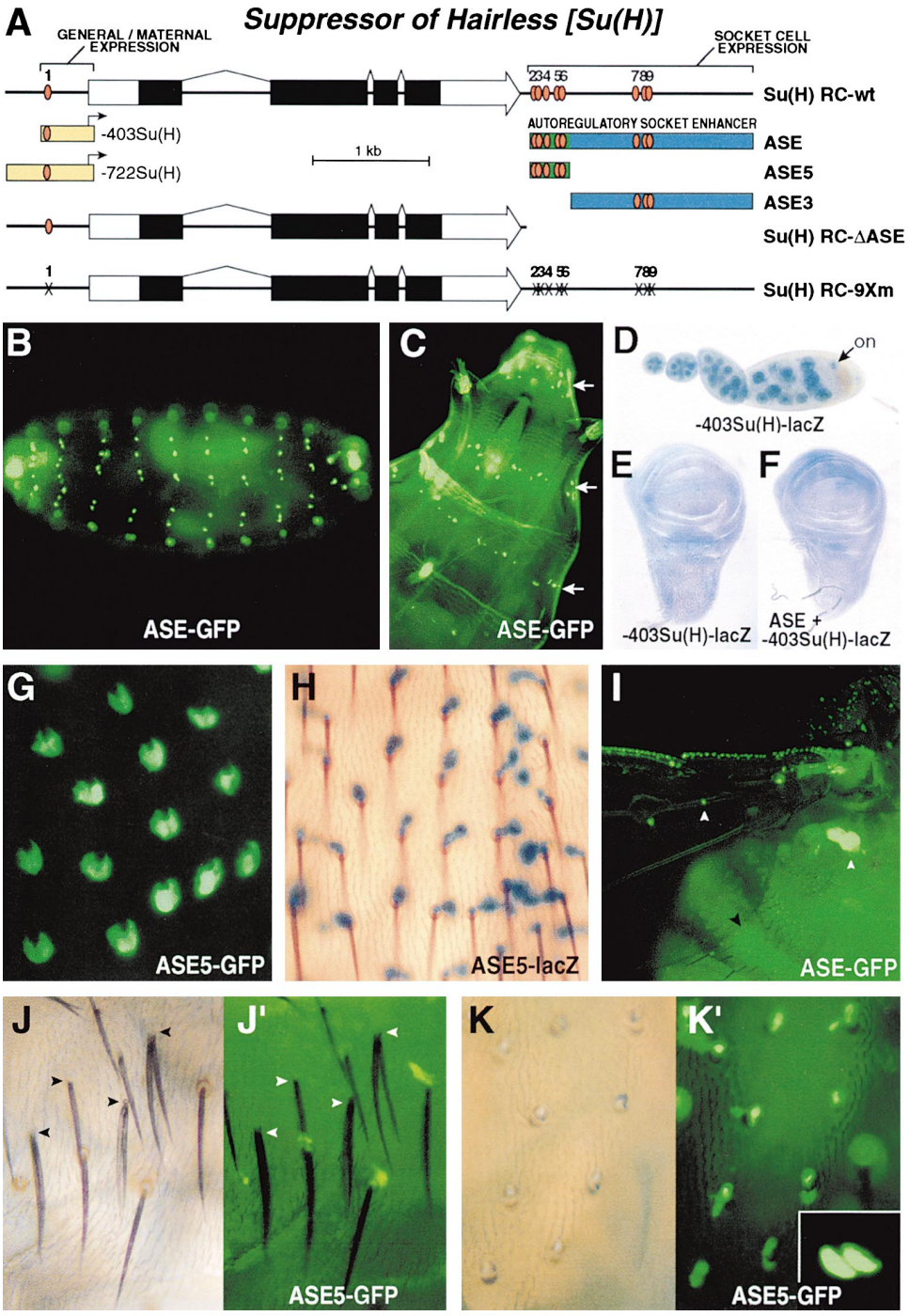

Figure 1. Socket Cell-Specific Transcription of *Su(H)* Is Driven by a Discrete Enhancer Module

(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 *lacZ* 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 β-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* (N^{ts1}/N^{null}), 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^{RP1}/H^{RP1}), 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

campaniform sensilla on the wing blade and haltere (white arrowheads). The ASE is not active in the socket-like structures of the nonsensory long hairs on the posterior wing margin (black arrowhead).

(J and K) ASE5 activity is a marker for the socket cell fate and is downstream of N signaling.

(J and J′) N^{ts}/N^{-} flies were subjected to the restrictive temperature (18 hr at 31°C) after 36 hr of pupal development at 18°C. Flies then developed to the pharate adult stage at 18°C and were assayed for GFP expression. Due to the partial loss of N signaling activity, some bristles lack a socket structure (arrowheads); these positions also lack GFP expression.

(K and K′) Flies homozygous for a partial loss-of-function allele of *H* (H^{RP1}) show shaft-to-socket cell fate transformations due to inappropriate N pathway activity in the trichogen. In this mutant background, *ASE5-GFP* is expressed in both the socket cell and the converted shaft cell. In the adult bristle, the tormogen appears to be tightly associated with the converted trichogen, making it difficult to distinguish the GFP-positive cells. However, in macrochaetes of pupae at 36 hr APF (inset in K′), two large GFP-expressing cells can be seen.

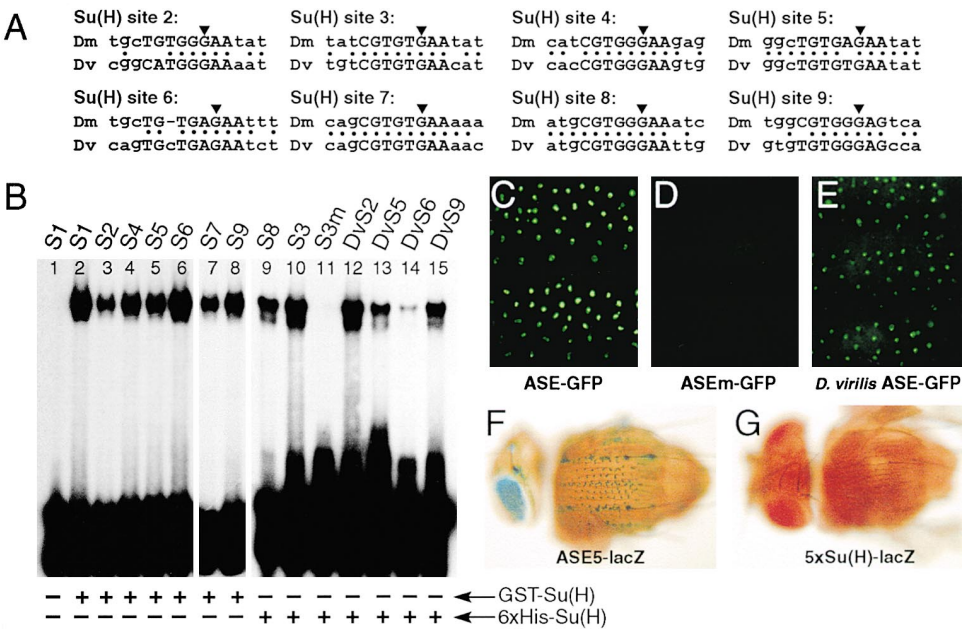

Figure 2. Su(H) Binds to and Activates the *Su(H)* ASE

(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). Autoactivation 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,

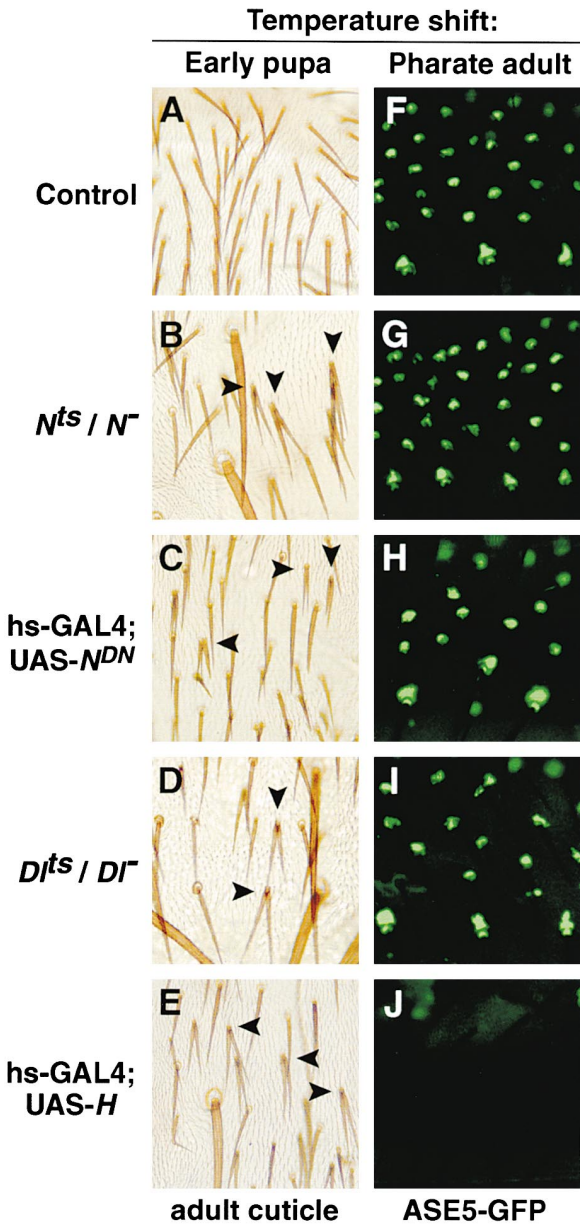

Figure 3. Auto-Activation of *Su(H)* in Adult Bristles Is Independent of N Signaling

(A–E) Adult thoracic cuticle preparations. All animals were subjected to the restrictive temperature or to heat shock after 36 hr of pupal development at 18°C (during the socket–shaft cell fate decision), and then developed to adulthood at 18°C.

(A) Wild-type (control) pupae placed at 31°C for 18 hr, following 36 hr of pupal development at 18°C, show normal socket and shaft cuticular structures.

(B) N^{ts}/N^{-} pupae subjected to the same temperature regime develop double-shaft bristles (arrowheads) due to a socket-to-shaft cell fate transformation.

(C) *hs-GAL4; UAS-*N^{DN} pupae, heat-shocked at 37°C for 2 hr during the tormogen–trichogen cell fate decision, show socket-to-shaft cell fate transformations (arrowheads).

(D) Dl^{ts}/Dl^{-} pupae, placed at 32°C for 18 hr at the same pupal stage, display double-shaft bristles (arrowheads).

(E) *hs-GAL4; UAS-H* pupae, heat-shocked as in (C), exhibit socket-to-shaft cell fate transformations (arrowheads).

(F–J) *ASE5-GFP* expression in socket cells of adult abdominal bristles. Animals were subjected to the same temperature regimes as in panels (A)–(E), but at the pharate adult stage.

(F–I) Loss of *N* or *Dl* function at the pharate adult stage does not affect ASE5 activity.

(J) Heat shock-induced overexpression of *H* in pharate adults irreversibly abolishes *ASE5-GFP* expression.

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 N^{ts1} 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 N^{ts1}/N^{null} 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 Dl^{ts}/Dl^{null} 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-H* 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)

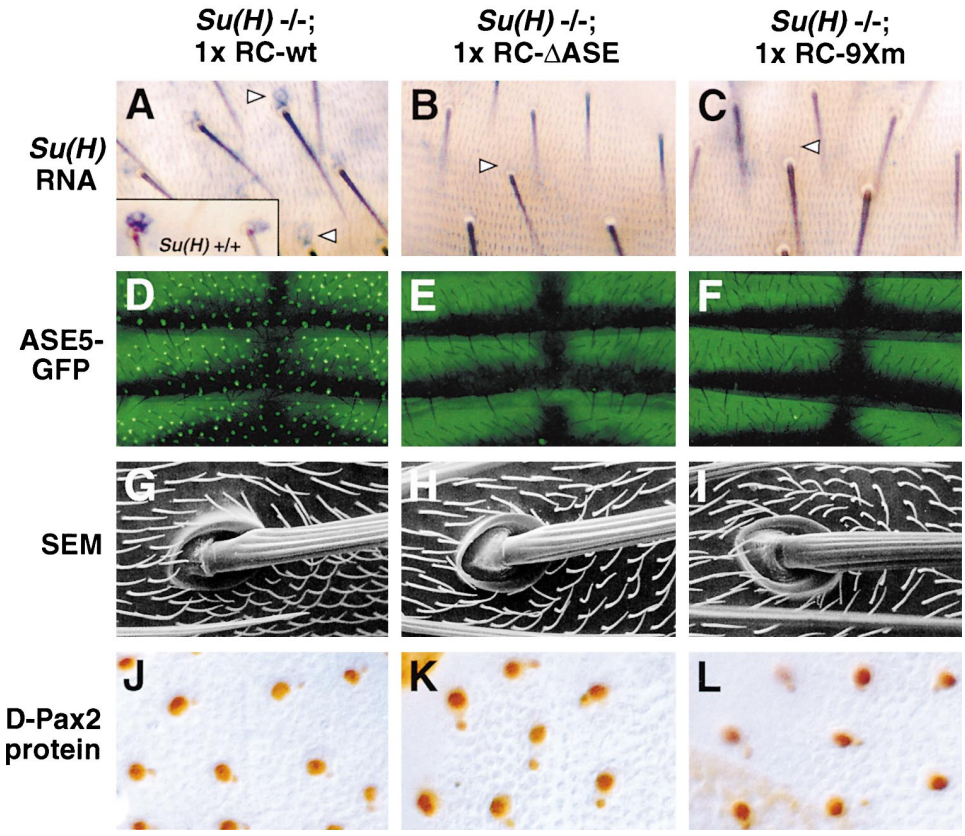

Figure 4. Autoregulation through the ASE Is Required for Socket Cell-Specific Elevation of *Su(H)* Expression but Not for Socket Cell Fate Specification

(A–C) In situ hybridization to detect *Su(H)* RNA in adult bristles. In *Su(H)−/−; 1x RC-wt* flies, *Su(H)* transcripts accumulate to high levels in adult socket cells ([A], arrowheads). *Su(H)* transcript accumulation in wild-type [*Su(H)+/+*] adult bristles is shown in the inset. *Su(H)* transcript is not detectable in bristles of ASE mutant [*Su(H)−/−; 1x RC-ΔASE* or *Su(H)−/−; 1x RC-9Xm*] adults ([B and C], arrowheads).

(D–F) *ASE5-GFP* expression in adult sockets. The wild-type *ASE5-GFP* reporter gene is active in *Su(H)−/−; 1x RC-wt* flies (D), but not in flies defective for *Su(H)* autoregulation (E and F).

(G–I) SEMs of adult bristles. Socket and shaft cuticular structures of mechanosensory bristles in adult flies lacking ASE-mediated autoregulation (H and I) are indistinguishable from those of *Su(H)−/−; 1x RC-wt* flies (G).

(J–L) Immunohistochemistry to detect D-Pax2 protein in developing thoracic bristles of pupae at 32 hr APF. As in the wild type (Kavaler et al., 1999), animals bearing either wild-type (J) or ASE mutant (K and L) *Su(H)* transgenes express D-Pax2 in the shaft cell (large nucleus) and sheath cell (small nucleus), but not in the socket cell.

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 did 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; polyploidy 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-*Δ*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-*Δ*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-*Δ*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 in 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),

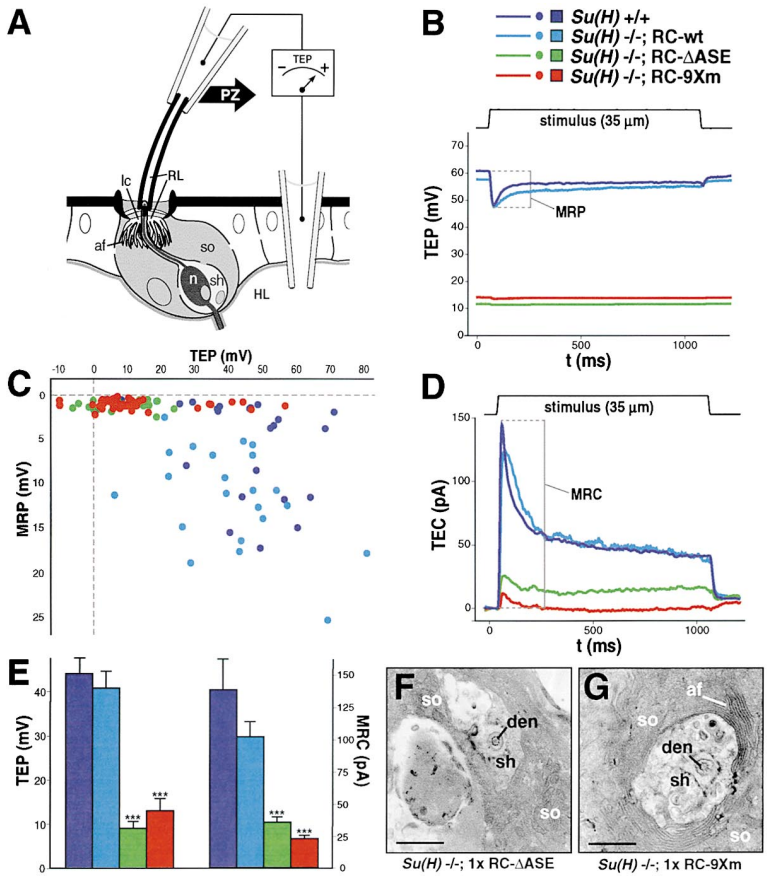

Figure 5. *Su(H)* Auto-Activation Is Required for Normal Mechanoreceptor Function

(A) Electrophysiological recording from *Drosophila* mechanosensory bristles. This diagram combines elements adapted from Kernan et al. (1994) and Keil (1997). See text for discussion. af, apical folds; HL, hemolymph; lc, lymph cavity; n, neuron; PZ, mechanical stimulus controlled by piezoelectric motor; RL, receptor lymph; sh, sheath cell; so, socket cell; TEP, transepithelial potential.

(B–E) Electrophysiological measurements of mechanoreception in adult thoracic macrochaete bristles.

(B) Voltage traces recorded from bristles during mechanical stimulation. Auto-regulatory mutants exhibit sharply reduced transepithelial potentials (TEP) and mechanoreceptor potentials (MRP).

(C) Scatter plot of TEP versus MRP. Sample size: *Su(H)+/+*, n = 23 bristles; *RC-wt*, n = 25; *RC-ΔASE*, n = 29; *RC-9Xm*, n = 43. Bristles of *RC-wt* flies show both TEPs and MRPs that resemble those of wild-type bristles, while ASE mutant bristles generally display very significantly reduced TEPs and MRPs.

(D) Current traces from mechanically stimulated bristles under voltage-clamp conditions (TEC: transepithelial current; MRC: mechanoreceptor current). Voltage was clamped at the previously recorded TEP for that bristle, unless the TEP was <40 mV, in which case voltage was clamped at 40 mV. In order to specifically measure the MRC, the resting current was set at zero.

(E) Bar graph of mean TEP and MRC in wild-type and mutant bristles; error bars show 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 macrochaete 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).

as well as flies in which *Su(H)* is overexpressed under control of a heat shock promoter (Schweisguth and Posakony, 1994). *RC-9Xm* lines show varying degrees of the double-socket effect (Figure 6E), most likely due to position effects at transgene insertion sites, but the average expressivity of the double-socket phenotype across all homozygous *RC-9Xm* lines is comparable to that of *Su(H)+/+; 6x RC-wt* (Figure 6F). *Su(H)+/+; 2x RC-wt* flies never show a double-socket phenotype (Figures 6A, 6E, and 6F); neither do flies carrying *2x RC-ΔASE* (Figures 6E and 6F).

The double-socket phenotype observed in *RC-9Xm* flies is clearly due to an excess of *Su(H)* activity: it is observed in a *Su(H)* wild-type background (Figure 6C); it is mitigated by reducing the dosage of the transgene (Figure 6D); and it is partially suppressed by null mutations in the endogenous copies of *Su(H)* (Figures 6G and 6H). *RC-9Xm* differs from *RC-wt* only in the nine nucleotides that constitute the point mutations in the Su(H) binding sites; since these mutations result in a *Su(H)* gain-of-function phenotype, we conclude that Su(H) binding sites in the ASE function as repressor sites in the trichogen, and that *Su(H)* auto-repression is important for commitment to the shaft cell fate.

Although *RC-9Xm* flies display a *Su(H)* gain-of-function phenotype in the trichogen, *RC-ΔASE* flies do not; thus, the double-socket effect of *RC-9Xm* is abrogated by removing the remaining ASE sequences. This indicates that sequences within ASEm are required for activating ectopic *Su(H)* expression in the *RC-9Xm* trichogen. We directly observed the expression pattern of ASEm driving a nuclear-localized form of GFP (*ASEm-GFP^{nuc}*) in wild-type pupae at 24 hr APF, and found that ASEm is active in both the tormogen (Figure 6I) and in the more basally located trichogen (Figure 6I′), although GFP accumulation is greater in the tormogen than in the trichogen. By comparison, the wild-type ASE is much more strongly active than ASEm in the tormogen, and does not function detectably in the trichogen (Figure 1G), indicating that Su(H) binding sites are acting both as activator sites in the tormogen and as repressor sites in the trichogen. The inferred ASE binding transcriptional activator(s) are clearly insufficient for ASE activation in adult flies, since *ASEm-GFP* is not expressed in adult bristles (Figure 2D). Nevertheless, binding sites for these or other factor(s) are still required for ASE activity in adults (Figures 2F and 2G).

Discussion

We have reported here that Su(H), a core component of the ubiquitous N signaling pathway, has been re-

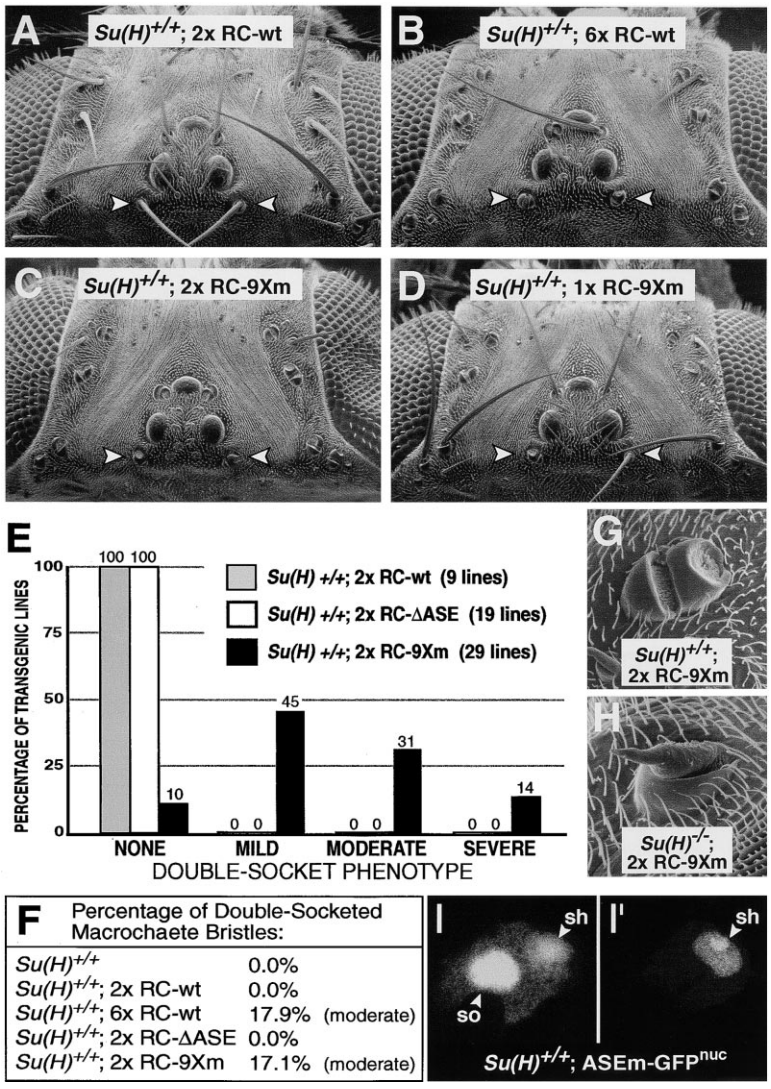

Figure 6. Su(H) Is a Transcriptional Auto-Repressor in the Shaft Cell

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

(C) *Su(H)*+/+ 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)*+/+ 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) Penetrance 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)*+/+*; 2x RC-9Xm* fly. The shaft cell (right) has undergone a complete phenotypic transformation to the socket fate. (H) Anterior orbital macrochaete of a $Su(H)^{AR9}/Su(H)^{SF8}$*; 2x RC-9Xm* fly. In the absence of endogenous *Su(H)* function, *RC-9Xm* causes a less complete shaft-to-socket transformation.

(I and I′) 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 $ASEm\text{-}GFP^{nuc}$ 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. (I′) Less intense GFP fluorescence in the more basal shaft cell nucleus (sh) indicates ectopic activity of the mutant enhancer.

cruited—via a discrete transcriptional enhancer module in the *Su(H)* gene itself—for a fundamental differentiative role specifically in one *Drosophila* cell type. The autoregulatory activity of Su(H) in the socket cells of external sensory organs represents, we suggest, an unusually direct link between initial cell fate specification and the physiological function of a differentiated cell type.

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-

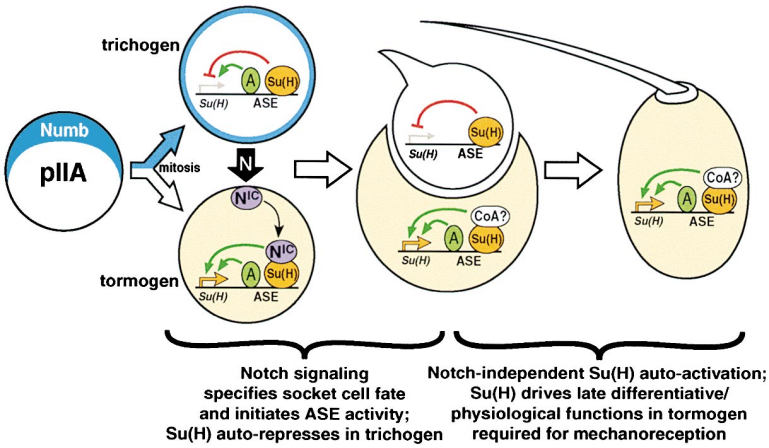

Figure 7. Novel Roles for Su(H) in the Development, Differentiation, and Mature Function of the Mechanosensory Bristle

Summary of Su(H) transcriptional regulatory activity in cell fate specification and differentiation during bristle development. Symbols: N = N signaling; A = ASE binding transcriptional activator protein(s); CoA = hypothetical coactivator for Su(H). See text for discussion.

nificant 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 N^{IC}/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 N^{ts1}/N^{null} 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 N^{IC} 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 N^{IC} 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 exerts 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) N^{IC} (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 (N^{IC}), Su(H) in the anterior cell acts as an auto-repressor via the ASE, preventing activation of *Su(H)* by the activity "A." The posterior sister cell, however, contains nuclear N^{IC}, 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; Kavaler 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 the *gypsy*-insulated P-element reporter vectors pPelican, pGreen Pelican, and pStinger, respectively (Barolo et al., 2000). The ASE is a 1874 bp Bsu36I-EcoRI restriction fragment of *Su(H)* genomic DNA. ASE5 is a 372 bp 5′ fragment of the ASE with Bsu36I and AseI termini, and ASE3 is the remaining 1504 bp 3′ fragment. Certain *lacZ* constructs (see Figures 1A, 1D–1F) contain the *Su(H)* promoter, either bases −722 to +51 or bases −403 to +51. All other β-galactosidase and eGFP reporter constructs contain a minimal promoter from the *Hsp70* gene (Barolo et al., 2000). *5xSu(H)-lacZ* contains the five Su(H) binding sites from ASE5, including a total of 12 bp for each site, but lacks all other enhancer sequences.

Bacteriophage clones containing *Drosophila virilis Su(H)* were isolated by screening a λEMBL3 *D. virilis* genomic DNA library (gift from R. Blackman).

Histochemistry and Immunohistochemistry

β-galactosidase activity staining was performed as described by Romani et al. (1989), and antibody staining as described by Kavaler et al. (1999).

DNA Binding Assays

GST-Su(H) fusion protein was purified as described by Bailey and Posakony (1995), and 6xHis-Su(H) protein as described by Jan-knecht et al. (1991). Electrophoretic mobility shift assays (EMSAs) were performed as described by Bailey and Posakony (1995). Sequences of oligonucleotide probes are available upon request.

Drosophila Stocks, Crosses, and Temperature Shifts

N *loss-of-function*: N^{ts1} females (Shellenbarger and Mohler, 1978) were crossed to w^a N^{81K1}*/Y; P[ASE5-GFP]/Cy Dp(*N^+,w^+*)* males. N^{81K1} is a deletion of the *N* locus (Grimwade et al., 1985). Progeny were kept at 18°C either for 36 hr after puparium 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; $Curly^+$ progeny had not inherited the N^+ duplication on the second chromosome. Dl *loss-of-function*: w^{1118}*;* Dl^{9P39}*/TM6B* males (Lehmann et al., 1983) were crossed to w^{1118}*; P[ASE5-GFP];* Dl^{RF}*/TM6B* females (Parody and Muskavitch, 1993). Non-Tubby larvae resulting from this cross (genotype w^{1118}*; P[ASE5-GFP]/+;* Dl^{RF}*/* Dl^{9P39}) 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^{RPI} is a hypomorphic allele of *H* (Bang et al., 1991). *Overexpression of* H *and* N^{DN}: w^{1118}*; P[ASE5-GFP]; P[hs-GAL4]/TM6B* (Brand et al., 1994) flies were crossed to w^{1118}*; P[UAS-H]* (Go et al., 1998) or w^{1118}*; P[UAS-ECN]* (Jacobsen 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 Kernan 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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