**INTRODUCTION**

Intercellular communication mediated by distinct signalling pathways is central to the proper specification and differentiation of a wide variety of cell and tissue types throughout metazoan development. One such intercellular signalling pathway, the Notch pathway, was first discovered in *Drosophila*, and is deployed during oogenesis, embryogenesis and metamorphosis in flies. The Notch pathway plays central roles in the partitioning of cell fates within equivalence groups throughout development (Muskavitch, 1994; Artavanis-Tsakonas et al., 1995). Notch signalling exhibits a high degree of conservation among vertebrates and invertebrates, in the individual genes that make up the signal transduction pathway and the developmental roles they play (Muskavitch, 1994; Artavanis-Tsakonas et al., 1995; de la Pompa et al., 1997).

In *Drosophila melanogaster*, many genes have been identified that play critical roles in Notch receptor signalling events. Components within the pathway include the ligands Delta and Serrate, Notch, and the downstream effectors Suppressor of Hairless [Su(H)] and the proteins encoded by the Enhancer of split-Complex [E(spl)-C]. The ligands Delta and Serrate bind to Notch molecules located on adjacent cells, initiating a cascade of events within the signal-receiving cell. In many cases, transduction of the Notch-mediated signal to the nucleus of the cell depends on the transcriptional activator Su(H), which physically interacts with the intracellular domain of Notch (Fortini and Artavanis-Tsakonas, 1994). In cell culture, and in some contexts in vivo, the Su(H) protein translocates from the cytoplasm to the nucleus subsequent to Notch activation (Fortini and Artavanis-Tsakonas, 1994; Gho et al., 1996). Among the targets of Su(H) transcriptional activation are the hairy-like helix-loop-helix proteins of the E(spl)-C, which act primarily as transcriptional repressors (Delidakis and Artavanis-Tsakonas, 1992; Bailey and Posakony, 1995; Lecourtois and Schweisguth, 1995; Oellers et al., 1994). In general, the effects of loss-of-function or gain-of-function mutations in any of these loci parallel one another, i.e., lead to reductions or increases in overall Notch signalling activity (Muskavitch, 1994; Artavanis-Tsakonas et al., 1995). The products of the *Hairless* (*H*) and *fringe* (*fng*) loci modulate Notch signalling. The Hairless protein interacts physically with Su(H) and antagonizes transduction of the Notch signal, possibly by sequestering Su(H) in the cytoplasm (Bang and Posakony, 1992; Brou et al., 1994; Bailey and Posakony, 1995). The Fringe protein appears to act at the level of ligand-receptor interactions within developing bristle organs, in contrast to previous reports of the converse effects of Fringe on Delta signalling in the developing wing.

**SUMMARY**

We find that ectopic expression of Delta or Serrate in neurons within developing bristle organs is capable of non-autonomously inducing the transformation of the pre-trichogen cell into a formen cell in a wide variety of developmental contexts. The frequencies at which Delta can induce these transformations are dependent on the level of ectopic Delta expression and the levels of endogenous Notch signalling pathway components. The pre-trichogen cell becomes more responsive to Delta- or Serrate-mediated transformation when the level of endogenous Delta is reduced and less responsive when the dosage of endogenous Delta is increased, supporting the hypothesis that Delta interferes autonomously with the ability of a cell to receive either signal. We also find that a dominant-negative form of Notch, ECN, is capable of autonomously interfering with the ability of a cell to generate the Delta signal. When the region of Notch that mediates trans-interactions between Delta and the Notch extracellular domain is removed from ECN, the ability of Delta to signal is restored. Our findings imply that cell-autonomous interactions between Delta and Notch can affect the ability of a cell to generate and to transduce a Delta-mediated signal. Finally, we present evidence that the Fringe protein can interfere with Delta- and Serrate-mediated signalling within developing bristle organs, in contrast to previous reports of the converse effects of Fringe on Delta signalling in the developing wing.

**Key words:** Delta, Notch, Serrate, cis-interaction, fringe, Drosophila, Neuron

**Cis-interactions between Delta and Notch modulate neurogenic signalling in *Drosophila***

Thomas L. Jacobsen¹, Keith Brennan², Alfonso Martinez Arias² and Marc A. T. Muskavitch¹, *²

¹Program in Genetics, Cell and Developmental Biology, Department of Biology, Indiana University, Bloomington, Indiana, USA
²Department of Zoology, University of Cambridge, Cambridge CB2 3E1, UK

*Author for correspondence (e-mail: muskavit@indiana.edu)

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binding, antagonizing the function of the Serrate ligand (Fleming et al., 1997; Panin et al., 1997) and possibly potentiating transmission of the Delta signal in the larval wing disc (Panin et al., 1997).

The event that initiates Notch signalling is the binding of the receptor to one of its ligands. The two identified Notch ligands in Drosophila, Delta and Serrate, are transmembrane proteins with extracellular domains that share extensive sequence homology within their amino-terminal domains (Vassil et al., 1987; Kopczynski et al., 1988; Fleming et al., 1990; Thomas et al., 1991). Cultured Drosophila cells that express either Delta (Delta+ cells) or Serrate (Serrate+ cells) are able to aggregate specifically with cells that express Notch (Notch+ cells; Fehon et al., 1990). Delta+ cells also display the ability to homotypically aggregate, implying that Delta, unlike Serrate or Notch, is able to self-associate in trans (Fehon et al., 1990). The ability of Delta+ or Serrate+ cells to aggregate with Notch+ cells is dependent on the presence of Notch EGF-like repeats (ELRs) 11 and 12, which are necessary and sufficient for the binding of either ligand (Rebay et al., 1991). While the activation of the full-length Notch receptor in vivo depends on the presence of ligand and the integrity of the ELR array, a truncated form of Notch composed of the intracellular domain acts as a constitutively active receptor (Lieber et al., 1993; Rebay et al., 1993; Struhl et al., 1993). This implies that the function of the Notch extracellular domain may be to restrict the activity of the intracellular domain prior to ligand binding. In contrast, removal of the intracellular domain from Notch yields a dominant-negative form of the receptor that inhibits Notch-mediated signalling in vivo (Rebay et al., 1993; Klein et al., 1997).

We are only beginning to understand interactions between Notch and its ligands. The multimterization states of the ligands and the receptor prior to and subsequent to ligand-receptor binding are unknown. It is also unclear whether ligand and receptor molecules located on the surface of the same cell are able to interact with each other. While interactions between Delta molecules on opposing cell surfaces have been observed in cultured cells (Fehon et al., 1990), the in vivo significance of this interaction, e.g. whether Delta on the surface of a signal-receiving cell can inhibit receipt of a Delta signal from an adjacent cell, has yet to be determined. Another unresolved question is whether Serrate and Delta activate Notch in the same fashion in all contexts, as they appear to be capable of doing during neuroblast specification (Gu et al., 1995).

In order to examine these and other aspects of Notch signalling, we chose to utilize one of the most studied contexts in which the Notch pathway functions, the specification and differentiation of the bristle sensory organs of the adult notum. Using the GAL4-UAS expression system (Brand and Perrimon, 1993), we have examined the effects of Delta and other Notch pathway components on a single cell within developing adult bristle organs. We find that Delta and Serrate are capable of non-autonomously inducing the transformation of the pre-trichogen cell into a tormogen cell, demonstrating for the first time the ability of the Notch ligands to specifically affect differentiation of the pre-trichogen cell. We show that altering the level of endogenous Delta present on a cell can affect the ability of that cell to respond to Delta and Serrate signals. We also present evidence that Notch can autonomously interfere with the ability of a cell to send a Delta-mediated signal and that this effect is dependent upon the same region of Notch that mediates Delta-Notch trans interactions. Finally, we demonstrate that Fringe is capable of interfering with Serrate- and Delta-mediated signalling in this context, in contrast to the situation within the developing wing margin.

MATERIALS AND METHODS

Drosophila strains

The Delta (Dl) alleles used in this study were Df(3R)Dl M2, a deficiency that removes the Dl locus, and Tp(3;3)bxad10 (DpDl+), which includes a copy of the Dl locus that has been inserted into bithoraxoid (Alton et al., 1988). Dp(1;2)w+51b7 (DpN+) includes a copy of the Notch (N) locus (Lindsley and Zimm, 1992). Df(3R)Espl8kb3 is a deficiency that removes the entire E(spl)-Complex (Preiss et al., 1988). St(H)mri is a P-element-induced hypomorphic allele (Schweisguth and Posakony, 1992). H1 is a loss-of-function mutation in the Hairless locus (Maier et al., 1992). The GAL4 driver chromosome 31-1::GAL4 (Brand and Perrimon, 1993), kept balanced over TM6C, cu b e Tb ca (TM6C), was used to direct ectopic expression from several UAS responder transposons. The UAS::Serrate (line D) strain, carrying a homozygous viable, second chromosome insertion of a Serrate responder construct (Gu et al., 1995), was kindly provided by R. Fleming (University of Rochester, Rochester, New York). The strain that we designate UAS::DeltaWT-2 (designated UAS::Delta in Seugnet et al., 1997) carries a homozygous viable insertion on the second chromosome. UAS::fng (line 27), a third chromosome insertion of P[UAS::fng] (Panin et al., 1997) was the gift of K. Irvine (Rutgers University, Piscataway, New Jersey). Strains were maintained at room temperature. Developmental times are based on growth at 25°C.

We assessed the specificity of 31-1::GAL4-mediated expression by determining the number of cells that exhibit β-galactosidase expression in developing bristle organs in 31-1::GAL4/+;UAS::βGalNUC/+ animals. Single-cell staining at the anterior postalar macrochaeta position, the position most sensitive to Delta-mediated trichogen-to-tormogen transformation, at 20 (n=11) or 24 (n=19) hours APF was observed in 30/30 bristle groups. A more general survey of expression in the scutellar and dorsocentral macrochaetae at 20 hours APF revealed single cell staining in 56/57 bristle groups. In the one exceptional instance expression was detected in only two adjacent cells.

Vector construction and germline transformation

The UAS::DeltaWT responder construct was made by inserting the D1 cDNA (Kopczynski et al., 1988) into the EcoRI site of the pUAST vector (Brand and Perrimon, 1993). P[UAS::β-GalNUC] was made by excising a 3.2 kb BamHI/SpeI fragment, containing sequences encoding an SV40 nuclear localization sequence fused to β-galactosidase, from pD8.02 (Fire et al., 1990) and then ligation this fragment into pUAST cut with SpeI and XhoI. P[UAS::ECN] was generated by subcloning the 5718 bp NraI fragment of the Notch cDNA (Lieber et al., 1993), which encodes for amino acids 1-1789, into the pUAST vector cut with EcoRI and KpnI and end-filled. The P[UAS::ECN10-12] construct was generated by creating a deletion within the KpnIBgIll fragment of the Notch cDNA subcloned into the pS72 vector (Promega) and then introducing this segment into P[UAS::ECN] as a KpnIBgIll fragment. The deletion removes amino acids 412-566, inclusive, and fuses EGF-like repeats 9 and 14 while maintaining the structural integrity of the remaining EGF-like repeats.
Further information regarding the generation of the deletion is available upon request from A. Martinez Arias (University of Cambridge, Cambridge, UK).

Germline transformation was carried out using embryos containing one copy of δ2-3 transposase (Robertson et al., 1988) balanced over either TM3, Sb e or TM6B, Hu e Tb ca (Lindsley and Zimm, 1992). Survivors were crossed to w; T(2;3) ap^82/CyO; TM3, which allows inserts to be mapped and placed into balanced or homozygous stocks simultaneously.

The specific UAS responder genotypes (unless otherwise noted) employed were: UAS::DeltaWT-1, which contains a homozygous viable insertion of P[UAS::DeltaWT] on the X chromosome, UAS::β-GalNUC8.2.1, which contains a homozygous viable insertion of P[UAS::β-GalNUC] on the second chromosome, UAS::ECN, which contains a second chromosome insertion of P[UAS::ECN] balanced over CyO, and UAS::ECNA10-12, which contains a homozygous viable insertion of P[UAS::ECNA10-12] on the second chromosome.

Fly crosses
All crosses were performed at 25°C in a w^1118 background. 31-1::GAL4/TM6C males were crossed to virgins homozygous or balanced for the appropriate responders. For crosses involving genetic interactions, 31-1::GAL4/TM6C males were crossed to virgins homozygous or balanced for a given responder and containing the appropriate mutation over a balancer. These schemes allowed unambiguous identification of animals carrying the 31-1::GAL4 driver, the responder(s) and the mutation (if any) of interest.

Counting of macrochaetae
For each genotype, the occurrence of shaft cell transformation was determined for a minimum of 25 female flies (i.e., 50 macrochaetae were assayed for each position). Homozygous UAS::Delta flies that do not carry the 31-1::GAL4 driver exhibit trichogen-to-tormogen transformation at the anterior postalar macrochaeta position at a frequency of 4% (2/50), but do not exhibit transformation at any other macrochaeta position. Heterozygous 31-1::GAL4/TM6C flies that do not carry the UAS::Delta responder exhibit transformation at the posterior notopleural macrochaeta position at a frequency of 2% (1/50), but do not exhibit transformation at any other macrochaeta position.

Immunohistochemistry
Animals were staged from white prepupa formation at 25°C. Notal dissection and antibody staining were performed as described in Parks and Muskavitch (1993). Primary antibodies included mouse anti-β-galactosidase (Promega, Madison, WI) at 1:450, rat anti-Su(H) (Gho et al., 1996) at 1:1000, kindly provided by F. Schweiguth (Ecole Normale Superieure; Paris, France), and mouse anti-Cut at 1:100 (Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA). Nota were mounted in glycerol and viewed using a Zeiss Axioskop light microscope.

Whole-mount microscopy
Legs and wings were removed from adults and mounted using Gary's Magic Mountant (Ashburner, 1989).

Scanning electron microscopy
Flies were stored in 70% EtOH and prepared for scanning electron microscopy as described in Shepard et al. (1989).

Macrochaeta abbreviations
The abbreviations used for macrochaetae are: PS, presutural; aNP and pNP, anterior and posterior notopleural; aSA and pSA, anterior and posterior supraalar; aPA and pPA, anterior and posterior postalar; aDC and pDC, anterior and posterior dorsocentral; aSC and pSC, anterior and posterior scutellar. See Fig. 1A for the location of each macrochaeta on the adult notum.

RESULTS
Each sensory bristle is formed from a single sensory organ precursor (SOP) cell that gives rise to the four cells, a neuron and three accessory cells, that constitute the mature bristle organ. The SOP (pI) divides to give two secondary precursors, pIIa and pIIb. The division of the pIIa cell occurs next; its progeny differentiate as the trichogen (shaft) and tormogen (socket) cells. The pIIb cell divides later to give rise to the neuron and the thecogen (glial) cell (Hartenstein and Posakony, 1989; Posakony, 1994). These events are roughly synchronous for microchaetae, while the initial times of SOP division vary for different macrochaetae (Hartenstein and Posakony, 1989; Huang et al., 1991). Delta is expressed during all stages of bristle development. Parks et al. (1997) report that, during development of the microchaetae, Delta is first expressed at higher levels in broad stripes wherein the microchaetae will develop and at much lower levels between these stripes. Subsequently, Delta is expressed in the microchaeta SOPs, in pIIa and pIIb, and in each individual cell of the bristle organ, with expression eventually being lost from all except the trichogen cell. For at least two macrochaeta positions, the pPA and the aDC (see Materials and Methods for macrochaeta abbreviations), the same expression patterns are observed (A. L. Parks and M. A. T. Muskavitch, unpublished data).

Notch signalling has been shown to play a role during each stage of bristle development. Loss of Notch function prior to the singling out of SOPs results in overcommitment of cells to the SOP fate, while excessive Notch activity can lead to a failure of any cell to commit to the SOP fate. Altering the level of Notch activity also causes defects during all subsequent stages of bristle organ development (Hartenstein and Posakony, 1990; Lyman and Yedulaobnick, 1995; Parks et al., 1997). Using heat-sensitive Dl alleles, requirements for Delta function in restricting adoption of the SOP fate to single cells within pronuclear clusters, in the specification of the pIIa and pIIb cell fates, and in restricting the neural fate to one cell within the four-cell bristle organ have been demonstrated (Parody and Muskavitch, 1993; Parks and Muskavitch, 1993).

Delta expression under control of the 31-1::GAL4 driver can cause trichogen-to-tormogen transformations
The 31-1::GAL4 driver directs GAL4 expression in neurons of the embryonic central and peripheral nervous systems (CNS and PNS; Brand and Perrimon, 1993). In light of this finding, we chose to determine whether this driver might also direct expression in the developing adult PNS and whether Delta expressed under control of this driver would affect bristle organ development. Flies of the genotype UAS::DeltaWT-1/+;31-1::GAL4/+ (31-1::GAL4;UAS::DeltaWT) exhibit a striking defect in the development of many of their bristles, namely, the appearance of a second socket-like structure in
many organs at the expense of the bristle shaft (i.e., the ‘double socket’ phenotype, Fig. 1C). Delta expression mediated by 31-1::GAL4 affects natal macrochaetae, wing hinge and wing margin bristles, leg bristles (including the male sex combs), interommatidial bristles (Fig. 1B-F), abdominal tergite bristles, and other sensory bristles as well (data not shown). In the notum, the trichogen-to-tormogen transformation induced by 31-1::GAL4-mediated Delta expression does not equally affect all macrochaetae examined. Among the 11 stereotypic natal macrochaeta positions, the frequency of bristle alteration observed at 25°C varies, from 99% alteration of the aPA macrochaeta to no alteration of the aDC macrochaeta (Table 1). In contrast to the effects seen for most macrochaetae, few effects on natal microchaetae development are observed for the UAS::DeltaWT responder. These results indicate that the 31-1::GAL4 driver must be expressed at some time during the development of many of the adult bristle sense organs and that 31-1::GAL4-mediated Delta expression is capable of affecting bristle organ development in most imaginal contexts.

We next performed crosses at a lower temperature and with differentially expressive UAS::DeltaWT responder lines, to assess the effects of varying Delta expression on the relative frequency of trichogen transformation at each macrochaeta position. The expressivity of the GAL4-UAS system has been shown to be temperature-dependent, with reductions in temperature leading to reduced driver-mediated expression (Speicher et al., 1994). Comparing outcomes at 25°C to those at 18°C reveals an overall drop in trichogen transformation frequencies at 18°C (data not shown). Some positions exhibit substantial reductions in transformation (e.g., aSC, pSC, aNP), while other positions exhibit little change (e.g., aPA, pDC). Similarly, when the 31-1::GAL4 line is crossed to other UAS::DeltaWT responders with varying expressivity (based on phenotypes induced by other unrelated GAL4 drivers, data not shown), the rank-ordered sensitivities of different bristles are basically unchanged (data not shown). Even in the case of the least expressive UAS::DeltaWT responder line, for which all other natal macrochaetae are unaffected, the aPA bristle still exhibits transformation at a frequency of 20%, indicating that even relatively low levels of 31-1::GAL4-mediated Delta expression are sufficient to induce trichogen transformation at the most sensitive macrochaeta position. In the case of a particularly strongly expressing Delta responder line, UAS::DeltaWT-2, Delta expression causes trichogen transformation of the aDC and pSA macrochaetae (data not shown), bristles rarely affected by UAS::DeltaWT-1, as well as a stronger effect on natal microchaetae. This implies that, although the aDC and pSA macrochaetae and the microchaetae appear to be inherently resistant to this Delta-mediated trichogen transformation, sufficiently high levels of the Delta signal can induce the transformation in these organs. Thus, altering the levels of 31-1::GAL4-mediated Delta expression produces graded effects on the frequency of trichogen transformation for different bristles.

We examined the exact cellular defects present in mutant bristle organs by staining developing natal macrochaetae.
from 31-1::GAL4;UAS::DeltaWT pupae with antibodies to the Su(H) protein. At the 3- and 4-cell stages of bristle development, Gho et al. (1996) report that only the socket cell expresses Su(H) at a high level, and that the protein is found in the cytoplasm and the nucleus of that cell. Because the high levels and nuclear localization of the protein are specific to the tormogen cell, these features can be used to distinguish the tormogen from the trichogen, which exhibits only low levels of cytoplasmic accumulation of Su(H) protein. When nota from 31-1::GAL4;UAS::DeltaWT pupae aged to 24 hours after puparium formation (APF) at 25°C were stained with anti-Su(H), only one such cell, along with one weakly staining cell, is observed at a macrochaeta position that almost never exhibits transformation (aDC, Fig. 2A). Two large cells that exhibit intense nuclear Su(H) staining are present consistently at a macrochaeta position (aPA, Fig. 2B) that almost always undergoes transformation. These results indicate that the phenotype observed in 31-1::GAL4;UAS::DeltaWT flies is due to the conversion of the weakly staining trichogen cell to one that exhibits a tormogen-like Su(H) staining profile, i.e., the trichogen cell is transformed into a tormogen cell in the presence of excess Delta signal.

31-1::GAL4-mediated expression is limited to one cell in developing thoracic bristle organs

We generated a nuclear β-galactosidase responder line (UAS::β-GalNUC) to assess expression of the 31-1::GAL4 driver during notal bristle development. In wing discs from 31-1::GAL4;UAS::β-GalNUC animals, expression is undetectable before 12 hours APF. By 16 hours APF, intense β-galactosidase accumulation is seen in the nucleus of one cell at each macrochaeta position and very slight accumulation is sometimes observed in an adjacent cell. Even in these infrequent situations, the adjacent cell in which 31-1::GAL4 activates expression is a diploid cell (see below), and therefore cannot be the pretrichogen cell, which is polyploid. At this time, all macrochaeta positions have undergone the terminal division that gives rise to the presumptive trichogen and tormogen cells, and each group contains four cells (Poodry, 1975). By 20 hours APF, β-galactosidase is detected in only one cell in each macrochaeta group. Around 24 hours APF, the microchaeta bristle organs in the notum, which are at the 4-cell stage and beginning to enter the ‘differentiative phase’ of bristle development (Hartenstein and Posakony, 1989), also begin to accumulate β-galactosidase in single cells (data not shown). When 24 hour APF nota from 31-1::GAL4;UAS::β-GalNUC flies are stained with antibody to the Cut protein, which stains all four cells of the bristle organ (Blochinger et al., 1993), and for β-galactosidase activity, β-galactosidase expression is detected at a stereotyped position corresponding to the neuronal cell of the bristle organ (Fig. 2D). The cell that exhibits β-galactosidase activity also stains with anti-Elav antibody, which marks neural cells (data not shown; Robinow and White, 1991). This neuronal cell is in direct contact with the pre-trichogen cell, the cell affected by 31-1::GAL4-mediated Delta expression.

Altering levels of Notch pathway components affects the frequency of trichogen-to-tormogen transformation

The 31-1::GAL4;UAS::DeltaWT cross was carried out in various heterozygous loss-of-function and duplication backgrounds to assess the phenotypic effects of altering the levels of various proteins involved in Notch signalling (Table 1). Increasing the level of available Notch (Dp\(\text{WT}^+\)) or decreasing the level of Hairless protein (\(H^1\)) results in strong enhancement of the transformation phenotype. Widespread microchaeta transformation can also be observed in these backgrounds (data not shown). Conversely, heterozygosity for a \(Su(H)\) loss-of-function mutation \([Su(H)^{MR}]\) or a deletion that removes the entire \(E(spl)-C\) \([E(spl)^{RX6}]\) leads to suppression of the trichogen transformation, with the \(E(spl)-C\) deletion yielding a stronger suppression phenotype. Therefore, increasing or

<table>
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<th>Responder and genetic background used in 31-1::GAL4 cross*</th>
<th>UAS-DI</th>
<th>UAS-Ser</th>
<th>UAS-DI;Dp(\text{N}^+)</th>
<th>UAS-DI;(H^1)</th>
<th>UAS-DI;(E(spl)^{RX6})</th>
<th>UAS-DI;(\text{Su}vH^{MR})</th>
<th>UAS-DI;(\text{Dm}^2)</th>
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\(^*\)All genotypes are heterozygous for the 31-1::GAL4 driver, the responder construct, and the mutation, if any, noted. 
\(^1\)UAS-DI refers to UAS::DeltaWT-1. 
\(^2\)UAS-DI;\(H^1\)/TM6C flies exhibit double sockets at these positions in absence of the 31-1::GAL4 driver (PS 67%;aPA 53%;aDC 45%;aSA 3%)
reducing the ability of the signal-receiving cell to transduce the Delta signal leads to enhancement or suppression, respectively, of the transformation of the presumptive trichogen cell in the 31-1::GAL4;UAS::DeltaWT background.

Changing the levels of endogenous Delta alters sensitivity of the presumptive trichogen cell to the ectopic Delta signal

The outcomes observed for the genetic backgrounds described above can be explained as the result of an increased or reduced capacity to transduce the 31-1::GAL4-mediated Delta signal within the signal-receiving cell. One might expect that reducing or increasing the levels of endogenous Delta expression would have analogous effects, by lowering or increasing the amount of endogenous signal generated by the 31-1::GAL4-expressing cell. However, this does not appear to be the case (Table 1). Removal of one copy of the endogenous Dl gene from 31-1::GAL4;UAS::DeltaWT flies, achieved by heterozygosity for Df(3R)DfM2, actually leads to a strong enhancement of the trichogen transformation phenotype, to an extent comparable to that seen for animals triploid for N. In 31-1::GAL4;UAS::DeltaWT flies that carry a duplication of the Dl locus (DpDl*), the opposite effect is observed. The extra copy of Dl suppresses transformation, although not to the extent observed in flies hemizygous for the E(spl)-C. These results imply that altering endogenous Delta levels can affect the capacity of the pre-trichogen cell to receive the Delta signal. One possible explanation for the observed effects of altering endogenous Delta levels is that Delta may be able to interact with Notch molecules on the same cell surface, rendering the cell less able to respond to Delta signal (see Discussion).

Expression of a dominant-negative form of Notch can autonomously affect the ability of a Delta-expressing cell to signal

To further examine interactions between Delta and Notch molecules, we coexpressed Delta and a Notch variant that lacks ELRs 10-12 (i.e., ECN (Table 2)). Together, these data imply that altering endogenous Delta levels can affect the capacity of the pre-trichogen cell to receive the Delta signal. The phenotypic effects observed appear to be additive. Most macrochaeta positions exhibit transformations in the 31-1::GAL4;UAS::DeltaWT;UAS::Serrate background at frequencies higher than those observed for either single responder background, with the aNP, aPA and aDC positions being the only exceptions (Table 2).

We also determined whether the effects of reducing or increasing endogenous Delta levels on the 31-1::GAL4 flies were coexpressed under control of the 31-1::GAL4 driver. In general, the phenotypic effects observed appear to be additive. Most macrochaetae positions exhibit transformations in the 31-1::GAL4;UAS::DeltaWT;UAS::Serrate flies at frequencies higher than those observed for either single responder background, with the aNP, aPA and aDC positions being the only exceptions (Table 2).

Because the Notch ligand Serrate appears to mimic Delta functionally in some developmental contexts (Gu et al., 1995), we determined whether Serrate could mimic the effects of Delta when expressed under control of the 31-1::GAL4 driver. We find that Serrate induces trichogen transformation in a fashion essentially indistinguishable from that of Delta, except several macrochaetae display different sensitivities to Serrate and Delta expression (Table 2). 31-1::GAL4;UAS::Serrate flies exhibit transformation at the aDC and PS positions over 50% of the time, positions rarely affected by Delta signalling. Furthermore, all 31-1::GAL4;UAS::Serrate flies examined exhibit some microchaetae transformation. Serrate effects are also seen in the same range of developmental contexts as for Delta. To determine whether the effects of Delta and Serrate on trichogen development are antagonistic or synergistic, Delta and Serrate were coexpressed under control of the 31-1::GAL4 driver. In general, the phenotypic effects observed appear to be additive. Most macrochaeta positions exhibit transformations in the 31-1::GAL4;UAS::DeltaWT;UAS::Serrate flies at frequencies higher than those observed for either single responder background, with the aNP, aPA and aDC positions being the only exceptions (Table 2).

Table 2. Frequency (%) of double sockets with the 31-1::GAL4 driver and different responders

<table>
<thead>
<tr>
<th>Responder and responder combinations with 31-1::GAL4*</th>
<th>UAS-Dl†</th>
<th>UAS-Ser</th>
<th>UAS-Dl; UAS-Ser</th>
<th>UAS-Dl; UAS-ECN</th>
<th>UAS-Dl; UAS-ECN Δ10-12</th>
<th>UAS-DC; UAS-fog</th>
<th>UAS-DC; UAS-fog</th>
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<tr>
<td>PS</td>
<td>7</td>
<td>86</td>
<td>92</td>
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<td>24</td>
<td>86</td>
<td>0</td>
<td>2</td>
<td>0</td>
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<td>46</td>
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*All genotypes are heterozygous for the 31-1::GAL4 driver and the responder(s)
†UAS-Dl refers to UAS::DeltaWT-1
endogenous Delta levels affect the Notch signalling pathway in this context is not specific to Delta-mediated signalling.

**The Fringe protein can reduce the frequency of Delta- and Serrate-mediated trichogen transformation**

Previous reports have shown that the Fringe protein is capable of interfering with Serrate activation of Notch (Fleming et al., 1997; Panin et al., 1997), and have suggested that Fringe may potentiate the response of cells to Delta in the developing wing disc (Panin et al., 1997). To assess the effects of Fringe on the trichogen transformation phenotype induced by Delta and Serrate under control of 31-1::GAL4, we coexpressed Fringe with either Serrate or Delta under control of the 31-1::GAL4 driver. Our results indicate that Fringe is capable of reducing the ability of Delta and Serrate to induce trichogen transformation (Table 2). Expression of Fringe, alone, under control of 31-1::GAL4 has no discernible effect on bristle development (data not shown). The effects of Fringe on Delta signalling in this context are even more pronounced than those on Serrate, although this could be due to relatively higher expression of the Serrate responder.

**DISCUSSION**

**Delta- and Serrate-mediated signalling can promote the socket cell fate in developing bristle organs**

Previous studies have defined roles for Delta in the specification of the SOP, pIIa and pIIb, and of the daughters of pIIb, the neuron and thecogen (Parody and Muskavitch, 1993; Parks and Muskavitch, 1993; Parks et al., 1997). We demonstrate that Delta is also capable of affecting cell fate choice by the daughters of pIIa, the trichogen and tormogen. Excess Delta expression in a cell contacting the progeny of pIIa can cause both cells to differentiate as tormogen cells, based on phenotypic and molecular criteria. This is consistent with the outcomes observed when the Notch intracellular domain (Lyman and Yedvobnick, 1995; Parks et al., 1997), a constitutively active form of Notch, or Su(H) (Schweisguth and Posakony, 1994), is ectopically expressed at the appropriate developmental times, as well as with the effects seen in backgrounds mutant for either H or numb, antagonists of Notch signalling (Bang et al., 1991; Rhuy et al., 1994). Furthermore, the trichogen-to-tormogen transformation ‘gain-of-function’ phenotype that results from 31-1::GAL4-mediated Delta expression is the converse of the tormogen-to-trichogen transformation that results from reduction in neurogenic signalling in vivo (Bang and Posakony, 1992; Frise et al., 1996). The observation that 31-1::GAL4-mediated Delta expression affects different macrochaeta positions at widely varying frequencies suggests that pre-trichogen cells at different macrochaeta positions may have intrinsically variable thresholds of sensitivity to Delta-mediated signalling. These differences are presumably due to local variations in the distribution of factors that increase or decrease the net efficacy of Delta-Notch signalling. The graded effects observed at different temperatures and with differentially expressive Delta responder lines provide further support for this premise.

Our findings imply that trichogen/tormogen specification constitutes another example of a developmental context in which Delta and Serrate are functionally interchangeable. Serrate is able to produce the same effects on the pre-trichogen cell as Delta, although the frequencies with which Delta and Serrate affect specific bristle positions vary. The fact that many notal macrochaeta positions, and microchaetae in general, are differentially sensitive to Delta and Serrate again suggests that the existence of local variations within the developing notum of different factors may specifically interfere with or enhance the net signalling activity of Notch ligands. If this were not the case, we would expect the rank order of position-specific trichogen-to-tormogen transformation frequencies associated with each ligand to be comparable. Yet, they are not. The frequency of trichogen transformation at most positions increases significantly when Delta and Serrate are coexpressed, indicating that Delta and Serrate can act additively and that neither ligand, in this context, interferes with the function of the converse ligand. We infer that this additive phenotype is due to an increase in the net activation of their mutual receptor, Notch, when both ligands are expressed.

**The level of Delta expressed by a cell can influence its ability to receive the Delta/Serrate signal**

Altering endogenous levels of different Notch pathway components within the cell receiving the 31-1::GAL4-mediated Delta signal has significant effects on the frequency with which the trichogen-to-tormogen transformation is observed. Increasing the capacity to receive and transduce the signal, either by increasing the amount of Notch or decreasing the amount of the Notch antagonist Hairless within the signal-receiving cell, results in enhancement of the 31-1::GAL4;UAS::DeltaWT phenotype. This is presumably due to either an increased capacity to receive the Delta signal, in the case of DpN*, or a reduced capacity to interfere with the transduction of the Notch signal, in the case of the H mutant. Similarly, reducing the ability of the receiving cell to transduce the Notch signal by decreasing the level of the Su(H) or E(spl)-C products, which act as downstream effectors of Notch signalling, results in a lower frequency of trichogen transformation at most bristle positions. In each of these cases, the observed effects can be explained by cell-autonomous events that occur either at the level of the receptor or downstream of the receptor within the signal-receiving cell. These data also imply that Delta-mediated effects on trichogen specification occur via the canonical Notch signalling pathway (Muskavitch, 1994; Artavanis-Tsakonas et al., 1995).

Heitzler and Simpson (1991) found that the levels of Notch or Delta present in adjacent populations of cells can influence whether cells within the developing notum adopt the epidermal or SOP fate. A lower level of Delta or a higher level of Notch biases a cell to differentiate as epidermis, the fate promoted by Notch activity in the notum. A higher level of Delta or a lower level of Notch promotes adoption of the SOP fate, the fate that Notch activity suppresses. This suggests that the amount of ligand as well as the amount of receptor present on the surface of a cell may be important in modulating receipt of the neurogenic signal by that cell. In accordance with these observations, our results demonstrate that increasing the endogenous Delta level suppresses the trichogen transformation, while decreasing the level of endogenous Delta strongly enhances the trichogen transformation induced by...
ectopic Delta expression. It appears that increased expression of Delta by the cell receiving the Delta signal makes that cell more refractory to the signal, while reduced Delta expression by the receiving cell makes that cell more receptive. This effect is also seen when Serrate-mediated signalling is examined, implying that the interactions that modulate signal reception in this context are not signal-dependent, and that the ability of the pre-trichogen cell to receive a Delta or Serrate signal is affected in a cell-autonomous manner by the level of Delta expressed by that cell.

Much evidence has accumulated recently to support the idea that high levels of Notch ligands within a cell may make that cell unresponsive to Notch signalling. Overexpression of Serrate or Delta due either to increased perdurance of the protein, as for the SerD mutation (Thomas et al., 1995) or via the GAL4-UAS system (Speicher et al., 1994; Thomas et al., 1995; Doherty et al., 1996; Jönsson and Knust, 1996; Klein et al., 1997), can autonomously inhibit the induction of genes within the developing wing that are dependent upon Notch activity. Michelli et al. (1997) report that in the cells that flank the presumptive wing margin, which express Delta and Serrate dorsally and Delta ventrally (Thomas et al., 1995; Doherty et al., 1996), endogenous levels of Delta and Serrate along the margin are sufficient to render these cells unresponsive to the Delta and Serrate signals expressed on the surfaces of their neighbors. In addition, they found that removing Delta and Serrate function from cells flanking the margin allows them to become responsive to Delta and Serrate signal from neighboring wild-type cells, resulting in Notch activation within the DI/Ser" cells. The above results are analogous to those that we observe when the level of Delta expression is increased or decreased within the context of the pre-trichogen cell.

It is unlikely that ligand-induced increases in Notch expression in pretrichogen cells, based on feedback regulatory activity (Heitzler et al., 1996; Huppert et al., 1997), contribute to the effect of 31-1::GAL4-mediated Delta or Serrate expression on the adoption of a fate by the pretrichogen cell. We infer this because up-regulation of Notch expression in the pupal notum is not observed when wild-type Delta is overexpressed under control of the hsp70 promoter and the Notch pathway is hyperactivated (Parody, 1998), or when constitutive Notch pathway hyperactivation is induced by expressing the Notch intracellular domain under control of the hsp70 promoter (A. L. Parks and M. A. T. M., unpublished data).

The level of Notch expressed by a cell can influence its ability to send the Delta signal

Coexpression of a dominant-negative form of Notch with Delta in the 31-1::GAL4 context results in the inability of the Delta-expressing cell to signal effectively, reducing the frequency at which trichogen transformation occurs. While an increased level of Notch protein on the surface of a cell may make that cell more responsive to incoming signals by increasing the amount of available receptor, as described by Heitzler and Simpson (1991), our data suggest that Notch is also able to autonomously interfere with the ability of a cell to send a Delta-mediated signal. Because the dominant-negative form of Notch lacks the intracellular domain, which is required for Notch activity (Rebay et al., 1993), the effect that we observe on signalling cannot be attributed to some consequence of increased Notch activity within the signalling cell.

Previous reports have described how increasing the levels of Notch can affect phenotypes resulting from misexpression of Delta or Serrate. Jönsson and Knust (1996) and Klein et al. (1997) report that dominant-negative effects of ectopic Serrate can be suppressed by coexpression of a full-length or truncated form of Notch. Additionally, extra copies of N can suppress the notching associated with the hypermorphic SerD allele (Fleming et al., 1990; Thomas et al., 1991). These observations are consistent with the premise that additional Notch protein can relieve the effects of excess ligand, i.e., the existence of cell-autonomous inhibition of signal reception. Doherty et al. (1996) report that, when Notch is coexpressed with Delta, under control of the ptc::GAL4 driver, ectopic cut induction within the wing disc occurs only in cells that express ectopic Delta and Notch. The increase in Notch expression apparently reduces the ability of Delta to signal to cells adjacent to the domain of ectopic expression. Similarly, development of supernumerary wing vein cells associated with the ‘Confluentes’ phenotype that results from hyperploidy for N (Lindsley and Zimm, 1992) can be attributed to interference of increased levels of Notch with the ability of Delta to signal effectively. Thus, it appears that the ability of a cell to either transmit or receive a Delta- or Serrate-mediated signal may be affected by the relative abundance of Delta or Serrate and Notch expressed by that cell, and that sufficiently high levels of receptor or ligand can autonomously interfere with the ability of the other member of the ligand-receptor pair to function.

Heitzler and Simpson (1993) postulated that cell-autonomous inhibition of signalling could be due to cis-interactions between Delta and Notch molecules present on the surface of the same cell. They suggested that, when the level of Notch in a cell is high relative to that of Delta, Notch could sequester Delta, preventing it from signalling to adjacent cells. We find that when Notch ELRs 11-12, which mediate trans-interactions between Notch and Delta (and Serrate; Rebay et al., 1991) in cultured cells, are deleted from the dominant-negative Notch ECN variant, the effects of ECN on the frequency of trichogen transformation by Delta are substantially mitigated (i.e., reduced suppression of transformation occurs). This implies that the same repeats that mediate trans-interactions between Notch and Delta in cultured cells may mediate cis-interactions between the two molecules during development. Fehon et al. (1990) showed that when Delta and Notch are coexpressed within the same cell, the two proteins appear to be colocalized on the membrane, further suggesting that Delta and Notch might interact in cis as well as in trans. Whether these cis-interactions have the same intermolecular polarity as the intercellular Delta-Notch trans-interaction is an open question. However, it is plausible that interactions between Delta and Notch extracellular domains within outbound vesicles in the protein export pathway, which could occur before such vesicles reach the cell surface, may also play a role in these phenomena. The apparent persistence of suppression by ECNA10-12 of the Delta-mediated trichogen transformation that we observe at some macrochaeta positions implies that regions of the Notch extracellular domain in addition to ELRs 11 and 12 may be capable of mediating Delta-Notch interactions in cis within the export pathway or at the cell surface.
Recent evidence has suggested the existence of a regulatory feedback loop between cells that express Notch and Delta (e.g., Heitzler et al., 1996; Huppert et al., 1997) such that the level of Notch activity within a cell influences the expression of Notch and Delta within that cell. High levels of Notch activity are associated with increased Notch expression and decreased Delta expression; lower relative levels of Notch activity are associated with converse expression patterns. This type of feedback loop and the effects of cis-interactions described above could cooperate in a mutually reinforcing manner, amplifying small initial differences between cells in Notch and Delta expression into larger differences that ultimately affect the adoption of alternative developmental fates.

Fringe is capable of interfering with signalling by Serrate and Delta

The fringe gene encodes a pioneer protein, predicted to be secreted, that plays a role in the development of the wing disc by modulating interactions between dorsal and ventral cells that establish the dorsal-ventral boundary and affect specification of the wing margin (Irvine and Wieschaus, 1994; Kim et al., 1995). One domain of the Fringe product contains motifs similar to the catalytic domain of glycosyltransferases (Yuan et al., 1997). The primary effect of Fringe on Notch signalling appears to be inhibition of the ability of the Serrate ligand to activate Notch, an effect observed during neuroblast specification within the neuroectoderm and in the developing wing disc (Fleming et al., 1997; Panin et al., 1997). Fleming et al. (1997) present evidence suggesting that Fringe may act by binding the amino-terminus of Serrate. Panin et al. (1997) also report that Fringe-expressing cells may be more responsive to Delta-mediated signalling.

We find that in the case of 31-1::GAL4-mediated expression of Delta and Serrate, coexpression of Fringe with either ligand can interfere with the ability of that ligand to induce trichogen transformation. In this context, Fringe impedes Serrate- and Delta-mediated signalling. The inhibition by Fringe of Delta-mediated signalling that we observe is in contrast to the effects on Delta signalling reported by Panin et al. (1997). Klein and Martinez Arias (1998), while presenting further evidence for possible enhancement of Delta function by Fringe, find that Fringe may interfere with Delta function in the developing wing. The inhibition of Delta and Serrate signalling that we observe in the developing bristle organ may be context-dependent, i.e., factors present at the wing margin that prevent Fringe from interfering with Delta-mediated signalling may be absent in developing macrochaetae. If Fringe is secreted by the neuron, it could act in a cell non-autonomous fashion to impede the ability of Notch on the pre-trichogen cell to receive ligand-mediated signals. Alternatively, Fringe could function in the neuron in a cell autonomous manner to impede signal generation by interacting with ligand. In either case, Fringe cannot be interfering with Notch-mediated signal reception in a cell autonomous manner in this context. The exact mechanism by which Fringe can operate in the context of bristle development must be the object of future experiments.

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