Delta and Serrate are redundant Notch ligands required for asymmetric cell divisions within the Drosophila sensory organ lineage

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Asymmetric divisions allow a precursor to produce four distinct cells of a Drosophila sensory organ lineage (SOL). Whereas this process requires cell–cell communication via Notch (N) receptor, mitotic recombination that removes the N ligand Delta (Dl) or Serrate (Ser) in the SOL had mild or no effect. Removal of both Dl and Ser, however, led to cell fate transformations similar to the N phenotype. Cell fate transformation occurred even when a single SOL cell lost both Dl and Ser. Thus, Dl and Ser are redundant in mediating signaling between daughter cells to specify their distinct cell fates.

Results and Discussion

Notch (N)-mediated signaling between cells is required for the formation of the adult external sensory organs (bristles) in two different developmental processes (Hartenstein and Posakony 1989, 1990; de Celis et al. 1991; Heitzler and Simpson 1991; Parks and Muskavitch 1993; Posakony 1994; Jan and Jan 1995; Parks et al. 1997; Wang et al. 1997). First, N mediates lateral inhibition, a process by which a single cell is selected from an equivalence group of competent cells to become the sensory organ precursor (SOP) (Heitzler and Simpson 1991, 1993). Second, N functions in the correct execution of three asymmetric cell divisions in a fixed sensory organ lineage (SOL), leading to the generation of four distinct cells that form a sensory organ (bristle). The SOP first divides into two different secondary precursor cells, IIA and IIB, which gives rise to one shaft-producing cell (trichogen) and one socket-producing cell (tormogen), and one neuron and one sheath cell (the cogon), respectively (see Fig. 4C, below). These four lineage-related cells constitute the SOL (Bodmer et al. 1989; Hartenstein and Posakony 1989; Posakony 1994; Rhyu et al. 1994).

Although N signaling is known to be required for specifying the SOL, two questions have not been adequately addressed: First, what is the identity of the relevant N ligand? Delta (Dl) and Serrate (Ser) are two known ligands for N, and Dl has been implicated as the N ligand that specifies cell fates of the SOL (Fehon et al. 1990; Fleming et al. 1990; Thomas et al. 1991; Parks and Muskavitch 1993; Parks et al. 1997). However, it is not known whether Dl is the only ligand involved. Second, which cells participate in the N-mediated signaling that enables the daughter cells to acquire distinct cell fates in these asymmetric divisions? A priori, the N ligand(s) could be provided by the neighboring epidermal cells (Fig. 1A), by the sibling cells within the SOL (Fig. 1B), or by both groups of cells (Fig. 1C). In the Drosophila CNS, the MP2 cell divides asymmetrically to produce a pair of distinct neurons, and both N and numb are required for this asymmetry. The N ligands required for this process are thought to be provided by the neighboring epidermal cells and not by the cells within the MP2 lineage (Spa and Doe 1996). In the SOL, there have been no experimental tests to distinguish among these scenarios.

To examine the source and identities of the N ligands during each step of the SOL, we induced mitotic recombination after SOP formation to specifically remove gene functions from marked mutant clones. Within the N mutant clone, both daughter cells adopted the same fate. Removal of Dl rarely gave rise to cell fate transformation in the mutant clones, and removal of Ser failed to produce detectable cell fate transformation. In contrast, removal of both Dl and Ser led to cell fate transformations reminiscent of the N phenotype. Thus Ser functions in the specification of daughter cell fates during the asymmetric divisions of the SOL, which is normally masked by the redundant function of Dl. Moreover, removal of both Dl and Ser function from a single cell within the SOL resulted in the transformation of the cell fate, indicating that daughter cell fates are determined mainly by signaling between the two siblings.

Results and Discussion

The phenotype of N clones is different from the phenotype of Dl clones

We used the yeast site-specific recombinase FLP to induce mitotic recombination (Golic and Lindquist 1989; Xu and Rubin 1993) and thereby produce clones of homozygous N mutant cells in otherwise heterozygous flies. N mutant clones generated on the central region of the adult scutum were devoid of any external bristle structures such as shafts and sockets (Fig. 2A), similar to the N5 mutant phenotype at restrictive temperature (Hartenstein and Posakony 1990; Wang et al. 1997). Whereas loss of N function during the process of lateral inhibition produces supernumerary SOPs (Heitzler and Simpson 1991, 1993), this balding phenotype is probably due to the requirement of N in asymmetric divisions. Without N activity the supernumerary SOPs divide symmetrically, giving rise to two IIB cells and, consequently,
no external sensory structures (Hartenstein and Posakony 1990; Heitzler and Simpson 1991, 1993; Wang et al. 1997).

**DI** is a ligand for **N** during bristle development (Fehon et al. 1990; Parks and Muskavitch 1993; Artavanis-Tsakonas et al. 1995; Parks et al. 1997). However, in contrast to N mutant clones, similarly induced DI clones typically gave rise to a tuft of densely packed bristles in the interior of the clone (Fig. 2B). These tufts of bristles are likely due to a failure of lateral inhibition, resulting in overproduction of SOPs (Heitzler and Simpson 1991, 1993). The presence of the external bristle structures in these DI mutant clones indicates that, unlike N clones, most of the supernumerary SOPs in the DI mutant clones produce IIA cells that divide to form shaft and socket cells.

To test for Ser involvement in bristle development, we generated mitotic clones of Ser, and clones of DI Ser double mutations. Clones homozygous for three Ser null alleles gave rise to normal external bristle structures (Fig. 2C). In contrast, clones with loss of both DI and Ser function produced epidermal cells but not external bristle structures (Fig. 2D). This balding phenotype is clearly different from the phenotypes of the DI or Ser mutant clones but is indistinguishable from that of N mutant clones, suggesting that Ser and DI have overlapping functions in the N signaling pathway.

Ser and DI are redundant signals to **N** for the specification of different daughter cell fates in the SOL.

To examine the role of DI and Ser in the SOL asymmetric divisions, we used SOL-specific Gal4 enhancer trap lines, Gal4
to-68 (Frise et al. 1996) or sca-Gal4 (Nakao and Campos-Ortega 1996), to drive FLP recombinase expression from a UAS–FLP transgene, which allows mitotic recombination mainly within the SOL, after the SOP cell is singled out via lateral inhibition. Because a typical SOL undergoes only two rounds of cell divisions (Bodmer et al. 1989; Hartenstein and Posakony 1989; Posakony 1994), each mutant “clone” consists of only one or two homozygous mutant cells depending on when the recombination takes place.

The loss of Ser function in such a mutant clone produced normal external bristle structures (Fig. 3A); of the 23 marked macrochaetae clones, no double shafts (i.e., macrochaetae with two shafts and no associated sockets) were seen. Similarly generated DI mutant clones were largely normal (Fig. 3B); only ∼5% of the marked mutant macrochaetae exhibited double shafts. In contrast, double mutant clones with loss of both DI and Ser function within the SOL frequently exhibited double shafts (Fig. 3C); ∼44% of the marked macrochaetae clones had double shafts. This is much more severe than the phenotype of DI or Ser clones but similar to the phenotype of N clones generated in similar fashion (Fig. 4B), where 39% of the single N macrochaetae clones had double shafts.

Unlike processes such as the wing blade formation where Ser serves a distinct function as a compartment-specific signal (Diaz-Benjumeda and Cohen 1995; Doherty et al. 1996; Jonsson and Knust 1996; de Celis et al. 1997; Michelli et al. 1997), our results indicate that the function of Ser in the SOL is similar to that of DI. Ser’s function in bristle development has not been identified previously (Fleming et al. 1990; Thomas et al. 1991; Speicher et al. 1994), because it is normally masked by the.
lutes that the N ligands are provided by daughter cells within the SOL to influence their sibling cell fates (Posakony 1994; Jan and Jan 1995; Frise et al. 1996). However, the source of the N ligands has not been identified experimentally. Either the daughter cells of the asymmetric divisions or their neighboring epidermal cells could be providing the Dl and/or Ser function for the N receptor (Fig. 1). To identify the cells that provide the Dl and Ser for the specification of the daughter cell fates, we analyzed UAS–FLP-induced clones with two distinct bristle morphology markers. As a result of FRT-mediated recombination, the daughter cell that was homozygous for both Dl and Ser mutations was homozygous for the bristle color mutation yellow (y), whereas its sibling cell, wild-type for Dl and Ser, was homozygous for the bristle mutation Stubble (Sb). The cells that did not undergo mitotic recombination were heterozygous for Dl, Ser, Sb, and y.

Our experiments produced clones with abnormal bristles composed of double shafts without associated sockets. Interestingly, some of these double shafts consisted of one yellow shaft and one stubble shaft (Fig. 4A, purple arrow). The double shafts most likely arose from a recombination event during the division of one IIA cell, producing one daughter cell homozygous for the y mutation and doubly mutant for Dl and Ser, plus another daughter cell homozygous for the marker Sb and wild-type for Dl and Ser (Fig. 4E). Similar results were obtained using the bristle and epidermal hair morphology marker forked (f) as an independent marker for Dl Ser double mutant clones (Fig. 3C). In these experiments, the presence of Dl and Ser gene function in the surrounding epidermal cells—which displayed a genetic marker indicative of cells heterozygous for Dl and Ser mutations—was not sufficient to rescue the cell fate transformation within the SOL. Thus, the elimination of Dl and Ser activities from only one of the two daughter cells of IIA could cause the transformation of a socket into a shaft cell.

We further examined the cell fate choice in the division of the IIB cell. Previous experiments with N+ indicate that loss of N function leads to the transformation of the sheath cells into neurons during the division of the IIB cell (Hartenstein and Posakony 1990; Parks and Muskavitch 1993; Wang et al. 1997). We used sca-Gal4 UAS–FLP, which appears to be more efficient in producing mitotic recombination than Gal4109D–68 UAS–FLP. Nota containing Dl Ser double mutant clones gave rise predominantly to normal clusters, each composed of one neuron and one sheath cell, as well as some abnormal clusters of cells with the following three phenotypes: three neurons and one sheath cell; two neurons with no sheath cells; and two neurons with two sheath cells (Fig. 3D). If a recombination event occurred during the division of the SOP cell, the lack of Dl and Ser function in one of its daughter cells could result in the appearance of two IIB cells (Fig. 4D). Upon subsequent divisions, the IIB cell with wild-type Dl and Ser function would produce one neuron and one sheath cell. The other sibling IIB cell, which was homozygous for Dl Ser mutations,
two shafts. Two light (parental IIA cell, which then divides symmetrically to produce

mutant shaft marked with type cannot be determined. (Fig. 4D). Alterna-

ning, thereby leading to the phenotype of three neu-

rons and one sheath cell in a cluster (Fig. 4D). Alternatively, the IIB cell homozygous for DI Ser mutations might produce one neuron and one sheath cell due to partial transformation or to the occasional supply of the N ligands by other cells such as the sibling IIB cell and its progeny, which were wild type for DI and Ser. This could account for the presence of two neurons and two sheath cells in a cluster. Finally, a cluster of two neurons with no sheath cells could be generated if the mitotic recom-

bination was induced later during the division of the IIB cell (Fig. 4F).

The requirement of N ligand function within the SOL demonstrates that interactions between sibling cells play an important role in specifying cell fates and rules out a situation depicted in model A (Fig. 1). However, our results do not exclude the possibility that surrounding epidermal cells might also contribute as a source of N ligands, in addition to the signaling between the sibling cells within the SOL. Thus, our results are compatible with either model B or C (Fig. 1). This is in contrast to the asymmetric division that gives rise to the embryonic MP2 cells of the CNS, where the N ligand is thought to be supplied exclusively from cells outside the lineage (Spana and Doe 1996).

Ser function is redundant to DI function during wing vein formation

The discovery of the redundant Ser and DI functions prompted us to ask whether Ser also plays a role in other DI–N signaling processes. The Gal4109–68 UAS-FLP also induces FRT recombination in longitudinal wing vein cells. Most members of the N signaling pathway are also involved in the restriction of the venation to a stripe of cells from larger vein-competent territories, a process similar to lateral inhibition during neurogenesis (Sturtevant and Bier 1995; de Celis et al. 1997; Huppert et al. 1997). Removal of DI in wing mosaics led to only modest expansions of the vein into the intervein area (Fig. 5B), whereas removal of Ser had no detectable phenotype (Fig. 5A). In contrast, simultaneous loss of both Ser and DI in wing vein mosaics brought about noticeably larger and more frequent thickening of the veins (Fig. 5C). We believe that Ser is also redundant to DI in wing vein pat-

terning.

The source and identity of the N ligands could be context dependent

Previous studies have shown that Ser can partially sub-

stitute for DI when expressed ectopically at high levels (Gu et al. 1995; Hukriede et al. 1997). In this study we show that these two N ligands serve redundant functions in specifying the fates of the SOP daughter cells, probably by acting in one daughter cell to promote the cell fate specification of the other daughter cell. We found that DI and Ser also have redundant functions in pattern-

ing wing veins. In contrast, DI and Ser are known to serve distinct functions in specifying dorsal–ventral compartment boundary of the wing (wing margin). Ser in dorsal cells signals to N in ventral cells, and DI in ventral cells signals to N in dorsal cells (Diaz-Benjumea and Cohen 1995; Doherty et al. 1996; Jonsson and Knust 1996; de Celis et al. 1997; Michelli et al. 1997). For DI and Ser to provide distinct signals from one compartment to the other without generating signals among cells within the same compartment, it may be necessary to involve other factors such as that encoded by the dorsally expressed gene fringe (fng), which inhibits a cell's ability to respond
heat shocked at 39°C for 1 hr. The genetic crosses were as follows: (1) For N clones, w N91E11 sn FRT[18A]/w Y N° males were crossed to w FRT[18A] FM6; hs-FLP/Cyo females (Fig. 2A). (2) For Dl clones, pr pwn+/ FRT[82B] DlRevF10 e/Ki males were crossed to pr pwn/pr pwn hs-FLP2; FRT[82B] kar ry/FRT[82B] kar ry Dp(2;3) P32[pr pwn+] females (Fig. 2B; Heitzler et al. 1996). Dl90S9, Dl82-23, Dl1G0114, and Dl83rev1 were also tested and found to give similar mosaic phenotype as DlRevF10 (data not shown). (3) For Ser clones, y; FRT[82B] e Ser82; /TM3 males were crossed to y w hs-FLP2; FRT[82B] Pr/y females (Fig. 2C). Ser82T106 and Ser82rev1 gave similar results (data not shown). (4) For DI Ser clones, pr pwn+/ FRT[82B] DlRevF10 e Ser82; /ki males were crossed to pr pwn/pr pwn hs-FLP2; FRT[82B] kar ry/FRT[82B] kar ry Dp(2;3) P32[pr pwn+] (Fig. 2D; Heitzler et al. 1996). Dl82T106, Dl82RevF10 Ser82T106, and DlRevF10 Ser82T106 were also tested (data not shown).

We obtained the same results when the DI, Ser, or DI Ser clones are marked by the loss of Pr/y Pr/y+/null or by Sm (data not shown).

**Materials and methods**

Generation of large mitotic clones

For making large clones, 24- to 48-hr AEL (after egg laying) larvae were

Figure 5. Ser serves a function that is redundant to DI in wing vein patterning. (A) Ser mutant clones; (B) DI mutant clones; (C) DI and Ser double mutant clones. (Arrowheads) Vein hypertrophy.

Ser to DI and pertinuates a cell’s response to DI (Fleming et al. 1997; Panin et al. 1997).

N signaling is used in many processes throughout Drosophila development, such as during oogenesis, neurogenesis, muscle formation, wing patterning, and eye development (for review, see Artavanis-Tsakonas et al. 1995). N homologs also play important roles in developmental processes in organisms ranging from Caenorhabditis elegans, Xenopus, chick, and mouse to man, which may utilize multiple N ligands that are similar to DI and Ser (for review, see Lewis 1996). Whereas some of these developmental processes may employ different N ligands for distinct signaling events, our studies underscore the possibility of multiple N ligands serving redundant functions. In both sensory organ cell fate specifica
tion and wing vein patterning, the function of Ser is revealed only after DI is removed. Thus, multiple N ligands serving redundant function may be more prevalent than previously appreciated. It will be of interest to determine the extent to which the redundant N signaling using multiple ligands is exploited in various developmental contexts.

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