RESEARCH COMMUNICATION

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.

Received January 12, 1998; revised version accepted February 26, 1998.

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 (thecogen), 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 (Spana 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 *D*l 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 N^{ts} 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,

[*Key Words*: *Notch*; *Delta*; *Serrate*; sensory organs; asymmetric cell divisions; *Drosophila*]
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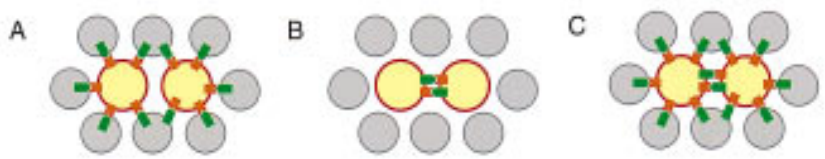

Figure 1. The three possible ways of supplying N ligands during the asymmetric cell divisions within the SOL (after Guo et al. 1996). (*A*) The N ligands could be supplied by non-SOL cells; (*B*) the sibling cells might be signaling to each other; (*C*) a combination of the two scenarios is also possible. (Gray circles) Epidermal cells; (yellow circles) SOL cells; (green ■) N ligands; (brown ■) N receptors.

no external sensory structures (Hartenstein and Posakony 1990; Heitzler and Simpson 1991, 1993; Wang et al. 1997).

Dl 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 *Dl* 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 *Dl* mutant clones indicates that, unlike *N* clones, most of the supernumerary SOPs in the *Dl* 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 *Dl 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 *Dl* and *Ser* function produced epidermal cells but not external bristle structures (Fig. 2D). This balding phenotype is clearly different from the phenotypes of the *Dl* or *Ser* mutant clones but is indistinguishable from that of *N* mutant clones, suggesting that *Ser* and *Dl* have overlapping functions in the *N* signaling pathway.

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

To examine the role of *Dl* and *Ser* in the SOL asymmetric divisions, we used SOL-specific Gal4 enhancer trap lines, Gal4$^{109\text{-}68}$ (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 *Dl* 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 *Dl* 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 *Dl* 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-Benjumea 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 *Dl*. *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

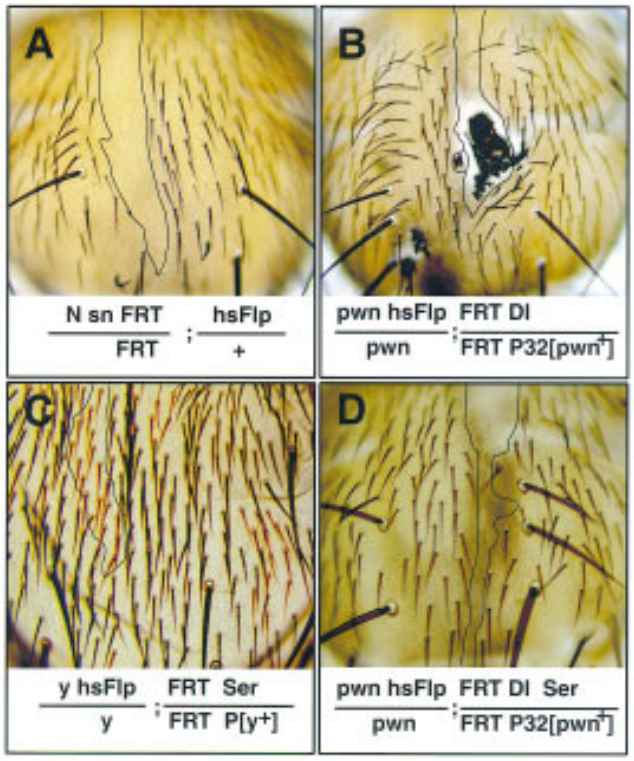

Figure 2. The contribution of *Ser* to cell fate specification of the SOL is uncovered when *Dl* is also removed. Doubly mutant clones of *Dl* and *Ser* (*D*) display a phenotype that is similar to that of *N* (*A*) but different from that of *Dl* (*B*) or *Ser* clones (*C*). For each genotype, a large representative clone (boundaries indicated by black lines) found on similar regions of the adult scutum is shown. (*A*) *N* clones; (*B*) Dl clones; (*C*) *Ser* mutant; (*D*) *Dl Ser* doubly mutant clones.

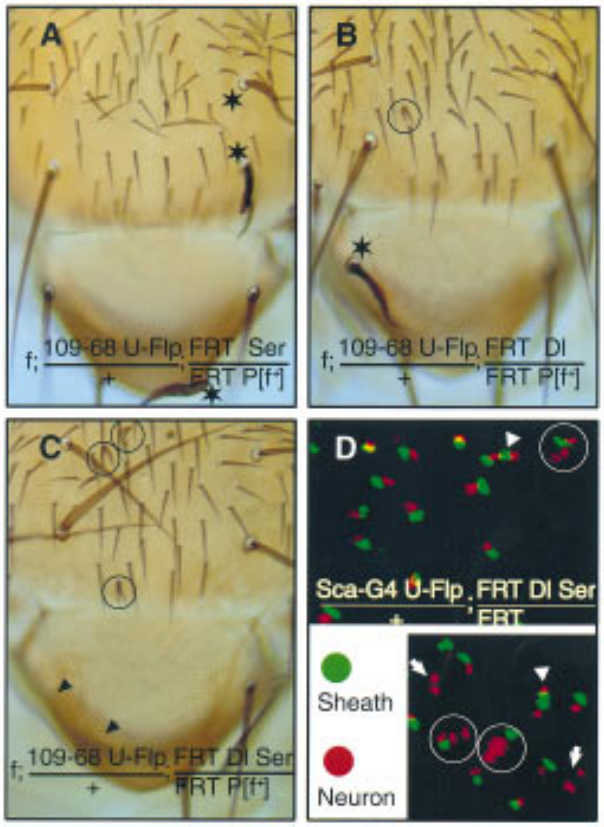

Figure 3. Both Dl and Ser can signal to N receptor during the asymmetric cell divisions within SOL. (*A*) *Ser* mutant macrochaetae, marked by *f*, have a normal complement of external structures (stars). (*B*) *Dl* mutant bristles, such as the macrochaetae (star), do not display cell fate transformation phenotypes, whereas some bristles have double shafts but no sockets (circled). (*C*) *Dl Ser* double mutant clones. (Arrowheads) Loss of external sensory structures, or balding; (circles) double-shaft bristles with no sockets. (*D*) Internal cell fate transformations in *Dl Ser* mutant clones as indicated by immunofluorescent staining of pupal nota 24–30 hr APF with neuron-specific anti-Elav (red) and sheath cell-specific anti-Prospero (green) antibodies. Closely associated clusters of 3 neurons and 1 sheath (encircled), 2 neurons and 2 sheath (arrowheads), and 2 neurons with no sheath (arrows) are indicated; the remaining clusters each have 1 neuron and 1 sheath.

redundant function of *Dl*. Only when *Dl* function is reduced or eliminated can we detect the contribution of *Ser* to SOL cell fate specification. Similarly, although *Dl* is essential for lateral inhibition and loss of *Dl* function produces a strong neurogenic phenotype, the function of *Dl* during the subsequent asymmetric cell divisions can be substituted almost entirely by *Ser*. These observations reinforce the notion that Ser and Dl are redundant signals to N for the specification of daughter cell fates of asymmetric cell divisions in the SOL.

The source of N ligands resides within the SOL during the asymmetric cell divisions

A current model of sensory organ development postulates 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^{ts} 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 $Gal4^{109\text{-}68}$ 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,

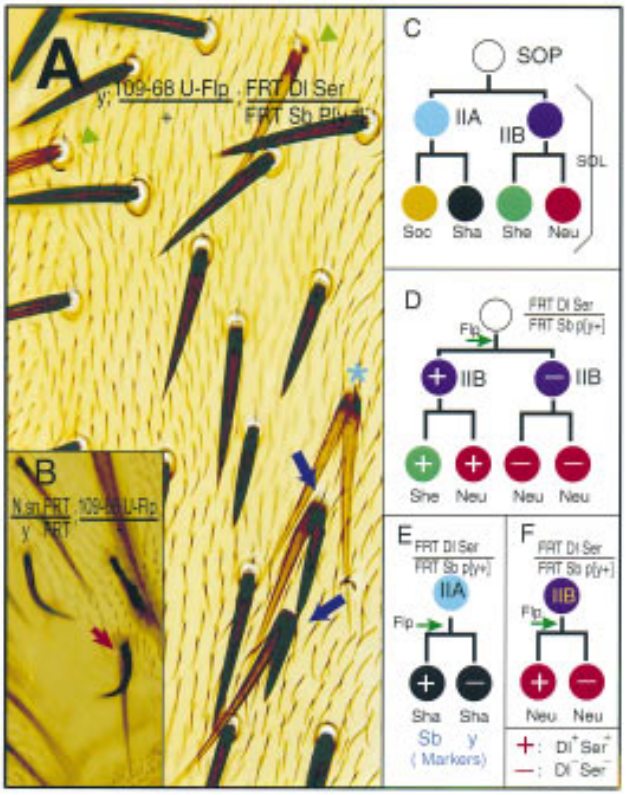

Figure 4. The sensory organ cell fate specification is mainly mediated by N signaling between the daughter cells of asymmetric divisions. (*A*) *Dl Ser* double-mutant clones. Cell fate transformations is manifested by two distinctly marked shafts without associated sockets (purple arrows). The *Dl Ser* mutant shaft is long (Sb^+) and light (*y*), whereas its sibling, the Dl^+ Ser^+ shaft is short (*Sb*) and dark (y^+). The other two shafts, both Sb^+ and *y* (star), could be due to an earlier loss of *Dl* and *Ser* from the parental IIA cell, which then divides symmetrically to produce two shafts. Two light (*y*) *Dl Ser* mutant shafts (green arrowheads) are found each associated with one socket, whose genotype cannot be determined. (*B*) A mutant bristle consists of one *N* mutant shaft marked with *sn* and y^+ and one shaft wild type for *N* marked with sn^+ and *y*. (*C*) The fixed pattern of cell divisions in a wild-type SOL. (*D*) One possible outcome of mitotic recombination during the SOP division. (*E*) A possible mechanism for the phenotype shown in *A*. (*F*) Mitotic recombination in the division of the IIB cell could lead to the two neurons with no sheath cell phenotype, as pictured in Fig. 3D. (+) The cell is wild type for *Dl* and *Ser;* (−) the cell is homozygous mutant for *Dl* and *Ser*.

could produce two neurons due to the failure in *N* signaling, thereby leading to the phenotype of three neurons and one sheath cell in a cluster (Fig. 4D). Alternatively, the IIB cell homozygous for *Dl 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 *Dl* 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 recombination 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* Dl *function during wing vein formation*

The discovery of the redundant *Ser* and *Dl* functions prompted us to ask whether *Ser* also plays a role in other Dl–N signaling processes. The $Gal4^{109\text{-}68}$ 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 *Dl* 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 *Dl* 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 *Dl* in wing vein patterning.

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

Previous studies have shown that Ser can partially substitute for Dl 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 Dl and Ser also have redundant functions in patterning wing veins. In contrast, Dl 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 Dl 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; Micchelli et al. 1997). For Dl 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

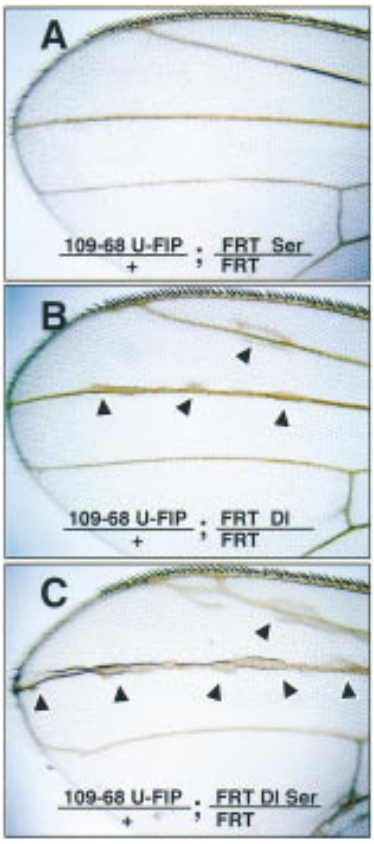

Figure 5. *Ser* serves a function that is redundant to *Dl* in wing vein patterning. (*A*) *Ser* mutant clones; (*B*) *Dl* mutant clones; (*C*) *Dl* and *Ser* double mutant clones. (Arrowheads) Vein hypertrophy.

to Ser and potentiates a cell's response to Dl (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 Dl 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 specification and wing vein patterning, the function of Ser is revealed only after Dl 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.

Materials and methods

Gereration of large mitotic clones

For making large clones, 24- to 48-hr AEL (after egg laying) larvae were heat shocked at 39°C for 1 hr. The genetic crosses were as follows: (1) For *N* clones, *w* N^{55e11} *sn* FRT[18A]/*w* Y N^{+} males were crossed to *w* FRT[18A]/FM6; hs–FLP^{2}/CyO females (Fig. 2A). (2) For *Dl* clones, *pr pwn*/+; FRT[82B] Dl^{RevF10} *e*/*Ki* males were crossed to *pr pwn*/*pr pwn* hs–FLP^{2}; FRT[82B] *kar ry*/FRT[82B] *kar ry* Dp(2;3) P32[pwn^{+}] females (Fig. 2B; Heitzler et al. 1996). Dl^{9p39}, $Dl^{82\text{-}23}$, Dl^{10G114}, and $Dl^{8c3rev1}$ were also tested and found to give similar mosaic phenotype as Dl^{RevF10} (data not shown). (3) For *Ser* clones, *y w;* FRT[82B] *e* Ser^{Rx82}/TM3 males were crossed to *y w* hs–FLP^{1}; FRT[82B] P[y^{+}] females (Fig. 2C). Ser^{Rx106} and $Ser^{rev6\text{-}1}$ gave similar results (data not shown). (4) For *Dl Ser* clones, *pr pwn*/+; FRT[82B] Dl^{RevF10} *e* Ser^{Rx82}/*Ki* males were crossed to *pr pwn*/*pr pwn* hs–FLP^{2}; FRT[82B] *kar ry*/ FRT[82B] *kar ry* Dp(2;3) P32[pwn^{+}] (Fig. 2D; Heitzler et al. 1996). Dl^{9p39} Ser^{RX106}, Dl^{RevF10} Ser^{Rx106}, and Dl^{RevF10} $Ser^{r6\text{-}1}$ were also tested (data not shown).

We obtained the same results when the *Dl*, *Ser*, or *Dl Ser* clones are marked by the lost of P[y^{+}], P[f^{+}], pwn^{+} or by *Sb* (data not shown).

Generation of SOL-specific mitotic clones

For making SOL-specific clones, *y w* f^{36a}; Gal4$^{109\text{-}68}$ P[UAS–FLP, w^{+}]^{2}; FRT[82B] P[f^{+}] females were crossed to the following males at 25°C, and male progeny was scored for phenotype. (1) For *Ser* mosaics, *y w;* FRT[82B] Ser^{Rx82}/TM3 (Fig. 3A). Ser^{Rx106} and $Ser^{rev6\text{-}1}$ gave similar results (data not shown). (2) For *Dl* mosaics, *y w;* FRT[82B] Dl^{RevF10}/TM3 (Fig. 3B). Dl^{9p39}, $Dl^{82\text{-}23}$, Dl^{10G114}, and $Dl^{8c3rev1}$ were also tested (data not shown). (3) For *Dl Ser* mosaics, *y w;* FRT[82B] Dl^{RevF10} Ser^{Rx82}/TM3 (Fig. 3C). Dl^{9p39} Ser^{RX106}, Dl^{RevF10} Ser^{Rx106}, and Dl^{RevF10} $Ser^{r6\text{-}1}$ were also tested (data not shown).

For doubly marked *Dl Ser* mosaics, *y w;* Gal4$^{109\text{-}68}$ P[UAS–FLP, w^{+}]^{2}; FRT[82B] Sb^{63b} P[y^{+}] females were crossed to *y w;* FRT[82B] Dl^{RevF10} Ser^{Rx82}/TM3 males (Fig. 4A).

For doubly marked *N* mosaics, *w* N^{55e11} *sn* FRT[18A]/FM6 females were crossed to *y w* FRT[18A]; Gal4$^{109\text{-}68}$ P[UAS–FLP, w^{+}]^{2}/CyO males (Fig. 4B).

For examining neuron–sheath cell fate decisions, *Dl Ser* mosaics were produced by crossing *y w;* Sca–Gal4 P[UAS–FLP, w^{+}]^{2}; FRT[82B] P[y^{+}] females to *y w;* FRT[82B] Dl^{RevF10} Ser^{Rx82}/TM6B males. Non-*Tb* pupae were collected at 24–30 hr APF (after pupal formation) for dissection.

Immunostaining of the dissected pupal nota

Pupal nota were dissected in PBS and fixed for 10 min in 5% formaldehyde. Neuronal maker mouse mAb 9F8A9 against Elav (obtained from Developmental Studies Hybridoma Bank, University of Iowa) was used at 1:100 dilution and detected by Cy3-conjugated secondary antibodies. Sheath cell marker rabbit anti-Prospero antibody (Vaessin et al. 1991) was used at 1:1000 dilution and detected by FITC-conjugated secondary antibodies. A Z-series was obtained from MRC-600 confocal microscope and assembled in Adobe Photoshop and Adobe Illustrator (Fig. 2D).

Acknowledgments

We thank J. Duffy, D. Harrison, and N. Perrimon for allowing us to use P[UAS–FLP, w^{+}]^{2} transgenic lines before publication; T. Xu, J.A. Campos-Ortega, R.J. Fleming, E. Knust, P. Simpson, P. Heitzler, M. Haenlin, S.B. Carroll, V.G. Corces, H.-M. Chung, G. Struhl, and the Bloomington Stock Center for fly strains, antibodies, and DNA sequences; and S. Abdelilah, D. Doherty, Y.M. Chan, J. Brenman, L. Ma and other members of the Jan laboratory for discussion and comments on the manuscript. C.Z. is supported by a postdoctoral fellowship from the National Institutes of Health (Public Health Service 416-5). S.Y.-S. is a research associate and L.Y.J. and Y.N.J. are investigators of the Howard Hughes Medical Institute.

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