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Implications of dynamic patterns of Delta and Notch expression for cellular interactions during *Drosophila* development

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SUMMARY

Delta and Notch function are required for cell fate specification in numerous tissues during embryonic and postembryonic *Drosophila* development. Delta is expressed by all members of interacting cell populations within which fates are being specified and is subsequently down-regulated as cells stably adopt particular fates. Multiphasic expression in the derivatives of many germ layers implies successive requirements for Delta function in a number of tissues. At the cellular level, Delta and Notch expression are generally coincident within developing tissues. At the subcellular level, Delta and Notch are localized in apparent endocytic vesicles during down-regulation from the surfaces of interacting cells, implying an interaction consistent with their proposed roles as signal and receptor in cellular interactions during development.

Key words: *Delta*, neurogenic gene, *Drosophila*

INTRODUCTION

Genetic and molecular data indicate that intercellular interactions are necessary for specification of the correct number of epidermal and neural derivatives during embryonic and postembryonic neurogenesis in insects (Doe and Goodman, 1985; Technau and Campos-Ortega, 1986; Simpson, 1990). Genetic analyses of the zygotic neurogenic gene, *Delta* (*Dl*), have demonstrated the importance of proper *Dl* function for the proper establishment of neuroblasts and dermoblasts within the embryonic neurogenic regions (Lehmann et al., 1983) and of bristle precursors and epidermal cells within imaginal proneural groups (Heitzler and Simpson, 1991; T. R. Parody and M. A. T. Muskavitch, unpublished data; A. L. Parks and M. A. T. Muskavitch, unpublished data). Mutational analyses have shown that *Dl* function is pleiotropic and is required in many tissues and during several developmental stages (Vässin et al., 1985; T. R. Parody and M. A. T. Muskavitch, unpublished data). Genetic interactions that involve *Dl* and *Notch* (*N*), as well as other neurogenic genes, imply the existence of direct interactions between the products of *Dl* and *N* in vivo (de la Concha et al., 1988; Shepard et al., 1989; Brand and Campos-Ortega, 1990; Xu et al., 1990). This hypothesis is further supported by the coincidence of numerous phenocritical periods for *Dl* and *N* function during metamorphosis (Shellenbarger and Mohler, 1978; T. R. Parody and M. A. T. Muskavitch, unpublished data).

Dl and *N* encode transmembrane cell surface proteins ('Delta'; Vässin et al., 1987; Kopczynski et al., 1988; and 'Notch'; Wharton et al., 1985; Kidd et al., 1986) with extracellular domains that include tandem repeats of a motif related to vertebrate epidermal growth factor (EGF). This motif is found in an array of proteins that are known to be involved in protein-protein interactions (Davis, 1990). Experiments using cultured cells (Fehon et al., 1990) provided evidence of physical interaction between Delta and Notch and suggested aspects of a mechanism by which these proteins mediate intercellular communication during development, but could not determine whether this interaction was significant in vivo. Furthermore, somatic mosaic analyses have revealed that Delta functions nonautonomously in establishing epidermal identity, whereas Notch functions autonomously, consistent with the hypothesis that Delta serves as a signal that interacts with a receptor encoded by *N* (Heitzler and Simpson, 1991; Artavanis-Tsakonas and Simpson, 1991).

To acquire a deeper understanding of Delta function in vivo, we have examined Delta expression at the cellular and subcellular levels throughout development, and compared Delta expression to that of Notch. Our results indicate that Delta is expressed within all three germ layers of the embryo, and in a variety of larval and pupal tissues, consistent with the myriad embryonic and imaginal defects observed in *Dl* mutants. All cells within neurogenic regions and proneural groups initially express Delta, and expression

is down-regulated as cells stably adopt 'neural' fates. Delta expression is reactivated in the late embryonic and larval central nervous systems, as well as in a variety of cells in the developing imaginal peripheral nervous system. Delta and Notch expression are generally coextensive at the cellular level within interacting cell populations. At the subcellular level, Delta is localized initially to the cell surface during embryonic and postembryonic development. Subsequent down-regulation of Delta from the surfaces of interacting cells involves endocytosis from the cell surface. We discuss the significance of the cellular and subcellular dynamics of Delta and Notch expression in relation to their functions in intercellular interactions that are central to the specification of cell fates in a variety of tissues during development.

MATERIALS AND METHODS

Antibodies

Mouse polyclonal sera M585 and M5 and rat polyclonal serum R135 were prepared essentially as described in Fehon et al. (1990). The 0.54 kb *Cla*I DNA fragment encoding Delta EGF-like repeats 4-9 (amino acids 350-529) was excised from the Dl1 cDNA (Kopczynski et al., 1988) and transferred into the pUR288 vector. Fusion protein prepared by isolation of inclusion bodies (Gilmer et al., 1982) was used to immunize mice or rats. The specificity of the anti-Delta antisera employed in this analysis is confirmed by three lines of evidence. First, anti-Delta polyclonal antisera were used to stain embryos collected from a stock heterozygous for a deficiency that eliminates the Delta gene [*Df(3R)Dl^{M2}/TM6C;* Alton et al., 1988]. All anti-Delta antisera employed for immunohistochemistry stained wild-type embryos and exhibited no staining, either associated with the cell surface or vesicular in character, of neurogenic mutant embryos homozygous for *Df(3R)Dl^{M2}*. Second, the anti-Delta antisera employed in this analysis exhibit staining patterns identical to those of Delta-specific monoclonal antibodies we have recently obtained (A. L. Parks and M. A. T. Muskavitch, unpublished data). Third, anti-Delta antisera stained *Drosophila* S2 tissue-culture cells that express Delta following transfection with pMTDl1 (Fehon et al., 1990) and did not stain control untransfected S2 cells. Notch mouse polyclonal antisera and rat polyclonal serum R1 were produced in the laboratory of S. Artavanis-Tsakonas, Yale University. Mouse polyclonal sera were raised against four different regions of the extracellular domain of Notch as described in Fehon et al. (1990). Rat polyclonal antiserum R1 was prepared from a rat injected with antigen that included the Notch EGF-like region contained within the 0.791 kb *Bst*YI fragment from the *N* gene (Fehon et al., 1990). Anti-Notch sera stained Notch-expressing S2 cells, while control cells remained unstained (Fehon et al., 1990) and stained wild-type embryos in the patterns expected for Notch, as described previously (e.g., Fehon et al., 1991).

Immunohistochemistry

Embryos were dechorionated, fixed for 20 minutes in a 1:1 (v/v) mixture of fresh 4% (w/v) paraformaldehyde in PBS:heptane, and then devitellinized with methanol, as described elsewhere (Johansen et al., 1989). After devitellinization, the embryos were rehydrated in PBS and incubated in block solution [10% (v/v) NGS (Sigma), 0.5% (v/v) Triton X-100 in PBS] for 1 hour. Fresh block solution containing the primary antibodies was added to the embryos and the mixture was incubated overnight (O/N) at 4°C with gentle rocking. Embryos were rinsed three times in PBS and incubated in block solution for 1 hour at room temperature (RT). Horseradish peroxidase (HRP)- or fluorochrome-conjugated secondary antibodies were reconstituted according to manufacturers instructions and were then added as described below.

For HRP staining, goat anti-mouse IgG conjugated to HRP (GAM-HRP, Jackson Immunoresearch) was added at a dilution of 1:1500 in block solution. All other incubations were as described in Johansen et al. (1989). Following development of the stain with diaminobenzidine (DAB) and H_2O_2 (Johansen et al., 1989), embryos were dehydrated in ethanol and cleared in methyl salicylate. Embryos were mounted in methyl salicylate and viewed by Nomarski optics. Some embryos were transferred to xylene from the ethanol, and then to paraffin and sectioned to 5μm thickness as described in Kopczynski and Muskavitch (1989).

For immunofluorescence, GAM-FITC (Jackson Immunoresearch, Multilabel grade) was added at a dilution of 1:250 in block solution, and the embryos were either incubated for 4 hours at RT or O/N at 4°C. After the incubation in secondary antibody, the embryos were rinsed three times with PBS, blocked for 1 hour, and rinsed once more in PBS. Embryos were mounted in 9:1 (v/v) glycerol:1 M Tris, pH 8.0, which contained 0.5% (w/v) *n*-propyl gallate. Imaginal discs were dissected in M3 tissue culture medium (Shields and Sang, 1977) containing 12.5% (v/v) fetal calf serum and fixed in 2% (w/v) paraformaldehyde in PBS for 5 minutes at RT. All other incubations were as described above, except that the blocking solution contained 10% (v/v) NGS and 0.1% (w/v) saponin in PBS. Larval brains were dissected in M3 medium, and incubated in 2 mg/ml collagenase (type II, Sigma) in M3 for 20 minutes. Brains were rinsed three times in PBS, fixed in 2% (w/v) paraformaldehyde for 10 minutes at RT, and incubated as described above for imaginal discs. For double-labelling, Delta mouse and Notch rat polyclonal sera were added simultaneously to the tissue. FITC-GAM (1:250, Jackson Immunoresearch, Multilabel grade) and TR-GAR (1:500, Jackson Immunoresearch, Multilabel grade) were used for detection.

Microscopy

Nomarski images were obtained from a Nikon Photoscope with DIC optics, and photographed using Kodacolor Gold 100 film. Most confocal images were collected using a BioRad MRC500 system and MRC-850 laser attached to a Zeiss Axiovert compound microscope, while some were collected using a BioRad MRC600 system and MRC-850 laser attached to a Nikon compound microscope. Merged images in Fig. 5 and the corresponding single channel images were obtained from a BioRad MRC600 system with a MRC-860 laser, attached to a Zeiss Axiovert compound microscope. Single channel fluorescein images were obtained using the 'BHS' filter block. Single channel images were collected from double-labelled preparations using the A1 and A2 filter blocks. The BioRad software 'som' and the programs 'base' and 'scale' were used to produce all images. Additive combination of sections to give linear projections was performed using the 'project' program. Image files were transferred via disc to a Macintosh IIcx computer using Apple File Exchange, and were reformatted to PICT format using IP Lab Spectrum (color version, IP Labs). The A1 and A2 channel images were merged using the IP Lab Spectrum program 'merge' command to produce a two-color image. Images produced using a MacIIcx microcomputer were recorded using Lasergraphics Film Recorder and the MacRascol RIP program (Lasergraphics) onto TMax 100 or Kodacolor 100 film.

Drosophila strains

All strains were grown on standard cornmeal-molasses-yeast-agar medium at 25°C unless other temperatures were mentioned. The wild-type strain was an Oregon-R isolate from Stanford Univer-

sity. Embryos were collected from stocks containing the following genotypes: (1) *Df(3R)Dl^{M2}/TM6C* (Alton et al., 1988); (2) *bib^{1D05}/CyO* (Lehmann et al., 1983); (3) *mam^{IB99}/CyO* (Lehmann et al., 1983); (4) *mam^{IJ113}/CyO* (Lehmann et al., 1983); (5) *neu^{IF65}/TM6B* (Lehmann et al., 1983); (6) *neu^{9L119}/TM6B* (Lehmann et al., 1983); (7) *Df(3R)E(spl)^{BX22} tx/TM6B* (Shepard et al., 1989); (8) *Df(3R)E(spl)^{BX36} tx/TM6B* (Shepard et al., 1989); (9) *w^a N^{81k1} rb/FM7C, y^{31d} sc^8 w^a sn^{x2} v B X FM7C, y^{31d} sc^8 w^a sn^{x2} v B/Y* (Grimwade et al., 1985); (10) *N^{81l4}/FM7C, y^{31d} sc^8 w^a sn^{x2} v B X FM7C, y^{31d} sc^8 w^a sn^{x2} v B/Y* (Grimwade et al., 1985); (11) *N^{81l6}/FM7C, y^{31d} sc^8 w^a sn^{x2} v B X FM7C, y^{31d} sc^8 w^a sn^{x2} v B/Y* (Grimwade et al., 1985); (12) *y w^a fa^g Ax^{59b8} N ^{x}/FM7C, y^{31d} sc^8 w^a sn^{x2} v B X FM7C, y^{31d} sc^8 w^a sn^{x2} v B/Y* (Portin, 1975; Kelley et al., 1987). One-quarter of the embryos collected from each of these stocks is hemizygous or homozygous for the chromosome that carries the mutated neurogenic gene and will display the neurogenic phenotype. Flies were presented with egg laying plates containing standard medium supplemented with a paste made of baker's yeast and water. The flies were conditioned on yeasted egg laying plates replaced daily for three days. Prior to the embryo collection, the flies were given a fresh egg laying plate for 1 hour to prevent them from holding eggs. Embryos were collected at 25˚C on freshly yeasted egg laying plates.

RESULTS

We have used Delta-specific polyclonal antibodies raised against a subset of the Delta extracellular domain EGF-like repeats to examine the distribution of Delta during development. These antisera are Delta-specific as judged by antibody binding in embryos and cultured cells (see Materials and Methods). We have also employed Notch-specific polyclonal antibodies, raised against a subset of the Notch extracellular domain EGF-like repeats (Fehon et al., 1990), to compare the distributions of Delta and Notch within the same tissues and cells. Embryonic stages referred to below have been defined by Campos-Ortega and Hartenstein (1985) and embryonic times correspond to hours of development after oviposition at 25°C.

Delta is dynamically regulated during gastrulation and germ band extension

Delta is first detectable in the cortical membrane of precellular blastoderm embryos and within invaginating cell membranes as cellularization proceeds (data not shown). This subcellular pattern of Delta accumulation results in a reticular network of stained cell membranes when the embryo is viewed in tangential sections (Fig. 1A), consistent with the prediction that *Dl* encodes a transmembrane protein (Vässin et al., 1987; Kopczynski et al., 1988) and observations of Delta subcellular localization in cultured cells (Fehon et al., 1990).

Another type of Delta subcellular localization becomes apparent shortly before the onset of gastrulation when levels of Delta rapidly decrease in a ventral band approximately 15-18 cells wide (Fig. 1A,C) that corresponds to the presumptive mesoderm (Leptin and Grunewald, 1990). This decrease in Delta expression coincides with the appearance of profuse vesicular subcellular staining (Fig. 1A-C), which is most prevalent in the cortical cytoplasm basal to nuclei (Fig. 1B). Two lines of evidence support the inference that this vesicular staining accurately reflects an aspect of Delta subcellular localization and does not represent general background staining. First, vesicular Delta staining is regionally and developmentally specific, appearing in the presumptive mesoderm, restricted regions of the posterior midgut, the larval central nervous system and specific regions in the eye-antennal imaginal disk (e.g., Fig. 1A,D). Second, similar vesicular staining has been described previously for the structurally related *Drosophila* Serrate protein in embryonic tissues (Thomas et al., 1991). The observation that vesicularization of Delta is associated with decreased expression suggests that these vesicles are associated with endocytosis from the cell membrane (Geuze et al., 1983; Stoorvogel et al., 1991), rather than synthesis and transport.

Delta is expressed in all ectodermal cells within the ventral neurogenic region (vNR, Fig. 1E), as well as mesectodermal cells, preceding and during the first and subsequent phases of neuroblast segregation (Campos-Ortega and Hartenstein, 1985). We do not detect any differences in Delta expression among cells in the vNR population, which includes emergent neuroblasts that will delaminate within the vNR at the beginning of embryonic neurogenesis (Fig. 1E). While Delta surface staining is prominent within the ectoderm during this interval, we also detect vesicles similar to those we observe in the mesodermal anlage preceding gastrulation (Fig. 1B,C). These vesicles appear to be uniformly distributed among cells within the vNR (Fig. 1E). Delta is also expressed apically and in subcellular vesicles within the posterior midgut primordium during germ band extension (Fig. 1D).

Delta expression is asymmetric among ectodermal and other germ layer derivatives in extended germ band embryos

While Delta is associated with the membranes of neuroblasts as they delaminate from the peripheral ectoderm (Fig. 1E), Delta is not apparent within the layer of neuroblasts established between the embryonic epidermis and mesoderm after neuroblast segregation (Fig. 1F,G; 6 hours post-oviposition, late stage 10). However, Delta continues to be expressed in the dermoblasts of the developing epidermis during and after neuroblast segregation (Fig. 1F-H). Delta expression is apparently reduced in metamerically arrayed groups of cells within the developing epidermis that correspond to the presumptive labial segment, salivary glands and tracheal pits, respectively (Fig. 1F, data not shown).

Delta remains undetectable within the mesoderm following ventral furrow formation and during early germ band extension. (Fig. 1E; 4 hours post-oviposition, stage 9). Transient Delta expression in the developing mesoderm during late neuroblast segregation results in a 'two-stripe' pattern of ectodermal and mesodermal expression in the extended germ band embryo (Fig. 1F,G). At this time, partitioning of mesoderm into somatopleura and splanchnopleura occurs (Campos-Ortega and Hartenstein, 1985). Delta is also expressed within the procephalic neurogenic region and within endodermal derivatives including the anterior midgut and posterior midgut invaginations and a portion of the hindgut (Fig. 1E,F,H). Delta expression is much reduced in slightly older embryos (6.5-7 hours post-oviposition, stage 11) and appears to be restricted to the

developing epidermis and posterior midgut (Fig. 1H). These data reveal the complex Delta expression pattern in derivatives of the three embryonic germ layers during early embryogenesis.

Delta expression was also assessed in extended germ band embryos homozygous for severe loss-of-function mutations in several neurogenic genes including *N*, *big brain*, *mastermind*, *neuralized* and *Enhancer of split* (alleles listed in Materials and Methods). This analysis revealed that Delta is expressed in the mesoderm and endoderm of

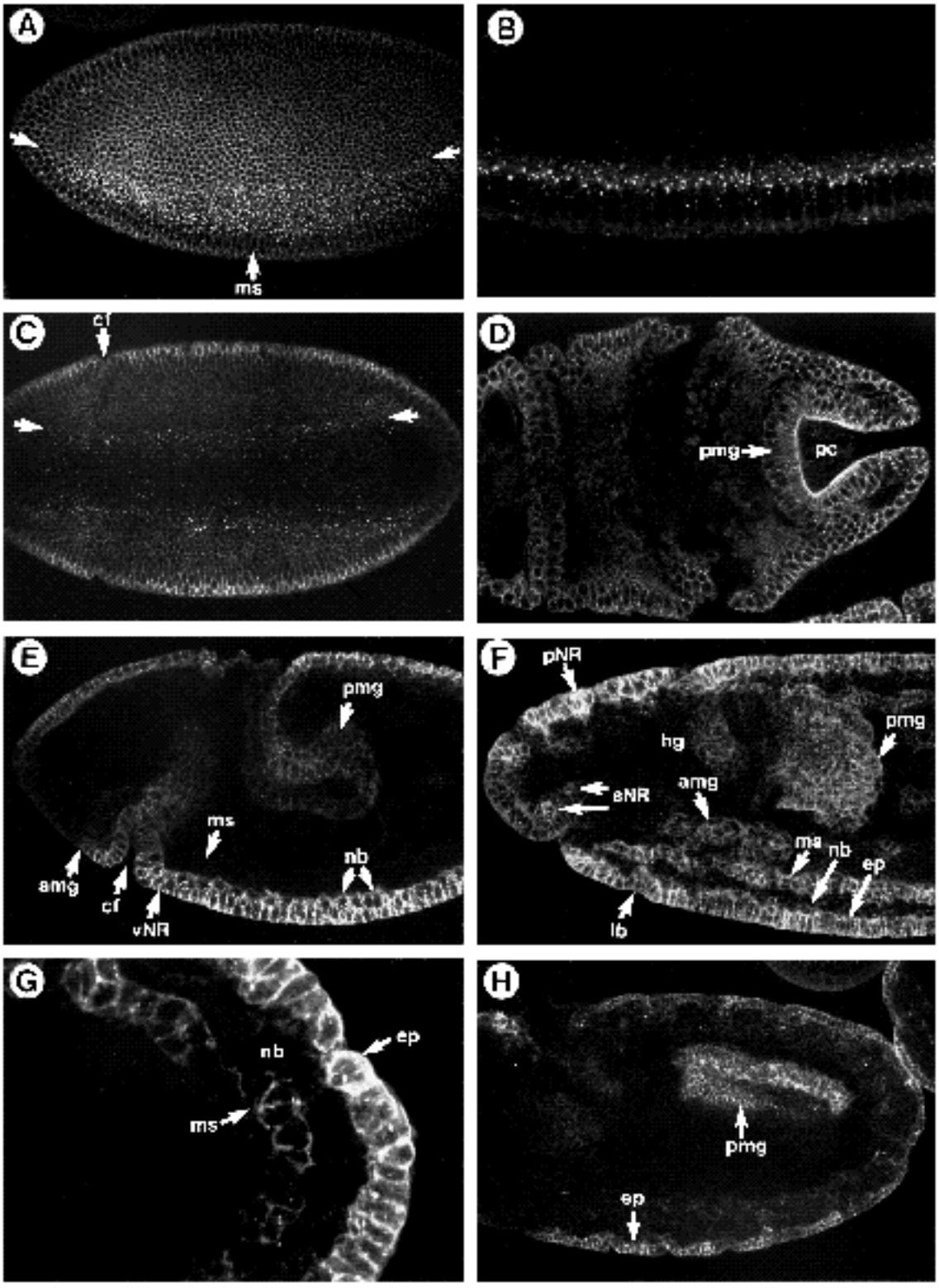


Fig. 1

extended germ band embryos, but not in neuroblasts, in each of the neurogenic mutants tested [e.g., *Df(3R)E-(spl)^{BX22}* homozygote, Fig. 2D; compare to wild type in Fig. 2C]. Thus, Delta expression is correctly regulated in all three germ layers in each of these neurogenic mutant backgrounds.

Delta expression is tissue-specific and highly restricted in contracted germ band embryos

Delta expression is complex in the developing nervous system and a number of other tissues during germ band contraction. Delta is expressed in several discrete clusters of neuronal cells (or sensory ganglia) within the cephalic region of the contracted germ band embryo (11 hours post-oviposition, stage 14; Fig. 2A,B) that appear to include the primordia of the optic lobes, the stomatogastric nervous system or antennomaxillary complex and the epiphysis (Fig. 2A,B). Delta is also expressed apically in the tracheal trunks, hindgut and pharynx, as well as in the proesophageal ganglion and anterior and posterior midgut (Fig. 2A,B).

Delta expression in the developing ventral nerve cord (VNC) of slightly older embryos (11.5 hours post-oviposition, late stage 14/early stage 15, Fig. 2B) occurs in metamerically arrayed groups of cells along the dorsal and ventral aspects of the VNC. Expression in the dorsal aspect of the VNC, which other views reveal to be along the midline of the central nervous system (CNS, data not shown), appears to correspond to dividing midline cells that will give rise to glia, the perineural sheath and neurons (Campos-Ortega and Hartenstein, 1985; Truman and Bate, 1988). Expression in the ventral aspect of the VNC apparently corresponds to dividing neuroblasts and/or their progeny (Campos-Ortega and Hartenstein, 1985). Delta expression is reduced or absent within the center of the VNC, where neuronal cell bodies are located (Fig. 2B).

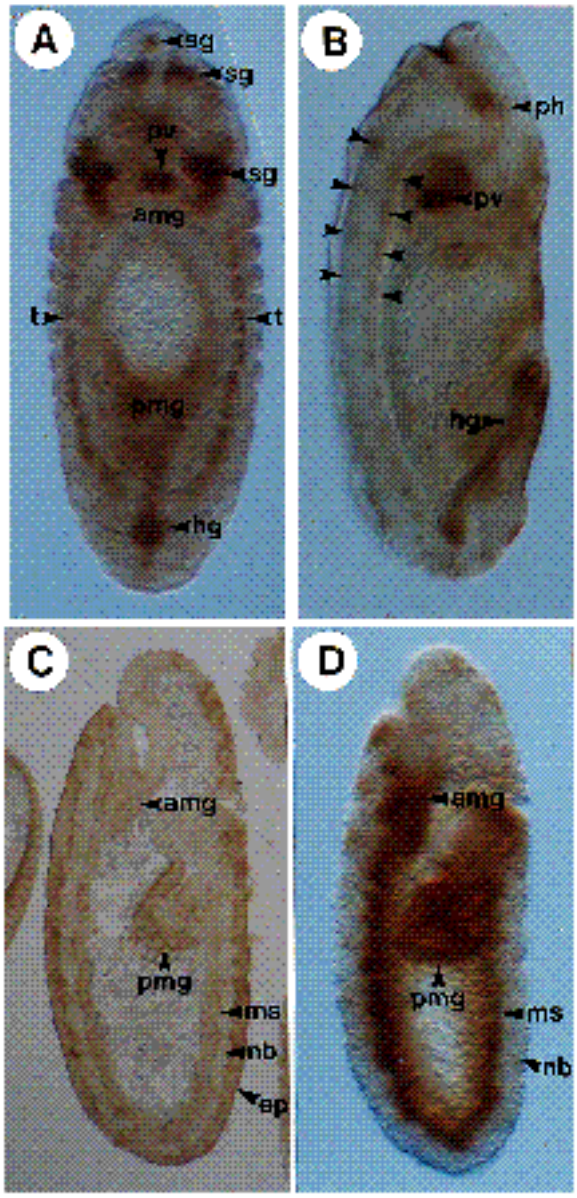
Delta is expressed in neuroblasts of the larval CNS and in ommatidial clusters in eye-antennal imaginal discs

Delta expression in the postembryonic nervous system is first evident in membranes of neuroblasts that begin to divide in brain hemispheres of first instar larvae (data not shown). Delta is later expressed in CNS neuroblasts and their progeny in second instar (L2, Fig. 3A) and third instar (L3, Fig. 3C) larvae and within the developing proliferation centers (data not shown). Neuroblast-derived cell clusters of the L3 CNS express Delta in membranes and cytoplasm, and exhibit a profusion of vesicles (Fig. 3C) that appear to reflect the same aspects of subcellular trafficking that produce vesicular staining observed in embryos (Fig. 1A-C). Delta is expressed in three segmentally arrayed 'rungs' within the ventral ganglion of the L3 CNS (Fig. 3E). Delta is also expressed at low levels in the cells located along the midline of the ventral ganglion (data not shown) that have been suggested to be glial in origin (Fehon et al., 1991).

Dl mutations can result in a variety of morphological defects in the eye, including reduction in eye size, eye scarring, glossiness, bristle multiplication and bristle loss (T. R. Parody and M. A. T. Muskavitch, unpublished data), suggesting that Delta function is required for correct development of most, if not all, cell types within the retina. Delta expression within eye discs isolated from L3 larvae is first evident on the surfaces of unpatterned cells ahead of the morphogenetic furrow (data not shown), consistent with the finding that Delta function is required for proper formation of photoreceptor clusters that emerge from the morphogenetic furrow (A. L. Parks and M. A. T. Muskavitch,

Fig. 1. Delta protein is expressed in specific tissues during early embryogenesis. Confocal optical sections through blastoderm (A-C) or extended germ band (D-H) embryos stained for Delta protein with mouse polyclonal serum M5. Anterior is left for all panels. Dorsal is up in A, B and E-H. (A) Delta staining in a lateral sagittal section through a precellular blastoderm embryo (stage 5). Ectodermal cells located dorsally in relation to the border between the mesoderm and ectoderm (arrows) exhibit membrane-association of Delta. The ventral-most cells, which constitute the mesodermal anlage (ms), accumulate less Delta than ectodermal cells. (B) High magnification image of vesicular Delta staining in the ventrolateral mesoderm from a precellular blastoderm embryo (stage 5). Vesicular staining is concentrated in cortical cytoplasm within the basal halves of the cells and is not obviously associated with developing cell membranes. (C) Delta staining in a horizontal section through the ventral surface of an embryo at the beginning of gastrulation (stage 6). Cephalic folds (cf) are forming. Vesicular Delta staining is concentrated at the border between the mesoderm and ventral ectoderm (arrows), and clearance of Delta from the membranes of mesodermal cells is almost complete. (D) Horizontal section through the dorsal aspect of an early extended germ band embryo (stage 7). Delta is expressed at high levels in the posterior midgut (pmg) invagination, but is present at much lower levels, or is absent, in pole cells (pc). Membrane-associated Delta is concentrated on the apical surfaces of the cells that surround the pole cells. (E) Delta staining in a mid-sagittal section through an extended germ band embryo at the start of neuroblast segregation (stage 9). Delta protein is present on the surfaces of all cells within the ventral neurogenic region (vNR), including delaminating neuroblasts (nb, arrows). Delta accumulation in vNR cells is composed of vesicular and cell surface-associated patterns. Delta is also expressed within the dorsal procephalic region, the cephalic furrow (cf) and the dorsal aspect of the posterior midgut (pmg). Delta accumulation is less intense within the ventral aspect of the procephalic region, containing the anterior midgut (amg) primordium and the stomodeum, the mesoderm (ms) overlying the ventral neurogenic region and the ventral half of the posterior midgut invagination. (F) Sagittal section through an extended germ band embryo (stage 10) after neuroblast segregation has begun. Delta is absent from the delaminated neuroblasts (nb), but is present in the mesoderm (ms) and epidermis (ep). Delta is present in the procephalic neurogenic region (pNR), developing stomatogastric nervous system (sNR), posterior midgut, anterior midgut and hindgut (hg). Delta accumulates to lower levels in presumptive labial ectoderm (lb) than in adjacent thoracic ectoderm or presumptive maxillary and mandibular ectoderm. (G) Higher magnification image of a sagittal section through the posterior pole of an extended germ band (late stage 10) embryo. Segregated neuroblasts (nb) contain little or no Delta, but high levels of Delta accumulate in mesoderm (ms) and epidermis (ep). (H) Sagittal section through an older extended germ band embryo (late stage 11). Delta persists in the posterior midgut (pmg) and the epidermis (ep), but expression in the mesoderm is drastically reduced compared to the previous stages. Delta is not expressed in neuroblasts or their progeny, located just above the epidermis, at this time. Magnification: A,C,D,E,F,H ×156; B,G ×517.

unpublished data). Delta expression is next apparent in clusters of cells that are first seen within the morphogenetic furrow and extend in a regular array throughout the developing retina that lies behind the furrow (Fig. 3G). Delta accumulation in cells within this region of the developing retina appears to be restricted to apical vesicles near the center of each developing ommatidium (Fig. 3H). Similar vesicular staining has been described for the sevenless protein by Cagan et al. (1992), who have identified these structures as multivesicular bodies. Based on the arrangement of the retinal cells that express Delta at this time and the concordance of their locations with cells that stain with the monoclonal antibody 22C10 (Zipursky et al., 1984), these Delta-expressing cells include the photoreceptors. In addition, we do not find a one-to-one correspondence between the number of vesicles and the number of photoreceptors expected in a given ommatidial cluster, suggesting that some photoreceptors contain multiple intracellular vesicles.



Approximately 24 hours after prepuparium formation, Delta is expressed in cone cells located centrally within developing ommatidia (Fig. 3J) and is expressed in the peripodial membrane (data not shown). By this time, photoreceptors, pigment cells, cone cells and bristle-forming cells have been determined (Cagan and Ready, 1989a,b). We also detect regional concentrations of Delta expression in cell surface-associated and vesicular patterns that extend from the base to the tip of the antennal anlage of the eye-antennal imaginal disc in late L3 larvae (Fig. 3L). These regions apparently correspond to proneural groups for innervated antennal bristles (Lienhard and Stocker, 1991). At this time, Delta is also expressed within regions of the peripodial membrane (Fig. 3L) that will eventually give rise to some head structures (Bryant, 1978).

Delta expression is spatially restricted in larval and pupal wing discs

The dependence of wing and notum development on *Dl* function is reflected in disrupted wing venation and alterations in sensory chaetae of the notum observed in flies heterozygous for *Dl* loss-of-function mutations (Lindsley and Grell, 1968) and in *Dl* temperature-sensitive mutants

Fig. 2. Delta protein is expressed in a variety of tissues during late embryonic stages, and in neurogenic mutants. Wild-type (A-C) and neurogenic mutant (D) embryos stained for Delta with the mouse polyclonal serum M585 and visualized with horseradish peroxidase-conjugated secondary antibody. Embryos were viewed as whole-mounted preparations using Nomarski optics (A, B, D) or embedded in paraffin and sectioned to 5 μm thickness following the development of the horseradish peroxidase-conjugated secondary antibody (C), and viewed with transmitted light. Anterior is up. A is a horizontal dorsal view. Dorsal is right in B-D. (A) Horizontal optical section through a contracted germ band embryo (stage 14) stained for Delta. Delta accumulates in clusters of cells within the procephalic region that have apparently arisen from the procephalic neurogenic region. The sensory ganglion (sg) located within the clypeolabrum is the epiphysis, more medial sensory ganglia are apparently correlated with the stomatogastric nervous system or the antennomaxillary complex, while the most posterior sensory ganglia appear to be the developing optic lobes. Delta is concentrated within the proventriculus (pv), trachea (t), hindgut (hg), and within the posterior midgut (pmg) that extends to fuse with the anterior midgut (amg) at this stage. (B) Sagittal section through an older extended germ band embryo (late stage 14/early 15). Delta accumulates along the dorsal and ventral aspects of the VNC at periodic intervals (arrowheads). Delta is also expressed in the ventral pharynx (ph), the proventriculus (pv), and the hindgut (hg). (C) Paraffin section of wild-type, late extended germ band embryo (stage 10) after neuroblast segregation has begun. Delta is expressed within the epidermis (ep), mesoderm (ms), anterior midgut (amg), and posterior midgut (pmg). Delta is absent from the delaminated neuroblasts (nb). (D) Optical section through a stage 10 neurogenic mutant embryo, homozygous for the *Df(3R)E(spl)*^{BX22} mutation (Shepard et al., 1989). The embryo exhibits disrupted development of the epidermis. Most ectodermal cells have adopted the neuroblast identity, so that little or no epidermis remains within this embryo. Delta is expressed in the mesoderm, anterior midgut and posterior midgut, but is absent from the neuroblast layer that occupies the remainder of the extended germ band. Magnification: ×182.

exposed to restrictive temperatures during larval and pupal development (T. R. Parody and M. A. T. Muskavitch, unpublished data; A. L. Parks and M. A. T. Muskavitch, unpublished data). Delta is expressed within most, if not all, cells in the wing imaginal discs and in the disc-associated peripodial membrane of L3 larvae. However, subsets of cells within the anlagen of the wing blade and the notum appear to express elevated levels of Delta relative to surrounding cells (Fig. 4A,B). The presumptive anterior wing margin (Fig. 4A) exhibits reduced levels of Delta expression and is flanked by two bands of cells that exhibit elevated levels of Delta expression. These bands correlate closely with the contiguous 'bristle equivalence groups' or 'proneural groups' (Simpson, 1990) that express products of the *achaete-scute* complex (Cubas et al., 1991; Skeath and Carroll, 1991). The sensory organ precursors (SOPs) of the anterior wing margin will be determined subsequently within these regions (Bryant, 1978; Huang et al., 1991). At the same time, the presumptive posterior wing margin is flanked by two bands of cells that exhibit higher levels of Delta expression (Fig. 4A,B). Later, cells within these regions give rise to non-innervated epidermal hairs (Bryant, 1978; Huang et al., 1991). Delta expression is greatly reduced slightly later during wing development in a row of cells that flank the presumptive anterior wing margin, yielding 'holes' within the strongly staining bands (Fig. 4B). These 'holes' are observed during late L3, when SOPs adopt the 'neural' fate (Bryant, 1978; Hartenstein and Posakony, 1989). A similar expression pattern has been described for Notch (Fehon et al., 1991). In that instance, cells that cease to express Notch correspond to the SOPs (Fehon et al., 1991).

Groups of cells that exhibit elevated levels of Delta expression relative to neighboring cells within the developing notum at this time (Fig. 4A) appear to overlap substantially with many of the notal macrochaeta proneural groups (Simpson, 1990), within which macrochaeta SOPs and epidermal cells will be specified subsequently (Cubas et al., 1991; Skeath and Carroll, 1991; Huang et al., 1991). Some of these groups have been identified tentatively as those for the tegular bristles, notopleural bristles, trichoidea sensilla 1 and 2 (within the anterior postalar bristle proneural group), dorsocentral bristles and scutellar bristles (Fig. 4A).

Six hours after prepuparium formation, Delta is expressed in cells adjacent to the wing margin in regions within which innervated bristles are developing along the anterior wing margin (Fig. 4C; Bryant, 1978; Hartenstein and Posakony, 1989) and non-innervated hairs are developing along the posterior wing margin (Bryant, 1978; Huang et al., 1991). Delta is also expressed at elevated levels, relative to surrounding cells within the wing blade, within the presumptive wing veins (Fig. 4C). We detect two groups of more intensely staining cells within the developing third longitudinal vein, which apparently correspond to developing campaniform sensilla, based on their locations and structures (Fig. 4C; Bryant, 1978; Fehon et al., 1991).

Delta and Notch expression are coincident and complementary during postembryonic development

To gain some insight into the molecular correlates of the various phenotypic interactions that have been described between *Dl* and *N* mutations (de la Concha et al., 1988; Shepard et al., 1989; Brand and Campos-Ortega, 1990; Xu et al., 1990), we have employed double-labelling with antibodies specific for Delta and Notch, respectively, to determine precisely their relative distributions in imaginal tissues during postembryonic development.

Larval CNS

Delta accumulates diffusely within the cytoplasm and plasma membranes of neuroblasts and their progeny in the brain lobes of L2 larvae and in the ventral thoracic ganglion of L3 larvae, and is also found in vesicles (Figs 3A,C, 5A). In contrast, Notch, which is expressed within the same cell clusters, is predominantly associated with the cell surface (Figs 3B,D, 5A; see also Fehon et al., 1991) and is only occasionally associated with intracellular vesicles that also contain Delta. More centrally within the thoracic ganglion of the L3 larval CNS, Notch is expressed in glial 'cups' (Fehon et al., 1991) that encompass the lateral termini of the three Delta-expressing 'rungs' (Figs 3E,F, 5B). These overall accumulation patterns of Delta and Notch within the central thoracic ganglion are strikingly dissimilar and are apparently complementary.

Wing imaginal disc

We define at least two qualitatively distinct patterns of Delta and Notch accumulation within the developing wing disc. First, Delta is more highly expressed in proneural groups along the wing margin, within the notum, and within wing veins than elsewhere in the wing disc (Figs 4A,C, 5C), whereas Notch is expressed relatively uniformly within these same regions of larval and pupal wing discs (Figs 4D, 5C; Fehon et al., 1991). Second, Delta and Notch are expressed throughout the developing late L3 larval and pupal wing blade (6 hours post-puparium formation), but Delta is more highly expressed within the developing wing veins, while Notch is more highly expressed in regions between the wing veins (Fig. 5C, Fehon et al., 1991). In this instance, the resulting overall expression pattern consists of complementary Delta and Notch accumulation. In addition, Delta and Notch expression coordinately cease within a number of SOPs along the anlage of the anterior margin of the developing wing disc (Fig. 4B; Fehon et al., 1991), as assessed in wing discs doubly stained for Delta and Notch (data not shown).

Eye-antennal imaginal disc

Delta and Notch expression and accumulation patterns exhibit substantial differences within larval and pupal imaginal eye discs. Delta and Notch are expressed within most, if not all, of the same cells of the developing L3 larval eye disc (Figs 3H,I, 5D). Delta is expressed in photoreceptors (Fig. 3I), accumulating on the apical surfaces of these cells and within subcellular vesicles that are most prevalent apically within the photoreceptors. Notch accumulates on the apical surfaces of photoreceptors during this period (Fehon et al., 1991) and is only occasionally colocalized with Delta in subcellular vesicles (Fig. 5D). Furthermore, colabelled vesicles are found only within the immediately subapical aspects of photoreceptors (Figs 3I, 5D). Delta and Notch accumulation patterns in L3 larval photoreceptors are there-

fore coincident on a cell-by-cell basis, but only partially coincident on a subcellular basis. During the same period, Delta and Notch expression also appear to coincide on a cell-by-cell basis within the peripodial membrane that surrounds the developing eye disc (Fig. 5D).

In contrast to these L3 larval patterns, Delta and Notch expression patterns appear to be substantially complementary on a cell-by-cell basis within the developing pupal eye disc (Figs 3J,K, 5E). While Delta is expressed in cone cells during this period, Notch is expressed in a ‘chain mail’ pat-

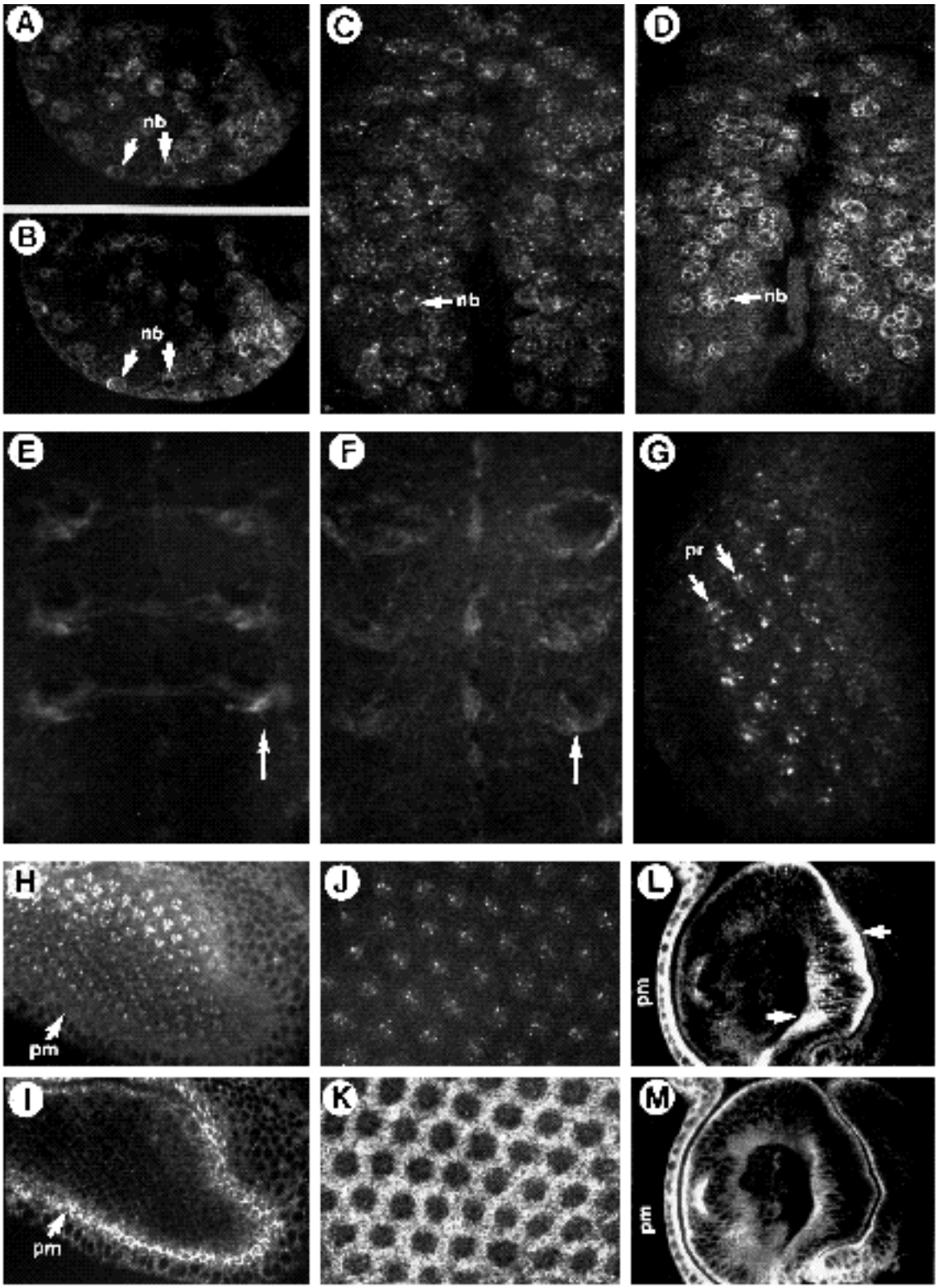


Fig. 3

tern composed of secondary and tertiary pigment cells and cells of the developing bristle apparatus (Fig. 5E; Fehon et al., 1991) that encircle the Delta-expressing cells. While these patterns appear to be complementary, it has been shown previously that Notch is expressed in cone and primary pigment cells during this stage, albeit at lower levels than in secondary and tertiary pigment and bristle apparatus cells (Fehon et al., 1991).

Comparison of Delta and Notch expression within the antennal anlage of the L3 larval eye-antennal imaginal disc reveals that Delta is expressed throughout the anlage, but is expressed at higher levels in regionally localized groups of cells that form 'stripes' which extend from the base to the tip of the anlage (Figs 3L, 5F). Notch is expressed more uniformly within the antennal anlage (Figs 3M, 5F). These patterns are reminiscent of those observed for Delta and Notch within proneural groups within the developing wing imaginal disc (Fig. 4B-D). Delta and Notch expression are also coincident on a cell-by-cell basis within a region of the eye-antennal disc peripodial membrane that will give rise to head structures, although Delta is expressed more highly within this region than in surrounding regions of the peripodial membrane (Fig. 5F).

DISCUSSION

We have employed polyclonal antisera specific for the extracellular domains of Delta or Notch to analyze their respective expression patterns in a variety of tissues during embryonic and postembryonic development. Since the Delta-specific antisera we utilized were directed against a subset of EGF-like repeats within the Delta extracellular domain, the patterns we have described may not reflect every aspect of protein modification and processing relevant to Delta function. We are in the process of generating antisera directed against other portions of the Delta protein to examine these questions.

Delta is expressed transiently within interacting cell groups preceding and during initial specification of cell fates

Phenotypic analyses of *Dl* mutants have demonstrated the existence of roles for Delta in the partitioning of neuroblasts and dermoblast fates within embryonic neurogenic regions (Lehmann et al., 1983) and of SOP and epidermal cell fates within imaginal proneural equivalence groups (Heitzler and Simpson, 1991; T. R. Parody and M. A. T. Muskavitch, unpublished data; A. L. Parks and M. A. T. Muskavitch, unpublished data). Our analysis reveals that Delta is expressed in embryonic neurogenic regions and proneural groups of the notum and wing preceding and during the specification of particular cell types within each of these tissues. There is no evident asymmetry in Delta expression among cells within the embryonic ventral neu-

Fig. 3. Delta protein is expressed in proliferation centers and neuroblasts of the larval central nervous system, and in the developing eye. (A-F) Confocal optical sections through L2 (A,B) and L3 (C-F) larval brains double-stained for Delta (A,C,E) with the mouse polyclonal serum M5 and for Notch (B,D,F) with the rat polyclonal serum R1. Anterior is left in A and B; anterior is up in C-F. (G-M) Confocal optical sections through eye-antennal imaginal discs stained for Delta (G) or double-stained for Delta and Notch (H-M). Discs from wandering L3 larvae stained for Delta (G,H,L) with mouse polyclonal serum M5 or for Notch (I,M) with rat polyclonal serum R1, and from a pupa (24 hours post-puparium formation) stained for Delta (J) and Notch (K) as above. (A,B) Tangential sections of the surface of an L2 larval brain hemisphere. Delta (A) and Notch (B) are found in the developing neuroblasts (nb). Delta accumulation is more diffuse than that of Notch, which appears restricted to membranes. (C,D) Frontal sections through the ventral surface of an L3 larval thoracic ganglion. Arrows indicate same position in C and D. Delta (C) accumulates diffusely within neuroblasts (nb) and their progeny, but vesicular accumulation of Delta is also apparent within these cells (arrow). Notch (D) is predominantly associated with membranes of neuroblasts (nb) and their progeny, clearly outlining these clusters (arrowhead) and infrequently exhibits vesicular accumulation in these cells. (E,F) Medial horizontal section through the same ganglion as in C and D. Arrows indicate same position in E and F. Anterior is up and the upper edge of the picture corresponds to the brain lobes, which are not visible in this plane of section. Delta (E) is expressed in cells that form three 'rungs', oriented perpendicular to the anterior-posterior midline. In some ganglion preparations, Delta is expressed within the midline cells, at much lower levels than in the 'rungs'. Notch (F) is expressed in more lateral structures or 'cups' (Fehon et al., 1991) and in cells of the midline. (G) Subapical horizontal section through an eye disc from an L3 larva. Anterior is left. Delta is detectable in vesicles within photoreceptors (pr). (H,I) Glancing section through an L3 larval eye disc. Anterior is up and right. This section passes through the peripodial membrane and the apical aspects of retinal cells in the upper portion of the panel; the section samples progressively more basal aspects of retinal cells as it progresses toward the center of the panel. Delta (H) is expressed in the peripodial membrane (pm), in membranes of cells that surround each photoreceptor cluster, and within photoreceptors. Vesicles are visible at the edge of this subapical section. Notch (I) is not detectable within photoreceptor clusters in this plane of section. Notch does accumulate in photoreceptors and in all cells of the eye, but is on the apical surfaces of cells in the developing retina and the peripodial membrane (pm; Fehon et al., 1991). (J,K) Subapical horizontal section through a flattened pupal eye disc (24 hours after prepuparium formation) within which other cell types found in the mature eye are developing. Delta accumulation (J) remains punctate and occurs predominantly within cone cells in this plane of section, despite the fact that each ommatidium now also contains pigment cells and presumptive bristle-forming cells. Notch (K) accumulates in cells that surround the photoreceptors in this plane of section. The resulting 'chain mail' pattern is composed of secondary and tertiary pigment cells and cells of the developing bristle apparatus. The primary pigment cells and the four cone cells within the ommatidium stain at very low levels (data not shown; Fehon et al., 1991). (L,M) Transverse section through the antennal anlage of an L3 larval eye-antennal disc. Anterior is up. Delta (L) is expressed in distinct regions within the disc (arrowheads) within which vesicular features are evident. Delta is also apparently concentrated in the portion of the peripodial membrane (pm) known to contribute to head structures (Bryant, 1978). Notch (M) is apically localized in cells within the antennal anlage and the surrounding peripodial membrane. Note that, as demonstrated in H,I and J,K, Delta and Notch signals obtained by double-labelling can be strikingly complementary, indicating that neither cross-channel bleed-through nor secondary antibody cross-reactivity is associated with our double-labelling protocol. Magnification: A,B ×503; C-F ×199; G-M ×239.

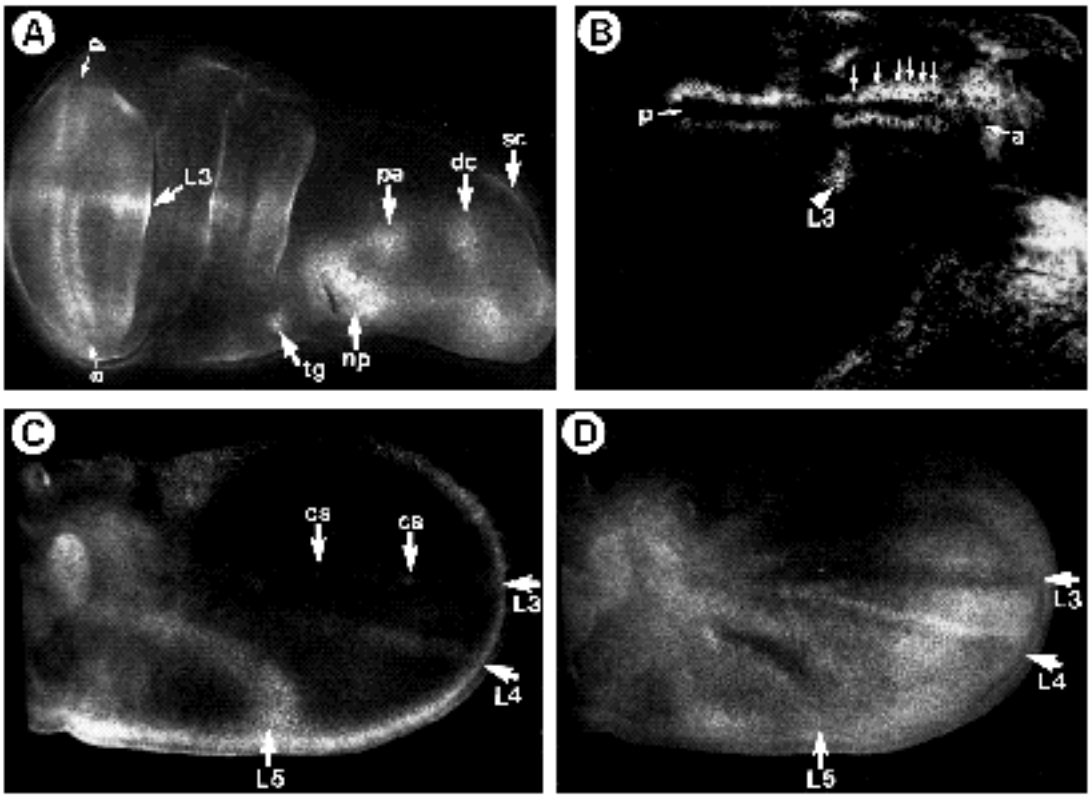


Fig. 4. Delta protein expression is spatially restricted in the developing wing and notum. Confocal optical sections through wing discs stained for Delta (A,B) or double-stained for Delta and Notch (C,D). Discs from L3 larvae stained for Delta (A,B) with mouse polyclonal serum M5, and from a pupa (6 hours post-puparium formation) stained for Delta (C), as above, and for Notch (D) with rat polyclonal serum R1. (A) A computer-generated projection of serial horizontal sections through an L3 larval wing disc and the surrounding peripodial membrane. Delta staining intensity is elevated in regions that flank the presumptive anterior wing margin (a, and small arrow) and encompass the proneural clusters that will give rise to bristles along the adult anterior wing margin (Bryant, 1978; Hartenstein and Posakony, 1989; Huang et al., 1991), and in regions that flank the posterior wing margin (p, and small arrow) and encompass the presumptive posterior row of hairs (Bryant, 1978; Huang et al., 1991). The presumptive third longitudinal wing vein (L3, and arrow) also exhibits more intense staining. Regions within the anlage of the notum (right) that exhibit more intense Delta staining overlap with the previously identified areas of achaete/scute transcript (Cubas et al., 1991) and protein (Skeath and Carroll, 1991) expression. These regions include anlage of a number of bristles: tegular (tg); notopleural (np); trichoid sensillae 1 and 2; postalar (pa); dorsocentral (dc); and scutellar (sc). (B) High magnification tangential section through the distal region of an L3 larval wing disc. Delta staining is elevated in regions that encompass proneural groups that flank the anterior wing margin (a, and arrow). Delta expression is reduced in SOPs (small arrows) that will give rise to bristle organs. Similar holes are not seen in regions of more intense Delta staining that flank the posterior wing margin (p, and arrow). (C,D) Computer-generated projections of serial horizontal sections through a pupal wing (6 hours post-puparium formation). Delta (C) is present throughout the disc, but is more concentrated in presumptive longitudinal wing veins (L3, L4 and L5). Delta expression is elevated along the anterior wing margin (upper right quadrant) where developing bristle organs reside. Within the L3 vein, arrows point to developing campaniform sensillae (cs) that exhibit elevated expression of Delta. Notch (D) is also present throughout the disc, but is apparently expressed less highly in wing veins (L3, L4, and L5). Notch protein appears to be expressed at higher levels in the regions of the blade between the presumptive longitudinal veins, effectively outlining the wing veins. Magnification: A ×149; B ×174; C,D ×192.

rogenic region (Campos-Ortega and Hartenstein, 1985) nor among cells within imaginal proneural groups (Simpson, 1990). We also find that Delta expression ceases in many cell types as they adopt particular fates. This is particularly evident for delaminating embryonic neuroblasts and imaginal anterior wing margin SOPs that are specified within embryonic and imaginal proneural equivalence groups (Skeath and Carroll, 1991, 1992). These patterns imply that Delta is expressed by all members of a group of interacting cells within which fates are being specified and that expression is then down-regulated in cells within the group that adopt particular fates.

The patterns of Delta protein expression we observe during embryogenesis are corroborated fully by analyses of *Dl* transcription in embryos (Vässin et al., 1987; Kopczynski and Muskavitch, 1989). For instance, *Dl* transcription is initially uniform in all cells within the neurogenic ectoderm, is down-regulated in neuroblasts and persists in dermoblasts following neuroblast delamination and is subsequently reactivated in the condensing VNC and the optic lobes later during embryogenesis. Similarly, Delta transcription occurs throughout the mesoderm before gastrulation, is down-regulated in the mesoderm immediately preceding gastrulation, and is transiently reactivated in the

mesoderm during germ band extension. For delaminating neuroblasts as well as mesodermal cells preceding gastrulation, clearance of Delta from cell surfaces follows closely after cessation of *Dl* transcription in these cells, as assessed by in situ hybridization performed using intron-specific *Dl* probes (Kopczynski, 1991). In fact, data on embryonic *Dl*

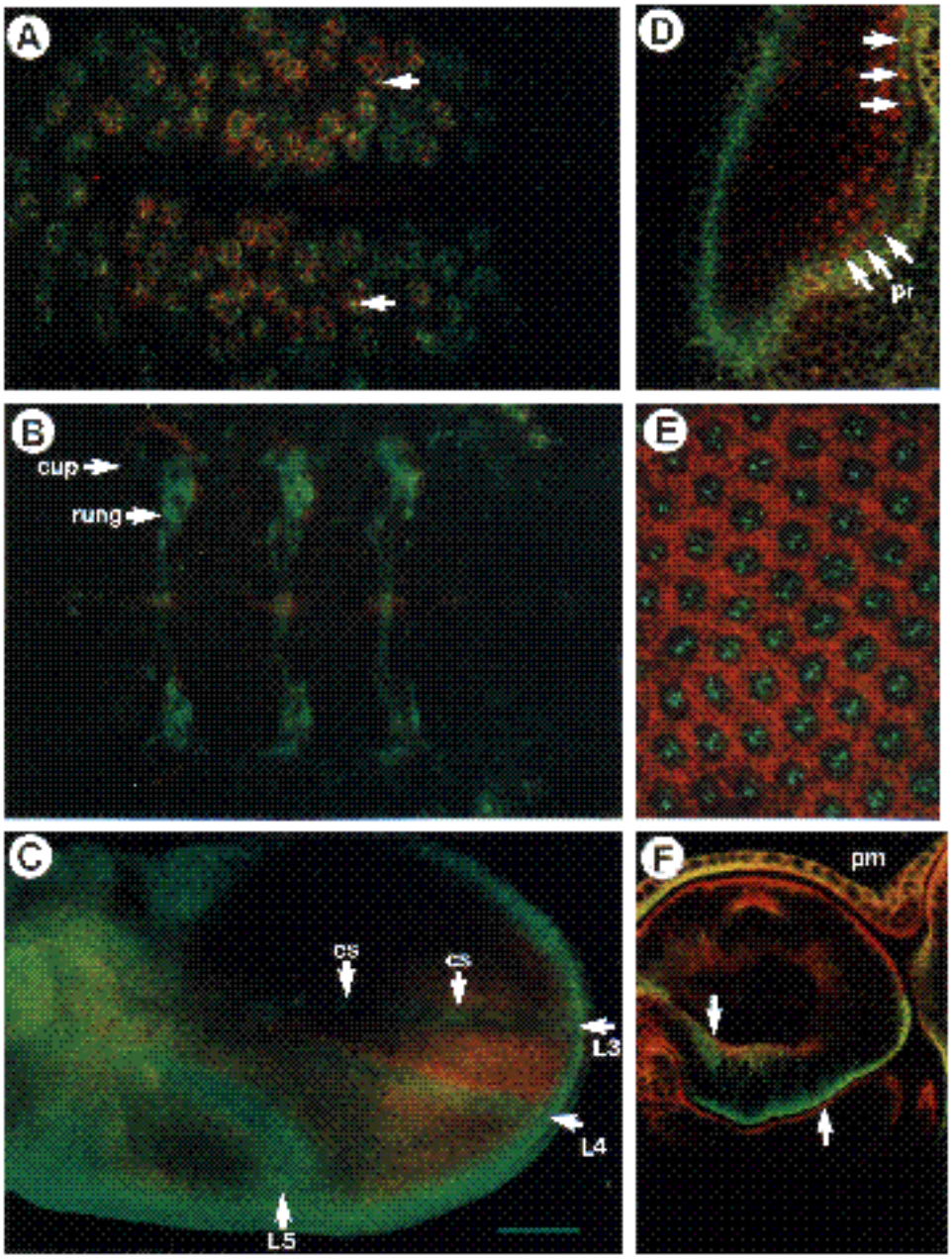


Fig. 5. Delta and Notch expression patterns are coincident and complementary during development. Tissues stained for Delta with mouse polyclonal serum M5 and FITC-GAM secondary antibodies and for Notch with rat polyclonal serum R1 and TR-GAR secondary antibodies. Single-channel images for Delta or Notch staining were obtained from a dual-channel confocal microscope system, and images were merged by computer (see Materials and Methods). Delta signals are green and Notch signals are red, except for panel D, in which Delta signals are red and Notch signals are green. Coincidence of Delta and Notch signals yields a yellow signal. (A) Frontal section through the ventral surface of an L3 larval thoracic ganglion. Anterior is left. Delta staining in neuroblasts and their progeny exhibits vesicular character, as well as more diffuse membrane association. Notch is generally associated with membranes in these cells, but is sometimes colocalized with Delta in intracellular vesicles (arrows). Single channel images from Fig. 3C,D. (B) Medial horizontal section through the same L3 larval thoracic ganglion. Anterior is left. The left edge of the picture corresponds to the brain lobes, which are not visible in this plane of section. Delta is present in three 'rungs' oriented perpendicular to the anterior-posterior midline. Notch 'cups' flank and encompass the lateral termini of the Delta 'rungs' in a complementary pattern. Single channel images from Fig. 3E,F. (C) A computer-generated projection of horizontal sections through a pupal wing disc (6 hours post-puparium formation). Delta and Notch accumulate throughout the wing blade surface. Delta is more concentrated in veins relative to the remainder of the wing blade. Notch is more concentrated in regions between and bordering the veins. cs, campaniform sensillum; L3, L4, and L5 are longitudinal wing veins three, four, and five, respectively. Single channel images in Fig. 4C,D. (D) Glancing section through an L3 larval eye disc. This section passes through the peripodial membrane and the apical aspects of retinal cells in the right-hand portion of the panel; the section samples progressively more basal aspects of retinal cells as it progresses toward the center of the panel. Delta and Notch are coincident in vesicles near the apical surfaces of photoreceptor clusters (horizontal arrows). Delta, but not Notch, accumulates within photoreceptors (pr) in subapical planes of section. Delta and Notch are expressed in the peripodial membrane and in the cell membranes surrounding the photoreceptors. Single channel images from Fig. 3H,I. (E) Subapical horizontal section through a pupal eye disc (24 hours post-puparium formation). Delta is expressed in cone cells, but is much reduced in or absent from other cell types. Notch accumulates in the secondary and tertiary pigment cells and cells of the developing bristle apparatus. Single channel images from Fig. 3J,K. (F) Transverse section through an L3 larval instar antennal disc. Delta is elevated in regions of the antennal anlage (arrows) and of the peripodial membrane (pm). Notch is expressed apically throughout the disc, and is therefore partially coincident with Delta, although Delta and Notch patterns are not identical. Single channel images from Fig. 3L,M. Magnification: A-C ×230; D-F ×319.

transcription provided the first indications that Delta protein expression is uniform within groups of interacting cells, and that Delta expression is down-regulated in cells as they adopt particular fates.

The cellular dynamics of Delta expression we observe for neuroblasts and anterior wing margin SOPs are directly relevant to the consideration of qualitative models for Delta function during the specification of these cell types. The lateral inhibition model for Delta function (Simpson, 1990; Heitzler and Simpson, 1991; Artavanis-Tsakonas and Simpson, 1991) implies that Delta acts as an inhibitory signal that is sent by the 'neural' cell (i.e., neuroblast or SOP) within a proneural equivalence group and inhibits adoption of the neural fate by other cells within the group. Our data refute the simplest prediction of this model: that Delta is expressed solely in the neural cell type. However, the resolution of our analysis does not exclude the existence of slight asymmetries in Delta expression among cells within embryonic neurogenic regions and imaginal proneural groups; and data presented by Heitzler and Simpson (1991) imply that slight differences in Delta expression by apposed cells can bias the adoption of fates by such cells. Even so, the apparent uniformity of Delta expression within embryonic and imaginal proneural equivalence groups may be more easily reconciled with the mutual inhibition model for Delta function recently presented by Goriely et al. (1991). This model holds that the functions of Delta and other neurogenic genes are required to establish an inhibitory field that prevents all cells within the proneural group from adopting the neural fate. Only those cells that escape from this field of mutual inhibition stably adopt the neural fate. Under this model, Delta expression by all members of the proneural group would reflect mutual inhibitory signalling among the interacting cells and down-regulation of Delta in neural cells would reflect escape from this field preceding stable adoption of the neural fate. The similar patterns of Notch expression and down-regulation that are observed within these cells (Fehon et al., 1991) further support such inferences, as discussed in greater detail below. If these inferences were true, persistent expression of Delta in emergent neuroblasts and SOPs should prevent stable adoption of neural fates by those cells. The availability of promoters that direct transcription in these cell types and germ line transformation should allow us to test this hypothesis in the future.

Delta expression is reactivated in many tissues following the initial specification of cell fates

The multiphasic character of Delta expression that we observe within the embryonic ectoderm and mesoderm and in developing eye imaginal discs implies that Delta function is required during multiple developmental stages in a number of embryonic and imaginal tissues. For instance, Delta expression is initially uniform within ventral and procephalic embryonic neurogenic regions and subsequently ceases in neuroblasts as their fates are specified. However, Delta expression resumes within the dorsal and ventral aspects of the ventral nerve cord and within a number of ganglia within the cephalic nervous system during later embryogenesis. Delta expression is also apparent in dividing neuroblasts and their progeny during postembryonic development of the larval central nervous system. The initial phase of Delta expression within embryonic neurogenic regions is clearly required for global partitioning of the ectoderm into neuroblast and dermoblast populations (Lehmann et al., 1983). We propose that the latter phases of expression are required for the specification of particular cell fates within the developing central nervous system, although we cannot formally exclude roles for Delta in the maintenance of neural fates or regulation of mitotic behavior within neuronal cell populations. Within the developing eye disc, Delta expression is evident within the morphogenetic furrow where photoreceptor clusters form, and continues within some or all photoreceptors as specific photoreceptor fates are adopted within developing ommatidial clusters arrayed behind the morphogenetic furrow. Again, Delta appears to be required first for global specification of cell fates (i.e., photoreceptor versus nonphotoreceptor; A. L. Parks and M. A. T. Muskavitch, unpublished data) and may subsequently be required for the adoption of particular fates within globally specified photoreceptor clusters (Tomlinson and Ready, 1987; Cagan and Ready, 1989a). Finally, Delta is expressed throughout the mesodermal anlage preceding gastrulation, and expression ceases in these cells as gastrulation proceeds and subsequently resumes in the mesoderm of extended germ band embryos. While the developmental significance of the initial phase of embryonic mesodermal Delta expression is unclear, the latter phase is apparently essential for specification of particular cell fates within the developing mesoderm (Corbin et al., 1991). In each of these cases, multiphasic Delta expression appears to reflect multiphasic requirements for Delta function within a given tissue.

Delta and Notch expression are generally coincident at the cellular level during cell fate specification

Comparison of the cellular geographies of Delta and Notch expression in a variety of embryonic and postembryonic tissues reveals that activation and cessation of expression of the two proteins is frequently coordinate at the cellular level. Delta and Notch are coordinately expressed within procephalic and ventral neurogenic regions preceding and during neuroblast segregation, the mesodermal anlage preceding gastrulation, the midembryonic epidermis, subsets of cells within the condensing embryonic ventral nerve cord, neuroblast clusters in the larval central nervous system, proneural groups of the notum and wing, and in developing anterior wing margin bristle organs, campaniform sensilla and photoreceptor clusters. Expression of the two proteins also ceases in embryonic neuroblasts and postembryonic anterior wing margin SOPs as they adopt specific fates. These data are consistent with the hypothesis that Delta functions coordinately with Notch to effect proper adoption of cell fates in a variety of tissues during development. Coordinate expression within interacting cell populations presumably reflects requirements for Delta and Notch in intercellular signalling during development, while coordinate down-regulation implies that requirements for Delta and Notch abate, at least transiently, once appropriate cell fates have been specified.

The finding that Notch expression is apparently uniform

among cells within each of the populations or groups listed above is consistent with the hypothesis that Notch may function as a receptor for developmental signals essential for proper cell fate specification (Fehon et al., 1991; Heitzler and Simpson, 1991; Artavanis-Tsakonas and Simpson, 1991). Our finding that Delta expression also appears to be uniform within these cell populations challenges hypotheses that propose that Delta is an asymmetrically disposed signal in cellular interactions mediated by Delta and Notch. As noted above, any asymmetries contingent on Delta function apparently would have to arise as the result of post-translational regulation of Delta activity. At present, we therefore favor hypotheses suggesting that cell fate specification mediated by coordinate Delta and Notch function involves interactions within groups of cells (e.g., mutual inhibition model) rather than polarized interactions between a single cell and its immediate neighbors (e.g., lateral inhibition model).

In contrast to the instances of spatially coordinate expression described above, Delta and Notch expression appear to be substantially or entirely complementary on a cell-by-cell basis within a number of other tissues. For instance, vein cells express Delta and blade tissue expresses Notch in the pupal wing, and cone cells express Delta and pigment and bristle cells express Notch in the pupal eye disk. In these situations, Delta may function as a polarized, instructive developmental signal that is transmitted between nonoverlapping, interacting cell populations that express Delta or Notch, respectively. Somatic mosaic analysis of the function of *glp-1*, which is required for specification of a number of cell fates in *Caenorhabditis elegans*, implies that this Notch homologue is also involved in the reception of polarized signals during development (Austin and Kimble, 1987).

The dynamics of subcellular trafficking imply that Delta and Notch function as a ligand-receptor pair during cell fate specification in vivo

The subcellular dynamics of Delta trafficking are consistent within most expressing cell populations during development. Delta initially accumulates on the surfaces of interacting cells and clearance of Delta from the cell surface is correlated with the accumulation of Delta in intracellular vesicles during and subsequent to the specification of cell fates. This pattern is observed within the neurogenic ectoderm, mesoderm, and posterior midgut of embryos, the larval neuroblasts and the developing photoreceptors. The temporal order of initial surface accumulation and later clearance implies that vesicular trafficking reflects endocytosis and targeting of Delta to degradative subcellular compartments, rather than synthesis, processing, and export of the protein. We propose that clearance of Delta from the cell surface involves two components. One component corresponds to intercellular signalling in cell populations within which fates are being specified, and another corresponds to generalized clearance of Delta from the cell surface once Delta-dependent signalling has ceased.

The nonautonomous character of Delta function and autonomous character of Notch function in the specification of epidermal cell fates within imaginal proneural groups support the hypothesis that Delta functions as an intercellular signal and Notch as a receptor of this signal during development (Heitzler and Simpson, 1991). The ability of cultured cells that express Delta to aggregate with those that express Notch provides compelling evidence for the direct physical interaction of Delta and Notch on cell surfaces (Fehon et al., 1990). The coimmunoprecipitation of Delta and Notch from embryonic extracts implies that interactions between Delta and Notch also occur in vivo (Fehon et al., 1990). Our comparative analysis of Delta and Notch subcellular trafficking during development provide further support for the hypothesis that dynamic interactions between Delta and Notch occur during the specification of cell fates in vivo.

The premise that Delta and Notch interact transiently as signal and receptor is supported by the occurrence of two classes of subcellular vesicles that contain Delta: those in which Delta and Notch are coresident and those that contain Delta, but not Notch. Vesicles that contain Delta and Notch are generally less prevalent than those that contain only Delta. Our observation that vesicles that contain Delta and Notch are restricted to the immediately subapical aspects of larval photoreceptor cells and that more basally disposed vesicles contain only Delta suggests a model for Delta-Notch interactions during intercellular signalling. We propose that the coresidence of Delta and Notch in subcellular vesicles reflects the occurrence of Delta-Notch interactions analogous to those in which ligands and receptors participate preceding ligand-receptor-mediated endocytosis from the cell surface (Geuze et al., 1983, 1987). This interaction of Delta and Notch at the cell surface generates information central to the specification of cell fates, and signalling by the Delta-Notch complex is down-regulated by endocytosis of the ligand-receptor complex from the cell surface. The predominance of vesicles that contain only Delta suggests that putative Delta-Notch complexes rapidly dissociate. Delta may then be targeted to the lysosomal compartment, in which it is degraded, and Notch rapidly degraded or recycled to the cell surface. The premise that Delta-Notch complexes can form and be cleared from cell surfaces is further supported by the observation that Delta and Notch expressed on the surfaces of apposed cultured cells appear to be coendocytosed into Notch-expressing cells (S. B. Shepard and M. A. T. Muskavitch, unpublished data; R. G. Fehon, M. E. Fortini and S. Artavanis-Tsakonas, unpublished data).

While many ligand-receptor interactions involve a free ligand and a membrane-associated receptor, the interaction we propose for Delta and Notch in vivo apparently involves a membrane-bound ligand. However, precedents for activation of receptors by membrane-bound ligands do exist. First, it has been shown that mammalian transforming growth factor-α is capable of activating signalling by the epidermal growth factor receptor even when the growth factor cannot be cleaved from its membrane-bound precursor (Wong et al., 1989; Brachmann et al., 1989). Second, recent analyses of interactions between the products of *bride of sevenless* (i.e., boss) and *sevenless* (i.e., sev) reveal that the membrane-bound boss ligand interacts with the cell surface sev receptor (Krämer et al., 1991), and that the entire boss protein is transferred between R8 and R7 photoreceptors during the interaction between these cells

required for specification of the R7 cell fate during ommatidial development (Krämer et al., 1991; Cagan et al., 1992). We propose that binding of Delta to Notch is a prerequisite for some forms of intercellular signalling central to the specification of cell fates during development, and that the subcellular dynamics of Delta and Notch trafficking we observe reflect the involvement of Delta and Notch in interactions analogous to those between boss and sev and between members of other ligand-receptor pairs involved in pattern formation during development.

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