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A new signalling pathway composed of numb, Notch and trantrack controls neuronal fate specification during asymmetric cell divisions

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A New Signalling Pathway Composed of numb, Notch and tramtrack Controls Neuronal Fate Specification During Asymmetric Cell Divisions

by

Ming Guo

DISSERTATION

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This dissertation is dedicated to my parents,

Ruilan Wang and Suofeng Guo

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A New Signalling Pathway Composed of *numb*, *Notch* and *tramtrack* Controls Neuronal Fate Specification During Asymmetric Cell Divisions

Ming Guo

ABSTRACT

During development, many cells divide to produce two daughter cells with different fates. A fundamental question in development is how these diverse fates are generated. I have addressed this question using the *Drosophila* peripheral nervous system as an experimental model. Each sensory organ precursor (SOP) divides twice to generate one neuron and three distinct non-neuronal cells. I have studied the role of *Notch* and identified a new signalling pathway which utilizes *numb*, *Notch* and *tramtrack* and is essential for specifying SOP progeny fate.

Both cell-intrinsic mechanisms (unequal distribution of cellular components) and cell-cell communication have been proposed to specify cell fates. I have shown that *Notch*-dependent cell-cell communication is essential for proper specification of the four distinct SOP progeny during multiple asymmetric divisions. Previously, it has been shown that Numb acts as a cell-intrinsic determinant during these divisions: Numb is asymmetrically distributed to one pole of the SOP, segregated to one daughter cell during SOP division, and it acts to specify this daughter cell fate. I have demonstrated that genetically *numb* negatively regulates *Notch*; biochemically Numb binds to Notch. These observations raise the possibility that Numb suppresses Notch-dependent signalling through a direct protein-protein interaction. Furthermore, I have identified the transcription factor Tramtrack as a downstream target of *Notch*.

Notch activates Tramtrack expression, and *numb* antagonizes this activation. In addition, I have shown that as with *Notch*, *tramtrack* is both necessary and sufficient for the non-neuronal fate. Tramtrack is expressed in non-neuronal cells but not in neurons, and ectopic expression of Tramtrack suppresses the neuronal fate. Based on these results, I propose that Numb suppresses the Notch activity in the daughter cell that receives Numb. As a consequence of this, *Notch*-dependent *tramtrack* activation does not occur. Thus the cell assumes the default cell fate of becoming a neuron. In the daughter cell that lacks Numb, *Notch* activity is sufficient to activate Tramtrack, suppressing the neuronal fate and forcing the cell to become non-neuronal.

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CHAPTER 1

INTRODUCTION

Organization

History and Overview

How a single fertilized egg gives rise to a whole organism with millions of differentiated cell types is a key question in developmental biology. During development, the progeny of a multipotential progenitor cell gradually limits their developmental potential through a process of cell fate determination or specification. Ultimately, this process results in overt changes in morphology and function as cells differentiate. Elucidating mechanisms of cell fate determination is thus crucial to understanding differentiation and generation of cell diversity.

Two models have been put forth to explain how cell diversity is generated. The cell-intrinsic model postulates that there is an unequal distribution of components in the cell that biases the cell fate decision. The cell-extrinsic model proposes that cell-cell communication leads to a particular cell fate. The dichotomy between cell-intrinsic and cell-extrinsic models of developmental control started as a mainly philosophical debate between preformists and epigeneticists. According to the preformistic view, which was formed in the 17th century, the organism begins with a complete but miniature organism within the egg (or sperm). Each miniature itself contains eggs for the next generation which again contain preexisting miniatures. From this prospective, development merely requires the growth and unfolding of the preexisting creature in the egg. In other words, the creature intrinsic to the egg determines the entire developmental outcome of the egg. The alternative epigenetic view, which was formed in the 18th century and supported by direct observation of organ formation, was that the organism, which is formed by germ layers, is generated anew from undifferentiated tissues. Epigenesis implies that interactions between parts of embryos generate new types of cells in organized patterns. These two extreme views foreshadowed the two mechanisms of cell

fate determination accepted by modern developmental biologists, cell-intrinsic (mediated by asymmetric segregation of cellular determinants) and cell-extrinsic (mediated by signals in the environment) mechanisms. However, at the time that they were originally proposed, neither view went much beyond philosophical contemplation. In the end, the two viewpoints were reconciled in a theory which states that development proceeds epigenetically through a predetermined force inherent in the matter of the embryo. In other words, epigenetic development is directed by preformed instructions in the egg. This view was summarized by Harrison in 1936 upon his reflection on this part of the debate (Harrison, 1969):

"This mode of development combines unfolding and interactions, thus according partly with each of the old theories of preformation and epigenesis. It may be described as epigenesis in that various characters of the future organism do not appear at first as such, but are realized through reaction between them and the environment. It is preformational in that there is something in the molecular configuration which determines the physical arrangement and the special chemical reactions that produce in the end the adult organism of the proper species."

In the late 19th century, early experimental embryologists addressed these arguments by examining developmental fates of dissociated cells, usually blastomeres. Experiments in different organisms favored two theories: mosaic and regulative development. In frog embryos an isolated blastomere forms a deformed partial embryo, corresponding to the parts of the embryos normally formed by that blastomere (Roux, 1888). This observation led to the theory of mosaic development, which focused on the presence of localized cytoplasmic determinants that generate distinct tissues in the mature organism. In sea urchins, on the other hand, a dissociated part of an embryo could form a complete, albeit smaller, organism (Driesch, 1892). This study led to the theory of regulation, which emphasized the effect of embryonic induction to set or reset the specific developmental program. Eventually, embryologists realized the

complexity of development and reached the conclusion that both mosaic and regulative processes play a role in all of the animals that they had tested.

New experimental technologies have been responsible for further advances. In recent years, the application of genetics and molecular biology has established molecular bases for cell fate specification. The mechanism of embryonic induction can be attributed to the transmission of signals from one cell to another via cell surface ligands and receptors, whereas cytoplasmic determinants have been identified as molecules that are differentially segregated to cells for specifying the fates of these cells. Furthermore, each somatic cell carries essentially the same set of genes, so the differences between cell types are due to the differential expression of these genes. The signals instructed by molecules responsible for cell-cell interaction or inherited determinants ultimately elicit the expression of transcription factors, which then regulate tissue-specific gene expression to execute particular developmental programs.

Diversity of cell fate can be generated by an asymmetric cell division which produces two distinct daughter cells. The unequal distribution of determinants to one daughter cell during division, cell-cell communication either between the daughter cells or between daughter cells and surrounding cells, or a combination of these mechanisms, may be responsible for the generation of the two different cell types (reviewed by Horvitz and Herskowitz, 1992). During development of *Drosophila* sensory organs, a single progenitor cell generates four progeny, one neuron and three different non-neuronal support cells, following multiple rounds of asymmetric cell division. These asymmetric divisions provide an excellent experimental system to investigate the mechanisms by which cell diversity is generated, as well as the mechanism by which neuronal fate is specified.

In this thesis, I will address how a cell-intrinsic determinant (Numb), a

transmembrane receptor (Notch), and a transcription factor (Tramtrack) all function in a signalling pathway to specify the neuronal fate in *Drosophila* sensory organs. Before I go into detail, I first give a brief review of neurogenesis in the *Drosophila* peripheral nervous system (PNS) and factors shown to be involved in these cell fate determination processes.

Development of the *Drosophila* Sensory Organs

Progressive development of the sensory organs

Development of the *Drosophila* PNS, as with that of the central nervous system (CNS), is a progressive process (Figure 1.1) (Ghysen and Dambly-Chaudiere, 1989; Jan and Jan, 1994). The expression of "proneural genes" in clusters of ectodermal cells, designated as the "proneural clusters", endows these cells with competence to become a sensory organ precursor (SOP) (reviewed by Ghysen and Dambly-Chaudiere, 1988; Campuzano and Modolell, 1992). Each cell in a proneural cluster inhibits its neighbors from adopting the SOP fate by cell-cell communication mediated by "neurogenic genes". As a consequence of this mutual inhibition, a single SOP is selected in each proneural cluster (Artavanis-Tsakonas and Simpson, 1991; Campos-Ortega, 1988). The SOP then undergoes rounds of asymmetric divisions to form a neuron and three distinct non-neuronal cells. In this thesis, I have addressed the molecular mechanisms of how cell fate is specified during SOP asymmetric divisions.

There are two main types of sensory organs in the *Drosophila* PNS, the external sensory (es) organ and the chodotonal (cho) organ. The development of es organ SOPs requires the proneural genes of the *achaete-scute* complex (Dambly-Chaudiere and Ghysen, 1987), and the development of the cho organ SOPs requires *atonal* (Jarman et al., 1993). These proneural genes all encode proteins with basic helix-loop-helix (bHLH) motifs (Campuzano et al., 1985; Jarman et al., 1993). Each of the proteins forms heterodimers with the ubiquitously expressed bHLH protein Daughterless (Murre et al., 1989; Cabrera and Alonso, 1991; Caudy et al., 1988; Jarman et al., 1993). The heterodimers can act as transcriptional activators *in vitro* (Cabrera and Alonso, 1991). The mutual inhibition process mediated by neurogenic genes results in an up regulation of the expression of proneural genes in the future SOP and down regulation of the

expression of these same genes in the adjacent cells (reviewed by Artavanis-Tsakonas and Simpson, 1991; Campos-Ortega, 1988; Ghysen et al., 1993). SOPs, once singled out, then undergo a stereotyped pattern of division to form four distinct progeny. To form an es organ (Figure 1.2A), the SOP gives rise to two distinct daughter cells, IIa and IIb. The IIa cell then divides to produce two outer support cells, the hair cell (tricogen) and the socket cell (tormogen). Shortly after the division of the IIa cell, the IIb cell divides to generate two inner cells, the neuron and the sheath cell (thecogen) (Bate, 1978; Hartenstein and Posakony, 1989). A different series of asymmetric divisions allows the cho organ SOP to give rise to the neuron and three different support cells (the sheath cell, the cap cell and the ligament cell) (Figure 1.2B) (Bodmer et al., 1989; Brewster and Bodmer, 1995).

A requirement for the *N* signalling pathway during development

A group of genes known as "neurogenic genes" including *Notch (N)*, *Delta (Dl)*, *Enhancer of Split* (*E(spl)*), *mastermind (mam)*, *neuralized*, and *big brain* mediate the mutual inhibition process that selects SOPs from proneural clusters (reviewed by Campos-Ortega, 1988). Loss of neurogenic gene function results in a significant increase in the number of neurons at the expense of epidermal cells. *N* encodes a transmembrane protein with EGF-like repeats in the extracellular domain and ankyrin repeats in the intracellular domain (Kidd et al., 1986; Wharton et al., 1985). *Dl* also encodes a transmembrane protein with EGF-like repeats (Kopczynski et al., 1988; Vaessin et al., 1987). N functions as a receptor and Dl acts as a ligand of N (Heitzler and Simpson, 1991; Rebay et al., 1991). Extensive studies have shown that *N* and *Dl* mediate cell-cell communication in multiple developmental processes in *Drosophila* , including oogenesis (Ruohola et al., 1991; Xu et al., 1992), eye development (Cagan and Ready, 1989),

development of ventral midline cells (Menne and Klambt, 1994), malpighian tubule formation (Hartenstein et al., 1992), axonal guidance (Giniger et al., 1993) and wing formation (de Celis et al., 1996; Doherty et al., 1996). Several *N* homologs have been identified in vertebrates including zebrafish, frogs, mice, rats, and humans (reviewed by Artavanis-Tsakonas et al., 1995).

Studies of *N* in various species have implicated *N* in two kinds of local signalling events. *N*-mediated cell-cell interactions may occur between equivalent cells with the result that some cells, with higher N signaling activity, adopt one cell fate, whereas the remaining cells adopt an alternative fate, such as in the process of singling out of neuronal precursor cells in *Drosophila* (Lehmann et al., 1983). *N*-mediated interactions may also occur as uni-directionally inductive events from one cell to another; the ablation of the signal results in failure of the receiving cell to adopt a particular cell fate instead of causing this cell to adopt the fate of the signalling cell. This sort of inductive cell fate decision occurs at the four-cell stage of the early *C elegans* embryo. Through the function of *glp-1*, a homolog of *N*, and *apx-1*, a homolog of *Dl*, the P2 cell induces the ABp cell to adopt a different fate from the ABa cell. Loss of *glp-1* results in the ABp cell adopting the fate of its sister cell, ABa, rather than that of the P2 cell (Mango et al., 1994; Mello et al., 1994; Mickey et al., 1996).

How does activated N function? Using the *Xenopus* homolog of *N*, *Xotch*, Coffman, et al. have shown that when the activated Xotch is expressed in the *Xenopus* embryo, it inhibits the early expression of epidermal and neural crest markers in cells, but allows these cells to respond to later mesodermal and neural inductive cues. This result suggests that *Xotch* functions to delay differentiation and leave undetermined cells competent to respond to later inductive signals (Coffman et al., 1993). This idea is supported by the study of *N* in *Drosophila* eye development; the expression of activated Notch arrests cell fate determination

and delays cell differentiation (Fortini et al., 1993). An alternative interpretation of these results is that *N* directly controls a series of binary cell-fate decisions (Greenwald, 1994).

Recent molecular and biochemical studies have identified several other genes as components in the *N* signalling pathway. *Suppressor of Hairless (Su(H))* acts downstream of *N*, and its protein product may activate transcription of *E(spl)* genes. *Hairless (H)* and *deltex (dx)* may function as regulators of this *N* signalling pathway (Figure 1.3) (reviewed by Artavanis-Tsakonas et al., 1995). Much of my thesis is focused on investigating the regulation of *N* and identifying targets of *N* during SOP progeny fate specification. In the next section, I review recent findings related to components of the *N* signalling pathway.

Su(H)* acts downstream of *N

Su(H) was originally isolated as a dominant suppressor of *H* (Ashburner, 1982). Although complete removal of zygotic function of *Su(H)* in the embryo does not result in a detectable PNS phenotype, eliminating both maternal and zygotic *Su(H)* function results in embryos with neuronal hypertrophy (Lecourtois and Schweisguth, 1995). This phenotype, at least in the CNS, is reminiscent of the classic neurogenic phenotype found in *N* null embryos. *Su(H)* encodes a protein homologous to the mouse recombination signal-binding protein (Furukawa et al., 1992; Schweisguth and Posakony, 1992); however, site-directed mutagenesis studies suggest that the sequence similarity has no functional significance *in vivo* (Schweisguth et al., 1994).

Fortini and Artavanis-Tsakonas (1994) demonstrated that *Su(H)* acts downstream of *N* using cultured *Drosophila* S2 cells (Figure 1.3). In cells transfected with Su(H) alone, Su(H) is found in the nucleus. When both N and Su(H) are expressed, Su(H) is found in the cytoplasm. When a group of cells is transfected with both N and Su(H) and aggregated with cells that express Dl,

Su(H) is found in the nucleus. Thus, when N is bound by Dl, Su(H) translocates from the cytoplasm into the nucleus (Fortini and Artavanis-Tsakonas, 1994). Initially, Su(H) was reported to bind to the ankyrin repeats of N (Fortini and Artavanis-Tsakonas, 1994). More recent work shows that Su(H) instead binds to the RAM23 region of N (Hsieh et al., 1996; Tamura et al., 1995). In any case, based on these *in vitro* results, Fortini and Artavanis-Tsakonas proposed that N, by binding Su(H), is capable of sequestering Su(H) protein into the cytoplasm and that the binding of Dl to N releases Su(H), resulting in the nuclear localization of Su(H) (Fortini and Artavanis-Tsakonas, 1994). However, this model predicts that Su(H) will be localized in the nucleus in the absence of *N* and in the presence of ligand-activated *N*. Since loss of *N* function and activated *N* generate opposite phenotypes, the localization of Su(H) to the nucleus does not imply active signalling. Thus, the regulation of *Su(H)* by *N* cannot be achieved just by translocating Su(H) into the nucleus.

In vivo antibody staining of Su(H) protein, however, indicates that neither differential expression nor differential subcellular localization of Su(H) is essential for Su(H) function during the time of SOP progeny fate determination (Gho et al., 1996). In wing imaginal discs, Su(H) is evenly distributed in the nuclei of all imaginal disc cells during the SOP selection and divisions. Although after the formation of the four SOP progeny, Su(H) specifically accumulates in the nucleus and later accumulates in both the nucleus and the cytoplasm of the future socket cell, Su(H) does not colocalize with N at the cell membrane in this cell (Gho et al., 1996). Two models may reconcile these *in vivo* results with those of Fortini and Artavanis-Tsakonas (1994). The physiologically significant interaction of N and Su(H) might occur in either the cytoplasm or the nucleus. The binding of N and Su(H) *in vivo* could occur in the cytoplasm, but the level of Su(H) may be below the detection limit of the anti-Su(H) antibody. The Su(H)/N

interaction in the cytoplasm may result in a modification of Su(H) by the activated *N*. If this model is correct, although Su(H) is found in the nucleus in the absence of *N* and in the presence of activated *N*, it may be that only the Su(H) modified by activated *N* is able to carry out downstream signalling events. Alternatively, the physiologically significant interaction of N and Su(H) might occur in the nucleus, and this N/Su(H) complex may be important for downstream signalling events. If this is correct, then the observation made in the tissue culture system that N sequesters Su(H) in the cytoplasm would then reflect the direct physical interaction of N and Su(H) before N was cleaved and able to enter the nucleus.

Does N signal in the nucleus?

A key question related to the regulation of Su(H) by N is whether N, a transmembrane protein, is cleaved in its intracellular domain, allowing this domain to enter the nucleus to carry out signalling activity. This intriguing possibility was first suggested by the detection of the N protein in the nucleus after overexpression of the intracellular domain of N (N^{intra}), which results in an activated *N* receptor (Fortini et al., 1993; Lieber et al., 1993; Struhl et al., 1993). In support of this view, two nuclear localization signals were identified in N^{intra} by examining the localization of fragments of N after these fragments were transfected into tissue culture cells (Lieber et al., 1993). The functional significance of the nuclear localization was first suggested by Kopan et. al. (1994), using a mammalian cell line that can undergo myogenesis. Overexpression of the intracellular domain of mNotch1, a mouse homolog of *N*, represses myogenesis. Removal of the nuclear localization signal reduces the nuclear localization of mNotch1 and diminishes its ability to inhibit myogenesis (Kopan et al., 1994). The physiological significance of the N nuclear localization was further suggested by another *in vitro* study. The activated forms of mNotch bind

to the human analog of Su(H), KBF2/RBP-J kappa. These protein complexes act as transcriptional activators through the KBF2-binding sites of the promoter of *HES-1*, the mammalian homolog of *E(spl)* (Jarriault et al., 1995). These studies thus suggest a model in which Su(H) binding to N results in a complex in the nucleus that activates transcription.

Is N cleaved? Using both an HA tag at the N-terminus and a Myc tag at the C-terminus of N fragments, Kopan et al. have shown that in tissue culture, the N fragment that lacks the extracellular domain but retains its membrane-spanning region becomes proteolytically processed on its intracellular surface (Kopan et al., 1994). As a result of this proteolytic event, the activated mNotch (intracellular domain) can move to the nucleus. This proteolytic cleavage at an intracellular site can be blocked by protease inhibitors (Kopan et al., 1994).

Taken together, these studies suggest that N can be cleaved and function in the nucleus. A major obstacle to acceptance of this model is that the N protein has not been detected in the nucleus *in vivo*, in either whole mount embryos or imaginal discs in *Drosophila*, nor has anyone detected N in the nucleus during cell fate specification in other species.

E(spl)* genes act as downstream targets of *N* and *Su(H)

Loss of *E(spl)* gene function causes hypertrophy of neural tissue similarly to the loss of *N* function. *E(spl)* is a gene complex of seven related genes that encode bHLH proteins characteristic of transcriptional regulators (Delidakis and Artavanis-Tsakonas, 1992; Knust et al., 1992). Epistasis analysis suggests that *E(spl)* genes act downstream of *N* (Vaessin et al., 1985). Also, expression of *E(spl)* genes is upregulated in the presence of activated *N* (Bailey and Posakony, 1995) and down regulated in the absence of *Su(H)* function (Lecourtois and Schweisguth, 1995), suggesting that *N*-dependent activation of *E(spl)* may be at least in part be mediated by *Su(H)* (Figure 1.3). In support of this finding, several

putative Su(H) binding sites have been found in the regulatory sequences of the *E(spl)* genes. Mutation of these Su(H) binding sites abolishes the expression of the lacZ transgene driven by this mutated *E(spl)* promoter (Bailey and Posakony, 1995; Lecourtois and Schweisguth, 1995). Thus Su(H) may directly activate the expression of *E(spl)* genes (Bailey and Posakony, 1995; Lecourtois and Schweisguth, 1995).

_H_ may negatively regulate _Su(H)_

Su(H) function is antagonized by the *H* gene. Phenotypes caused by loss of *H* function and overexpression of *H* are opposite to those caused by loss of *Su(H)* and *Su(H)* overexpression (Bang et al., 1991; Bang and Posakony, 1992; Lees and Waddington, 1942; Ashburner, 1982). Epistasis studies between *H* and *Su(H)* reveal no strict epistatic relationship between these two genes (Schweisguth and Posakony, 1994). *H* also antagonizes *N* and *Dl* function in the embryo, although removal of zygotic *H* shows no obvious nervous system phenotype on its own. Thus, H behaves as an "anti-neurogenic gene" (Vaessin et al., 1985). *H* encodes a novel basic protein, and the *H* mRNA is expressed ubiquitously in the embryo and the imaginal disc (Bang and Posakony, 1992; Maier, 1992). H physically binds Su(H) and suppresses Su(H) binding to DNA *in vitro* (Brou et al., 1994). The dynamics of the subcellular localization of Su(H) and the intimate genetic and biochemical interaction between *H* and *Su(H)* suggest the following questions: What is the subcellular localization of *H*? Are there signals that regulate *H*? Does H act to block Su(H) from activating *E(spl)* transcriptionally? Can H block the physical interaction of N and Su(H)?

_dx_ may positively regulate _N_

dx encodes a basic cytoplasmic protein which is ubiquitously expressed throughout development (Busseau et al., 1994). Dx binds to the ankyrin repeats of N (Diederich et al., 1994; Matsuno et al., 1995). Genetically, *dx* interacts with

N, *Delta*, *mam* (Xu and Artavanis-Tsakonas, 1990), and *Su(H)* (Fortini and Artavanis-Tsakonas, 1994). Overexpression of *dx* causes phenotypes of missing bristle and "double socket", similar to those due to *N* activation. Expression of a constitutively activated *N* receptor rescues the phenotype of a *dx* hypomorphic mutation. Using *Drosophila* S2 tissue culture cells, Matsuno et. al. (1995) have shown that when Dx is coexpressed with Su(H) in N-expressing cells, Su(H) is detected in the nucleus, as opposed to in the cytoplasm. Thus, Dx prevents the N-dependent cytoplasmic retention of Su(H) in the absence of Dl (Matsuno et al., 1995). Therefore, *dx* may act upstream of *N* and positively regulate *N* signalling (Matsuno et al., 1995). Alternatively, *dx* could enhance the nuclear localization of Su(H) or retain Su(H) in the nucleus.

A requirement for the *N* signaling pathway during specification of SOP progeny fates

The section above described the function and components of the *N* signalling pathway during development. In the next section, I review the critical role that *N* signalling plays in specifying SOP progeny fates. The first indication that the *N* signalling pathway was involved in the specification of SOP progeny fate came from the study of a temperature-sensitive allele of *N* (N^{ts}). Using N^{ts}, Hartenstein and Posakony were able to separate the function of *N* in SOP selection from the later requirement of *N* in sense organ differentiation (Hartenstein and Posakony, 1990). When N^{ts} pupae were shifted to the restrictive temperature during SOP divisions and differentiation, extra neurons developed at the expense of the three support cells. This result was interpreted to suggest that the four cells in the es lineage constitute a "miniature neurogenic region", and that the role of *N* is to select one neuron from this four-cell proneural cluster (Hartenstein and Posakony, 1990). A more thorough

phenotypic analysis combining both loss of function of *N* and overexpression of *N* (Chapter 3) leads us to a different interpretation: *N* specifies four distinct SOP progeny fates by acting during all three asymmetric divisions of the es organ. Nevertheless, this insightful yet limited study by Hartenstein and Posakony (1990) provided the first evidence that cell-cell interaction plays an important role in the proper fate determination of the adult es organ.

In similar experiments using Dl^{ts}, Parks et al. (1993) demonstrated a requirement for *Dl* for the specification of SOP progeny fate (Parks and Muskavitch, 1993). Reduction of *Dl* activity during SOP progeny formation results in phenotypes of four neurons and no support cells, as well as two neurons and two sheath cells. Thus, these results suggest that *Dl* specifies four distinct progeny in a stepwise fashion, first during the SOP division and then during the IIb cell division (Parks and Muskavitch, 1993).

Other components of the N pathway, *Su(H)*, *H*, and *E(spl)*, also function in SOP progeny fate determination. Loss of *Su(H)* function results in loss of outer support cells associated with multiple neurons (Schweisguth, 1995), as well as twin hairs without sockets (Ashburner, 1982; Schweisguth and Posakony, 1994). Thus, *Su(H)* is required for specification of the IIa and IIb cell fate and specification of the hair and socket cell fate. *H* is required for the hair/socket decision, since loss of *H* function causes double sockets without hairs (Bang et al., 1991; Lees and Waddington, 1942), and overexpression of *H* results in double hairs without sockets (Bang and Posakony, 1992). Whether *H* also functions in specifying the IIa/IIb cell fate remains to be investigated. *E(spl)* genes may also act in specifying the IIa/IIb cell fates, since loss of *E(spl)* function leads to bristle balding associated with supernumerary neurons (Tata and Hartley, 1995). Also, overexpression of *E(spl)* genes during SOP differentiation produces bristles with aberrant outer support cells indicative of cell fate transformations (Tata and

Hartley, 1995). E(spl) genes may act as a neuronal suppressors, since ectopic expression of *E(spl)* causes reduction in the number of neuronal precursors including SOPs (Nakao and Campos-Ortega, 1996).

The *N* signalling pathways are utilized repeatedly as "genetic cassettes" (Jan and Jan, 1993; Ruohola-Baker et al., 1994) during development of many different tissues (reviewed by Artavanis-Tsakonas et al., 1995). It is likely that *N* is regulated in different ways in these diverse developmental processes to allow the control of specific decisions. Furthermore, it is likely that different transcriptional targets mediate different *N*-dependent decisions. How is *N* regulated? How is the regulator able to access *N*? What are these different targets? In this thesis, I examined the role of *N* function in a specific set of cell fate decisions and identified a new regulator of *N*, *numb*, as well as a new downstream target of *N*, *ttk*. My results suggest that Numb may block *N* signalling directly at the level of the receptor through a direct protein-protein interaction. Moreover, *ttk* may constitute a readout independent of *Su(H)*. These results provide a molecular mechanism for how the specificity of *N* signalling can be achieved (Chapter 2, 3, 4 and 5). Other proteins, Phyl and Sina, may regulate the ability of *ttk* to carry out *N* signalling (Chapter 4). Before I present the data, I first review published findings related to factors including *numb*, *ttk*, *phyl* and *sina*.

Numb acts as a cell-intrinsic determinant

numb acts in all three divisions of the es organ. Loss of *numb* function and overexpression of *numb* result in the opposite cell fate transformation (Uemura et al., 1989; Rhyu et al., 1994; S.W. Wang, personal communication). Immunocytochemical studies have shown that Numb is membrane-associated, asymmetrically localized to one pole of the SOP and is segregated to one

daughter cell (Rhyu et al., 1994; Knoblich et al., 1995). Thus, *numb* acts as an intrinsic determinant for cell fate decision during multiple asymmetric divisions. Similarly, asymmetric localization of Numb is also required to specify two distinct neuronal fates following the asymmetric division of the MP2 neuroblast in the central nervous system (CNS) (Spana et al., 1995). The sequence of *numb* (Uemura et al., 1989) reveals a protein with a motif known as the phosphotyrosine binding (PTB) (Kavanaugh and Williams, 1994), or phosphotyrosine interaction (PID) domain (Bork and Margolis, 1995).

How does the initial asymmetric distribution of Numb regulate SOP progeny fate decision? How do *numb*-dependent signals interact with those transduced by the *N* signalling pathway? As shown in Chapters 2 and 3, Numb antagonizes N, probably through a direct physical binding. As a consequence of this antagonism, Ttk is differentially expressed in one of the daughter cells, which may result in the regulation of cell-specific gene expression.

Tramtrack, a transcription factor required in many developmental processes

The *ttk* gene encodes two alternatively spliced proteins, 69KD and 88KD, with different pairs of zinc fingers and a common N-terminal region (Read and Manley, 1992) including a 120-amino-acid conserved motif called a POZ or BTB domain (Bardwell and Treisman, 1994; Godt et al., 1993). This domain is evolutionarily conserved in genes from viruses to mammals (Zollman et al., 1994) and is shared by multiple genes in *Drosophila* including the *Broad-complex* (DiBello et al., 1991), *bric-a-brac* (Godt et al., 1993), *lola* (Giniger et al., 1994), *pipsqueak* (Horowitz and Berg, 1996; Siegel et al., 1993), *fruitless* (Ito et al., 1996), *abrupt* (Hu et al., 1995) and *gaga* (Soeller et al., 1993). This domain may be involved in protein dimerization and transcriptional regulation (Bardwell and Treisman, 1994; Chen et al., 1995).

Ttk was originally identified as a protein that binds the regulatory regions

of the segmentation genes *fushi tarazu* (*ftz*) and *even-skipped* (*eve*) (Brown et al., 1991; Harrison and Travers, 1990; Read and Manley, 1992). Removal of the Ttk binding site from a *ftz* promoter-lacZ reporter construct causes ectopic ß-gal expression in cells between the usual *ftz* stripes (Brown et al., 1991). Overexpression of *ttk* suppresses the expression of Ftz and Eve (Brown and Wu, 1993; Read et al., 1992). Thus, *ttk* may act as a transcriptional repressor.

sina, phyl, twins* and *musashi

Other genes have also been shown to play important roles in specifying SOP progeny fates. *twins* encodes a regulatory subunit of serine/threonine phosphatase 2A (PP2A) (Uemura et al., 1993). *musashi* encodes an RNA-binding protein (Nakamura et al., 1994). Genes that function in the *Ras* signalling pathway during eye development, *seven-in-absentia (sina)* and *phyllopod (phyl)*, also play a role in PNS development. *phyl* encodes a novel nuclear protein and acts as a downstream target gene of the *Ras* pathway (Chang et al., 1995; Dickson et al., 1995). *sina* encodes a protein with a zinc finger motif characteristic of a nuclear protein, and it may act in parallel to the *Ras* pathway (Carthew and Rubin, 1990). Interestingly, the function of *sina* in photoreceptor R7 specification depends on *ttk* (Lai et al., 1996). Loss of *sina* or *phyl* function causes missing bristles and aberrant hairs and sockets. (Carthew and Rubin, 1990; Chang et al., 1995; Dickson et al., 1995). In Chapter 4 I characterize the function of *sina* and *phyl* during PNS development, and discuss the potential regulation of *ttk* by *phyl* and *sina*.

When I started my thesis project in the spring of 1993, several studies pointed to SOP progeny fate determination as an interesting problem. Both *H* and *Su(H)* had been long shown to play a pivotal role in specifying hair and socket fates. Studies of N^{ts} (Hartenstein and Posakony, 1990) and Dl^{ts} (Parks and

Muskavitch, 1993) revealed the requirement for *N*-dependent cell-cell interaction in SOP specification.

In our lab, two mutants that appear to act in this process were isolated from a mutagenesis screen, *numb* (for loss of neurons) and *oversensitive* (*osn*, for overproduction of neurons) (Bier et al., 1988; Jan and Jan, 1990). When I started working on *osn*, I asked the following questions. What role does *osn* play in cell fate determination? What is the epistatic relationship between these two genes? Immediately, I found that the sequence of *osn* (determined by Ethan Bier) is identical to that of the *ttk* gene. I then showed that *ttk* acts as a binary switch between the two SOP daughter fates and likely between the neuron and the sheath cell fates. Furthermore, during these divisions Ttk is expressed in only one of the two daughter cells (also see Raemarker et al., submitted). In addition, *ttk* acts in the same genetic pathway as *numb* and is negatively regulated by *numb* (Chapter 2).

By the end of 1994, considerable progress had been made in the study of the *N* signalling pathway. The previously isolated "players", *Su(H)*, *E(spl)*, and *H* were placed in a signalling cascade (Figure 1.3). The dependence of asymmetric division on *numb* and genes in the *N* signalling pathway raised several questions. How does the cell-intrinsic signal interact with signals arising from cell-cell interaction? How are these different instructions to the two daughter cells implemented to secure their distinct fates? I addressed these questions by identifying a signalling pathway composed of *N*, *numb*, and *ttk*. Genetically *numb* negatively regulates *N*; biochemically Numb binds N. *N* positively regulates *ttk*, leading to Ttk expression in the daughter cell that does not inherit Numb (Chapter 2 and 3). How does *ttk*, the target of *numb* and *N*, exert its function to specify cell fate? In Chapter 4, I suggest that *ttk* suppresses the neural fate and may be negatively regulated by two nuclear proteins, Sina and Phyl. In

Figure 1.1. Neurogenesis in the PNS is a progressive process.

Proneural genes are expressed in clusters of ectodermal cells designated as "proneural clusters" (lightly shaded); this endows these cells with the potential to become SOPs. A single SOP is selected in each proneural cluster by a process of "mutual inhibition" mediated by neurogenic genes. This process results in an up regulation of the expression of proneural genes in the future SOP (darkly shaded), and down regulation of the expression of these same genes in the adjacent cells which will adopt epidermal fates. The SOP then begins to express neuronal precursor genes (also known as "pan-neural genes"), which may confer neural characteristics. The SOP then delaminates from the epidermis and undergoes stereotypical cell divisions to produce a fixed number of progeny (four for the es organ). These four cells adopt distinct fates and subsequently differentiate into one neuron and three different non-neuronal cells.

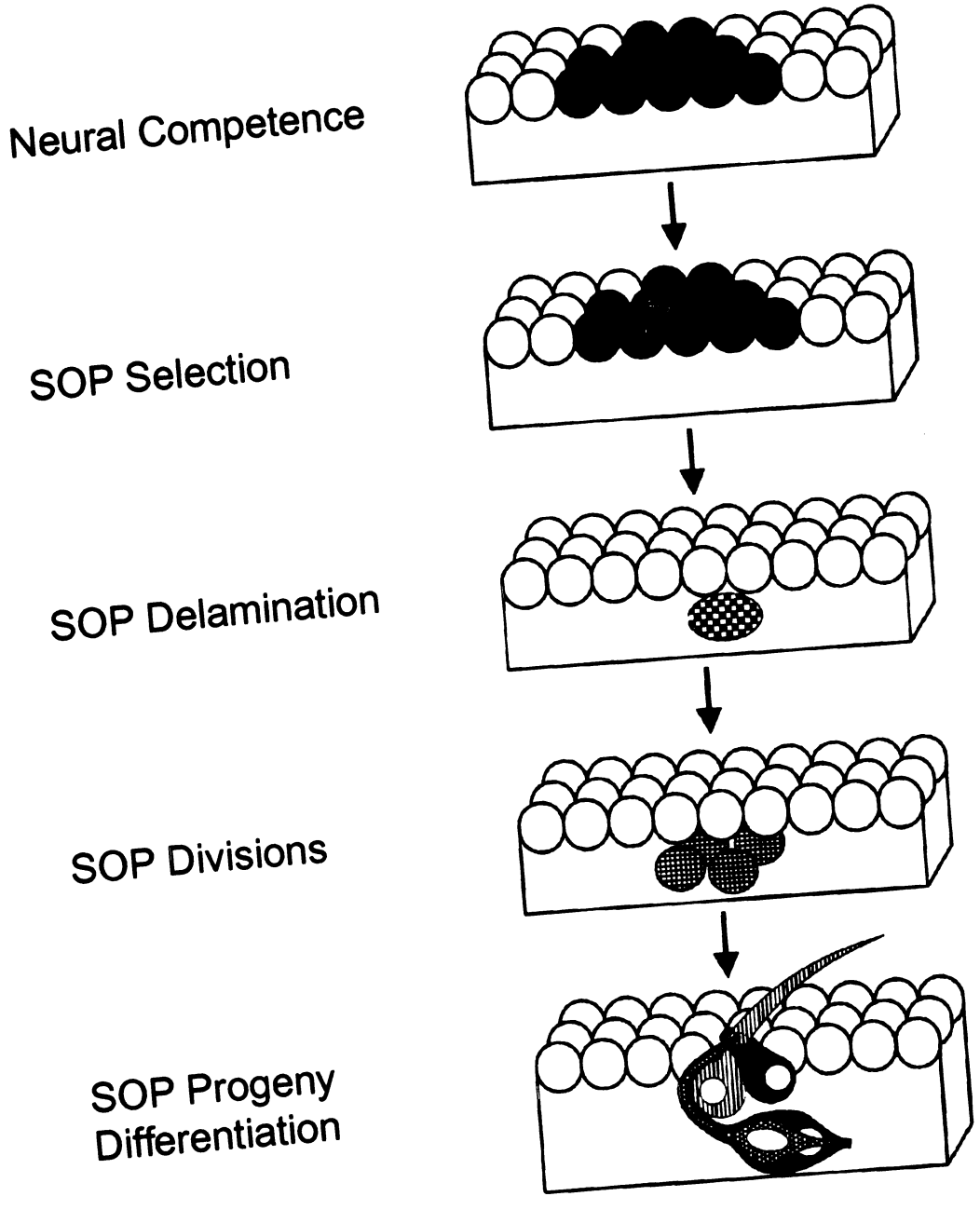
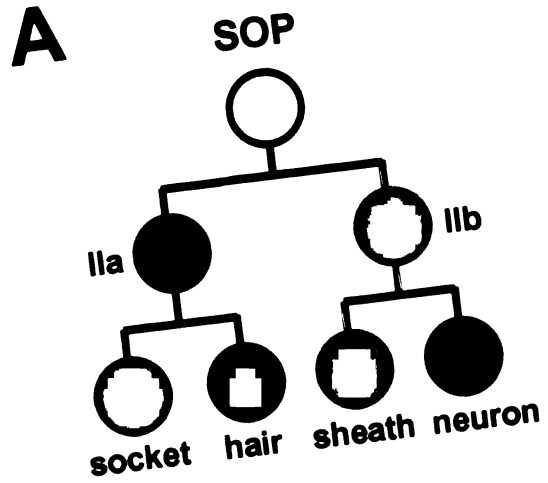

Neural Competence
SOP Selection
SOP Delamination
SOP Divisions
SOP Progeny
Differentiation

Figure 1.1

Figure 1.2. A schematic drawing of cell lineages for the es organ and cho organ.

(A) SOPs undergo a stereotyped pattern of division to form four distinct progeny. To form an es organ, the SOP gives rise to two distinct daughter cells, IIa and IIb. The IIa cell then divides to produce two outer support cells, the hair cell and the socket cell. Shortly after the division of the IIa cell, the IIb cell divides to generate two inner cells, the neuron and the sheath cell.

(B) To produce a cho organ, a different series of asymmetric divisions allows the SOP to give rise to one neuron and three different support cells, the sheath cell, the cap cell and the ligament cell (B). The cho lineage was modified by Brewster and Bodmer in 1995 (Bodmer et al., 1989; Brewster and Bodmer, 1995).


A
SOP
IIa
IIb
socket
hair
sheath
neuron

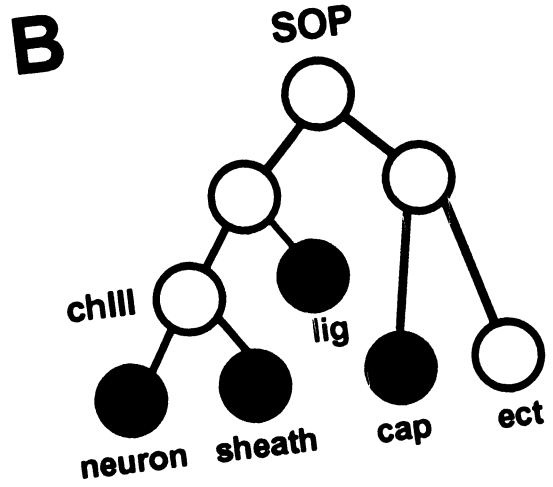

B
SOP
chIII
lig
neuron
sheath
cap
ect

Figure 1.2

Figure 1.3. A schematic drawing of the *N* signalling pathway.

Recent molecular and biochemical studies have demonstrated that N functions as a receptor and that Dl acts as a ligand of N. *Su(H)* acts downstream of *N*, and its protein product may activate transcription of *E(spl)* genes. H physically binds Su(H) and prevents Su(H) from binding to DNA. *dx* may positively regulate the *Notch* signalling.

The *Notch* Signalling Pathway

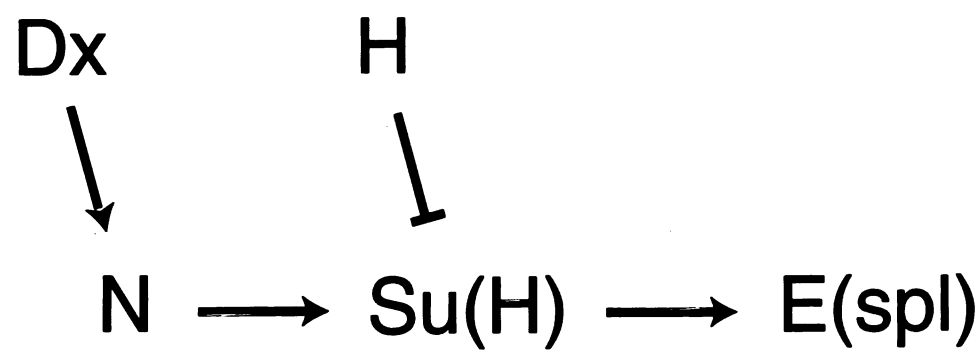


Figure 1.3

CHAPTER 2

tramtrack acts downstream of *numb* to specify distinct daughter cell fates during asymmetric cell divisions

tramtrack Acts Downstream of *numb* to Specify Distinct Daughter Cell Fates during Asymmetric Cell Divisions in the Drosophila PNS

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Summary

Asymmetric cell divisions allow a sensory organ precursor (SOP) cell to generate a neuron and its support cells in the Drosophila PNS. We demonstrate a role of *tramtrack* (*ttk*), previously identified as a zinc finger-containing putative transcription factor, in the determination of different daughter cell fates. Both loss of function and overexpression of *ttk* affect the fates of the SOP progeny. Whereas loss of *ttk* function transforms support cells to neurons, *ttk* overexpression results in the reverse transformation. ttk is expressed in support cells but not in neurons. It has been shown that numb, a membrane-associated protein asymmetrically distributed during the SOP division, confers different daughter cell fates. Loss of *ttk* or *numb* function results in reciprocal cell fate transformation. Epistatic studies suggest that *ttk* acts downstream of *numb*. We propose that ttk executes the command dictated by asymmetrically localized numb to specify distinct daughter cell fates during multiple asymmetric divisions.

Introduction

In the nervous system, cell lineage plays an important role in specifying the fate of some of the neurons. In such cases, cell lineage is highly determinate, i.e., the fate of a neuron can be predicted from its ancestry. A determinate cell lineage generally involves asymmetric cell division, i.e., a type of division that produces two daughter cells that ultimately differ in their developmental fates (reviewed by Horvitz and Herskowitz, 1992). The basic mechanism of generating an asymmetric division is fundamental to the understanding of not only neuronal cell fate specification, but also how cell diversity is generated during development in general. The simple and stereotyped division pattern of the developing Drosophila PNS provides a very useful model system to study the mechanism of asymmetric divisions.

The Drosophila PNS contains several types of sensory organs, including external sense (es) organs and chordotonal (cho) organs (Campos-Ortega and Hartenstein, 1985; Ghysen et al., 1986; Bodmer and Jan, 1987). A simple es (see Figure 4A) or cho organ is composed of one neuron and three different nonneuronal support cells. To generate a simple es organ, a sensory organ precursor (SOP) cell divides to produce two secondary precursor cells, IIa and IIb (see Figure 3A). The IIa cell then divides to form two outer support cells, a hair cell (tricogen) and a socket cell (tormogen), whereas the IIb cell divides to form a neuron and a sheath cell (thecogen) (Bate, 1978; Bodmer et al., 1989; Hartenstein and Posakony, 1989). A cho organ, which consists of a neuron, a scolopale cell, a cap cell, and a ligament cell, is derived from a SOP through three rounds of asymmetric divisions (see Figure 3D; Bodmer et al., 1989).

The *numb* gene has been shown to play an important role in the fate determination of the SOP progeny cells. In es organs, loss of *numb* function transforms one daughter cell of the SOP, IIb, into the other, IIa (Uemura et al., 1989), whereas overexpression of *numb* causes the opposite cell fate transformation (Rhyu et al., 1994). *numb* is also required for progeny of cho organs to adopt proper fates (Uemura et al., 1989; Rhyu et al., 1994). Immunocytochemical study has shown that numb is membrane associated and asymmetrically distributed within the SOP prior to its division and is predominantly partitioned to one of the daughter cells (Rhyu et al., 1994). Thus, the asymmetric distribution of numb confers distinct fates to SOP daughter cells.

The *tramtrack* (*ttk*) gene product was originally identified biochemically on the basis of its ability to bind the regulatory regions of the pair-rule genes *fushi tarazu* (*ftz*) and *even-skipped* (*eve*), and has been postulated to be a transcriptional repressor (Harrison and Travers, 1990; Read and Manley, 1992; Brown et al., 1991). The *ttk* gene encodes two alternatively spliced proteins, 69 kDa and 88 kDa, with different pairs of zinc fingers and a common N-terminal region (Read and Manley, 1992), including a 120 amino acid conserved motif called POZ domain (Bardwell and Treisman, 1994), or BTB domain (Godt et al., 1993). *ttk* has been shown to be required for the specification of cell fate in eye development and for cuticle formation (Xiong and Montell, 1993). The complex expression of *ttk* in the embryo, however, suggests that *ttk* has other functions (Read et al., 1992; Brown and Wu, 1993).

In this report, we describe a role of *ttk* in the determination of distinct daughter cell fate during asymmetric divisions in the Drosophila PNS. We have characterized the phenotypes of *ttk* loss of function and *ttk* overexpression in both embryonic and adult PNS development. Loss of *ttk* function and *ttk* overexpression result in opposite cell fate transformation in both es organs and cho organs at the time of the formation and differentiation of the SOP progeny. Furthermore, we have shown that the ttk protein is expressed in support cells but not in neurons. In addition, we have obtained genetic and immunocytochemical evidence to indicate that *ttk* most likely acts downstream of *numb*. Thus, we conclude that the proper amount of ttk protein is required for the asymmetrically localized numb protein to endow distinct daughter cell fates during multiple asymmetric divisions in the Drosophila PNS.

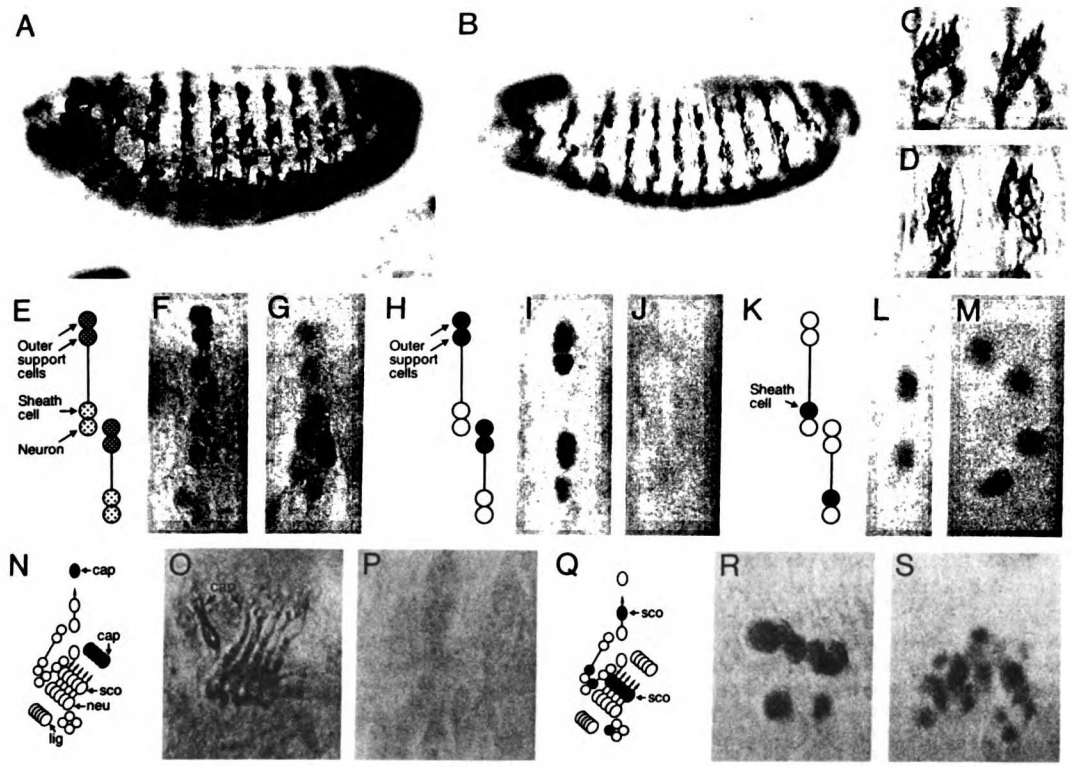

Figure 1. Loss of *ttk* Function Transforms Support Cells into Neurons

(A–D) The *ttk* mutant (B and D) exhibits an overproduction of Mab22C10-positive cells (Mab22C10 labels sensory neurons) as compared with wild type (A and C). Whole-mount embryos are shown in (A) and (B), and the lateral clusters are shown at higher magnification in (C) and (D).

(E–M) Cell fate transformation in es organs of *ttk* mutants. Schematics of two simple es organs at the dorsal-most cluster of an abdominal segment represent stainings with anti-cut (E), anti-β-gal of A1-2-29 (H), and anti-pros (K). Shadings of the nuclei reflect the cells stained with the antibodies. In wild type (F), anti-cut antibody stains neurons and sheath cells less strongly than outer support cells. In the *ttk* mutant (G), the number of cells stained is not altered. However, all cells within each es organ show weak staining indicative of neurons and sheath cells. Unlike wild type (I), anti-β-gal staining for the outer support cells is absent in *ttk* mutant (J). The anti-β-gal staining of the Keilin organs, which are complex es organs, is not absent. In the *ttk* mutant (M), the number of anti-pros positive sheath cells is doubled compared with wild type (L).

(N–S) Cell fate transformation in cho organs of *ttk* mutants. Schematics of cells in the lateral region of an abdominal segment represent staining with antibody 1188, which recognizes cap cells (N), and anti-pros, which labels scolopale (sco) and sheath cells (Q). Antibody 1188 staining is evident in the wild type (O) but not in the *ttk* mutant (P). The number of pros-positive cells is doubled in the *ttk* mutant (S) compared with wild type (R).

Results

Loss of *ttk* Function Transforms Support Cells to Neurons in Embryos

In a P element mutagenesis screen (Bier et al., 1989), we isolated a mutation, *oversensitive* (*osn*; Jan and Jan, 1990), with an overproduction of sensory neurons. We cloned and sequenced *osn* and found it to be identical to *ttk*. The original *osn* allele, named ttk^{osn}, and a small deficiency in the region, *Df(3R)Kpn-rev7* (Biggs et al., 1988), were used for the study. The ttk^{osn} mutation results in embryonic lethality and an embryonic phenotype in the PNS apparently as strong as that of *Df(3R)Kpn-rev7*, which removes both 69 kDa and 88 kDa transcripts. In ttk^{osn}, the ttk 69 kDa protein expression is absent except in salivary glands, and the level of the 88 kDa ttk protein is reduced, as revealed by immunocytochemistry (data not shown).

In the *ttk* mutant embryo, the number of peripheral neurons is significantly increased, as shown by a neuronal specific antibody Mab22C10 (Zipursky et al., 1984; Figures 1B and 1D). Compared with the wild type (Figures 1A and 1C), the number of es and cho neurons is approximately doubled (Figures 1B and 1D). Various markers (antibodies and enhancer trap lines) that label different cell types within es and cho lineage were used to investigate the cause of this defect.

For es organs, we focused our studies at the dorsal-most region of abdominal segments in the embryo. At this region, two simple es organs are separated from the rest of the cells in the dorsal cluster, thus making the cells in the organs easy to identify. Figures 1E, 1H, and 1K are the schematics of these two es organs stained with various markers, with each circle representing an individual cell, and the shadings marking the cells recognized by that

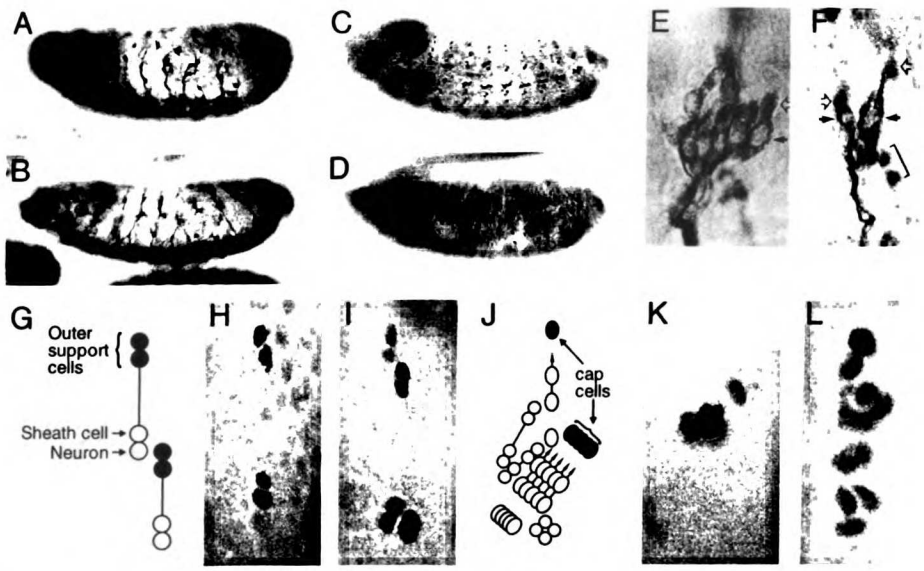

Figure 2. *ttk* Overexpression Transforms Neurons to Support Cells

A significant reduction of the Mab22C10-positive peripheral neurons in the hs-*ttk* 69 kDa embryo (A) is similar to that in the *numb* loss of function mutant (B). The number of anti-pros positive cells is severely reduced in a hs-*ttk* embryo (D) as compared with wild type (C). Double labeling with Mab22C10 (arrows) and anti-pros antibody (open arrows) in wild type (E) and a hs-*ttk* 69 kDa embryo (F) reveal that in most cases the loss of neurons is accompanied by the loss of sheath or scolopale cells. Occasionally, two pros-positive sheath or scolopale cells (bracket) were found without affiliation with Mab22C10-positive neurons. The schematic in (G) shows two es organs in the dorsal-most cluster of an abdominal segment; the outer support cells marked by anti-β-gal in the A1-2-29 enhancer trap line are shaded. In the hs-*ttk* 69 kDa embryo (I), the number of outer support cells is increased by up to 2-fold compared with wild type (H). The schematic in (J) shows the lateral cluster in an abdominal segment. The cap cells, which are shaded, are labeled with anti-β-gal in the B7-2-7 enhancer trap line. An up to 2-fold increase in the number of cap cells is revealed in hs-*ttk* (L) compared with wild type (K).

particular marker. As shown in Figures 1E and 1F, the nuclei of all four cells can be recognized by the anti-cut antibody; the staining intensity is stronger for outer support cells than for the neuron and sheath cell (Blochlinger et al., 1990). In the *ttk* mutant, the number of cut-positive cells in an es organ is not altered; however, the relatively weak staining intensity indicates that all of the cut-positive cells are neurons and/or sheath cells (Figure 1G). *ttk* mutant embryos also exhibit an approximate doubling of sheath cells as indicated by a sheath cell marker, anti-prospero (pros) antibody (Vaessin et al., 1991; Figures 1K–1M). Furthermore, a specific marker for outer support cells, the enhancer trap line A1-2-29 (Bier et al., 1989; Hartenstein and Posakony, 1990b; Figure 1H and 1I), reveals no outer support cells in the *ttk* mutant (Figure 1J). Taken together, loss of *ttk* function appears to cause a transformation of outer support cells to neurons and sheath cells in es organs (see Figure 3B). These data are consistent with those described in an independent mutagenesis screen conducted by Salzberg et al. (1994).

For cho organs, we focused our investigations at the lateral region of abdominal segments, where five cho organs are aligned side by side as shown by schematics (Figures 1N and 1Q). In *ttk* mutant embryos, no cap cells are detected using the cap cell–specific antibody 1188 (Jarman et al., 1993; Figures 1N–1P). The number of scolopale cells recognized by anti-pros antibody is doubled (Vaessin et al., 1991; Figure 1Q–1S). Therefore, extra neurons and scolopale cells that are derived from the sister cell of the cap cell, chIII, are produced at the expense of the cap cells (see Figure 3E).

We believe that the *ttk* loss of function phenotype is due to transformation of cell fate rather than overproduction of SOPs, accompanied by a concomitant death of IIa cells or the outer support cells. This is because in the *ttk* mutant, we examined the number of SOP cells and did not detect any alteration in the number of SOPs recognized by anti-asense antibody (Brand et al., 1993; data not shown).

Overexpression of *ttk* in the Embryo Results in Cell Fate Transformation Opposite to That Due to Loss of *ttk* Function

To determine whether *ttk* acts as a genetic switch for alternative cell fates, we examined the phenotype of *ttk* overexpression. *ttk* overexpression was achieved by putting the *ttk* cDNA under heat-shock promoter control (Read et al., 1992) and inducing ubiquitous expression of the ttk protein by shifting the embryos to 39°C for a certain period of time (see Experimental Procedures). We overexpressed ttk at 6.5–8.5 hr of embryonic development at 25°C, the time around the formation and differentiation of the SOP progeny in the embryo (Bodmer et al., 1989), and indeed observed the reverse cell fate transformation.

Following overexpression of the ttk 69 kDa, the number

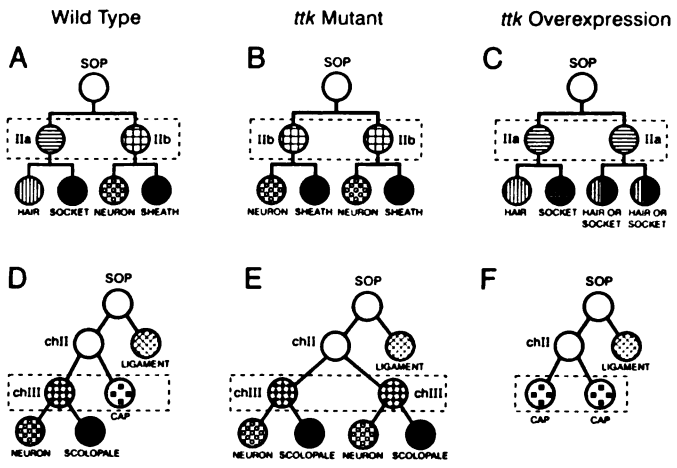

Figure 3. Wild Type and Proposed SOP Cell Lineages in the Embryo Due to *ttk* Loss of Function Mutations or *ttk* Overexpression

(A–C) Cell lineages for the es organ in wild type (A), *ttk* loss of function mutant (B), and embryos with *ttk* 69 kDa overexpression around the time of the formation and the differentiation of the SOP progeny (C). Within each lineage, we have boxed two daughters whose fates are indicated to be affected by *ttk*. In the *ttk* mutant (B), the IIa cell is transformed into IIb cell, yielding two IIb cells and subsequently two neurons and two sheath cells. Upon *ttk* overexpression (C), the IIb cell is transformed into IIa cell, resulting in two IIa cells, which give rise to four outer support cells.

(D–F) Cell lineages for the cho organ in wild type (D), *ttk* loss of function mutant (E), and embryos with *ttk* overexpression (F). In a cho organ, two neurons and two sheath cells are produced at the expense of the cap cell in the *ttk* mutant (E), possibly the consequence of a transformation from the cap cell to chIII, the precursor cell for the neuron and scolopale cell. Upon *ttk* overexpression (F), two cap cells are formed coincident with the loss of both the neuron and sheath cell, which could result from a transformation of the chIII cell to an extra cap cell. Both loss of function and overexpression of *ttk* at the time of the formation and differentiation of SOP progeny cells appear to disrupt the normally asymmetric division, producing two identical daughter cells. Note that the lineages for the *ttk* overexpression only represent the effects of hs-ttk 69 kDa at the time of formation and differentiation of SOP progeny. Overexpression of ttk 69 kDa at an earlier stage seems to produce additional defects (see text); thus, these lineages do not apply to all the defects of *ttk* overexpression.

of peripheral neurons is significantly reduced, as revealed by Mab22C10 staining (Figure 2A). The number of sheath cells in es organs and scolopale cells in cho organs that are recognized by anti-pros is also severely reduced (Figure 2D) compared with the wild type (Figure 2C). Double labeling experiment with both Mab22C10 and anti-pros demonstrates that the absence of the sheath or scolopale cell is usually associated with the absence of the neuron in the same sensory organ (Figures 2E and 2F). In about 10% of the cases, we also observed two sheath or scolopale cells that are not associated with any neurons (Figure 2F), suggestive of a transformation of a neuron into a sheath or scolopale cell. Of two dorsal-most es organs, the loss of neuron and sheath cell is not associated with any change in the total number of cut-positive cells (data not shown), but the number of outer support cells (Figures 2G–2I) labeled by A1-2-29 is increased by up to 2-fold. Of the lateral cho organs, the number of cap cells is also increased by up to 2-fold upon *ttk* overexpression, as demonstrated by a cap cell–specific enhancer trap line B7-2-7 (Bier et al., 1989; Figures 2J–2L). Therefore, in both es and cho organs, overexpression of ttk 69 kDa results in cell fate transformation (Figures 3C and 3F) opposite to that in the *ttk* loss of function mutant (Figures 3B and 3E). Furthermore, the *ttk* overexpression displays a phenotype strikingly similar to that of the *numb* loss of function mutant (Figure 2B).

Overexpression of ttk 69 kDa at an earlier stage appears to result in the reduction in the number of cut-positive cells in the PNS (data not shown) and the defect of germ band retraction, suggesting that overexpression of *ttk* may cause some additional defects in the formation of SOP. Overexpression of ttk 88 kDa at the time of SOP progeny formation and differentiation also leads to the reduction of neurons (as judged by Mab22C10 staining) without changing the number of cells in the dorsal-most es organs (as judged by anti-cut staining; data not shown). These data suggest that ttk 88 kDa overexpression can produce similar (albeit milder) phenotypes as ttk 69 kDa overexpression.

Loss of *ttk* Function in the Adult Leads to the Appearance of Supernumerary Neurons at the Expense of Outer Support Cells

To study the role of *ttk* in es organ development further, we examined the requirement of *ttk* in the adult. The es organs in the adult are generated by division patterns analogous to those in the embryo (Hartenstein and Posakony, 1989). The SOPs for adult es organs in the nota divide during pupal development, giving rise to macrochaetes (large bristles) at fixed positions and microchaetes (small bristles) that are evenly spaced (Figure 4B). Because the ttk^{ts} allele is embryonic lethal, we carried out mosaic analysis to investigate the requirement of *ttk* function in the adult es organ development. The mosaics were generated by FLP/FRT method (Golic and Lindquist, 1989; Golic, 1991).

In *ttk* mosaics, we observed bald patches of cuticle that have no outer support cells (hair and socket cells; Figure 4C). The es organs affected included both microchaetes and macrochaetes on the nota, all three types of bristles (stout, slender, and recurved) in the anterior wing margin, as well as interommatidial bristles. By dissecting the nota and staining them with Mab22C10, we observed multiple neurons underneath the bald patches in *ttk* mosaic flies (Figure 4D). These neurons tend to bundle together, presumably owing to a lack of outer support cells as attachment sites. Thus, loss of *ttk* function in the adult results

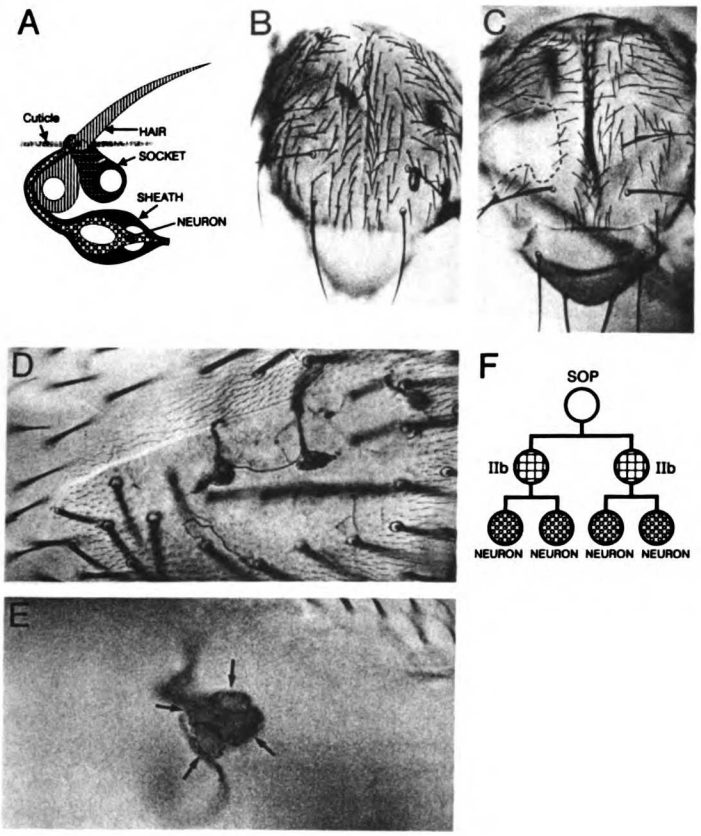

Figure 4. Loss of *ttk* Function in the Adult Produces Supernumerary Neurons at the Expense of Hair and Socket Cells

(A) A schematic of an es organ, which consists of a neuron and three nonneuronal support cells: a hair cell, a socket cell, and a sheath cell.

(B) The notum of a wild-type fly.

(C) The notum of a *ttk* mosaic fly. The outlined area is the mosaic patch within which both hairs and sockets are missing.

(D) Underneath the mosaic patches, Mab22C10-positive neurons tend to bundle together.

(E) Four neurons, each marked by an arrow, can be discerned in small clones.

(F) The proposed lineage for the four neurons seen in *ttk* mosaics. Two steps of transformation take place, first from IIa to IIb, producing two IIb cells, and then from the sheath cells to the neurons, resulting in four neurons.

in the transformation of hair and socket cells into supernumerary neurons. In regions where individual neuronal bundles can be separated, or in very small clones in which a single bristle seems to be missing, we frequently discerned four neurons (Figure 4E). It is likely that these four neurons are caused by transformation of the sheath cell, as well as hair and socket cells, to neurons, though in the absence of any sheath cell markers in the adult fly, the fate of the sheath cell cannot be determined. Besides the phenotype described above, we also observed isolated single sockets at a very low frequency (data not shown).

ttk Overexpression in the Adult Results in Duplicated Bristles and Multiple Sockets

Overexpression of *ttk* was induced in most or all pupal cells around the time of formation and differentiation of the SOP progeny in es organs. For the microchaetes on nota, we collected the white pupae (marked as 0 hr after puparium formation [APF]) of heat-shocked (hs)-ttk 88 kDa flies at a 2 hr interval and aged these pupae until some time between 10 and 24 hr APF. We then induced *ttk* overexpression by a heat-shock treatment of these pupae and allowed them to develop into adult flies. Following the heat-shock treatment of the pupae of the hs-ttk 88 kDa flies, the bristle phenotypes (described in the next paragraph) were observed starting from 12–14 hr APF, peaking at 14–20 hr APF, subsiding at 20–22 hr APF, and no longer observed at 22–24 hr APF. This time window corresponds to the time of SOP divisions for the microchaetes on nota (Hartenstein and Posakony, 1989).

A range of phenotypes was observed, and the two most prominent phenotypes are the following. The first class is "duplicated bristles," with either two shafts and two sockets or two shafts coming out of a seemingly merged socket (Figure 5A). No neurons were found to be associated with these duplicated bristles, as determined by the Mab22C10 staining (Figure 5D). This phenotype is analogous to the phenotype observed in *ttk* overexpression in embryos. The phenotype of two hairs coming out of a seemingly single socket may reflect the partial transformation of IIb to IIa cell or the fusion of two socket cells. This class of phenotype is evident for over 85% of the flies with *ttk* overexpression induced during 14–18 hr APF. Among those flies, about three-fourths have more than 30% of the microchaetes duplicated. The second class of phenotype is multiple sockets. This phenotype happens less frequently; less than 20% of bristles in each of the 50% flies affected show multiple sockets. As shown in Figure 5B, there appears to be "double sockets" without hairs in a location where a single es organ normally resides, indicative of a transformation of the hair cell to the socket cell. Mab22C10 staining demonstrates that no neurons are associated with the double sockets (Figure 5D). Although no sheath cell

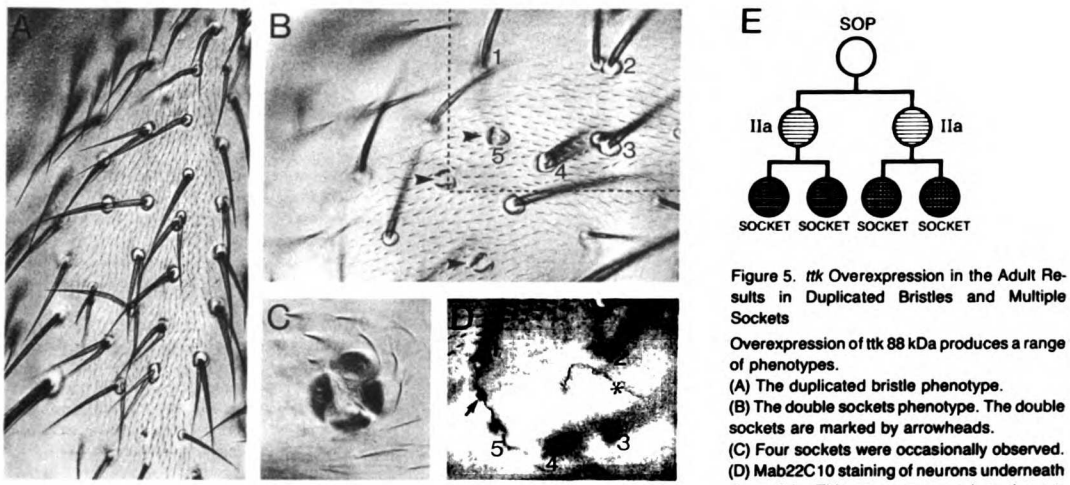

Figure 5. *ttk* Overexpression in the Adult Results in Duplicated Bristles and Multiple Sockets

Overexpression of ttk 88 kDa produces a range of phenotypes.

(A) The duplicated bristle phenotype.

(B) The double sockets phenotype. The double sockets are marked by arrowheads.

(C) Four sockets were occasionally observed.

(D) Mab22C10 staining of neurons underneath the cuticle. This area corresponds to the outlined area shown in (B). Each bristle in (D) is marked by a number (1–5). The numbers correspond to the bristles marked in (B). The wild-type bristle (1) is innervated by a neuron (arrow), whereas the mutant bristles (2–4 for duplicated bristles and 5 for double sockets) have no neurons associated with them. The asterisk marks a strayed nerve process that is not associated with any of the bristles shown in the region.

(E) The proposed lineage for four sockets, the extreme phenotype observed upon *ttk* overexpression. There appears to be a two-step transformation event, first from IIb to IIa and then from hair cells to socket cells.

marker is available for adult flies to confirm the transformation, one possibility is that the phenotype results from a transformation from a neuron to a sheath cell, as well as from a hair cell to a socket cell. In extreme cases, four sockets can be clearly discerned in a location where a single es organ normally resides, as shown in Figure 5C. A single hair coming out of two or three sockets was also observed (data not shown), presumably owing to the combinatory effect of the duplicated bristles and the double sockets. We occasionally observed an apparently normal bristle without the association of a neuron as well as double sockets with a neuron (data not shown). These may reflect occasions of *ttk* overexpression affecting the division of IIb or the division of IIa. Furthermore, three hairs coming out of one or two sockets were detected at a frequency of about 5% of total bristles affected.

These phenotypes were also observed in other bristles, including the macrochaetes on the notum and head and bristles in anterior wing margin, legs, abdomen, and eye. Overexpression of ttk 69 kDa during late larval or early pupal stage led to a high lethality rate. Of the few individuals that did develop into adult fly, similar phenotypes of duplicated bristles were observed to that of hs-ttk 88 kDa. However, the high rate of lethality prevented us from a detailed analysis of the effect of the hs-ttk 69 kDa.

ttk Is Expressed in Support Cells but Not in Neurons of Both es and cho Organs

We investigated the ttk protein expression pattern by immunofluorescence in the embryo. Expression of both ttk 69 kDa and 88 kDa has been shown in most of the cells in the epidermis but not in the nervous system (Read et al., 1992; Brown and Wu, 1993). Because the general epidermal staining of ttk beyond stage 12 may obscure the epidermal and subepidermal staining of the cells in the PNS, we attempted to examine the ttk expression by immunofluorescence and confocal microscopy. By double labeling cells with various markers that recognize cells in the sensory organs, we indeed observed cells in the es and cho organs that are recognized by anti-ttk 69 kDa antibodies. As shown in Figure 6C, at the dorsal-most region of an abdominal segment (Figure 6A), a total of eight cells from two es organs are labeled with anti-cut antibody. Of these eight cells, we observed six cells that are recognized by anti-ttk antibody (Figures 6B and 6D), whereas the other two weakly cut-positive cells are not. By double labeling with the neuronal marker Mab44C11, which labels nuclei of all neurons (Bier et al., 1988; Figure 6F), we observed that ttk 69 kDa is not expressed in Mab44C11-positive neurons (Figures 6E and 6G).

At the lateral region of the embryo, five cho organs are aligned side by side in each abdominal segment (Figure 6H). By double-labeling with the neuronal marker Mab22C10 (Figure 6I), we found that ttk is expressed in scolopale cells and cap cells, but not in Mab22C10-positive neurons (Figures 6J and 6K). Therefore, in both es and cho organs, ttk 69 kDa is expressed in support cells, but not in neurons. By double labeling with anti-asense antibody, which recognizes SOP and its daughters shortly after the SOP division, we have not been able to detect a signal with anti-ttk 69 kDa antibody in SOPs and its daughters (IIa and IIb) shortly after the SOP division. Owing to the rapid cell division time in the embryo, we have not been able to determine whether

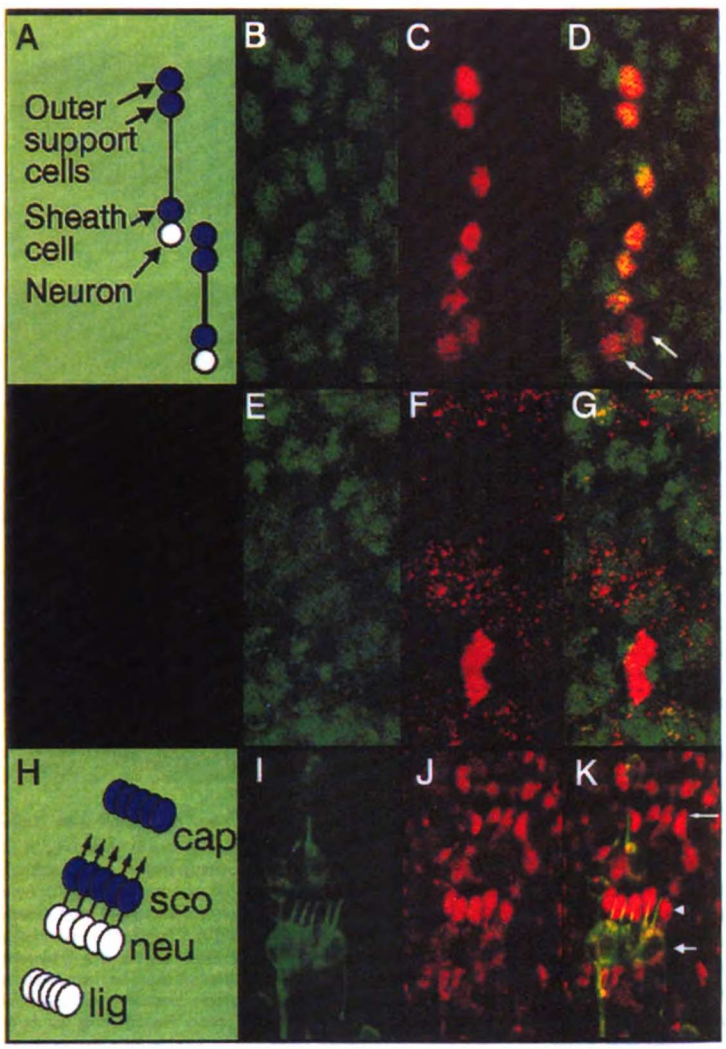

Figure 6. ttk Is Expressed in Support Cells but Not in Neurons

(A) A schematic drawing of the dorsal-most region of an abdominal segment with two isolated es organs. Shadings of nuclei reflect the corresponding cells recognized by anti-ttk 69 kDa antibody.

(B–D) ttk 69 kDa is expressed in three of the four cells in an es organ. ttk 69 kDa is expressed in most of the cells in the epidermis (B). Anti-cut antibody recognizes all four cells in each es organ (C). Superimposition of (B) and (C) shows that six cut-positive cells, but not two weakly cut-positive cells (noted by two arrows), are recognized by anti-ttk 69 kDa antibody (D).

(E–G) ttk 69 kDa is not expressed in neurons of es organs. Double staining with anti-ttk 69 kDa (E) and Mab44C11, which labels all neurons (F), and the superimposition of both stainings (G) show that the Mab44C11-positive neurons are not recognized by anti-ttk 69 kDa.

(H–K) In cho organs, ttk 69 kDa is expressed in support cells but not in neurons. A schematic of cho organs at the lateral region of an abdominal segment with the shadings of nuclei corresponding to the cells recognized by anti-ttk 69 kDa antibody (H). (K) shows the superimposition of Mab22C10 staining (I) and anti-ttk 69 kDa staining (J), demonstrating that ttk 69 kDa is not expressed in Mab22C10-positive neurons (wider arrow). The position of the other cells stained by anti-ttk 69 kDa in subepidermal layer shows that the scolopale cells (arrowhead) and cap cells (thinner arrow) are labeled by anti-ttk 69 kDa.

IIa and/or IIb expresses ttk 69 kDa later on. However, in pupal es organ development, in which cell cycle time is longer, Ramaekers et al. was able to detect ttk 69 kDa expression in IIa prior to its division (Ramaekers et al., personal communication). Anti-ttk 88 kDa results in seemingly identical expression pattern in the es and cho organ (data not shown).

ttk Acts Downstream of *numb*

The *ttk* gene appears to be essential for cells within the lineage of the es and cho organs to adopt their proper identities. *numb* has also been shown to play an important role in this fate specification process. Loss of *numb* function in the embryo results in extra outer support cells at the expense of neurons and sheath cells in es organs and extra cap cells at the expense of neurons and scolopale cells in cho organs (Uemura et al., 1989). Overexpression of *numb* results in the opposite cell fate transformation (Rhyu et al., 1994). In the adult, four sockets can be detected in the *numb* mosaic animals, and *numb* overexpression results in phenotypes of "balding" (no hair and no socket) with extra neurons as well as "twinning" of hairs without socket (Rhyu et al., 1994). Thus, removal of the *ttk* function results in cell fate transformation opposite to that due to loss of *numb* function in most of the asymmetric divisions that depend on *numb* function. Indeed, loss of *ttk* function shows phenotypes similar to those due to *numb* overexpression, whereas *ttk* overexpression (see Figure 2A) shows phenotypes similar to the *numb* loss of function phenotypes (see Figure 2B). Based on these observations, it seems likely that these two genes function in the same genetic pathway, and presumably, one negatively regulates the other.

To test this hypothesis, we constructed double mutants lacking both *ttk* and *numb* function and stained them with neuronal marker Mab22C10. Whereas the *numb* mutant lacks neurons (Figure 7A) and the *ttk* mutant overproduces neurons (Figure 7B), the double mutant overproduces

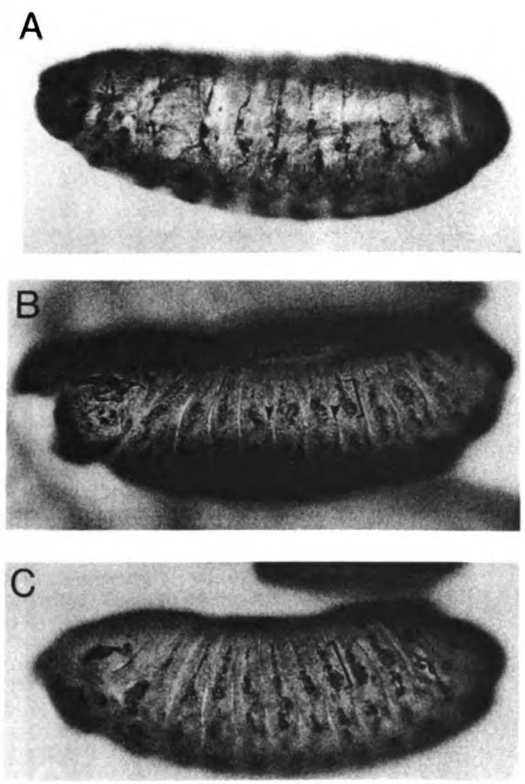

Figure 7. *ttk* Is Epistatic to *numb*

Mab22C10 staining of a *numb* mutant embryo (A), *ttk* mutant embryo (B), and a double mutant embryo (C). The *numb;ttk* double mutant embryo (C) shows overproduction of neurons, a phenotype indistinguishable from that of the *ttk* mutant (B). The dorsal cluster in the double mutant (bracketed in [C]) and the dorsal cluster in *ttk* mutant (bracketed in [B]) show a similar extent of neuronal overproduction, whereas no neurons in the dorsal cluster can be discerned in the *numb* mutant (A). The *ttk* mutant embryo and the double mutant embryo are from the same genetic cross; epidermal staining with anti-numb antibody, as indicated with arrowhead in (B), allows us to distinguish the *ttk* mutant that carries the wildtype *numb*$^+$ allele (B) from the double mutant (C).

neurons (Figure 7C). This phenotype of the double mutant is indistinguishable from that of the *ttk* mutants. Thus, *ttk* and *numb* most likely function in the same genetic pathway, in which *ttk* acts downstream of *numb*.

To evaluate the epistatic relationship between *ttk* and *numb* further, we examined ttk expression in *numb* mutant embryos and ttk expression in *numb* overexpression embryos. We focused our studies at the dorsal-most region of the abdominal segments of the embryos. In *numb* mutant embryos, all four cells in an es organ are recognized by anti-ttk 69 kDa antibody (Figures 8A and 8C). These cells appear to be outer support cells, as they are strongly stained with anti-cut antibody (Figures 8B and 8C), consistent with a transformation from a neuron and a sheath cell (two weakly cut-positive cells) to outer support cells (two strongly cut-positive cells) in *numb* loss of function mutants. We then examined the ttk 69 kDa expression in *numb* overexpression embryos. We induced *numb* overexpression by a heat-shock treatment of the 5–9 hr embryos carrying hs-*numb* and allowed them to develop to stage 16–17 (see Experimental Procedures). Following the heat-shock treatment of hs-*numb* embryos, we observed 3–6 cells that are not stained by anti-ttk 69 kDa. Thus, fewer than normal number of the cells within the es lineage are stained with anti-ttk 69 kDa. Figures 8D–8F show that at least four cells are not labeled with anti-ttk in a hs-*numb* embryo upon *numb* overexpression. This is consistent with hs-*numb* resulting in two neurons and two sheath cells at the expense of the outer support cells (Rhyu et al., 1994). The variation in the number of anti-ttk-positive cells may be partly due to the occasions that *numb* overexpression only affects one of the two es organs, and partly due to cases in which *numb* overexpression causes a transformation from sheath cells to neurons.

We also examined numb distribution in *ttk* mutant embryos. Shortly before the SOP divides, the membrane-associated numb protein is localized to one pole, forming a crescent-like staining pattern as revealed by anti-numb antibody staining (Rhyu et al., 1994). Furthermore, during division of neuroblasts in CNS, numb is also localized to one side of the neuroblast membrane (Rhyu et al., 1994). In the *ttk* mutant, the asymmetric localization of numb in the SOPs (Figures 8G–8I) and in the neuroblasts (data not shown) does not appear to be affected. Taken together. these immunocytochemical data are consistent with *ttk* functioning downstream of *numb*.

Other Embryonic Phenotypes

Besides the defects in PNS, we noticed other embryonic defects in *ttk* mutants. By using the muscle marker 6D5 antibody, we detected a muscle defect in the *ttk* mutant (data not shown). In addition, there seem to be some defects in Malpighian tubules and midgut constriction. Furthermore, *ttk* mutant embryos failed to undergo proper head involution and dorsal closure. These observations suggest that *ttk* functions in a variety of tissues during development.

Discussion

ttk Specifies Distinct Daughter Cell Fate during Multiple Asymmetric Divisions in PNS

We have described a role of the *ttk* gene in specifying cell fates of the SOP progeny. In embryonic es organs, loss of *ttk* function transforms outer support cells to neurons and sheath cells. Conversely, overexpression of *ttk* results in the opposite cell fate transformation. The cell fate transformation probably takes place between two daughter cells of the SOP, IIa and IIb, because the doubling of neurons is coupled to the doubling of sheath cells, and *ttk* overexpression often causes loss of both neuron and sheath cell in the same es organ. Thus, the *ttk* embryonic phenotype can be interpreted as a transformation from IIa

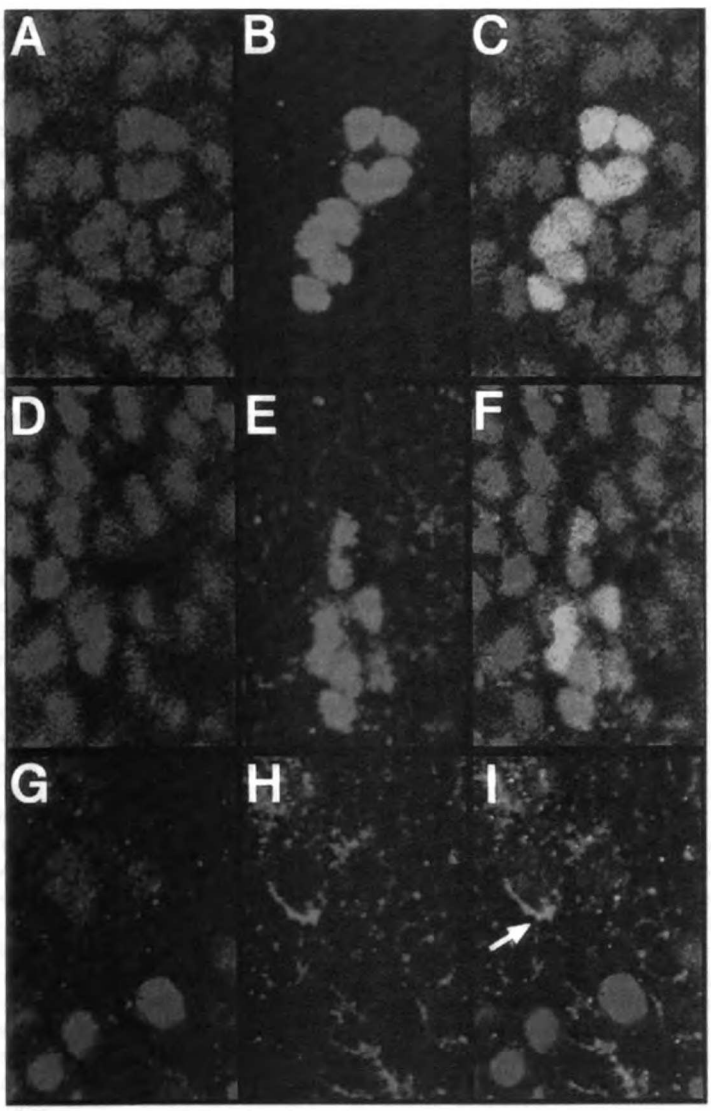

Figure 8. ttk Expression in *numb* Loss of Function Mutant and *numb* Overexpression Embryo; Numb Expression in *ttk* Mutant

(A–C) Double staining of anti-ttk 69 kDa (A) and anti-cut (B) and superimposition of these two (C) at the dorsal-most region of an abdominal segment in a *numb* mutant. All eight cells that are labeled by anti-cut are recognized by anti-ttk. These cells appear to be the outer support cells, since they are strongly stained with anti-cut antibody.

(D–F) Double staining of anti-ttk 69 kDa (D) and anti-cut (E), and superimposition of these two (F) at the dorsal-most region of an abdominal segment following heatshock treatment of hs-*numb* embryos. At least four out of eight cells that are labeled by anti-cut are not recognized by anti-ttk.

(G–I) Double staining of anti-asense (G) and anti-numb (H) and superimposition of these two images (I) in a stage 10 *ttk* mutant embryo. In the SOP cell labeled by anti-asense, numb is asymmetrically localized to one side of the membrane (noted by an arrow in [I]). The cytoplasmic localization of the nuclear protein asense in this SOP cell indicates that the SOP cell is in mitosis and the nuclear membrane has broken down. This is similar to the numb localization in SOP cells in the wild type (Rhyu et al., 1994).

to IIb, producing two IIb cells (see Figure 3B), whereas *ttk* overexpression results in two IIa cells (see Figure 3C). A proper amount of *ttk* is therefore essential for two SOP daughters to adopt different cell fates. Loss of *ttk* function and *ttk* overexpression both seem to disrupt the asymmetry of the SOP division, resulting in an apparently symmetric division.

Besides its requirement for SOP division, *ttk* may be involved in the division of IIb cells in the es lineage. Overexpression of ttk in the embryo sometimes causes a transformation of a neuron to a sheath cell, whereas loss of *ttk* function in mosaic flies results in supernumerary neurons. We interpret the *ttk* mosaic phenotype as a two-step transformation, first from IIa to IIb and subsequently from a sheath cell to a neuron (see Figure 4F). Thus, the proper amount of ttk is likely to be required for the division of IIb.

Besides es organs, *ttk* is required for cell fate specification in cho organs. Loss of *ttk* function and *ttk* overexpression cause transformations between precursor chIII and its sister cell, the cap cell (see Figures 3E and 3F), indicating that *ttk* is required for these two daughters of the chII precursor cell to adopt their correct identities. In addition, transformation from neurons to scolopale cells is occasionally observed upon *ttk* overexpression in the embryo, suggesting that the final division of the cho lineage is also disrupted. Taken together, *ttk* appears to act as a genetic switch between two alternative daughter cell fates. Rather than specifying the fate for one particular cell type, *ttk* is required for various divisions involving different cell types in two types of sensory organs. Thus, *ttk* seems to be a general element employed in multiple asymmetric divisions in the Drosophila PNS.

The role of *ttk* in the asymmetric divisions of SOP, IIb, and chIII is reflected by the differential distribution of ttk. ttk is expressed in the IIa cell but not in the IIb cell (Ramaekers et al., personal communication), in the sheath cell but not in the neuron of the es organ, and in the scolopale cell but not in the neuron of the cho organ. The fact that ttk is expressed in one of the daughter cells that is dependent on *ttk* function suggests that ttk promotes this particular daughter cell fate. ttk could thus serve either as a positive factor to activate this daughter cell fate or as a negative regulator to suppress the alternative daughter fate. Considering that ttk functions as a transcription repressor of segmentation genes *eve* and *ftz* (Harrison and Travers, 1990; Read and Manley, 1992; Brown et al., 1991), it seems more likely that ttk acts as a transcriptional repressor to suppress the particular daughter cell fate.

We do not know whether *ttk* is required in the division of IIa cells. Since loss of *ttk* function transforms IIa to IIb, the fates of the daughters of IIa cells cannot be examined in *ttk* mutant. *ttk* overexpression, on the other hand, results in double sockets, and in extreme cases, four sockets. The four sockets phenotype due to *ttk* overexpression can be viewed as a transformation first from IIb to IIa, and subsequently from hair cells to socket cells (see Figure 5E). ttk, however, is expressed in both of the two daughter cells of IIa. It remains possible that *ttk* is normally not involved in specifying daughter cell fates of IIa cells, even though overexpression of *ttk* affects these cells. Alternatively, it could be that *ttk* is indeed required in the specification of IIa daughters. Furthermore, there is a difference in ttk expression between hair and socket cells, but this difference is not readily resolved by immunocytochemistry.

Although we suggest that *ttk* is essential for cell fate specification within the lineage of es and cho organs, we do not assume that this is the sole function of *ttk*. Overexpression of *ttk* in the embryo and adult at the stage prior to divisions and differentiation of SOP progeny cells appears to result in the reduction of total number of cells in the PNS (unpublished data; Ramaekers et al., personal communication). This may suggest an additional function of *ttk* on SOP formation, reflecting the pleotropic effect of *ttk*. Alternatively, overexpression of *ttk* ubiquitously may affect some processes that normally are not dependent on *ttk* function.

Both Alternatively Spliced Forms of ttk May Be Involved

The two alternatively spliced forms of ttk, the 69 kDa and 88 kDa proteins, have been shown to have distinct DNA binding activities in vitro (Read and Manley, 1992). Only overexpression of the ttk 69 kDa protein is capable of suppressing the expression of pair-rule genes, including *eve* and *ftz* (Read et al., 1992; Brown and Wu, 1993). In the eye development, two forms of ttk may also have different roles (Xiong and Montell, 1993). In the development of PNS, both ttk 69 kDa and 88 kDa proteins are expressed in the PNS. The ttk^{oan} mutation, which removes ttk 69 kDa protein in the periphery and reduces the level of 88 kDa protein expression, produces an embryonic phenotype as strong as that of the deficiency that eliminates both 69 kDa and 88 kDa proteins. By contrast, in another *ttk* allele, ttk^{1}, which removes 88 kDa transcript completely but does not affect the *ttk* 69 kDa transcript (Xiong and Montell, 1993), a mild embryonic phenotype of neuronal overproduction was observed (unpublished data). Further, overexpressing either 69 kDa or 88 kDa at the time of SOP progeny differentiation appears to display similar cell fate transformation phenotypes in es organs of both the embryo and the adult. These observations indicate that both alternatively spliced forms of ttk may be involved in specifying progeny cell fate in the embryonic PNS and possibly in the adult es organ, too.

ttk Acts Downstream of *numb*

numb has been shown to be essential for asymmetric divisions in the PNS. In almost all the asymmetric divisions of es and cho organs that *ttk* is required, *numb* is also involved. Loss of *ttk* and loss of *numb* function result in reciprocal cell fate transformation between daughters in these asymmetric divisions. A double mutant lacking both *numb* and *ttk* function exhibits *ttk*-like phenotype. Moreover, extra ttk-positive cells within the es lineage are produced in *numb* mutant embryos, whereas fewer ttk-positive cells within the es lineage are produced in hs-*numb* embryos. The asymmetric localization of numb in the SOPs and in the neuroblasts, on the other hand, does not appear to be affected in the *ttk* mutant embryo. Thus, *ttk* and *numb* most likely function in the same genetic pathway, in which *ttk* acts downstream and is negatively regulated by *numb*.

The numb protein, which is partitioned to one of the daughter cells (Rhyu et al., 1994), might suppress *ttk* activity in the same daughter cells. In this model, loss of *numb* function leads to the failure of suppression of *ttk* in the daughter cell that normally exhibits predominant *numb* expression, resulting in *ttk* activity in both of the daughter cells. Conversely, overexpression of *numb* may cause the removal of *ttk* activity in the daughter cell that normally does not have predominant numb expression. The differential expression of ttk in one of the daughter cells of asymmetric division is consistent with this model.

Therefore, we propose that ttk, as a downstream transcription factor, executes the command dictated by asymmetrically localized numb by way of directly regulating cell type–specific gene expression in one of the daughter cells during asymmetric divisions.

Other Genes Involved in Asymmetric Cell Fate Specification

Asymmetric divisions in the Drosophila PNS involve both cell-intrinsic factors, such as *numb*, and cell–cell interaction (cell-extrinsic mechanism) mediated by the neurogenic genes *Notch* (*N*) and *Delta* (*Dl*; reviewed by Posakony, 1994; Jan and Jan, 1994, 1995). Loss of *N* or *Dl* function during SOP cell divisions results in either two neurons and two sheath cells or four neurons (Hartenstein and Posakony, 1990a; Parks and Muskavitch, 1993). One approach in understanding how information from cell–cell interaction is integrated with that from cell-intrinsic determinant is to investigate the interaction between genes

such as *N* and *Dl* and genes such as *ttk* and *numb*. Although we suggest that *ttk* is downstream of *numb*, we do not assume that *ttk* is necessarily more closely related to *numb* than *N* and *Dl*. Formally, *ttk* could serve either as a regulator of *N* and *Dl* or as a final readout point by integrating information derived from both the cell-intrinsic determinants and cell–cell interaction.

Suppressor-of-Hairless (*Su(H)*) has been shown to act downstream of *N* (Fortini and Artavanis-Tsakonas, 1994). Loss of *Su(H)* function results in the transformation from socket to hair cells (Ashburner, 1982; Schweisguth and Posakony, 1994). Partial loss of function of *Hairless (H)*, the gene product of which inhibits the DNA binding of Su(H) through direct protein–protein interaction (Brou et al., 1994), results in the opposite cell fate transformation from hair to socket cells (Lees and Waddington, 1942; Bang et al., 1991). How *ttk* and *numb* interact with *Su(H)* and *H* is therefore one avenue for investigation that may reveal how cell-intrinsic and cell-extrinsic mechanisms interface.

Other genes have been shown to be involved in the asymmetric cell fate decisions in the adult es organs. Partial loss of function of *twins*, which encodes a regulator of serine/threonine protein phosphatase 2A (Uemura et al., 1993), results in duplicated bristles at the expense of neurons and sheath cells (Shiomi et al., 1994). The *musashi* gene encodes a RNA-binding protein, and loss of *musashi* function results in extra outer support cells, frequently at the expense of neurons (Nakamura et al., 1994). It would be interesting to investigate whether these genes function in the same genetic pathway as *numb* and *ttk*, and/or *N* and *Dl*, and if so, whether they are involved in establishing the asymmetric localization of numb, mediating a process from the membrane-associated numb to ttk in the nucleus, or regulating cell type–specific differentiation.

Experimental Procedures

Genetics and Drosophila Strains

Drosophila strains were raised on standard cornmeal–yeast agar medium at room temperature or 25°C. For characterizing the embryonic phenotype, both *ttk*^{ves}/*TM3 Sb* and *Df(3R)Kpn-rev7/TM3 Sb* strains were used. When enhancer trap lines were used as markers, flies carrying various enhancer trap lines were crossed to the flies carrying *Df(3R)Kpn-rev7*. The progeny carrying one copy of *Df(3R)Kpn-rev7* and one copy of the P-lacZ insertion were mated to one another. The expression pattern of these marker lines in *ttk* mutant embryos were examined immunocytochemically using anti-β-galactosidase (anti-β-gal) antibody in homozygous *Df(3R)Kpn-rev7* embryos that contain at least one copy of the P-lacZ insertion. The *ttk* mutant embryos can be unambiguously identified, as those embryos that have defects in head involution and muscle development.

For constructing the double mutants, flies carrying a copy of a *numb* null allele, *numb'* (Uemura et al., 1989), and a copy of *Df(3)Kpn-rev7* were crossed to each other. Embryos from this cross were collected and double labeled with Mab22C10 and anti-numb antiserum, which also stains general epidermis. Out of those embryos that do not have anti-numb epidermal staining, a quarter of the embryos (double mutants) showed *ttk*-like phenotype.

For examining the numb localization, 4.5–7 hr embryos of *Df(3R) Kpn-rev7/TM6, p[Ubx-lacZ]* flies were double stained using both anti-numb and anti-β-gal. *ttk* mutant embryos were unambiguously identified as those embryos that lack the stripe staining from the *p[Ubx-lacZ]*.

Immunocytochemistry

Embryos were collected, dechorionated in 50% bleach, fixed for 20–30 min in a 1:1 mixture of fixative, 5% formaldehyde in PBS (10 mM phosphate buffer [pH 7.0], 0.15 M NaCl), and heptane. The fixative was replaced by methanol to remove the vitelline membrane. The embryos were then rinsed with PBT (0.1% Triton X-100 in PBS), blocked with PBT plus 2% bovine serum albumin for 1 hr, and incubated with the primary antibody overnight at 4°C. After washes with PBT, embryos were incubated with the secondary antibody for 2 hr. After rinses with PBT and 0.12 M Tris (pH 7.6), the reaction was performed in the presence of 10 mg/ml of diaminobenzidine and 0.0015% hydrogen peroxide (H_2O_2). The embryos were then dehydrated through an ethanol series (30%, 50%, 70%, 95%, and 100%), transferred to toluene, and mounted in permount. All the antibodies used in this study have been described previously: Mab22C10 (Zipursky et al., 1984), rat or rabbit anti-cut antibody (Blochlinger et al., 1990), anti-pros antibody (Vaessin et al., 1991), antibody 1188 (Jarman et al., 1993), anti-asense antibody (Brand et al., 1993), rat anti-ttk 69 kDa antibody and guinea pig anti-ttk 88 kDa antibodies (Read et al., 1992), Mab44C11 (Bier et al., 1988), and anti-numb antibody (Rhyu et al., 1994). Avidin–Biotin amplification (Elite ABC kit, Vectastain) was used in some cases for anti-asense and anti-ttk antibodies.

Adult nota were dissected from newly hatched flies in PBS and fixed in a 1:1 mixture of fixative (5% formaldehyde in PBS) for 30–45 min. The remaining steps were the same as described for the embryos, except that in the end, the nota were not dehydrated through ethanol but mounted in Hoyer's solution (Ashburner, 1989).

Immunofluorescence and Confocal Microscopy

Embryo preparation and antibody incubation were the same as described above. The secondary antibodies were either fluorescein conjugated or rhodamine conjugated. Embryos were washed once using PBS after normal PBT washes and subsequently mounted in 90% glycerol with 2% n-propyl-gallate. A Zeiss microscope with a Bio-Rad Krypton Argon laser was used to view and analyze the Confocal images.

Mosaic Analysis

The mosaic flies that have patches of homozygous *ttk* mutant tissues in otherwise heterozygous *ttk* background were generated using FLP/FRT method (Golic and Lindquist, 1989; Golic, 1991). Females carrying *yw p[hs-FLP]; p[FRT] y*$^{+}$ were crossed to males carrying *yw; FRT ttk*^{ves}*/ TM3 Sb*. (The *yw p[hs-FLP]; p[FRT] y*$^{+}$ and *yw; FRT ttk*^{ves}*/ TM3 Sb* were constructed from *p[hs-FLP]* and *p[FRT]* [Golic and Lindquist, 1989; Golic, 1991].) The progeny of this cross was heat-shocked (to induce the expression of FLP) from the first to early third instar larval stage. In this genetic scheme, any bristles in the *ttk* mutant patches would be marked by the *yellow* mutation. Instead, bald patches without hair and sockets were observed. The heat-shock regime is incubation at 39°C for 45 min twice, with 1 hr at room temperature in between. The progeny that are non-*Stubble* were scored for mosaic phenotypes whereas the *Stubble* flies served as control.

Heat-Shock Treatment

The hs-*ttk* strains were obtained from D. Read (Read et al., 1992) For hs-*ttk*, the embryos were collected, aged for 6.5–8.5 hr at 25°C, heat-shocked in a 39°C water bath (15 min for hs-ttk 69 kDa and 30 min for hs-ttk 88 kDa), then aged to stage 16–17 to be fixed for subsequent immunocytochemistry. For hs-*numb*, the embryos were aged for 5–9 hr and heat-shocked for 30 min (Rhyu et al., 1994). For heat shock of pupae, white pupae were collected, aged at 25°C, and then heat-shocked at 39°C (20 min for hs-ttk 69 kDa and 40 min for hs-ttk 88 kDa). Overexpression of ttk 69 kDa is associated with a high rate of pupal lethality. Wild-type flies were heat-shocked in parallel for control.

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References

Ashburner, M. (1982). The genetics of a small autosomal region of *Drosophila melanogaster* containing the structural gene for alcohol dehydrogenase III: hypomorphic mutations affecting the expression of *Hairless*. Genetics *101*, 447–459.

Ashburner, M. (1989). Drosophila: A Laboratory Handbook (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press).

Bang, A., Hartenstein, V., and Posakony, J. (1991). *Hairless* is required for the development of adult sensory organ precursor cells in Drosophila. Development *111*, 89–104.

Bardwell, V., and Treisman, R. (1994). The POZ domain: A conserved protein–protein interaction motif. Genes Dev. *8*, 1664–1677.

Bate, C. M. (1978). Development of sensory systems in arthropods. In Handbook of Sensory Physiology, M. Jacobson, ed. (Berlin: Springer-Verlag), 1–53.

Bier, E., Ackerman, L., Barbel, S., Jan, L. Y., and Jan, Y. N. (1988). Identification and characterization of a neuron-specific nuclear antigen in Drosophila. Science *240*, 913–916.

Bier, E., Vaessin, H., Shepherd, S., Lee, K., McCall, K., Barbel, S., Ackerman, L., Carretto, R., Uemura, T., Grell, E., Jan, L. Y., and Jan, Y. N. (1989). Searching for pattern and mutation in the Drosophila genome with a P-lacZ vector. Genes Dev. *3*, 1273–1287.

Biggs, J., Tripoulas, N., Hersperger, E., Dearolf, C., and Shearn, A. (1988). Analysis of the lethal interaction between the *prune* and *killer of prune* mutations of Drosophila. Genes Dev. *2*, 1333–1343.

Blochlinger, K., Bodmer, R., Jan, L. Y., and Jan, Y. N. (1990). Patterns of expression of Cut, a protein required for external sensory organ development in wild-type and *cut* mutant Drosophila embryos. Genes Dev. *4*, 1322–1331.

Bodmer, R., Carretto, R., and Jan, Y. N. (1989). Neurogenesis of the peripheral nervous system in *Drosophila melanogaster* embryos: DNA replication patterns and cell lineages. Neuron *3*, 21–32.

Bodmer, R., and Jan, Y. N. (1987). Morphological differentiation of the embryonic peripheral neurons in Drosophila. Rouxs Arch. Dev. Biol. *196*, 69–77.

Brou, C., Logeat, F., Lecourtois, M., Vandekerckhove, J., Kourilsky, P., Schweisguth, F., and Israel, A. (1994). Inhibition of the DNA-binding activity of Drosophila Suppressor of Hairless and of its human homolog, KBF2/RBP-J kappa, by direct protein–protein interaction with Drosophila Hairless. Genes Dev. *8*, 2491–2503.

Brand, M., Jarman., A. P., Jan, L. Y., and Jan, Y. N. (1993). *asense* is a *Drosophila* neural precursor gene and is capable of initiating sense organ formation. Development *119*, 1–17.

Brown, J. L., Sonoda, S., Ueda, H., Scott, M., and Wu, C. (1991). Repression of the *Drosophila fushi tarazu (ftz)* segmentation gene. EMBO J. *10*, 665–674.

Brown, J. L., and Wu, C. (1993). Repression of Drosophila pair-rule segmentation genes by ectopic expression of *tramtrack*. Development *113*, 45–58.

Campos-Ortega, J. A., and Hartenstein, V. (1985). The embryonic development of *Drosophila melanogaster*. (New York: Springer-Verlag).

Fortini, M. E., and Artavanis-Tsakonas, S. (1994). The Suppressor of Hairless protein participates in Notch receptor signaling. Cell *79*, 273–282.

Ghysen, A., Dambly-Chaudiere, C., Aceves, E., Jan, L. Y., and Jan Y. N. (1986). Sensory neurons and peripheral pathways in Drosophila embryos. Rouxs Arch. Dev. Biol. *195*, 281–289.

Godt, D., Couderc, J. L., Cramton, S. E., and Laski, F. A. (1993) Pattern formation in the limbs of Drosophila: bric-a-brac is expressed in both a gradient and a wave-like pattern and is required for specification and proper segmentation of the tarsus. Development *119*, 799–812.

Golic, K. G. (1991). Site-specific recombination between homologous chromosomes in Drosophila. Science *252*, 958–961.

Golic, K. G., and Lindquist, S. (1989). The FLP recombinase of yeast catalyzes site-specific recombination in the Drosophila genome. Cell *59*, 499–509.

Harrison, S., and Travers, A. (1990). The *tramtrack* gene encodes a Drosophila finger protein that interacts with the *ftz* transcriptional regulatory region and shows a novel embryonic expression pattern. EMBO J. *9*, 207–216.

Hartenstein, V., and Posakony, J. W. (1989). Development of adult sensilla on the wing and notum of *Drosophila melanogaster*. Development *107*, 389–405

Hartenstein, V., and Posakony, J. W. (1990a). A dual function of the *Notch* gene in Drosophila sensillum development. Dev. Biol. *142*, 13–30.

Hartenstein, V., and Posakony., J. W. (1990b). Sensillum development in the absence of cell division: the sensillum phenotype of the Drosophila mutant *string*. Dev. Biol. *138*, 147–158.

Horvitz, H. R., and Herskowitz. I. (1992). Mechanisms of asymmetric cell division: two Bs or not two Bs, that is the question. Cell *68*, 237–255.

Jan, Y. N., and Jan, L. Y. (1990). Genes required for specifying cell fates in Drosophila embryonic sensory nervous system. Trends Neurosci. *13*, 493–498.

Jan, Y. N., and Jan, L. Y. (1994). Genetic control of cell fate specification in Drosophila peripheral nervous system. Annu. Rev. Genet. *28*, 373–393.

Jan, Y. N., and Jan, L. Y. (1995). Maggot's hair and Bug's eye: role of cell interactions and intrinsic factors in cell fate specification. Neuron *14*, 1–5.

Jarman, A., Grau, Y., Jan, L. Y., and Jan, Y. N. (1993). *atonal* is a proneural gene that directs chordotonal organ formation in the Drosophila peripheral nervous system. Cell *73*, 1307–1321.

Lees, A. D., and Waddington, C. H. (1942). The development of the bristles in normal and some mutant types of *Drosophila melanogaster*. Proc. Roy. Soc. Lond. (B) *131*, 87–110.

Nakamura, M., Okano, H., Blendy, J. A., and Montell, C. (1994). Musashi, a neural RNA-binding protein required for Drosophila adult external sensory organ development. Neuron *13*, 67–81.

Parks, A., and Muskavitch, M. A. (1993). *Delta* function is required for bristle organ determination and morphogenesis in Drosophila. Dev. Biol. *157*, 484–496.

Posakony, J. W. (1994). Nature versus nurture: asymmetric cell divisions in Drosophila bristle development. Cell *76*, 415–418.

Read, D., Levine, M., and Manley, J. (1992). Ectopic expression of the Drosophila *tramtrack* gene results in multiple embryonic defects, including repression of *even-skipped* and *fushi tarazu*. Mech. Dev. *38*, 183–196.

Read, D., and Manley, J. (1992). Alternatively spliced transcripts of the Drosophila *tramtrack* gene encode zinc finger proteins with distinct DNA binding specificities. EMBO J. *11*, 1035–1044.

Rhyu, M., Jan, L., and Jan, Y. N. (1994). Asymmetric distribution of numb protein during division of the sensory organ precursor confers distinct fates to daughter cells. Cell *76*, 477–491.

Salzberg, A., D'Evelyn, D., Schulze, K., Lee, J.-K., Strumpf, D., Tsai, L., and Bellen, H. (1994). Mutations affecting the pattern of the PNS in Drosophila reveal novel aspects of neuronal development. Neuron *13*, 269–287.

Schweisguth, F., and Posakony, J. W. (1994). Antagonistic activities of *Suppressor-of-Hairless* and *Hairless* control alternative cell fates in the Drosophila adult epidermis. Development *120*, 1433–1441.

Shiomi, K., Takeichi, M., Nishida, Y., Nishi, Y., and Uemura, T. (1994). Alternative cell fate choice induced by low-level expression of a regulator of protein phosphatase 2A in the Drosophila peripheral nervous system. Development *120*, 1591–1599.

Uemura, T., Shepherd, S., Ackerman, L., Jan, L. Y., and Jan, Y. N. (1989). *numb*, a gene required in determination of cell fate during sensory organ formation in Drosophila embryos. Cell *58*, 349–360.

Uemura, T., Shiomi, K., Togashi, S., and Takeichi, M. (1993). Mutation of *twins* encoding a regulator of protein phosphatase 2A leads to pattern duplication in Drosophila imaginal discs. Genes Dev. *7*, 429–440.

Vaessin, H., Grell, E., Wolff, E., Bier, E., Jan, L. Y., and Jan., Y. N. (1991). *prospero* is expressed in neuronal precursors and encodes a nuclear protein that is involved in the control of axonal outgrowth in Drosophila. Cell *67*, 941–953.

Xiong, W. C., and Montell, C. (1993). *tramtrack* is a transcriptional repressor required for cell fate determination in the Drosophila eye. Genes Dev. *7*, 1085–1096.

Zipursky, S. L., Venkatesh, T. R., Teplow, D. B., and Benzer, S. (1984). Neuronal development in the Drosophila retina: monoclonal antibodies as molecular probes. Cell *36*, 15–26.

CHAPTER 3

Control of daughter cell fates during asymmetric division: interaction of Numb, Notch and Tramtrack

Control of Daughter Cell Fates during Asymmetric Division: Interaction of Numb and Notch

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Summary

During development of the Drosophila peripheral nervous system, a sensory organ precursor (SOP) cell undergoes rounds of asymmetric divisions to generate four distinct cells of a sensory organ. Numb, a membrane-associated protein, is asymmetrically segregated into one daughter cell during SOP division and acts as an inherited determinant of cell fate. Here, we show that Notch, a transmembrane receptor mediating cell–cell communication, functions as a binary switch in cell fate specification during asymmetric divisions of the SOP and its daughter cells in embryogenesis. Moreover, *numb* negatively regulates *Notch*, probably through direct protein–protein interaction that requires the phosphotyrosine-binding (PTB) domain of Numb and either the RAM23 region or the very C-terminal end of Notch. *Notch* then positively regulates a transcription factor encoded by *tramtrack* (*ttk*). This leads to Ttk expression in the daughter cell that does not inherit Numb. Thus, the inherited determinant Numb bestows a bias in the machinery for cell–cell communication to allow the specification of distinct daughter cell fates.

Introduction

In asymmetric division, an important process in the generation of cell diversity during development, a mother cell produces two daughter cells that ultimately adopt distinct fates. Two mechanisms may be responsible for making the two daughter cells different. One, the cell-intrinsic mechanism, involves an inherited determinant that is asymmetrically segregated to one daughter cell at the time of cell division. The other, the cell-extrinsic mechanism, entails communication of the daughter cells with each other or with surrounding cells (reviewed by Horvitz and Herskowitz, 1992). In the Drosophila peripheral nervous system (PNS), both mechanisms are used to generate the distinct cell types of a sensory organ (Uemura et al., 1989; Hartenstein and Posakony, 1990; Parks and Muskavitch, 1993; Rhyu et al., 1994). It thus provides an opportunity to assess how these two mechanisms interact in the process of daughter cell fate specification during asymmetric divisions.

Two main types of sensory organs in the larval and adult Drosophila PNS, the external sense (es) organ and chodotonal (cho) organ, are formed in a progressive process during embryogenesis and pupal development (Bodmer and Jan, 1987; Campos-Ortega and Hartenstein, 1985; Ghysen et al., 1986). The "proneural genes," including those in the *achaete–scute* complex and *atonal*, are first expressed in clusters of ectodermal cells to endow these cells with the competence to adopt neuronal fates (reviewed by Ghysen and Dambly-Chaudiere, 1988; Campuzano and Modolell, 1992). One sensory organ precursor (SOP) is then singled out from each cluster through a process of "mutual inhibition" mediated by "neurogenic genes" including *Notch* and *Delta* (reviewed by Campos-Ortega, 1988; Artavanis-Tsakonas and Simpson, 1991; Ghysen et al., 1993). To form an es organ, the SOP gives rise to two distinct daughter cells, IIa and IIb (see Figure 3A). The IIa cell then divides to produce the hair cell (tricogen) and the socket cell (tormogen), the outer support cells. Shortly after the division of IIa, IIb divides to generate the neuron and the sheath cell (thecogen), the inner cells (Bate, 1978; Hartenstein and Posakony, 1989). A different series of asymmetric divisions allows the SOP of a cho organ (see Figure 3D) to give rise to the neuron and three different support cells (the sheath cell, the cap cell, and the ligament cell) (Bodmer et al., 1989; Brewster and Bodmer, 1995).

Notch and *Delta* have been shown to mediate cell–cell communication in eye development (Cagan and Ready, 1989), muscle development (Corbin et al., 1991), oogenesis (Ruohola et al., 1991; Xu et al., 1992), and neurogenesis (Lehmann et al., 1983) in Drosophila. Homologs of *Notch* and *Delta* have been characterized in various species ranging from worm to human (reviewed by Artavanis-Tsakonas et al., 1995). *Notch* encodes a transmembrane protein with EGF-like repeats in the extracellular domain and tandem ankyrin repeats in the intracellular domain (Wharton et al., 1985; Kidd et al., 1986). The *Notch* product has been postulated to act as a receptor, whereas the *Delta* gene product, another transmembrane protein with EGF repeats (Vaessin et al., 1987; Kopczynski et al., 1988), may function as a ligand for the Notch receptor (Heitzler and Simpson, 1991; Rebay et al., 1991).

Cell–cell interaction is required not only for singling out SOPs, but also for the proper cell fate determination of SOP progeny, as indicated by studies of temperature-sensitive alleles of *Notch* and *Delta*. Reduction of *Notch* or *Delta* function during SOP progeny formation in pupal development causes an adult es organ (bristle) to contain four neurons and no support cells (Hartenstein and Posakony, 1990; Parks and Muskavitch, 1993). A slightly earlier temperature shift, presumably prior to IIa and IIb division, leads to two neurons and two sheath cells in $Delta^{ts}$ flies (Parks and Muskavitch, 1993). Thus, it was postulated that *Delta* specifies four distinct progeny cells in a stepwise fashion during the SOP division and the IIb cell division (Parks and Muskavitch, 1993).

Besides cell–cell interaction, a cell-intrinsic factor, the *numb* gene product, is also essential for cell fate determination of SOP progeny. *numb* encodes a protein with a motif called the phosphotyrosine-binding (PTB) domain (Kavanaugh and Williams, 1994) or phosphotyrosine interaction (PID) domain (Bork and Margolis, 1995). Loss of *numb* function transforms the IIb cell into the IIa cell during embryogenesis (Uemura et al., 1989), and

overexpression of *numb* results in reciprocal cell fate transformation (Rhyu et al., 1994). Immunocytochemical studies have shown that Numb is asymmetrically localized to one pole of the SOP and segregated to one of the daughter cells (Rhyu et al., 1994; Knoblich et al., 1995). Thus, asymmetrically distributed Numb confers distinct daughter cell fates (Rhyu et al., 1994). Asymmetric divisions of the SOP and its daughter cells that give rise to the adult es organ (bristle) also depend on *numb* function (Rhyu et al., 1994); the cell fate transformation due to overexpression of *numb* via heat shock (*hs-numb*) is similar to the phenotype caused by reduction of *Notch* function. Similarly, asymmetric division of the MP2 neural precursor in the central nervous system (CNS) depends on asymmetric localization of Numb (Spana et al., 1995).

numb exerts its function at least in part via a downstream target gene called *tramtrack* (*ttk*) (Guo et al., 1995). *ttk* encodes two zinc finger proteins due to alternative splicing (Harrison and Travers, 1990; Brown et al., 1991; Read and Manley, 1992). The 69 kDa Ttk protein has been shown to act as a transcriptional repressor in the process of segmentation (Brown et al., 1991; Read et al., 1992). Like *numb*, *ttk* acts as a genetic switch. Loss of *ttk* function causes the SOP daughter cell IIa to be transformed into IIb, a phenotype opposite to the loss of *numb* function phenotype. Overexpression of *ttk*, on the other hand, results in the same cell fate transformation as does loss of *numb* function (Guo et al., 1995). Genetic and immunocytochemical studies indicate that *ttk* is negatively regulated by *numb* (Guo et al., 1995).

Several questions are raised by the dependence of asymmetric division on a gene encoding a cell-intrinsic determinant (Numb) as well as genes such as *Notch* and *Delta*, which mediate cell–cell interaction. How does the information derived from cell-intrinsic signals interface with signals arising from cell–cell interaction? How are these different instructions to the two daughter cells implemented to secure their distinct fates? In this paper, we first show that *Notch* plays a critical role in asymmetric divisions during embryogenesis. We then demonstrate that genetically *Notch* is most likely negatively regulated by *numb*, and biochemically Notch binds to Numb. This direct protein–protein interaction requires either the RAM23 region or the very C-terminal end of Notch and the PTB domain of Numb. Finally, we have identified *ttk* as a downstream target gene of *Notch*. Taken together, we propose that Ttk acts as a readout to integrate the information derived from both cell-intrinsic mechanism and cell–cell communication; the inherited determinant Numb sets up or biases the direction of cell–cell interaction to allow the specification of distinct daughter cell fates during asymmetric divisions.

Results

Reduction of *Notch* Function during SOP Division Causes Support Cells to Be Transformed into Neurons

Although there have been extensive studies of *Notch* function in the process of lateral inhibition to single out a neuronal precursor from a proneural cluster (Campos-Ortega, 1988; Artavanis-Tsakonas and Simpson, 1991; Ghysen et al., 1993), the role of *Notch* in asymmetric divisions of the SOP has only been examined in the adult (Hartenstein and Posakony, 1990), but not in the embryo. Hence, we characterized the embryonic phenotypes due to reduction of *Notch* function or overexpression of *Notch* and showed that they corresponded to reciprocal daughter cell fate transformations.

Elimination of *Notch* function throughout development results in a significant overproduction of neuronal precursors (Lehmann et al., 1983). The general disorganization of the PNS in these null mutants, however, makes it difficult to ascertain any cell fate alterations within the lineage of a single sensory organ. To overcome this difficulty, a temperature-sensitive allele of *Notch* (*Notch*^{ts}) was used. Embryos of *Notch*^{ts} were first raised at the permissive temperature (18°C) to allow the normal development of the embryo and then shifted to the nonpermissive temperature (30°C) at certain developmental stages to disrupt the function of *Notch* (see Experimental Procedures).

SOPs are singled out and then divide asymmetrically during 4–7 hr of embryogenesis at 25°C (Bodmer et al., 1989). Shifting *Notch*^{ts} embryos to the nonpermissive temperature at stages corresponding to 4–5 hr of embryogenesis at 25°C resulted in significant overproduction of neurons in most embryos, as revealed by staining with a neuronal marker MAb22C10 (Zipursky et al., 1984). Using anti-Cut antibody, which recognizes all four cells of the es organ (Blochlinger et al., 1990), we examined the site of emergence of normally a single es organ and found that the number of Cut-positive cells increased by a factor of two to three (data not shown). Thus, most likely supernumerary SOPs emerged from the disruption of *Notch* function at this early stage due to failure of singling out of SOPs.

When *Notch*^{ts} embryos were shifted to the nonpermissive temperature at stages corresponding to 5–7 hr of embryogenesis at 25°C, we observed phenotypes of cell fate transformation within both es and cho lineages. We focused our studies in the dorsal-most region of an abdominal hemisegment, which normally harbors two es organs, and doubly stained the embryos with anti-Cut antibody and MAb22C10. In wild type, there are eight Cut-positive cells and two MAb22C10-positive neurons. Among younger embryos in a *Notch*^{ts} embryo collection, which would have their *Notch* function disrupted at an earlier stage, some had ten to twelve Cut-positive cells; most of these cells expressed MAb22C10. The rest of the embryos had a complement of eight Cut-positive cells, though up to six of these cells also expressed MAb22C10. It thus appears that even after the singling out of the SOPs, the number of neurons within an es organ increases with the disruption of *Notch* function. This could be interpreted as a cell fate transformation during asymmetric divisions of SOP, analogous to what has been observed in the adult bristle formation (Hartenstein and Posakony, 1990).

To test this further, we doubly stained the *Notch*^{ts} embryos with MAb22C10, a neuronal marker, and anti-Prospero antibody, which labels the nuclei of sheath cells in both es and cho organs (Vaessin et al., 1991). We focused our studies on the older embryos in the collection that would have their *Notch* function disrupted at a later stage. In the wild-type embryo, the two

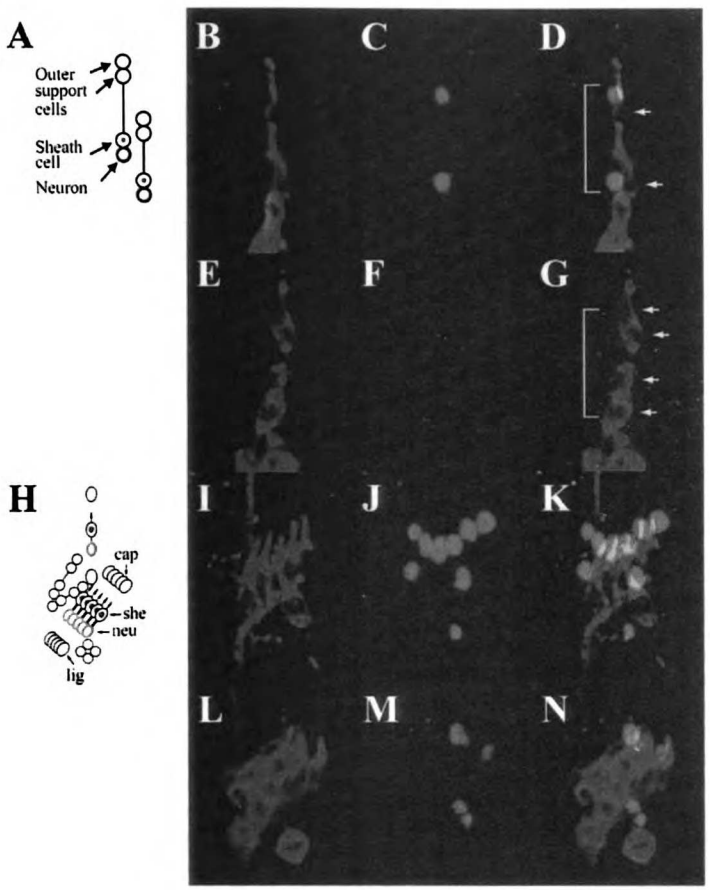

Figure 1. Reduction of *Notch* Function Transforms Sheath Cells to Neurons

(A–D) Two simple es organs at the dorsal-most region of an abdominal segment in wild-type embryo. (A) A schematic drawing of the two isolated es organs, with each circle representing an individual cell. Green circles correspond to the MAb22C10 staining that recognizes the cytoplasm of neurons shown in (B) and (D), whereas red dots correspond to the anti-Prospero staining that labels the nuclei of sheath cells shown in (C) and (D). In wild type, two neurons (B) (two arrows in [D]) and two sheath cells (C) can be observed (bracket in [D]).

(E–G) In *Notch*^{ts}, four neurons ([E] and four arows in [G]) can be detected without the associated sheath cells ([F] and bracket in [G]).

(H) Lateral region of an abdominal segment where five cho organs are aligned side by side. In this region, there are also other cells, one v′ cho organ and three es organs. Similarly to (A), green circles and red dots correspond to the MAb22C10 and anti-Prospero staining respectively. she, sheath cell; neu, neuron; lig, ligament cell; cap, cap cell.

(I–K) In wild type, five cho neurons (I and K) have five sheath cells associated with them (J and K).

(L–N) In *Notch*^{ts}, the number of MAb22C10-positive neurons increases (L and N), while the number of anti-Prospero positive sheath cells decreases (M and N). Moreover, the number of neuronal overproduction is equivalent to that of the sheath cell reduction (N).

isolated dorsal-most es organs have two MAb22C10-positive neurons and two Prospero-expressing sheath cells (Figures 1A–1D). In contrast, the *Notch*^{ts} embryos contained four neurons without associated sheath cells at the corresponding location (Figures 1E–1G), indicating that the sheath cells are transformed into neurons (see Figure 3B). As expected from such a cell fate transformation, staining with anti-Cut antibody revealed four strongly staining cells (hairs or sockets) and four weakly staining cells (neurons or sheath cells) (data not shown). Similar cell fate transformation from MAb22C10-positive neurons to Prospero-positive sheath cells in cho organs was observed (Figures 1L–1N). This cell fate transformation from support cells to neurons in both es and cho organs further substantiates the role of *Notch* in cell fate specification during asymmetric divisions. These embryonic phenotypes were then used to investigate the epistatic genetic relationships between *Notch* and *ttk*, as well as between *Notch* and *numb* (see below).

Overexpression of Activated Notch Results in the Reverse Cell Fate Transformation

When a truncated form of *Notch* (intracellular domain) is ubiquitously overexpressed during the stage when neuronal precursors are singled out, there is a reduction of neuronal precursors (Lieber et al., 1993; Rebay et al., 1993; Struhl et al., 1993; Lyman and Yedvobnick, 1995; Bang et al., 1995). Since these phenotypes are opposite to that due to loss of *Notch* function during the process of singling out of neuronal precursors, it is suggested that the Notch intracellular domain corresponds to a constitutively active form of the Notch receptor (Lieber et al., 1993; Rebay et al., 1993; Struhl et al., 1993).

We used the UAS–GAL4 system (Brand and Perrimon, 1993) and studied the effect of overexpression of the constitutively active form of Notch (Notch^{ACT}) (Doherty et al., 1996) that contains a truncated Notch (with the extracellular domain deleted but with the transmembrane domain and the intracellular domain retained) during SOP divisions. Transgenic flies carrying UAS–Notch^{ACT} were mated to transgenic flies carrying a GAL4 enhancer–trap line, 1407 GAL4, which expresses the reporter gene in all neurons of the PNS (Luo et al., 1994). The embryos carrying UAS–Notch^{ACT} as well as the 1407 GAL4 line would then express Notch^{ACT} primarily in neurons. At the dorsal-most region of abdominal segments of these embryos, anti-Cut staining showed the expression pattern of four strongly stained and four weakly stained cells in approximately 90% of the segments (data not shown). Double labeling of both MAb22C10 and anti-Prospero, however, revealed a cell fate transformation in at least one of these two es organs in about 30%–40% of the abdominal segments examined. A typical example is shown in Figures 2A–2C, in which the es organ on the top contains two anti-Prospero-positive cells but no neurons, suggestive of a transformation

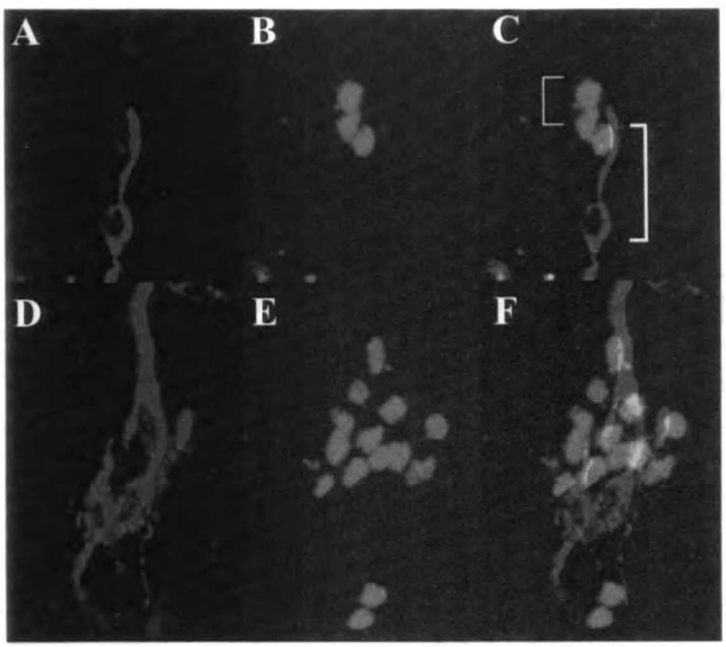

Figure 2. Overexpression of Notch^{ACT} Transforms Neurons to Sheath Cells

(A–C) Of the two dorsal-most es organs, the top es organ (the bracket on the left in [C]) has two anti-Prospero positive sheath cells (B), without the associated MAb22C10-positive neuron (A). (C) The lower es organ (the bracket on the right in [C]) is not transformed. (D–F) In the lateral region of an abdominal segment, the number of anti-Prospero positive sheath cells increases (E and F), while the number of MAb22C10-positive neurons decreases (D and F). Moreover, the overproduction of sheath cells matches in number with the reduction of neurons (F).

from neurons to sheath cells (Figure 3C). The lower es organ, on the other hand, appears normal. In the cho organs at the lateral region, we also observed an increase in the number of Prospero-positive sheath cells and a concurrent decrease in the number of MAb22C10-positive neurons (Figures 2D–2F), indicative of a cell fate transformation from neurons to sheath cells (Figure 3F). In this region, at least three of the five cho organs in most segments showed transformation. Thus, overexpression of Notch^{ACT} results in a cell fate transformation opposite to that caused by reduction of *Notch* function.

Overexpression of the Wild-Type Notch Results in Cell Fate Transformation Similar to That Caused by Activated Notch

If overexpressing the truncated Notch, Notch^{ACT}, indeed reveals the activity of the Notch receptor upon activation, it should produce phenotypes qualitatively similar to those due to overexpression of the wild-type Notch (Notch^{WT}). It has been reported that overexpression of wild-type Notch via the heat shock promoter does not result in any significant phenotypes (Lieber et al., 1993; Rebay et al., 1993; Fortini et al., 1993). We found that Notch overexpression due to UAS–Notch^{WT} in combination with various GAL4 enhancer–trap lines resulted in a very mild phenotype of transformation of neurons to sheath cells in the embryo (data not shown), but much stronger phenotype in adult es organs (bristles).

Overexpression of Notch^{ACT} in the 109-68 GAL4 enhancer–trap line, which expresses GAL4 in all four cells of an adult es organ, caused 90% of the bristles in the notum to form double sockets (Figure 4A) or, less frequently, triple sockets or four sockets (Figure 4B). The double socket phenotype is similar to the phenotype due to overexpression of the intracellular domain of Notch (Lieber et al., 1993; Rebay et al., 1993; Struhl et al., 1993; Lyman and Yedvobnick, 1995; Bang et al., 1995) and is indicative of a transformation of the hair cell into the socket cell. The four sockets most likely arise from a transformation of the IIb cell into the IIa cell, followed with a transformation of the hair cell into the socket cell (Figure 4E). Overexpression of Notch^{WT} using the same GAL4 enhancer–trap line caused about 20% of the bristles to form double sockets (Figure 4C) or four sockets (Figure 4D). Thus, Notch^{ACT} overexpression causes qualitatively similar, albeit stronger, phenotypes, as compared with those due to overexpression of the wild-type Notch product.

Sensitive Genetic Interaction between *numb* and *Notch*

The effects of reducing *Notch* function on adult bristle formation (Hartenstein and Posakony, 1990) are similar to those due to overexpression of *numb* (Rhyu et al., 1994). To determine whether *Notch* and *numb* function in the same genetic pathway, we first tested to see whether there is a synergistic enhancement of the adult bristle phenotypes due to both reduction of *Notch* function and *numb* overexpression.

Whereas heat shock during larval development causes flies carrying one or two copies of the *hs-numb* transgene to exhibit double hairs, as a result of transformation of the socket cell into the hair cell (Rhyu et al., 1994), without heat shock these flies did not have any bristle phenotype (Figure 5B). Flies carrying only one copy of the functional *Notch* gene, *Notch^{55e11}*/+, also showed no obvious hair or socket abnormalities (Figure 5C). By contrast, even in the absence of any heat shock treatment, *Notch^{55e11}*/+; *hs-numb*/+ flies had double hairs without sockets at the anterior wing margin (Figure 5D). This phenotype was further enhanced in *Notch^{55e11}*/+ flies that carried two copies of *hs-numb* (Figure 5E). In addition to finding double hairs with no sockets in place of a normal bristle (Figure 5F), we also observed double hairs right next to a normal looking bristle (Figure 5G) and two sets of double hairs right

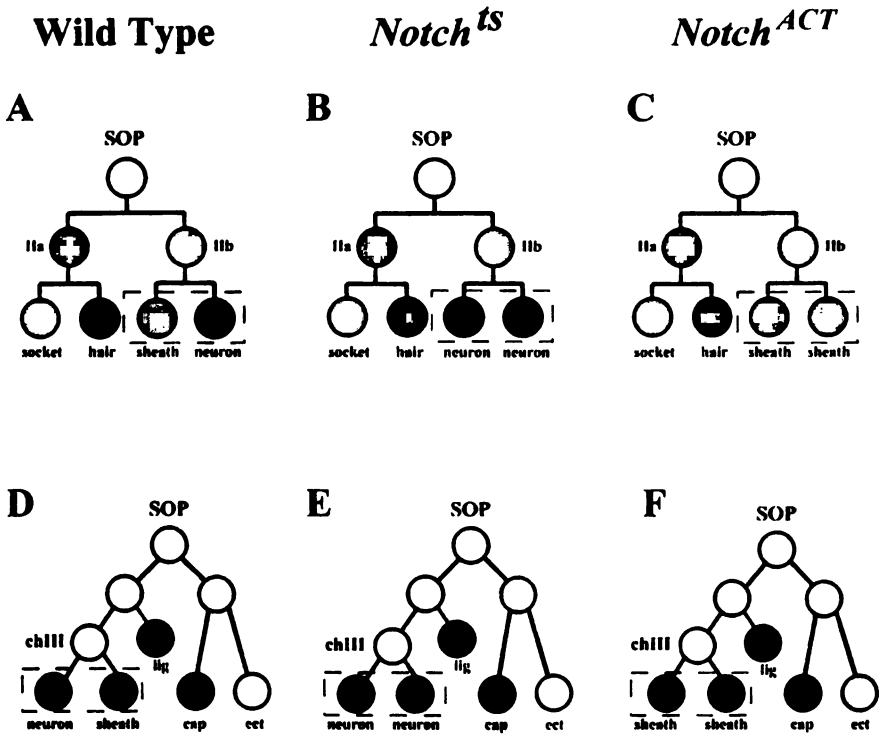

Figure 3. Wild-Type and Proposed SOP Cell Lineages in the Embryo due to Reduction of *Notch* Function or Notch^{ACT} Overexpression

(A–C) Cell lineages for the es organ in wild type (A), *Notch*^{ts} (B), and Notch^{ACT} overexpression (C). Within each lineage, we have boxed two daughters whose fates are indicated to be affected by *Notch*. In the *Notch*^{ts} (B), the sheath cell is transformed into the neuron, yielding two neurons. Following Notch^{ACT} overexpression (C), the neuron is transformed into the sheath cell.

(D–F) Cell lineages for the cho organ in wild type (D), *Notch*^{ts} (E), and Notch^{ACT} overexpression (F). We have modified drawings of the cho lineage according to Brewster and Bodmer (1995). Lig, the ligament cell; ect, the ectodermal cell. In a cho organ, two neurons are produced at the expense of the sheath cell in the *Notch*^{ts} (E). Following Notch^{ACT} overexpression (F), two sheath cells are formed coincident with the loss of the neuron. Both reduction of *Notch* function and overexpression of Notch^{ACT} appear to disrupt the normally asymmetric division, producing two identical daughter cells. Only the effects of reduction of *Notch* function and Notch^{ACT} overexpression on the formation and differentiation of the IIb lineage in the es organ and of the chIII lineage in the cho organ are depicted in this figure; similar cell fate transformations in the IIa lineage and between IIa and IIb have been observed but are not indicated here.

next to each other (Figure 5H). Two independent *Notch* alleles and two independent insertion lines of *hs-numb* were tested, and similar synergistic enhancement of the bristle phenotype was observed (data not shown). Thus, it is unlikely that the observed phenotypes could be due to the site of insertion of the *hs-numb* transgene or unknown genetic background. These bristle phenotypes therefore represent a strong synergistic effect of reducing *Notch* function in the *Notch* heterozygote and slightly elevating Numb expression due to the basal activity of *hs-numb* without heat shock; neither alone generated any detectable phenotype. This suggests, though does not prove, that *numb* and *Notch* function in the same genetic pathway.

Epistatic Relationship between *Notch* and *numb*

To investigate the epistatic relationship between *numb* and *Notch*, we examined whether reduction of *Notch* function is capable of suppressing the phenotypes resulting from loss of *numb* function. Embryos homozygous for the null mutation *numb*^{1} and *Notch*^{ts} were shifted to the nonpermissive temperature at the time of cell fate specification within the lineage of the sensory organs. Compared with the wild type (Figures 6A and 6B), loss of *numb* function reduced the number of neurons to less than 10% of that in the wild type (Uemura et al., 1989), as revealed by the neuronal marker MAb22C10 (Figures 6C and 6D). Reduction of *Notch* function, on the other hand, caused a neuronal overproduction as described earlier (Figures 6E and 6F). The double mutants of *numb* and *Notch*^{ts} showed a range of phenotypes; 10% of the embryos demonstrated a neuronal overproduction approaching the *Notch*^{ts} phenotype (Figures 6G and 6H). The rest of the embryos did not exhibit as extensive an overproduction, but the number of neurons was much larger than that in *numb* null mutants (Figures 6I and 6J). Since a reduction of *Notch* function partially suppressed the *numb* null phenotypes, *Notch* might act downstream of *numb* and appears to be negatively regulated by *numb*. We then examined Numb expression when *Notch* function is disrupted. The asymmetric Numb localization was still present in at least some of the dividing precursor cells in both CNS and PNS of the *Notch*^{55e11} null mutant embryo (data not shown), consistent with *Notch* functioning downstream of *numb*.

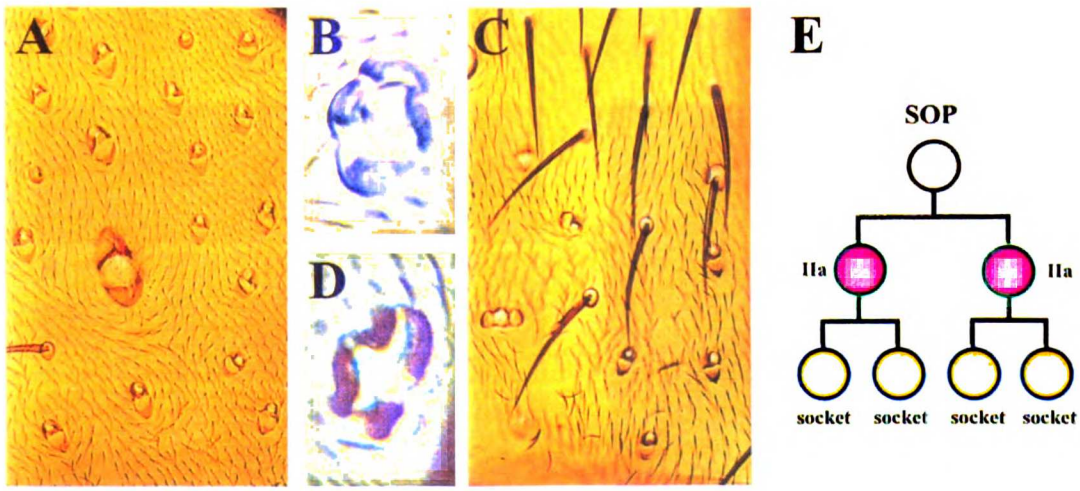

Figure 4. Overexpression of Notch^{ACT} and Notch^{WT} Results in Similar Phenotypes of Multiple Sockets

(A) Double sockets phenotypes of both macrochaetes and microchaetes on the notum due to Notch^{ACT} overexpression.

(B) Four sockets observed in a Notch^{ACT}-expressing fly.

(C) Double sockets due to overexpression of Notch^{WT} observed at a lower frequency.

(D) Four sockets were also seen in Notch^{WT} overexpression.

(E) The proposed lineage for the four socket phenotype observed in both Notch^{ACT} overexpression and Notch^{WT} overexpression. There is a cell fate transformation first from the IIb cell to the IIa cell and then from the hair cell to the socket cell.

Direct Protein–Protein Interaction between Notch and Numb

Since we detected a very strong synergistic interaction in transheterozygous flies carrying *Notch^{55e11}*/+; *hs-numb*/+ (without heat shock) (see Figure 5), we wondered whether there could be direct physical interaction between these two proteins. We assessed this possibility first using the yeast two-hybrid interaction assay (Bartel et al., 1993). Full-length as well as fragments of Numb or Notch were fused with either the LexA DNA-binding domain or the GAL4 transcriptional activation domain. If Numb and Notch physically interact, cotransformation of these two fusion proteins in yeast cells would result in the reconstitution of the "hybrid" transcription factor that can be assessed by the β-galactosidase activity. As described below, this assay revealed interaction between Notch and Numb. These two proteins were then subdivided into fragments to localize the regions involved in this protein–protein interaction.

To identify the portions of the Notch protein involved in the interaction with Numb, we subdivided the Notch intracellular domain into three regions, A, M, and P (Figure 7), and assayed their interactions with Numb individually. Two regions, A and P, interacted with Numb, whereas the M region did not (Figure 7A and Table 1). This interaction appears to be specific, since neither Numb nor Notch-A bound to the control protein Lamin. Moreover, Suppressor of Hairless (Su(H)), a known Notch-binding protein (Tamura et al., 1995; Hsieh et al., 1996), bound to Notch-A but not Notch-P (Table 1). Both regions A and P display a high degree of amino acid conservation among vertebrate Notch homologs (Weinmaster et al., 1991, 1992; Bierkamp and Campos-Ortega, 1993) and include several functional motifs. Region P is at the very C-terminal end of Notch and includes the PEST region; it has been suggested to play an important role in Notch functioning (Xu et al., 1990; Lieber et al., 1993). Region A includes RAM23, a Su(H)-binding region, and ankyrin repeats, a Deltex-binding region (Diederich et al., 1994; Matsuno et al., 1995). Since Su(H) and possibly Deltex are involved in the cell fate decision in the es lineage (Ashburner, 1982; Schweisguth and Posakony, 1994; Matsuno et al., 1995), we wondered whether Numb binds to RAM23 and/or ankyrin repeats. As shown in Table 1 and Figure 7A, Numb bound to RAM23, but not ankyrin repeats of Notch.

Two lines of evidence implicate the N-terminal portion but not the C-terminal portion of Numb in Notch binding. As described later, a glutathione S-transferase (GST) fusion protein of Notch bound to Numb-N but not to Numb-C (Figure 7C). In the yeast two-hybrid assay, Notch-A also showed interaction with Numb-N but not Numb-C (Figure 7B and Table 1). Consistent with the finding, Notch-A also bound to Numb-P, which includes Numb-N and is conserved between Drosophila Numb and mouse Numb (Zhong et al., 1996 [this issue of *Neuron*]; Figure 7B and Table 1). We then tested whether the PTB domain alone, part of Numb-N, is sufficient to confer the binding to Notch-A. Indeed, we detected an interaction as shown in Figure 7B and Table 1.

To confirm the physical interaction between Notch and Numb, we used the in vitro binding assay as an independent test. The Notch intracellular domain was fused to GST, and this fusion protein was expressed in bacteria. The GST–Notch fusion protein was immobilized on glutathione–Sepharose beads and then mixed with in vitro translated ^{35}S-labeled Numb-N or Numb-C. As shown in Figure 7C, Numb-N, but not Numb-C, was retained with GST–Notch on beads. Neither Numb-N nor Numb-C was retained with GST protein alone on the glutathione–Sepharose beads. The ability of Notch intracellular domain to bind Numb-N in vitro as well as in yeast two-hybrid assay suggests that direct protein–

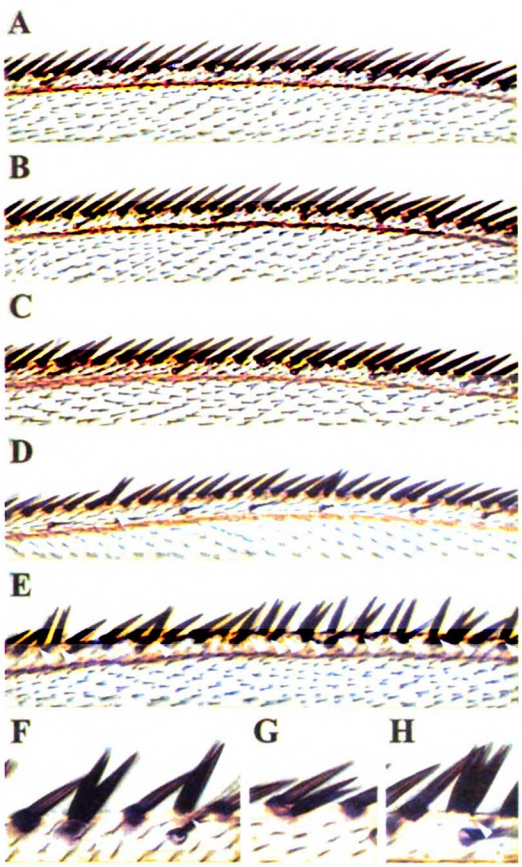

Figure 5. Synergistic Interaction between Reduction of *Notch* Function and Slight Elevation of Numb Expression

The bristles in anterior wing margin of wild type (A), *hs-numb* (B), *Notch*^{55e11}/+ (C), *Notch*^{55e11}/+; *hs-numb*/+ (D), and *Notch*^{55e11}/+; *hs-numb* (E–H). The stout bristles are in focus. Bristles in *hs-numb* (B) and *Notch*^{55e11}/+ (C) are essentially wild type in appearance (A). In *Notch*^{55e11}/+; *hs-numb*/+ (D), there is a phenotype of "double hairs" (arrowhead) at low frequency. In *Notch*^{55e11}/+; *hs-numb*, there is a high frequency of abnormal bristles (E), including "double hairs with no socket" (arrowhead in [F]), "double hairs next to a bristle with a normal looking bristle" (arrowhead in [G]), as well as "four hairs next to one another" (arrowhead in [H]).

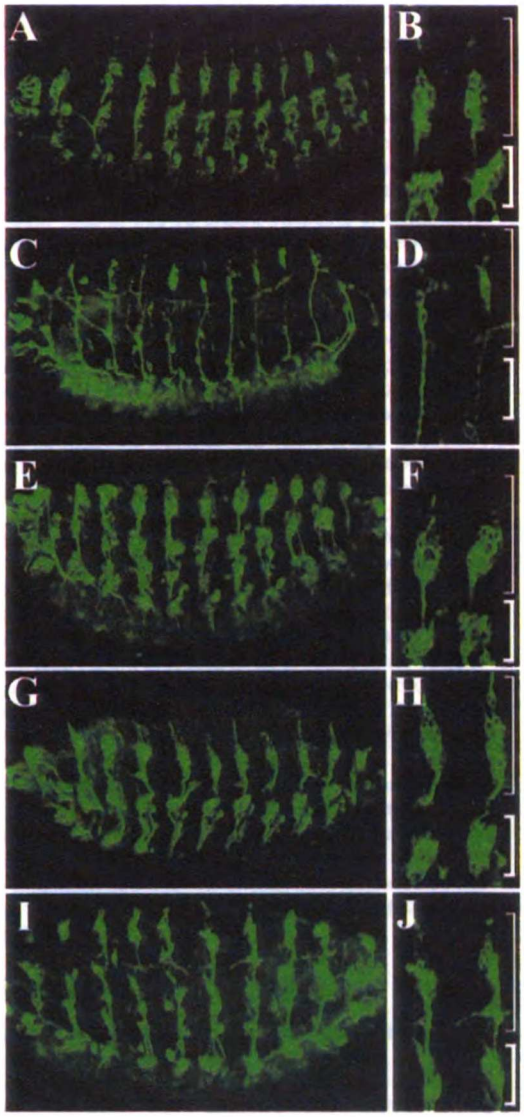

Figure 6. Epistasis of *numb* and *Notch*: Reduction of *Notch* Function Suppresses the Neuronal Phenotype of *numb* Null Allele

MAb22C10 staining of a wild-type embryo (A and B), a *numb*^{1} embryo (C and D), a *Notch*^{ts} embryo (E and F), and the double mutant *numb*^{1}; *Notch*^{ts} embryos (G–J). Whole-mount embryos are in (A), (C), (E), (G), and (I), while the dorsal cluster (thin bracket) and lateral cluster (thick bracket) of two abdominal segments are shown at higher magnification in (B), (D), (F), (H), and (J). Compared with the wild type (A and B), the *numb*^{1} embryo shows a severe reduction in the number of neurons (C and D), whereas the *Notch*^{ts} embryo exhibits a significant increase in the number of neurons (E and F). About 10% of the double mutant *numb*^{1}; *Notch*^{ts} embryos such as the one in (G) and (H) show neuronal overproduction almost to the same extent as *Notch*^{ts} (E and F), whereas the majority of the double mutants such as the one in (I) and (J) shows the "intermediate phenotype," the number of neurons is more than that in *numb*^{1} embryo (C and D), but fewer than that in *Notch*^{ts} embryo (E and F).

protein interaction may provide a molecular mechanism for the negative regulation of *Notch* by *numb*.

ttk Acts Downstream of *Notch*

Since *Notch* and *ttk* are both downstream of *numb* and cause similar phenotypes in the cell fate specification in the es organ lineage, we asked whether *Notch* and *ttk* act in the same genetic pathway and, if so, whether *Notch* acts upstream of *ttk*.

We first examined whether there is any alteration of the Ttk expression due to either reduction of *Notch* function or overexpression of Notch^{ACT}. We focused our investigation on the IIb lineage of the es organ, because Ttk is expressed in the sheath cell that also expresses Prospero, but not in the other daughter cell of IIb, the

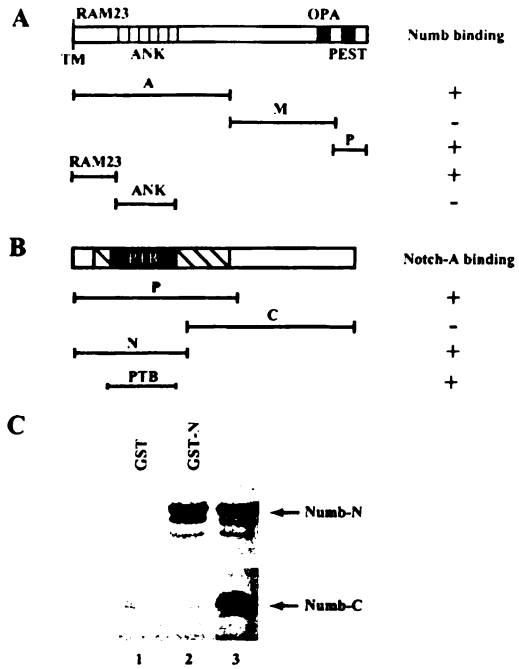

Figure 7. Direct Protein–Protein Interaction between Numb and Notch

(A) and (B) depict the region of Numb and Notch used in two-hybrid assay.

(A) Notch intracellular domain. The RAM23 region, the ankyrin repeats (ANK), the OPA repeats, and the PEST region are indicated. The fusion protein containing regions A, P, or RAM23 binds to Numb (indicated by a plus sign), whereas region M and ANK fail to bind (indicated by a minus sign) (see Table 1).

(B) Numb protein. The conserved region between Drosophila Numb and mouse Numb is illustrated as a striped area and includes the PTB domain. Regions P, N, and PTB, but not C, bind to Notch-A (see Table 1).

(C) In vitro binding of [^{35}S]Numb-N but not [^{35}S]Numb-C to GST–Notch (intracellular domain). [^{35}S]Numb-N or [^{35}S]Numb-C was mixed with the control GST protein (lane 1), or GST–Notch fusion protein (lane 2), as well as glutathione–Sepharose beads. Glutathione–Sepharose beads were used to isolate GST-containing protein complex, which were then analyzed by SDS–PAGE and autoradiography (top, [^{35}S]Numb-N; bottom, [^{35}S]Numb-C). An aliquot of [^{35}S]Numb-N or [^{35}S]Numb-C was run on the same gel (lane 3) to indicate the size of the in vitro translated Numb protein fragments.

neuron that expresses anti-Elav (Guo et al., 1995; Ramaekers et al., submitted). Figures 8A–8F show the double labeling of *Notch*^{ts} embryos using anti-Ttk and anti-Elav antibodies. In the dorsal-most region that normally contains two es organs and hence two neurons, we detected four Elav-expressing neurons (Figure 8B). As shown earlier, the two extra neurons arose from a transformation of the sheath cells into neurons. We found that Ttk was expressed in most of the cells in the epidermis of the *Notch*^{ts} embryo (Figure 8A), but not in neurons, including the supernumerary neurons derived from transformation of sheath cells (Figure 8C). Similar observations were made in the lateral cho organs. None of the neurons, including those sheath cells that had transformed into neurons, were recognized by anti-Ttk antibody (Figures 8D–8F). Thus, *Notch* function is required not only to specify the sheath cell but also to express Ttk in this daughter cell of an asymmetric division.

In a reciprocal experiment, we examined Ttk expression in embryos with Notch^{ACT} overexpression (Figures 8G–8L). In these embryos, we found that often one es organ in the dorsal-most region showed transformation (Figures 8G–8I). Anti-Prospero antibody labeled two sheath cells in this es organ, including one that arose from a transformation of the neuron into a sheath cell (Figure 8H). The superimposition of anti-Ttk staining and anti-Prospero staining reveals that Ttk is expressed in all the sheath cells, including those transformed from neurons (Figure 8I). Similarly, in the cho organs, Ttk is expressed in all the sheath cells, including those that are transformed from neurons (Figures 8J–8L). Thus, activated Notch is able to turn on Ttk expression in cells that normally do not express Ttk. The up- and down-regulation of Ttk expression due to overexpression of activated Notch and reduction of *Notch* function, respectively, indicates that *ttk* is very likely downstream of *Notch* and is positively regulated by *Notch*.

To test this hypothesis further, we asked whether constitutively active Notch requires *ttk* in mediating cell fate transformation. We therefore overexpressed Notch^{ACT} in the *ttk* loss-of-function mutant. Compared with the wild type (see Figure 6A), loss of *ttk* resulted in an overproduction of neurons (Figure 9B), whereas Notch^{ACT} expression resulted in a reduction of neurons (Figure 9A). We found that overexpression of Notch^{ACT} in *ttk* mutant background resulted in an overproduction of neurons (Figure 9C). The extent of this neuronal overproduction is indistinguishable from that due to loss of *ttk* function; without *ttk* function, Notch^{ACT} fails to transform neurons into sheath cells. Therefore, the capability of Notch^{ACT} to transform neurons into sheath cells depends on normal *ttk* function, suggesting that *ttk* acts downstream of *Notch*.

Discussion

Notch Specifies Distinct Daughter Cell Fates during Multiple Asymmetric Divisions in the Formation of Es and Cho Organs

Notch has been shown to play an important role during the process of singling out of SOPs from proneural clusters, a cell fate decision between neuronal fate and epidermal fate. In this paper, by studying the effect of both reduction of Notch function and overexpression of Notch^{ACT} and Notch^{WT}, we show that Notch serves as a binary switch between two daughter cells during SOP divisions in both the es organ and the cho organ.

Notch appears to be important in specifying sheath cell fate in the IIb cell lineage of es organs and the chIII cell lineage of cho organs. Reduction of *Notch* function transforms sheath cells to neurons, whereas overexpression of Notch^{ACT} results in the opposite cell fate transformation in the embryo. Moreover, *Notch* is also involved in the choice between the hair and the socket cell fates. In adult es organs, reduction of *Notch* function in *Notch*^{ts} mutants results in twinning (two hairs without socket) (data not shown), whereas overexpression of either Notch^{ACT} or Notch^{WT} results in double sockets with-

Table 1. Notch and Numb Interact in the Yeast Two-Hybrid Assay

LexA DNA-Binding Domain Fushion	GAL4-Transcriptional Activation Domain Fusion	β-Galactosidase Activity
1. Notch-A	Numb	+ +
2. Notch-A	Vector alone	-/+
3. Vector alone	Numb	-
4. Lamin	Notch-A	-
5. Lamin	Numb	-
6. Numb	Notch-A	+~+ +
7. Numb	Vector alone	-/+
8. Vector alone	Notch-A	-
9. Notch-A	Su(H)	+ +
10. Vector alone	Su(H)	-
11. Numb	Notch-M	-/+
12. Vector alone	Notch-M	-
13. Notch-P	Numb	+~+ +
14. Notch-P	Vector alone	-
15. Notch-P	Su(H)	-
16. Notch-RAM23	Numb	+ +
17. Notch-RAM23	Vector alone	-/+
18. Notch-RAM23	Su(H)	+ +
19. Numb	Notch-ANK	-/+
20. Vector alone	Notch-ANK	-/+
21. Numb-P	Notch-A	+ +
22. Numb-P	Vector alone	-
23. Numb-P	Notch-M	-
24. Notch-A	Numb-P	+ +
25. Vector alone	Numb-P	-
26. Numb-N	Notch-A	+ +
27. Numb-N	Vector alone	-
28. Numb-PTB	Notch-A	+ +
29. Numb-PTB	Vector alone	-
30. Notch-RAM23	Numb-C	-/+
31. Vector alone	Numb-C	-

Plasmids containing fusion protein with LexA–DNA-binding domain and plasmids containing fusion protein with GAL4 activation domain were cotransformed into L40 yeast strain containing the *lacZ* reporter gene under the control of GAL4 elements. The β-galactosidase activity was monitored semiquantitatively by filter lift assay: double plus, turning blue in 1.5 hr; plus, turning blue in 1.5–6 hr; minus/plus, some or all colonies turning blue in 6–24 hr; minus, remaining white over 24 hr.

out hair, the reverse cell fate transformation. Thus, *Notch* seems to promote the socket cell fate in the IIa lineage and the sheath cell fate in the IIb lineage.

Notch also appears to be involved in the choice between the IIa and IIb cell fates during SOP divisions. Reduction of *Notch* function during formation of the adult es organs results in the formation of four neurons at the expense of three support cells (Hartenstein and Posakony, 1990), whereas four sockets are formed in the extreme cases of overexpression of $Notch^{ACT}$ or $Notch^{WT}$. These phenotypes most likely are produced by first a switch of cell fate between IIa and IIb and then a switch of cell fate between hair and socket or between neuron and sheath. In other words, *Notch* function is required first to specify IIa and then to specify socket (a daughter of IIa) and sheath cell (a daughter of IIb). Reducing or removing *Notch* function results in two IIb cells and their altered divisions leading to the production of four neurons, whereas elevating *Notch* activity leads to two IIa cells, which then divide abnormally and give rise to four sockets. Thus, *Notch* appears to be required for all three asymmetric divisions of the es organ and for at least the final division in the cho organ lineage. The asymmetry is disrupted by either reduction of *Notch* function or *Notch* overexpression, resulting in symmetric divisions.

The Interaction between *Notch* and *numb*

Like *Notch*, *numb* is involved in multiple asymmetric divisions during the formation of a sensory organ (Rhyu et al., 1994). There are several possible scenarios concerning interactions between *numb* and *Notch*. *Notch* could act upstream of *numb*, regulating the asymmetric localization of Numb in the SOP, the segregation of Numb to one of the daughter cells, or Numb activity. Alternatively, Numb, once segregated into one of the daughter cells, could bias subsequent cell–cell interaction by modulating *Notch* signaling. It is also conceivable that *numb* and *Notch* function in parallel to ensure the proper cell fate specification. The possibility that *Notch* acts entirely upstream of *numb* has been ruled out, because a reduction of *Notch* function in double mutants of $Notch^{ts}$ and *numb* null mutation partially suppressed the *numb* mutant phenotype. Since these double mutants eliminate *numb* function and yet some of the embryos show overproduction of neurons as do $Notch^{ts}$ embryos, the action of *Notch* does not appear to require *numb* gene function. Rather, manifestation of the *numb* mutant phenotype depends on *Notch* gene activity, indicating that *numb* is upstream of *Notch* and negatively regulates *Notch*. In this scenario, loss of *numb* function may lead to an increase of *Notch* activity in the daughter cell that normally inherits Numb. In the double mutant

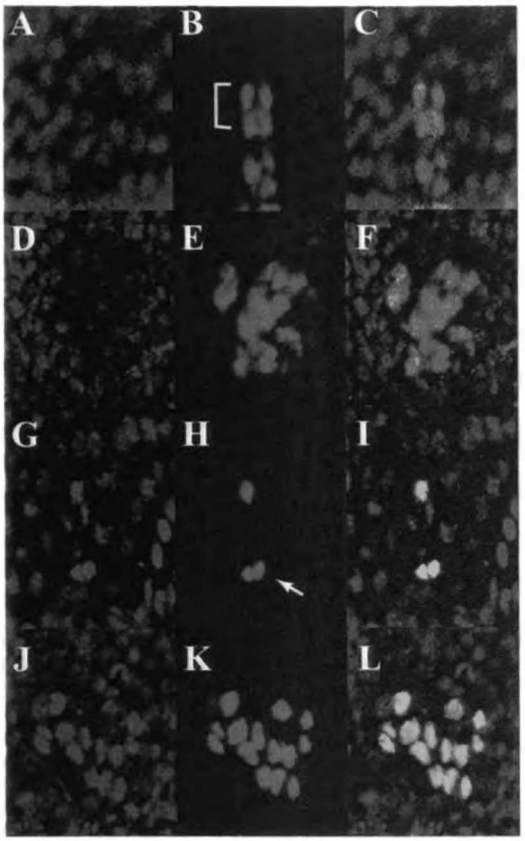

Figure 8. Ttk Expression Is Altered in Opposing Ways in *Notch*^{ts} Mutants and Flies Expressing Notch^{ACT}

(A–C) Double labeling of *Notch*^{ts} embryos using anti-Ttk 88 kDa (A), anti-Elav (B) antibodies, and the overlay of these two images (C) for two dorsal-most es organs. In contrast with the presence of two neurons in wild type, we detected four Elav-expressing neurons (bracketed in [B]). As shown earlier, the two extra neurons arose from a transformation of the sheath cells into neurons. Although Ttk is expressed in most of the cells in the epidermis of the *Notch*^{ts} embryo (A), it is not expressed in any neurons, including the supernumerary neurons derived from transformation of sheath cells (C).

(D–F) The double labeling of *Notch*^{ts} embryos using anti-Ttk 88 kDa (D), anti-Elav (E) antibodies, and the overlay of these two images (F) in five lateral and one v′ cho organs. Similarly, none of the supernumerary neurons (E), including those sheath cells that had transformed into neurons, were recognized by anti-Ttk antibody (D and F).

(G–I) Double labeling of embryos with Notch^{ACT} overexpression using anti-Ttk 88 kDa (G), anti-Prospero (H) antibodies, and the overlay of these two images (I) for the two dorsal-most es organs. Often, one es organ shows transformation. The arrow in (H) points to two sheath cells, including one that is transformed from a neuron. (Top es organ is not transformed.) Ttk is expressed in all sheath cells (G and I), including one that is transformed from a neuron.

(J–L) Double labeling of embryos with Notch^{ACT} overexpression using anti-Ttk 88 kDa (J), anti-Prospero (K) antibodies, and the overlay of these two images (L) for the lateral and v′ cho organs. Similarly, all sheath cells (K), including those that are transformed from neurons, express Ttk (J and L).

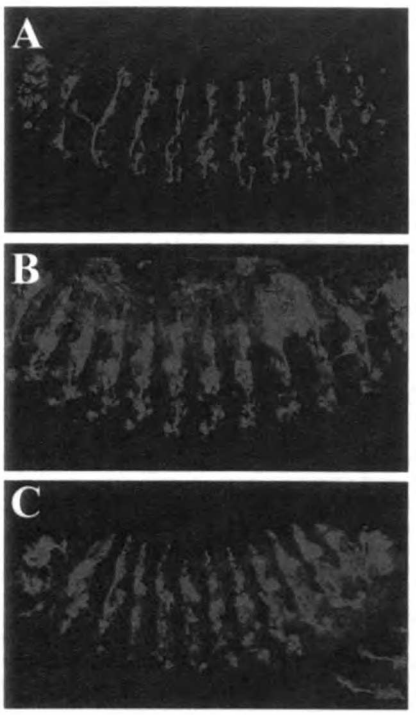

Figure 9. Loss of *ttk* Function Prevents Notch^{ACT} from Transforming Neurons to Sheath Cells

MAb22C10 staining of an embryo expressing Notch^{ACT} (A), a *ttk*^{osn} embryo (B), and a *ttk*^{osn} embryo expressing Notch^{ACT} (C). Compared with the wild type (Figure 6A), there is a reduction in the number of sensory neurons in Notch^{ACT} expressing embryos (A), whereas loss of *ttk* function causes an overproduction of sensory neurons (B). The *ttk*^{osn} embryo that expresses Notch^{ACT} (C) shows the neuronal overproduction phenotype indistinguishable from that due to *ttk* loss of function (B).

that carries the null mutation *numb*^{1} and the hypomorphic allele *Notch*^{ts}, the residual *Notch* activity is likely to be higher than that in the *Notch*^{ts} single mutant. This could account for the milder *Notch* mutant phenotype in the double mutant. Consistent with the possibility that *numb* is upstream of *Notch*, Numb is still asymmetrically localized in *Notch* null alleles. We also detected a very strong synergistic enhancement of the effects due to a partial loss of *Notch* function and a slight elevation of *numb* expression. While these findings suggest that *Notch* acts downstream of *numb*, as proposed previously by Posakony (1994), Rhyu (1994), and Jan and Jan (1995), they do not exclude the possibility of more complex and elaborate schemes of action of *Notch* and *numb*. For example, *numb* could also be involved in an additional pathway parallel to the *Notch* signaling pathway. Models that incorporate our findings are illustrated in Figure 10. Numb, depicted in red, is asymmetrically distributed to one pole of the SOP during SOP division and segregated into one of the daughter cells so as to bias or set up the direction of cell–cell interaction, causing that cell to adopt the cell fate of IIb. This may be achieved in at least two ways, as indicated by the following two models. In model one (left), cell–cell communication occurs between the two daughter cells. Numb may suppress the Notch (in brown) activity directly, possibly via direct protein–protein interactions between Notch and Numb. Numb may also suppress Notch activity indirectly by regulating Delta (in green)

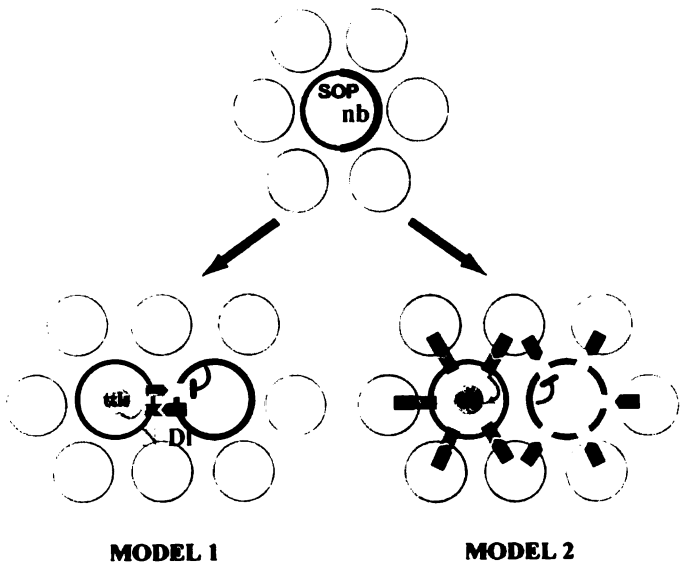

Figure 10. Models for the Interaction among *numb*, *ttk*, and *Notch*

Each circle represents an individual cell. Whereas circles with gray shading represent epidermal cells surrounding the SOP and its daughter cells, circles with yellow shading illustrate the SOP and its daughter cells. The membrane-associated Numb (nb), depicted in red, is segregated into one daughter cell so as to bias or set up the direction of cell–cell interaction. This may be achieved in at least two ways, as indicated by the following two models. In model one (left), cell–cell communication occurs between the two daughter cells. Whereas the Notch activity is illustrated in orange, with the reduced activity shown as the light orange, the Delta activity is demonstrated in green, with the reduced activity in light green. Numb may suppress the Notch activity directly, or indirectly by regulating Delta activity, which renders the Numb-containing daughter cell less effective in receiving signals and more effective in sending signals to its sister cell. In model two (right), the cell–cell signaling mediated by Notch and Delta occurs between SOP daughter cells and the surrounding epidermal cells which express Delta. The suppression of *Notch* by *numb* results in a difference in Notch activity between two daughter cells. These two models are not mutually exclusive. In either model, the Notch activity in the cell without Numb would be sufficiently high to cause Ttk expression (in blue), whereas the Notch activity in the other daughter cell is suppressed by Numb to such an extent that it can no longer induce Ttk expression.

activity. Suppression of Notch activity by Numb renders the Numb-containing daughter cell less effective in receiving signals and more effective in sending signals to its sister cell, so that it adopts the cell fate of IIb and its sister adopts the cell fate of IIa. In model two (right), the cell–cell signaling mediated by Notch and Delta occurs between SOP daughter cells and the surrounding epidermal cells that express Delta. The segregation of Numb into one of the daughter cells causes Notch activity in that cell to be suppressed. The daughter cell that lacks Numb has higher Notch activity and becomes IIa, whereas the daughter cell that has inherited Numb has lower Notch activity, thereby assuming the default cell fate of IIb. These two models are not mutually exclusive. In either model, the Notch activity in the IIa cell would be sufficiently high to cause Ttk expression (in blue), whereas the Notch activity in IIb is suppressed by Numb to such an extent that it can no longer induce Ttk expression.

Our observation of binding of Numb to Notch suggests that Numb may suppress Notch activity through direct protein–protein interaction. One can envision a variety of scenarios as to how this may take place. For instance, the binding of Numb to Notch may prevent Notch from being activated. Alternatively, Numb could inhibit the coupling of activated Notch with its downstream effectors, such as Su(H), or prevent the activation of Su(H) by Notch. Two regions of Notch show interactions with Numb and are highly conserved among Notch homologs in different species. The RAM23 region of Notch physically interacts with Su(H) (Tamura et al., 1995; Hsieh et al., 1996) as well as Numb, whereas Notch-P binds to Numb but not Su(H). Recently, Dishevelled has been shown to interact with Notch C-terminus (Axelrod et al., 1996). It will be interesting to see whether Dishevelled binds to Notch-P, as does Numb.

We have identified the PTB domain of Numb as the Notch-binding domain. PTB domain in other proteins including Shc binds to phosphotyrosine in proteins such as EGF receptor and NGF receptor and is involved in signaling through tyrosine phosphorylation (Kavanaugh and Williams, 1994; Kavanaugh et al., 1995; reviewed by van der Geer and Pawson, 1995; Laminet et al., 1996). This raises the possibility that signaling from Numb to Notch may be regulated by tyrosine phosphorylation. The PTB domain between Drosophila Numb and mouse Numb shares 63.3% amino acid identity (Zhong et al., 1996). We note also that similar protein–protein interaction has been observed between mouse Numb and mouse Notch1 (Zhong et al., 1996), suggesting an evolutionarily conserved mechanism.

ttk Is Regulated by Both *Notch* and *numb*

We have previously demonstrated that *ttk* is involved in multiple asymmetric divisions in both es and cho organs (Guo et al., 1995). Transformation of neurons into sheath cells is caused either by *ttk* overexpression or by $Notch^{ACT}$ overexpression in the presence of normal *ttk* gene function. In the absence of *ttk* function, $Notch^{ACT}$ overexpression fails to transform neurons into sheath cells. This suggests a requirement of *ttk* for the sheath cell fate, though loss of zygotic *ttk* function does not cause sheath cells to be transformed into neurons (Guo et al., 1995), presumably due to the presence of redundant pathways. The dependence of $Notch^{ACT}$ action on *ttk* also indicates that *ttk* is a downstream target of *Notch*. Indeed, whereas Ttk is normally expressed in only one of the IIb daughters, reduction of *Notch* function apparently prevented *ttk* expression in either daughter cell, and overexpression of $Notch^{ACT}$ leads to abnormal *ttk* expression in both daughter cells. Previous studies have shown that *ttk* is negatively regulated by

numb (Guo et al., 1995). This is consistent with the model shown in Figure 10 in which *ttk* is positively regulated by *Notch*, which is in turn negatively regulated by *numb*. We propose that Ttk, as a transcription factor, acts as a readout to integrate signals derived both from a cell-intrinsic determinant and from cell–cell communication.

Besides *ttk*, other downstream targets of *Notch* have been identified, such as *Su(H)* and *Enhancer of split (E(spl))*. Su(H) and Notch exhibit direct protein–protein interaction in the yeast two-hybrid assay (Fortini and Artavanis-Tsakonas, 1994; Tamura et al., 1995; Hsieh et al., 1996). In tissue culture cells, the binding of Delta and Notch allows the translocation of Su(H) from the cytoplasm into the nucleus (Fortini and Artavanis-Tsakonas, 1994). Su(H) then acts as a transcriptional activator to regulate directly *E(spl)* (Lecourtois and Schweiguth, 1995; Bailey and Posakony, 1995), which is a gene complex of seven related genes that encode basic helix-loop-helix proteins characteristic of transcriptional regulators (Delidakis and Artavanis-Tsakonas, 1992; Knust et al., 1992). It is interesting to note that the overexpression of Notch^{ACT} or *Su(H)* results in phenotypes that are slightly different from the phenotype due to the overexpression of *E(spl)*. Whereas overexpression of Notch^{ACT} and *Su(H)* results in predominantly double sockets (Schweisguth and Posakony, 1994), overexpression of the m5 and m8 genes in the *E(spl)* complex results in duplicated bristles, double sockets as well as other aberrant outer support cells (Tata and Hartley, 1995). The phenotype due to overexpression of *ttk* is similar to that of overexpression of *E(spl)* (Guo et al., 1995; Ramaekers et al., submitted). It would be interesting to examine the interaction between *ttk*, *Su(H)*, and *E(spl)* and to investigate how *Notch* signaling eventually leads to the cell fate decision.

Experimental Procedures

Genetics and Drosophila Strains

Drosophila strains were raised on standard cornmeal-yeast agar medium at room temperature or 25°C except those with *Notch^{ts}* (18°C).

For overexpressing the Notch^{ACT} in *ttk* mutant background, males with a genotype of *yw; 1407 GAL4; ttk^{one}/TM6, Ubx* were mated with females with a genotype of *yw; UAS–Notch^{ACT} ttk^{one}/TM3, Sb*. The embryos were collected and stained with markers. All the *ttk* mutant should also have Notch^{ACT} expression driven by *1407 GAL4*. The *ttk* mutant embryos can be unambiguously identified as those embryos that have defects in head involution and dorsal closure (Guo et al., 1995).

For examining the dosage-sensitive interaction between *Notch^{55e11}/+* and *hs-numb*, *w^{a}Notch^{55e11}/FM6* females were crossed to *w* males or *yw, hs-numb* (line 2.4C) males, respectively. Female progeny with *Bar$^+$* eye shape, thus with a genotype of *w^{a}Notch^{55e11}/+* or *w^{a}Notch^{55e11}/+; hs-numb/+*, were collected, and their phenotypes were examined. For examining the phenotype of the *w^{a}Notch^{55e11}/+; hs-numb* flies, *w^{a}Notch^{55e11}/+; hs-numb/CyO* stock was first constructed. *w^{a}Notch^{55e11}/+; hs-numb* flies were unambiguously identified as those with wing notching (due to the haploinsufficiency of the *Notch^{55e11}* allele) and straight wings. All crosses described above were set up in parallel. The same experiment was performed using homozygotes of *nd^{Gne2}*, an independent viable *Notch* allele, and one or two copies of *hs-numb* (line 11.1) located on the third chromosome. Similar synergistic enhancement was observed (see text).

For constructing the double mutants between *numb* and *Notch*, we constructed flies with a genotype of *wNotch^{ts}, numb^1 pr cn Bc/ CyO, P[T8–lacZ]*. Embryos from this stock were collected and double labeled with MAb22C10 and anti-Numb or anti-β-galactosidase antiserum. Double mutants were identified as those embryos that show no anti-Numb epidermal staining nor anti-β-galactosidase staining.

For examining the numb localization, 4.5–6.5 hr embryos of *Notch^{55e11}/FM6* flies were doubly stained using both anti-Asense and anti-Numb antibodies. *Notch* mutant embryos were identified as those embryos that have overproduction of neurons in the CNS.

Temperature Shift of *Notch^{ts}* Embryos

wNotch^{ts} stock was kept at 18°C. The developmental time in 18°C was estimated to be twice the amount of time at 25°C (Giniger et al., 1993). Embryos collected between 8–14 hr at 18°C were put into either a 30°C incubator, or a water bath at 32°C. Embryos were kept at these nonpermissive temperatures for 5 hr. After the temperature shift, embryos were placed in an 18°C incubator for 0.5–4 hr prior to fixation.

Immunofluorescence and Confocal Microscopy

Embryos were fixed and stained as described by Guo et al. (1995). A Zeiss microscope and a Bio-Rad MRC-600 confocal were used to view and obtain the images. All the antibodies used in this study have been described previously: MAb22C10 (Zipursky et al., 1984), rat or rabbit anti-Cut antibody (Blochlinger et al., 1990), anti-Prospero antibody (Vaessin et al., 1991), anti-Asense antibody (Brand et al., 1993), guinea pig anti-Ttk 88 kDa antibodies (Read et al., 1992), anti-Elav (Mab44C11) (Bier et al., 1988), and anti-Numb antibody (Rhyu et al., 1994).

Adult nota and wings were dissected from flies in 80% isopropanol and mounted in Hoyer's solution (Ashburner, 1989).

Yeast Two-Hybrid Interaction Assay

Fragments of the *Notch* cDNA were inserted into either the pBHA vector (obtained from C. T. Chien), pGAD424 vector, or pGADGH vector (obtained from Clontech). Region A includes the area from the SalI site to the PstI site (amino acids 1792–2269), or the area from the beginning of the intracellular domain to the EcoRI site (amino acids 1766–2263). Region M spans the area from the EcoRI site (amino acid 1767) to the SalI site (amino acid 2612). Region P includes the C-terminal fragment from amino acid 2602 to the end of the protein. RAM23 construct contains amino acids 1766–1897, whereas the ANK construct amino acids 1895–2109. The amino acid coordinates shown here are as in Wharton et al. (1985). The *numb* full-length cDNA was inserted into either the pGAD vector or the pBHA vector (generated by C. T. Chien). Numb-P starts from the beginning of the protein to the PstI site (amino acids 1–330). Numb-N spans amino acids 1–223 (the BamHI site), whereas Numb-C spans amino acids 224 to the end of the protein. pBHA Numb-N, pBHA Numb-C, and pBHA Numb-P were obtained from C. T. Chien and S. W. Wang. Full-length Su(H) was cloned into pGAD424 (generated by S. W. Wang).

The yeast transformation was performed according to Bartel et al. (1993). The β-galactosidase enzymatic activity was evaluated by filter-lifting assay. Nitrocellulose filters were used to lift up the colonies, and then frozen in liquid nitrogen. Subsequently, the filters were overlaid on the Whatman paper presoaked with 5–45 μl of X-Gal in 3 ml of Z buffer. Colonies producing β-galactosidase turned blue with time. For each set of experiments, a single colony from the transformants carrying either the two fusion proteins to be tested or proper controls (Figure 7B) was then streaked to the same plate and assayed again to ensure the equivalence of experimental conditions.

GST Fusion Proteins and In Vitro Protein Binding Assays

The whole region of Notch intracellular domain (1766–2703) was fused in frame with GST in GST-4T vector (Pharmacia). For protein expression, overnight bacterial culture was diluted and grown until OD at 260 nm reached 1.0. After adding IPTG, the culture was grown for another 3–7 hr. The bacteria were then harvested and lysed by sonication in PBS in the presence of protease inhibitors (aprotinin, leupeptin and pepstatin). Commassie blue staining showed the production of the protein whose size corresponds to the predicted fusion between GST and Notch intracellular domain.

^{35}S-labeled Numb was synthesized in vitro from pNAC-Numb-N or pNAC-Numb-C (generated by C. P. Shen from the same regions of Numb used in two-hybrid assays) using the TNT-coupled rabbit reticulocyte lysate system (Promega); 10 μl of this lysate containing ^{35}S-labeled Numb-N or Numb-C was mixed with GST fusion proteins

immobilized on the glutathione–Sepharose 4B beads (Pharmacia), and the mixture was incubated at room temperature for 1–2 hr. The beads were then washed with cold 0.05% Triton X-100 in PBS and collected by centrifugation. To release the bound protein, the beads were boiled in gel sample buffer and analyzed by SDS–polyacrylamide gel electrophoresis (SDS–PAGE) followed by autoradiography.

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References

Artavanis-Tsakonas, S., and Simpson, P. (1991). Choosing a cell fate: a view from the Notch locus. Trends Genet. *7*, 403–408.

Artavanis-Tsakonas, S., Matsuno, K., and Fortini, M.E. (1995). Notch signaling. Science *268*, 225–232.

Ashburner, M. (1982). The genetics of a small autosomal region of *Drosophila melanogaster* containing the structural gene for alcohol dehydrogenase III: hypomorphic mutations affecting the expression of *Hairless*. Genetics *101*, 447–459.

Ashburner, M. (1989). *Drosophila*: A Laboratory Handbook (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press).

Axelrod, J.D., Matsuno, K., Artavanis-Tsakonas, S., and Perrimon, N. (1996). Interaction between wingless and Notch signaling pathways mediated by Dishevelled. Science *271*, 1826–1832.

Bailey, A., and Posakony, J. (1995). Suppressor of Hairless directly activates transcription of *Enhancer of split* complex genes in response to Notch receptor activity. Genes Dev. *9*, 2609–2622.

Bang, A., Bailey, A., and Posakony, J. (1995). Hairless promotes stable commitment to the sensory organ precursor cell fate by negatively regulates the activity of the *Notch* signaling pathway. Dev. Biol. *172*, 479–494.

Bartel, P., Chien, C.T., Sternglanz, R., and Fields, S. (1993). Using the two-hybrid system to detect protein–protein interactions. In Cellular Interactions in Development: A Practical Approach, D.A. Hartley, ed. (Oxford: Oxford University Press), pp. 153–179.

Bate, C.M. (1978). Development of sensory systems in arthropods. In Handbook of Sensory Physiology, M. Jacobson, ed. (New York: Springer-Verlag), pp. 1–53.

Bier, E., Ackerman, L., Barbel, S., Jan, L.Y., and Jan, Y.N. (1988). Identification and characterization of a neuron-specific nuclear antigen in *Drosophila*. Science *240*, 913–916.

Bierkamp, C., and Campos-Ortega, J.A. (1993). A zebrafish homologue of the *Drosophila* neurogenic gene Notch and its pattern of transcription during early embryogenesis. Mech. Dev. *43*, 87–100.

Blochlinger, K., Bodmer, R., Jan, L.Y., and Jan, Y.N. (1990). Patterns of expression of Cut, a protein required for external sensory organ development in wild-type and *cut* mutant *Drosophila* embryos. Genes Dev. *4*, 1322–1331.

Bodmer, R., and Jan, Y.N. (1987). Morphological differentiation of the embryonic peripheral neurons in *Drosophila*. Roux's Arch. Dev. Biol. *196*, 69–77.

Bodmer, R., Carretto, R., and Jan, Y.N. (1989). Neurogenesis of the peripheral nervous system in Drosophila melanogaster embryos: DNA replication patterns and cell lineages. Neuron *3*, 21–32.

Bork, P., and Margolis, B. (1995). A phosphotyrosine interaction domain. Cell *80*, 693–694.

Brand, A.H., and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development *118*, 401–415.

Brand, M., Jarman, A.P., Jan, L.Y., and Jan, Y.N. (1993). *asense* is a *Drosophila* neural precursor gene and is capable of initiating sense organ formation. Development *119*, 1–17.

Brewster, R., and Bodmer, R. (1995). Origin and specification of type II sensory neurons in *Drosophila*. Development *121*, 2923–2936.

Brown, J.L., Sonoda, S., Ueda, H., Scott, M., and Wu, C. (1991). Repression of the *Drosophila fushi tarazu* (*ftz*) segmentation gene. EMBO J. *10*, 665–674.

Cagan, R.L., and Ready, D.F. (1989). *Notch* is required for successive cell decisions in the developing *Drosophila* retina. Genes Dev. *3*, 1099–1112.

Campos-Ortega, J.A. (1988). Cellular interactions during early neurogenesis in *Drosophila melanogaster*. Trends Neurosci. *11*, 400–405.

Campos-Ortega, J.A., and Hartenstein, V. (1985). The Embryonic Development of *Drosophila melanogaster* (New York: Springer-Verlag).

Campuzano, S., and Modolell, J. (1992). Patterning of the *Drosophila* nervous system: the *achaete–scute* gene complex. Trends Genet. *8*, 202–208.

Corbin, V., Michelson, A.M., Abmayr, S.M., Neel, V., Alcamo, E., Maniatis, T., and Young, M.W. (1991). A role for the Drosophila neurogenic genes in mesoderm differentiation. Cell *67*, 311–323.

Delidakis, C., and Artavanis-Tsakonas, S. (1992). The *Enhancer of split* [*E(spl)*] locus of *Drosophila* encodes seven independent helix-loop-helix proteins. Proc. Natl. Acad. Sci. USA *89*, 8731–8735.

Diederich, R.J., Matsuno, K., Hing, H., and Artavanis-Tsakonas, S. (1994). Cytosolic interaction between deltex and Notch ankyrin repeats implicates deltex in the Notch signaling pathway. Development *120*, 473–481.

Doherty, D., Feger, G., Younger-Shepherd, S., Jan, L.Y., and Jan, Y.N. (1996). Delta is a ventral to dorsal signal complementary to Serrate, another Notch ligand, in *Drosophila* wing formation. Genes Dev. *10*, 421–434.

Fortini, M.E., and Artavanis-Tsakonas, S. (1994). The Suppressor of Hairless protein participates in Notch receptor signaling. Cell *79*, 273–282.

Fortini, M.E., Rebay, I., Caron, L.A., and Artavanis-Tsakonas, S. (1993). An activated *Notch* receptor blocks cell fate commitment in the developing *Drosophila* eye. Nature *365*, 555–557.

Ghysen, A., and Dambly-Chaudiere, C. (1988). From DNA to form: the *achaete–scute* complex. Genes Dev. *7*, 723–733.

Ghysen, A., Dambly-Chaudiere, C., Aceves, E., Jan, L.Y., and Jan, Y.N. (1986). Sensory neurons and peripheral pathways in *Drosophila* embryos. Roux's Arch. Dev. Biol. *195*, 281–289.

Ghysen, A., Dambly-Chaudiere, C., Jan, L.Y., and Jan, Y.N. (1993). Cell interactions and gene interactions in peripheral neurogenesis. Genes Dev. *7*, 723–733.

Giniger, E., Jan, L.Y., and Jan, Y.N. (1993). Specifying the path of the intersegmental nerve of the *Drosophila* embryo: a role for *Delta* and *Notch*. Development *117*, 431–440.

Guo, M., Bier, E., Jan, L.Y., and Jan, Y.N. (1995). *tramtrack* acts downstream of *numb* to specify distinct daughter cell fates during asymmetric cell divisions in the Drosophila PNS. Neuron *14*, 913–925.

Harrison, S., and Travers, A. (1990). The *tramtrack* gene encodes a *Drosophila* finger protein that interacts with the *ftz* transcriptional regulatory region and shows a novel embryonic expression pattern. EMBO J. *9*, 207–216.

Hartenstein, V., and Posakony, J.W. (1989). Development of adult sensilla on the wing and notum of *Drosophila melanogaster*. Development *107*, 389–405.

Hartenstein, V., and Posakony, J.W. (1990). A dual function of the *Notch* gene in *Drosophila* sensillum development. Dev. Biol. *142*, 13–30.

Heitzler, P., and Simpson, P. (1991). The choice of cell fate in the epidermis of Drosophila. Cell *64*, 1083–1092.

Horvitz, H.R., and Herskowitz, I. (1992). Mechanisms of asymmetric cell division: two Bs or not two Bs, that is the question. Cell *68*, 237–255.

Hsieh, J.J.-D., Henkel, T., Salmon, P., Robey, E., Peterson, M.G., and Hayward, S.D. (1996). Truncated mammalian Notch1 activates CBF1/RBPJk-repressed genes by a mechanism resembling that of Epstein–Barr virus EBNA2. Mol. Cell. Biol. *16*, 952–959.

Jan, Y.N., and Jan L. Y. (1995). Maggot's hair and bug's eye: role of cell interactions and intrinsic factors in cell fate specification. Neuron *14*, 1–5.

Kavanaugh, W.M., and Williams, L.T. (1994). An alternative to SH2 domains for binding tyrosine-phosphorylated proteins. Science *266*, 1862–1865.

Kavanaugh, W.M., Turck, C.W., and Williams, L.T. (1995). PTB domain binding to signaling proteins through a sequence motif containing phosphotyrosine. Science *268* , 1177–1179.

Kidd, S., Kelley, M.R., and Young, M.W. (1986). Sequence of the *Notch* locus of *Drosophila melanogaster*: relationship of the encoded protein to mammalian clotting and growth factors. Mol. Cell. Biol. *6*, 3094–3108.

Knoblich, J., Jan, L.Y., and Jan, Y.N. (1995). Localization of Numb and Prospero via a novel mechanism for asymmetric protein segregation. Nature *377*, 624–627.

Knust, E., Schrons, H., Grawe, F., and Campos-Ortega, J.A. (1992). Seven genes of the *Enhancer of split* complex of *Drosophila melanogaster* encode helix-loop-helix proteins. Genetics *132*, 505–518.

Kopczynski, C.C., Alton, A.K., Fechtel, K., Kooh, P.J., and Muskavitch, M.A.T. (1988). *Delta*, a *Drosophila* neurogenic gene, is transcriptionally complex and encodes a protein related to blood coagulation factors and epidermal growth factor of vertebrates. Genes Dev. *2*, 1723–1735.

Laminet, A.A., Apell, G., Conroy, L., and Kavanaugh, W.M. (1996). Affinity, specificity, and kinetics of the interaction of the SHC phosphotyrosine binding domain with asparagine-X-X-phosphotyrosine motifs of growth factor receptors. J. Biol. Chem. *271*, 264–269.

Lecourtois, M., and Schweiguth, F. (1995). The neurogenic Suppressor of Hairless DNA-binding protein mediates the transcriptional activation of the *Enhancer of split* complex genes triggered by Notch signaling. Genes Dev. *9*, 2598–2608.

Lehmann, R., Jimenez, F., Dietrich, U., and Campos-Ortega, J.A. (1983). On the phenotype and development of mutants of early neurogenesis in *Drosophila melanogaster*. Roux's Arch. Dev. Biol. *192*, 62–74.

Lieber, T., Kidd, S., Alcamo, E., Corbin, V., and Young, M.W. (1993). Antineurogenic phenotypes induced by truncated Notch proteins indicate a role in signal transduction and may point to a novel function for Notch in nuclei. Genes Dev. *7*, 1949–1965.

Luo, L., Liao, Y.J., Jan, L.Y., and Jan, Y.N. (1994). Distinct morphogenetic functions of similar small GTPases: *Drosophila Drac1* is involved in axonal outgrowth and myoblast fusion. Genes Dev. *8*, 1787–1802.

Lyman, D. and Yedvobnick, B. (1995). Drosophila *Notch* receptor activity suppresses Hairless function during adult external sensory organ development. Genetics *141*, 1491–1505.

Matsuno, K., Diederich, R.J., Go, M.J., Blaumueller, C.M., and Artavanis-Tsakonas, S. (1995). Deltex acts as a positive regulator of Notch signaling through interactions with the Notch ankyrin repeats. Development *121*, 2633–2644.

Parks, A., and Muskavitch, M.A.T. (1993). *Delta* function is required for bristle organ determination and morphogenesis in *Drosophila*. Dev. Biol. *157*, 484–496.

Posakony, J.W. (1994). Nature versus nurture: asymmetric cell divisions in Drosophila bristle development. Cell *76*, 415–418.

Read, D., and Manley, J. (1992). Alternatively spliced transcripts of the *Drosophila tramtrack* gene encode zinc finger proteins with distinct DNA binding specificities. EMBO J. *11*, 1035–1044.

Read, D., Levine, M., and Manley, J. (1992). Ectopic expression of the *Drosophila tramtrack* gene results in multiple embryonic defects, including repression of *even-skipped* and *fushi tarazu*. Mech. Dev. *38*, 183–196.

Rebay, I., Fleming, R.J., Fehon, R.G., Cherbas, L., Cherbas, P., and Artavanis-Tsakonas, S. (1991). Specific EGF repeats of Notch mediate interactions with Delta and Serrate: implications for Notch as a multifunctional receptor. Cell *67*, 687–699.

Rebay, I., Fehon, R.G., and Artavanis-Tsakonas, S. (1993). Specific truncations of Drosophila Notch define dominant activated and dominant negative forms of the receptor. Cell *74*, 319–329.

Rhyu, M. (1994). How sister cells acquire different fates: the role of *numb* in asymmetric divisions. PhD thesis, University of California, San Francisco, California.

Rhyu, M., Jan, L., and Jan, Y.N. (1994). Asymmetric distribution of numb protein during division of the sensory organ precursor confers distinct fates to daughter cells. Cell *76*, 477–491.

Ruohola, H., Bremer, K.A., Baker, D., Swedlow, J.R., Jan, L.Y., and Jan, Y.N. (1991). Role of neurogenic genes in establishment of follicle cell fate and oocyte polarity during oogenesis in Drosophila. Cell *66*, 433–449.

Schweisguth, F., and Posakony, J.W. (1994). Antagonistic activities of *Suppressor-of-Hairless* and *Hairless* control alternative cell fates in the *Drosophila* adult epidermis. Development *120*, 1433–1441.

Spana, E., Kopczynski, C., Goodman, C., and Doe, C. (1995). Asymmetric localization of numb autonomously determines sibling neuron identity in the *Drosophila* CNS. Development *121*, 3187–3195.

Struhl, G., Fitzgerald, K., and Greenwald, I. (1993). Intrinsic activity of the *lin-12* and *Notch* intracellular domains in vivo. Cell *74*, 331–345.

Tamura, K., Taniguchi, Y., Minoguchi, S., Saka, T., Tun, T., Furukawa, T., and Honjo, T. (1995). Physical interaction between a novel domain of the receptor Notch and the transcription factor RBP-Jk/ Su(H). Curr. Biol. *5*, 1416–1423.

Tata, F., and Hartley, D.A. (1995). Inhibition of cell fate in Drosophila by *Enhancer of split* genes. Mech. Dev. *51*, 305–315.

Uemura, T., Shepherd, S., Ackerman, L., Jan, L.Y., and Jan, Y.N. (1989). *numb*, a gene required in determination of cell fate during sensory organ formation in Drosophila embryos. Cell *58*, 349–360.

Wharton, K.A., Johansen, K.M., Xu, T., and Artavanis-Tsakonas, S. (1985). Nucleotide sequence from the neurogenic locus *Notch* implies a gene product that shares homology with proteins containing EGF-like repeats. Cell *43*, 567–581.

Vaessin, H., Bremer, K., Knust, E., and Campos-Ortega, J.A. (1987). The neurogenic gene *Delta* of *Drosophila melanogaster* is expressed in neurogenic territories and encodes a putative transmembrane protein with EGF-like repeats. EMBO J. *6*, 3431–3440.

Vaessin, H., Grell, E., Wolff, E., Bier, E., Jan, L.Y., and Jan., Y.N. (1991). *prospero* is expressed in neuronal precursors and encodes a nuclear protein that is involved in the control of axonal outgrowth in Drosophila. Cell *67*, 941–953.

van der Geer, P., and Pawson, T. (1995). The PTB domain: a new protein module implicated in signal transduction. Trends Biochem. Sci. *20*, 277–280.

Weinmaster, G., Roberts, V.J., and Lemke, G. (1991). A homolog of *Drosophila Notch* expressed during mammalian development. Development *113*, 199–205.

Weinmaster, G., Roberts, V.J., and Lemke, G. (1992). Notch2: a second mammalian Notch gene. Development *116*, 931–941.

Xu, T., Rebay, I., Fleming, R.J., Scottgale, T.N., and Artavanis-Tsakonas, S. (1990). The *Notch* locus and the genetic circuitry involved in early *Drosophila* neurogenesis. Genes Dev. *4*, 464–475.

Xu, T., Caron, L.A., Fehon, R.G., and Artavanis-Tsakonas, S. (1992).

The involvement of the *Notch* locus in *Drosophila* oogenesis. Development *115*, 913–922.

Zhong, W.M., Feder, J.N., Jiang, M.M., Jan, L.Y., and Jan, Y.N. (1996). Asymmetric localization of a mammalian Numb homolog during mouse cortical neurogenesis. Neuron *17*, this issue.

Zipursky, S.L., Venkatesh, T.R., Teplow, D.B., and Benzer, S. (1984). Neuronal development in the *Drosophila* retina: monoclonal antibodies as molecular probes. Cell *36*, 15–26.

CHAPTER 4

ttk suppresses neural fate and may be negatively regulated by *sina* and *phyl*

INTRODUCTION

ttk is essential for the specification of SOP daughter cell fates, as well as of the neuron and sheath cell fate. *ttk* is a target of *N* signaling, and *N* in turn is suppressed by *numb*. How does *ttk* exert its function to specify cell fate? I approach this question by investigating the effect of ectopic expression of *ttk* on neural development and by examining the requirement of *ttk* in the hair/socket decision. I also identify genetic and biochemical interactions between *ttk* and *phyl*, and between *ttk* and *sina*. The results suggest that *ttk* acts to suppress neural fates and is most likely negatively regulated by *sina* and *phyl*.

MATERIALS AND METHODS

Genetics and *Drosophila* strains

Drosophila strains were raised on standard cornmeal-yeast agar medium at room temperature or 25°C.

Strains used for *ttk* overexpression, *hs-ttk69KD* and *UAS-ttk69KD*, were obtained from D. Read (Read et al., 1992) and C. Klambt, respectively. To overexpress *ttk* in the eye, *w; UAS-ttk*$^{3-1}$ flies were mated to *Cyo/+; sev-GAL4* flies. Both *Cyo* and non-*Cyo* progeny have a phenotype of small eyes. Homozygous flies for a *sina* null allele were identified as non-*Tb* in the stock of *sina*^{CC1} */TM6b Tb* (Carthew and Rubin, 1990). A *phyl* null allele, *phyl2245*, was obtained from the lab of G. Rubin (Chang et al., 1995). The *phyl4* allele was from E. Hafen (Dickson et al., 1995).

To examine the dosage-sensitive interaction between *ttk* and *sina*, the recombinant chromosomes carrying *sinaCC1 ttkosn*, or *sinaCC1 ttkB1* were constructed. *sina*^{CC1} *ttk*^{osn} */TM3 Sb* flies were mated to *sina*^{CC1}*/TM3 Sb* flies, and the *sina*^{CC1} *ttk*^{osn}*/sina*^{CC1} progeny were identified as non-*Sb* flies. Similarly, *sina*^{CC1} *ttk*^{osn} */TM3 Sb* flies were mated to *sina*^{CC1} *ttk*^{B1}*/TM3 Sb* flies, and the *sina*^{CC1} *ttk*^{osn}*/sinaCC1 ttkB1* progeny were identified as non-*Sb* flies.

To examine the dosage-sensitive interaction between *ttk* and *phyl*, flies with a genotype of *yw, phyl*^{2245}*/Cyo, ttk*^{osn}*/+* were mated to flies with a genotype of *yw, phyl2245/Cyo, TM6 Ubx p[y+]/+*. All progeny with straight wings (non-*Cyo*) have the red eye color from the *ttk*^{osn} (a P element mutation). Thus only in the absence of one copy of *ttk*, do *phyl*^{2245} flies survive. Control experiments with *phyl*^{2245}*/Cyo* were set up in parallel and no non-*CyO* flies were observed. Thus the reduction of one copy of *ttk* results in a rescue of lethality of *phyl*^{2245}.

Yeast two-hybrid interaction assay

The yeast interaction assay was performed according to Bartel et al. (1993). The *ttk69KD* cDNA (from D. Read) was inserted into the pGAD vector as a fusion with the transcriptional activation domain. The *phyl* cDNA (from H. Chang) was inserted into the pBHA vector as a fusion with the Lex-A DNA binding domain.

For yeast transformation, yeast cells were collected and resuspended in 1xTE/LiAC, and treated with the plasmid DNAs and carrier DNA. After adding 40% PEG solution in 1 x TE and 1 x LiAC, the cells were incubated at 30^{O} C for 30 minutes. Afterwards, 10% dimethyl sulfoxide was added and cells were heat-shocked for 15 minutes in a 42^{O} C water bath. The cells were then spun down, resuspended, and plated on yeast minimal plates, which were then incubated for 2-3 days at 30^{O} C.

The ß-galactosidase enzymatic activity was evaluated by a filter-lift assay. Nitrocellulose filters were used to lift the colonies, which were then frozen in liquid nitrogen. Subsequently, the filters were placed at 30^{O}C on Whatman paper presoaked with 45µl of X-gal in 3ml Z buffer (Na_2HPO_4, NaH_2PO_4, KCl and $MgSO_4$). Colonies producing ß-galactosidase turned blue with time.

Mosaic analysis

For late mosaics, females carrying *yw p[hs-FLP]; p[FRT] y*$^{+}$ were crossed to males carrying *yw; [FRT] ttk*^{osn}*/ TM3 Sb*. The progeny of this cross were heat-shocked (to induce the expression of *FLP*) at stages from late third instar larval to early pupae. The heat shock regime is incubation at 39^{O}C for 45 minutes twice, with an hour at room temperature in between. In this genetic scheme, any bristles in the *ttk* mutant patches would be marked by the *yellow* mutation. Instead, missing bristle and empty socket phenotypes were observed. As a control, females carrying *yw p[hs-FLP]; p[FRT] y*$^{+}$ were crossed to males carrying *yw;p[FRT] neu/ TM3 Sb*. In this case, yellow twin bristles at the expense of socket were observed.

To eliminate *ttk* function in single es organs, *yw; 109(68)GAL4 p[UAS-FLP]; p[FRT] y^+/ TM3 Sb* flies (constructed by C.Y. Zeng) were crossed to flies that were *yw; p[FRT] ttk^{osn}/ TM3 Sb*. *109(68)GAL4* expresses GAL4 in all four cells of an adult es organ (Chapter 3). Non-*Sb* progeny were collected and their phenotype examined.

Heat-shock treatment

Embryos were collected, aged for 4-7 hours at 25°C, heat-shocked in a 39°C water bath for fifteen minutes, then aged to stage 16-17 to be fixed for subsequent immunocytochemistry. For heat-shock of pupae, white pupae were collected, aged to 6-8 hours at 25°C, and then heat-shocked at 39°C for three to five minutes. Overexpression of *ttk 69KD* is associated with a high rate of pupal lethality. Wildtype flies were heat-shocked in parallel as controls.

Immunocytochemistry

Immunocytochemistry on embryos and larvae was performed as described in Chapters 2 and 3.

RESULTS

Ectopic expression of *ttk* suppresses neural fate.

I examined the effect of *ttk* overexpression on neural development. Ubiquitous expression of *ttk* before the formation of SOPs results in a significant reduction in the number of bristles (Figure 4.1B) compared with the wildtype (Figure 4.1A). No SOPs were detected using anti-Asense as a marker (data not shown). Similarly, when *ttk* was overexpressed before the formation of SOPs in the embryo, a significant reduction in the number of neurons in both the CNS and the PNS was observed (data not shown). *ttk* overexpression driven by sev-GAL4, which is expressed in a subset of photoreceptors, caused a significant reduction in eye size (Figure 4.1D) compared with the wildtype (Figure 4.1C). Anti-Elav staining revealed a reduction in the number of neurons (data not shown). Thus *ttk* overexpression suppresses the fates of neuron and/or neuronal precursor.

The requirement of *ttk* in specifying hair and socket cell fates

Eliminating *ttk* function caused cell fate transformation from the IIa cell to the IIb cell, thus precluding our ability to identify a *ttk* function in the hair/socket decision. To overcome this difficulty, FLP-mediated mitotic recombination was used to induce homozygous *ttk* cells at specific developmental stages. By inducing FLP expression specifically in the SOP and its progeny, I removed *ttk* function within a single es organ (see materials and methods). If *ttk* acts in the hair/socket decision, removing *ttk* function after the first SOP division would be expected to cause a cell fate transformation from the socket to the hair cell. As shown in Figure 4.2E, both missing bristle and empty socket phenotypes were observed. A phenotype of double hairs without sockets was not observed. As an alternative approach, I attempted to remove *ttk* function in the lineage by inducing FLP expression ubiquitously around the time

of SOP divisions. Again, loss of *ttk* function resulted in phenotypes of missing bristles and empty sockets, but not that of twin hairs without sockets (data not shown). A parallel control experiment, in which *neuralized* (*neu*) function was removed, on the other hand, revealed twinning hairs without sockets, indicative of cell fate transformation from the socket to the hair cell (data not shown; S. Younger, personal communication). Thus, *ttk* may not function in specifying the hair and socket cell fates.

To further assess the requirement of *ttk* in the hair and socket decision, I examined the phenotype of hypomorphic allelic combinations of *ttk*. B1-3-51 (ttk^{B1}) and E7-3-3 (ttk^{E7}) are two enhancer trap lines in the *ttk* region (Bier, 1988) that are fully viable with no discernible phenotypes. Flies with allelic combinations of ttk^{osn}/ttk^{B1}, ttk^{osn}/ttk^{E7}, $Df(3R)Kpn^{Rev7}/ttk^{B1}$, and $Df(3R)Kpn^{Rev7}/ttk^{E7}$ all have reduced viability and a variable degree of bristle defects. As shown in Figure 4.2A and 4.2B, these flies display empty socket and missing bristle phenotypes. Anti-MAb22C10 staining reveals two neurons associated with the single socket (Figure 4.2D). Preliminary results of double staining with anti-Pros and anti-Elav showed that at least in some cases two neurons were associated with one sheath cell (data not shown). Thus, it is possible that reduction of *ttk* function results in a partial cell fate transformation from the IIa to the IIb cell. Again, I failed to observe the socket to hair transformation in these flies. Taking these results together, we conclude that *ttk* may not be required for hair and socket fate specification.

The adult phenotypes observed with *ttk* viable allelic combinations are sensitive to genetic modification. Reducing one copy of *numb*, the gene antagonizing *ttk* function, suppressed this phenotype, whereas reducing one copy of *N*, the gene activating *ttk*, enhanced it (data not shown). Unfortunately, the variability of this phenotype prevented us from conducting an enhancer or

suppressor screen in this genetic background (S. Younger and S. Ralls, personal communications).

Interaction of *ttk*, *phyl*, and *sina*

Removal of *sina* function results in a significantly reduced viability. The few escapers that survive to adulthood showed missing bristle and aberrant hair and socket phenotypes. These phenotypes were observed in bristles on the notum (Figure 4.3A), in the anterior wing margin, and in the abdomen (Figure 4.3D). Anti-MAb22C10 staining showed no neurons associated with the aberrant bristles, and there were no neurons underneath the bald cuticle (Figure 4.3B and 4.3C). Similarly, flies with a combination of *phyl* alleles had missing and aberrant bristle phenotypes (Figure 4.4A, 4.4C and 4.4D). No neurons were detected underneath the cuticle (Chang et al., 1995; Dickson et al., 1995). Further experiments examining the number of SOPs in flies with loss of *sina* or *phyl* function should reveal if *sina* or *phyl* functions in SOP selection, SOP differentiation, or both.

Since loss of *sina* or *phyl* function showed a phenotype similar to that due to *ttk* overexpression, I asked if I could identify genetic interaction among these genes. Both the lethality and the bristle phenotype due to lack of *sina* or *phyl* function were rescued by a simultaneous reduction of *ttk* function. In the absence of *sina* function, a simultaneous reduction of one copy of *ttk* increased the number of bristles and reduced the number of aberrant bristles. This rescuing effect is most striking in bristles in the abdomen (Figure 4.3E). Further reduction of *ttk* function using ttk^{osn}/ttk^{B1} in the absence of *sina* resulted in more bristles reappearing. Moreover, the bristle phenotypes of ttk^{osn}/ttk^{B1} were also rescued by the simultaneous removal of *sina* function. Similarly, a reduction of *ttk* function rescued the lethality of a *phyl* null allele. Thus, there is a sensitive dosage-dependent genetic interaction between *ttk* and *sina*, as well as between *ttk*

and *phyl*. Since reduction of *ttk* function rescues *sina* and *phyl* null phenotypes, *ttk* most likely acts downstream of *sina* or *phyl*, though the possibility that *sina* and/or *phyl* might act in parallel cannot be excluded.

The strong genetic interaction between *ttk* and *phyl* suggested that they may bind each other directly. Using the yeast two-hybrid assay, I indeed detected an interaction between Ttk and Phyl (Table 4.1). Furthermore, an interaction between Ttk was also detected, which could be mediated by the BTB/POZ domain shown to be involved in protein dimerization (Bardwell and Treisman, 1994; Chen et al., 1995).

DISCUSSION

Ectopic expression of *ttk* suppresses neural fate.

Overexpression of *ttk* results in a significant reduction in the number of SOPs and neurons in the PNS, the CNS, and in the eye. Loss of *ttk* in the embryo causes epidermal and muscle cells to express a low level of the neuron-specific antigen recognized by MAb22C10 (data not shown). Thus *ttk* may function to suppress the neural fate. The expression pattern of Ttk seems to be consistent with this hypothesis. Though Ttk is expressed in many tissues including cone cells in ommatidia (Lai et al., 1996), epidermal cells, midline glia, trachea, muscle, and follicle cells, it is not expressed in any neurons including those in the CNS, PNS and photoreceptors (Chapter 2 and unpublished results). Detailed studies of Ttk expression in both the es and cho lineage (Chapter 2 and Ramaekers, submitted) suggest that Ttk is expressed in non-neuronal cells or in precursors that will give rise to non-neuronal cells; it is not expressed in neurons, nor in neuronal precursors.

***ttk* most likely is negatively regulated by *phyl* and *sina*.**

Loss of *phyl* or *sina* function results in a bristle phenotype reminiscent of that due to *ttk* overexpression (Figure 1B, Chapter 2). I have also demonstrated a very sensitive dosage-dependent genetic interaction between *ttk* and *sina*, as well as between *ttk* and *phyl*. Furthermore, a protein-protein interaction was detected between Ttk and Phyl using the yeast two-hybrid assay. These results raise the possibility that *phyl* may negatively regulates *ttk* at the protein level. Recently, Sina and Phyl have been shown to physically bind to each other (Kauffmann et al., 1996). Future experiments should include the following. Does Ttk bind Sina? What is the expression pattern of Sina and Phyl in the PNS? Does this expression pattern change in *ttk* mutant backgrounds? Does *phyl* regulate *ttk* at the protein level, and if so, does this process depend on *sina*?

Table 1. Ttk and Phyl Interact in the Yeast Two-hybrid Assay.

LexA DNA-binding Domain Fusion	GAL4-Transcriptional Activation Domain Fusion	ß-galactosidase Activity
1. Ttk	Ttk	++
2. Ttk	vector alone	-
3. vector alone	Ttk	-
4. Phyl	Ttk	++++
5. Phyl	vector alone	-

Plasmids containing a fusion protein with the LexA-DNA binding domain and plasmids containing a fusion protein with the GAL4 activation domain were cotransformed into the L40 yeast strain containing the lacZ reporter gene under the control of GAL4 elements. The ß-galactosidase activity was monitored semi-quantitatively by a filter lift assay: ++++, turns blue in 20 minutes; ++, turns blue in 1 hour; -, remains white over 24 hours.

Figure 4.1. Ectopic expression of *ttk* causes a reduction in the number of neurons.

(A) The notum of a wildtype fly.

(B) Overexpression of *ttk 69KD* between 6-8 hour after puparium formation (APF) results in a reduction in the number of bristles.

(C) The eye of a wildtype fly.

(D) The eye of a fly with a genotype of *Sev-GAL4/+; UAS-ttk 69KD/+*. Overexpression of *ttk 69KD* results in a small-eye phenotype, which is caused by a reduction in the number of photoreceptors (data not shown).

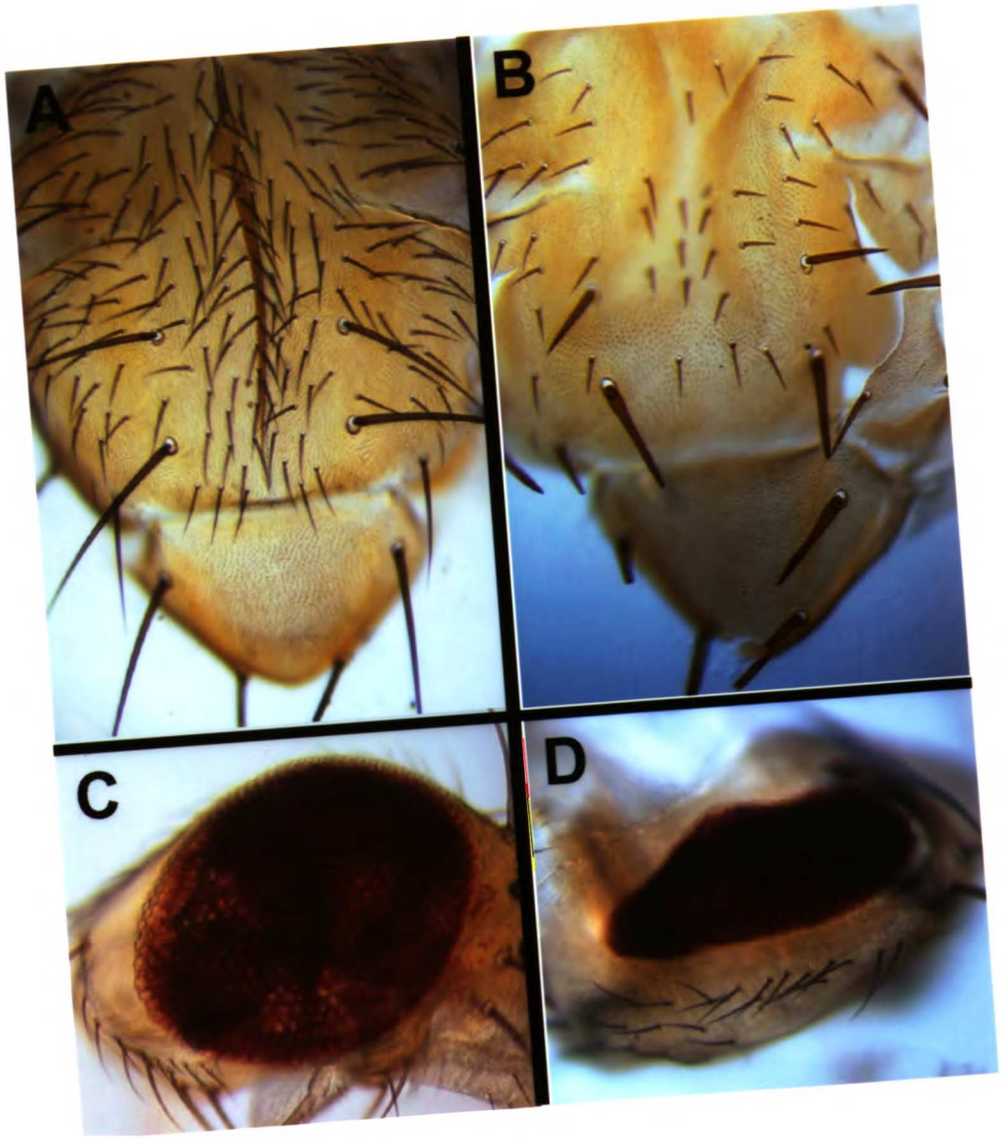


Figure 4.1

Figure 4.2. Loss of *ttk* function causes bristle loss and empty socket phenotypes.

(A) The notum of a fly with a genotype of ttk^{osn}/ttk^{B1}. There are missing bristles (x marks missing dorso-central macrochaetes) and empty sockets (arrowhead marks a dorso-central bristle).

(B) The abdomen of a fly with a genotype of $Df(3R)Kpn^{Rev7}/ttk^{E7}$. Similar bristle phenotypes were observed.

(C and D) MAb22C10 staining of neurons underneath the cuticle. A wildtype bristle is innervated by a single neuron. A bristle with a hair and an aberrant socket is also innervated by a single neuron (C). In contrast, the empty socket is innervated by two neurons (D).

(E) The notum of a fly with a genotype of *yw; 109(68)GAL4 p[UAS-FLP]/+; p[FRT]* y^+*/ p[FRT]* ttk^{osn}. It shows missing bristles (some of them marked by x) and empty sockets (arrowhead). No hair twinning at the expense of sockets was observed.

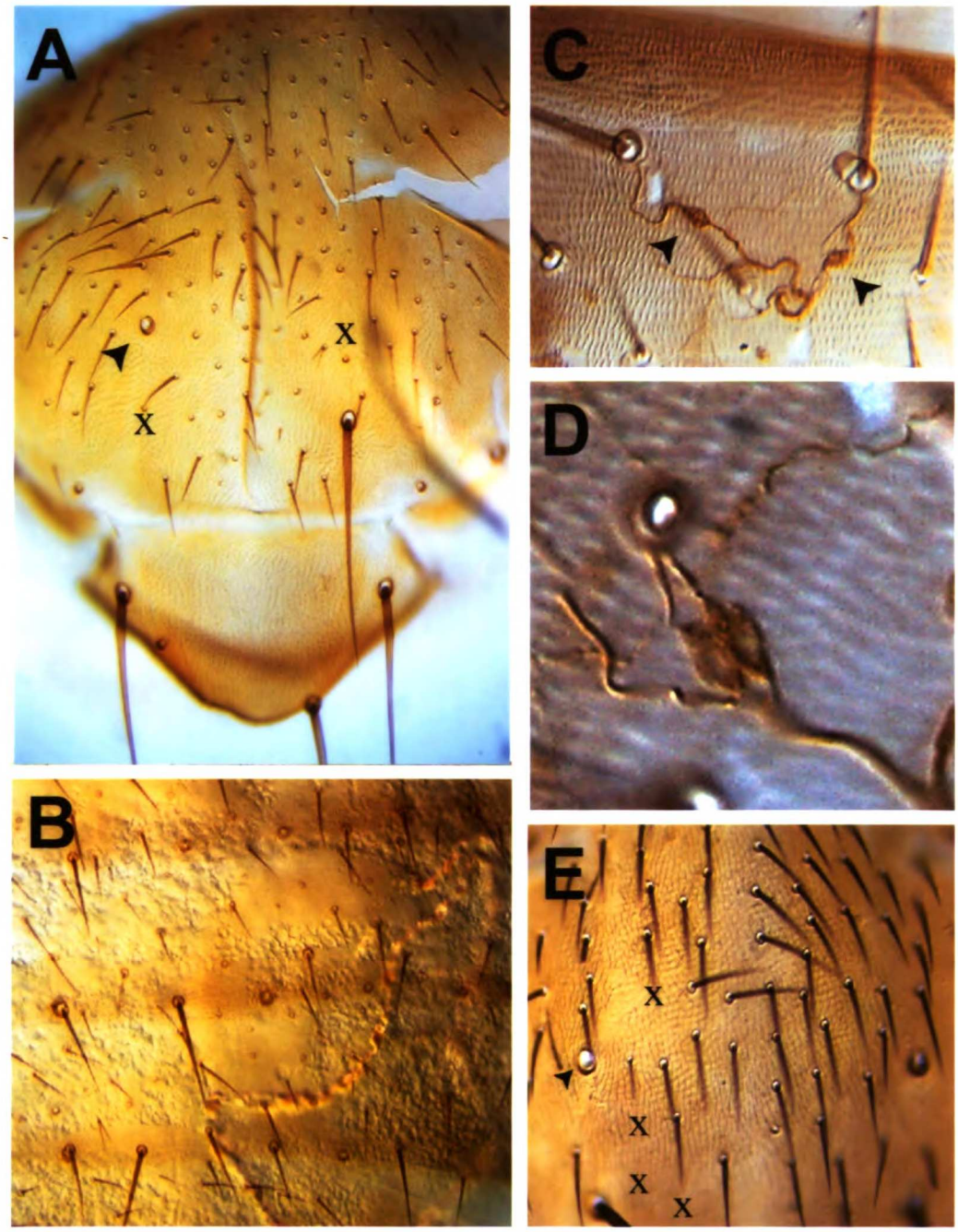


Figure 4.2

Figure 4.3. Reduction of *ttk* function suppresses bristle phenotypes caused by lack of *sina* function.

(A and D) The notum (A) or abdomen (D) of a $sina^{CC1}$ fly shows missing bristles and aberrant bristle phenotypes.

(B and C) MAb22C10 staining of neurons underneath the cuticle of a $sina^{CC1}$ fly. (B) and (C) are the same image in two different focal planes. A wildtype bristle is innervated by one neuron (white arrowhead). The aberrant bristle has no neurons associated with it. No neurons were detected underneath the bald cuticle.

(E) Compared with (D), a fly with a genotype of $sina^{CC1}$ $ttk^{osn}/sina^{CC1}$ increases the number of bristles.

(F) Compared with (E), a $sina^{CC1}$ $ttk^{osn}/sina^{CC1}ttk^{B1}$ fly with a further reduction of *ttk* function replaces more bristles. Furthermore, the bristle missing and empty socket phenotypes due to ttk^{osn}/ttk^{B1} are also rescued.

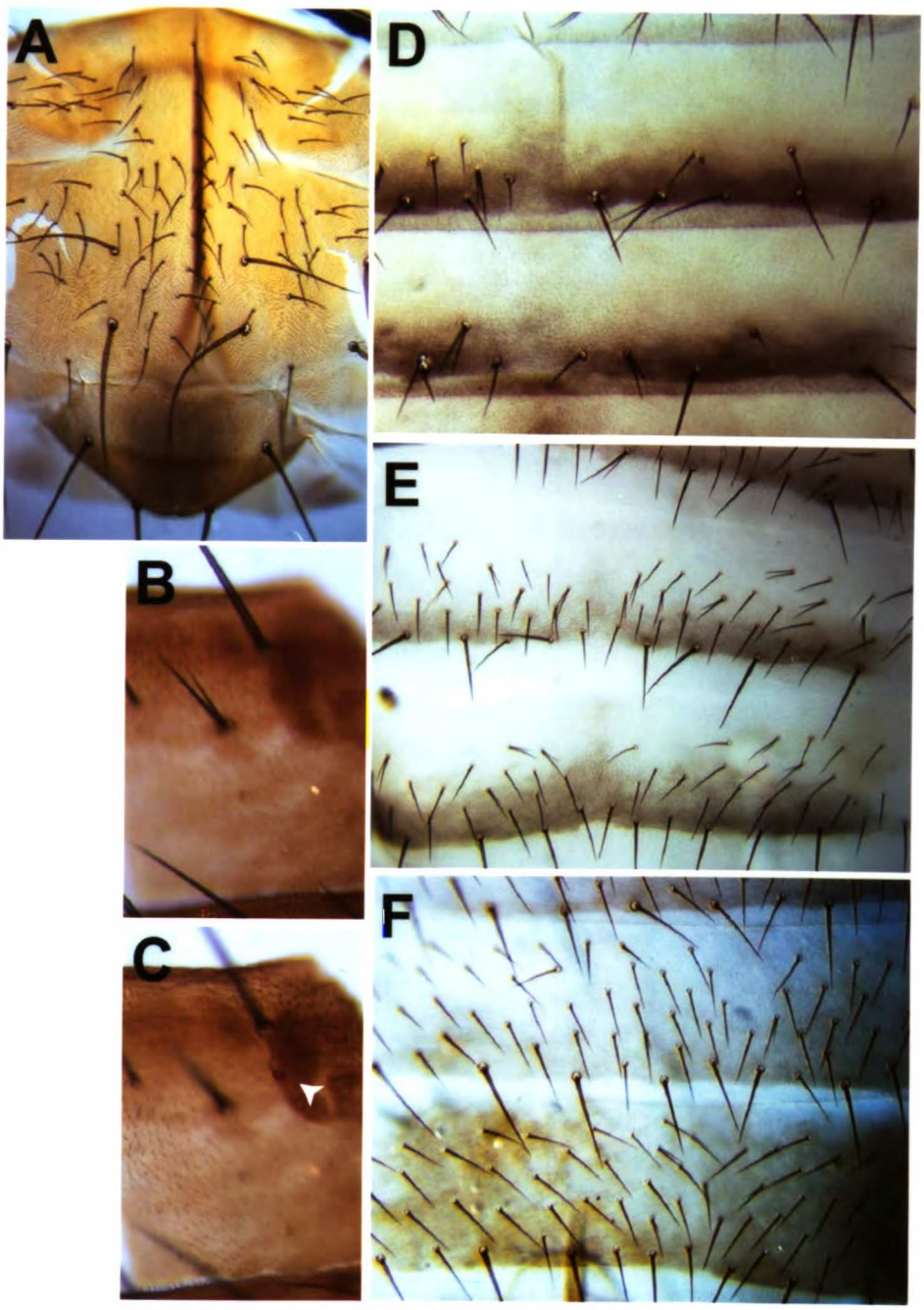


Figure 4.3

Figure 4.4. Reduction of *ttk* function rescues the lethality and suppresses bristle phenotypes caused by lack of *phyl* function.

(A, C, and D) On the notum (A and C) and abdomen (D) of a $phyl^{2245}/phyl^{4}$ fly hypomorphic for *phyl*, there are missing bristles, duplicated bristles, and double hair-single socket phenotypes.

(B and E) Flies with a genotype of $phyl^{2245}$ are larval lethal (Chang et al., 1995). In contrast, flies with a genotype of $phyl^{2245}/phyl^{2245}$; $ttk^{osn}/+$ survive to adulthood and have more bristles than flies hypomorphic for *phyl* (A and D).

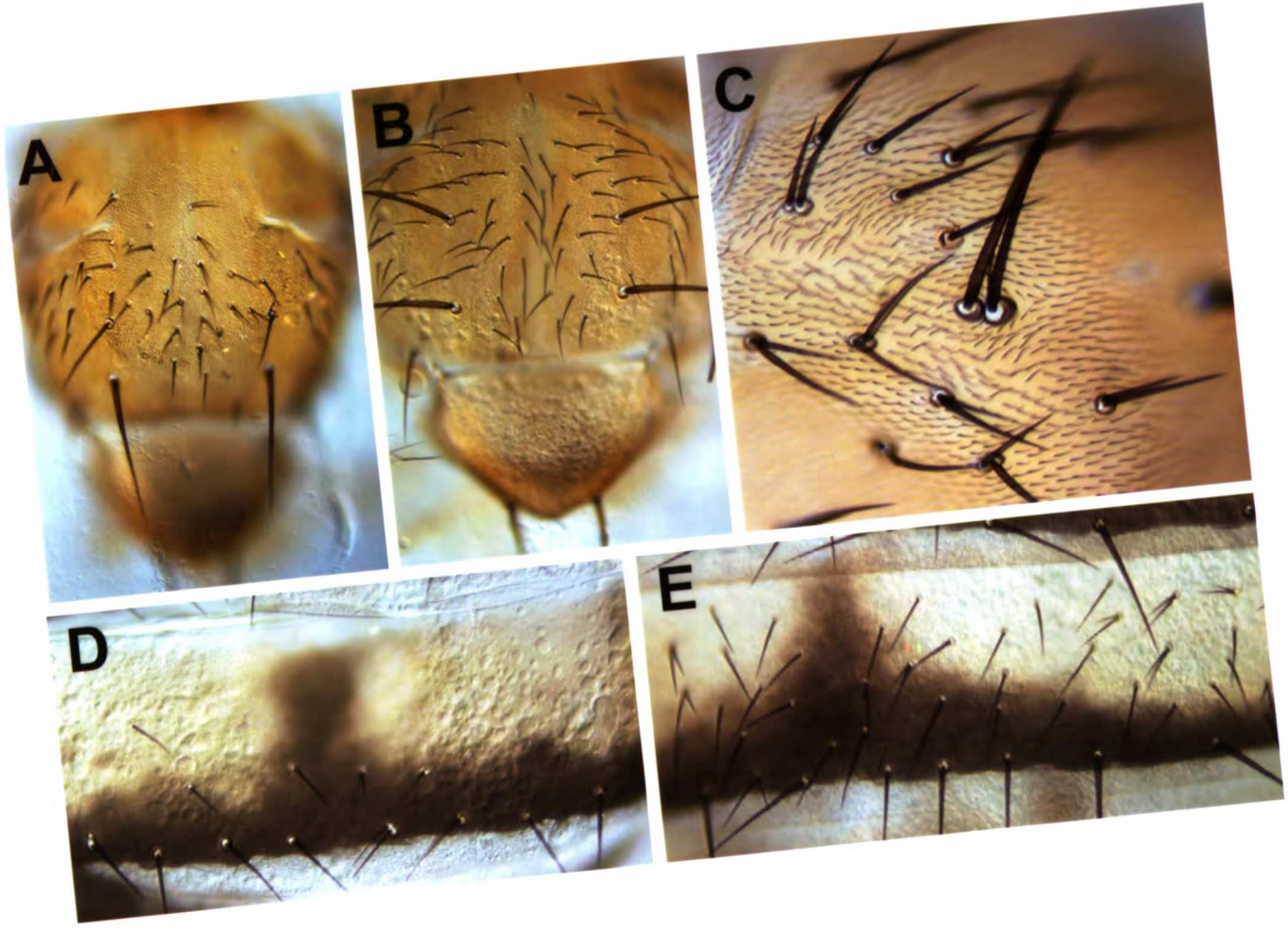


Figure 4.4

CHAPTER 5

CONCLUSION

SUMMARY

This thesis describes the molecular mechanisms by which different cell fates are generated following asymmetric divisions. Using genetic, molecular and biochemical approaches, I have identified a signalling pathway which utilizes Numb (a cell-intrinsic determinant), N (a transmembrane receptor mediating cell-cell signalling), and Ttk (a transcription factor) to specify the neuronal versus non-neuronal decision in the *Drosophila* PNS. In this pathway, *numb* negatively regulates *N*, and *N* positively regulates *ttk* (Figure 5.1).

A model that incorporates these results is illustrated in Figure 5.1. Numb is asymmetrically distributed to one pole of the SOP and is segregated to one daughter cell during SOP division. Numb suppresses N activity in the daughter cell that receives Numb, probably through a direct protein-protein interaction. As a consequence of this suppression, *N* fails to activate the expression of Ttk, a downstream transcription factor. Thus the cell assumes the default cell fate of becoming a neuron. In the daughter cell which lacks Numb, *N* activity is sufficient to activate Ttk, suppressing the neuronal fate and forcing the cell to become non-neuronal. The signal from the ligand of N, Dl, may arise from the other daughter cell and/or the surrounding epidermal cells.

By identifying *numb* as a regulator of *N* and *ttk* as a target gene regulated by *N*, these results add a new dimension to understanding the *N* signaling pathway (Figure 5.2). The mechanisms by which cell fate is regulated by the *numb/N/ttk* signalling pathway are very likely to be conserved in mammals. Thus, my studies of the *Drosophila* signalling pathway may have implications for understanding human development and disease mechanisms.

PERSPECTIVES

1. The function of *Notch*

In Chapter 3, I show that *N* functions as a binary switch between two distinct daughter cell fates during all three asymmetric divisions of the es organ, and during at least the final division of the cho organ. These studies extend previous work by Hartenstein and Posakony (Hartenstein and Posakony, 1990) to suggest that *N* specifies four distinct SOP progeny by progressive binary cell fate decisions. In this context, *N* mediates cell-cell communication between non-equivalent cells after the asymmetric segregation of Numb.

Studies of the function of activated N in *Xenopus* and *Drosophila* suggest that N activation may keep cells in an undetermined state and cause a delay in cell differentiation (Coffman et al., 1993; Fortini et al., 1993). During SOP progeny fate specification, *N* functions to promote formation of the IIa cell and activated N results in a cell fate transformation of the IIb to the IIa cell. In wildtype, the IIa cell divides earlier, and thus differentiates earlier, than the IIb cell. Although we have not examined the division timing of SOP progeny in the presence of activated N, the final cell fate transformation from the IIb cell (which should differentiate later) to the IIa cell (which should differentiate earlier) suggests that activated N may not function to delay differentiation during cell fate specification of SOP progeny.

2. How does *numb* regulate *N*?

In Chapter 3, I show that Numb negatively regulates N. The suppression of *N* by *numb* is also observed in specification of two distinct neurons following the MP2 neuroblast division in the *Drosophila* CNS (Spana and Doe, 1996). Using *Drosophila* S2 tissue culture cells, Frise et al. have shown that after *numb* is transfected into cells expressing N and aggregated with cells that express Dl,

Su(H) fails to translocate into the nucleus. Furthermore, expression of Numb prevents activated N (N^{intra}) from entering the nucleus (Frise et al., 1996). In addition, Numb binds the RAM23 region of N, the same region that binds Su(H) (Chapter 3). These results raise the possibility that Numb is capable of blocking cell-cell signaling directly at the receptor. Does this physical interaction between Numb and N reflect a regulatory mechanism utilized *in vivo*? Several observations support this. The trans-heterozygous genetic interaction between *N* and *numb* supports this possibility, though it does not prove this possibility (Chapter 3). Deletion of the PTB domain of Numb, which binds to N (Chapter 3), abolishes the ability of Numb to abort translocation of Su(H) into the nucleus (Frise et al., 1996). One way to further address this question is to overexpress the parts of the Numb or N protein which mediate their physical interaction, and examine if they can generate any dominant negative phenotypes. Future questions as to how *numb* regulates *N* include the following: Does Numb regulate N by competing for a binding site in the RAM23 region of N that also binds to Su(H)? Does Numb binding perturb the modification of Su(H)? Does Numb binding recruit other signalling molecules that mediate distinct signals to N? Does Numb binding to N prevent N or a N/Su(H) complex from entering the nucleus?

3. How does *N* activate Ttk expression?

A pathway composed of *N*, *Su(H)*, and *E(spl)* has been identified during the process of singling out neuronal precursors, which may also be utilized in specifying SOP progeny fate. Where does *ttk*, another downstream target of *N*, act in this regulatory cascade (Figure 5.2)? This question may be addressed by investigating the epistatic relationship between *Su(H)* and *ttk*, and between *E(spl)* and *ttk*.

Loss of function of either *ttk*, *Su(H)* or *E(spl)* results in an adult phenotype of bristle "balding" associated with supernumerary neurons. These phenotypes can be explained as a result of cell fate transformation from the IIa to the IIb cell. The division of the SOP therefore allows us to study how these three genes function together to specify the IIa and the IIb cell fate.

Both *N* (Chapter 3; Hartenstein and Posakony, 1990) and *numb* (Rhyu et al., 1994; Uemura et al., 1989; and S.W. Wang, personal communication) function in all three divisions of the es organ and at least in one division of the cho organ. Other factors, however, only function in some of these divisions. *ttk* does not seem to be involved in the division of IIa (Chapter 4). Removal of *ttk* function around the time of SOP divisions and differentiation fails to transform sockets to hairs. Furthermore, Ttk is expressed at the same level in both hair and socket cells. *Su(H)*, on the other hand, plays a pivotal role in the hair/socket decision. Both loss of function and overexpression of *Su(H)* suggest that *Su(H)* acts as a binary switch in the hair/socket decision. Moreover, after the IIa division, Su(H) specifically accumulates in the future socket cell but not in the hair cell. Thus, at least in the division of the IIa cell, *ttk* does not act as a readout for *N* signalling through *Su(H)*.

In the division of the IIb cell, removal of *Su(H)* function by mosaic analysis does not cause a cell fate transformation between the neuron and sheath cell (S.W. Wang, personal communication). *ttk* does play a role in this division (Chapter 2). Thus, *ttk* may act as another readout of the *N* signalling independent of *Su(H)*. Several questions become important: Do genes in the *E(spl)* complex play a role in the decision to become a neuron or a sheath cell? If so, does *ttk* function require *E(spl)*, or does *E(spl)* function depend on *ttk*? If *E(spl)* is not involved, does *ttk* constitute another readout of *N* independent of *Su(H)* and *E(spl)* (Figure 5.2)?

In summary, the signalling pathways that regulate three asymmetric divisions of the es organ are not identical. By further characterizing how these different lineages arise, we may learn more about how different *N*-dependent signals are transduced.

4. How does *ttk* work?

ttk may act to suppress the neural fate (Chapter 4). *ttk* has previously been shown to act as a transcriptional repressor during segmentation. It remains to be determined if *ttk* transcriptionally represses expression of genes in non-neuronal cells to prevent them from becoming neurons.

During development, both activation and repression signals may be required. The final developmental outcome results from a balance between these two kinds of signals. In an attempt to search for neuralizing signals antagonizing the non-neuralizing signal mediated by *ttk*, I found two genes, *sina* and *phyl*, that most likely negatively regulate *ttk* (Chapter 4). It will be interesting to examine if *sina* and *phyl* indeed serve as a neuralizing signal, and if so, how they regulate *ttk* function.

Besides its role in PNS and eye development, mutant phenotypes suggest that *ttk* functions in many other developmental contexts including myogenesis, development of Malphigian tubules, midgut constriction, midline cell formation, dorsal closure and head involution (Chapter 2; also see a recent review by Badenhorst et al., 1996). How *ttk* functions in these processes is unknown. Does *ttk* act as a downstream target of *N* in these decisions? Can *ttk* also function as a readout for other signalling pathways?

5. What can we learn for human development and disease?

A theme that has emerged over the past decade is that many genes and signalling pathways are highly evolutionarily conserved. Homologs of *N* have been found in *C. elegans*, zebra fish, *Xenopus*, mouse, rat, and human (reviewed by Artavanis-Tsakonas et al., 1995). Regions of *N* important for signalling are highly conserved across species, suggesting that *N*-dependent signals may be similarly regulated. Vertebrate homologs in other components of the *N* pathway including *Dl* (Chitnis et al., 1995; Henrique et al., 1995), *Su(H)* (Oka et al., 1995), and *E(spl)* (Ishibashi et al., 1995; Tomita et al., 1996) have been identified. Recently, a mouse homolog of *numb* has been isolated (Zhong et al., 1996). Mouse Numb, like *Drosophila* Numb, is also asymmetrically localized in neuronal progenitor cells and physically binds to mouse N, suggesting that it may play a similar role in neurogenesis (Zhong et al., 1996).

N homologs have been implicated in several human diseases. Tan-1 has been implicated in the T-cell lymphoblastic leukemia (Ellisen et al., 1991) and Int-3 during tumorigenesis in mammary glands (Jhappan et al., 1992). Recently, human *Notch3* has been associated with the CADASIL syndrome, which causes stroke and dementia (Joutel et al., 1996). In *C. elegans*, the genetic interaction between *sel-12*, a homolog of the presenilin genes implicated in Alzheimer's disease, and *lin-12*, a *N* homolog, suggests a potential link between *N* signalling and pathogenesis of Alzheimer's disease (Levitan and Greenwald, 1995). Studies of *N* signalling may shed light on crucial biological processes shared by many species, including cell-cell signalling, proliferation, and differentiation. Thus, my studies of how *N* functions, how *N* is regulated, and how *N* regulates downstream target genes should help elucidate the molecular mechanisms of regulating these fundamental processes, and may eventually help understand mechanisms of human development and disease.

Figure 5.1. Schematic drawings of the *numb/N/ttk* pathway and a model for how it specifies neuronal and non-neuronal fates.

Numb, N, and Ttk interact in a signalling pathway to specify the neuronal versus non-neuronal decision. In this pathway, *numb* negatively regulates *N*, and *N* positively regulates *ttk*.

Numb (nb), depicted in red, is asymmetrically distributed to one pole of the SOP and is segregated to one daughter cell during SOP division. Numb suppresses N activity (in green) in the daughter cell that receives Numb, probably through a direct protein-protein interaction. As a consequence of this interaction, *N* fails to activate the expression of Ttk, a downstream transcription factor. Thus the cell assumes the default cell fate of becoming a neuron. In the daughter cell which lacks Numb, *N* activity is sufficient to activate Ttk (in pink), suppressing the neuronal fate and forcing the cell to become non-neuronal. The Dl signal (in blue) may arise from the other daughter cell and/or the surrounding epidermal cells.

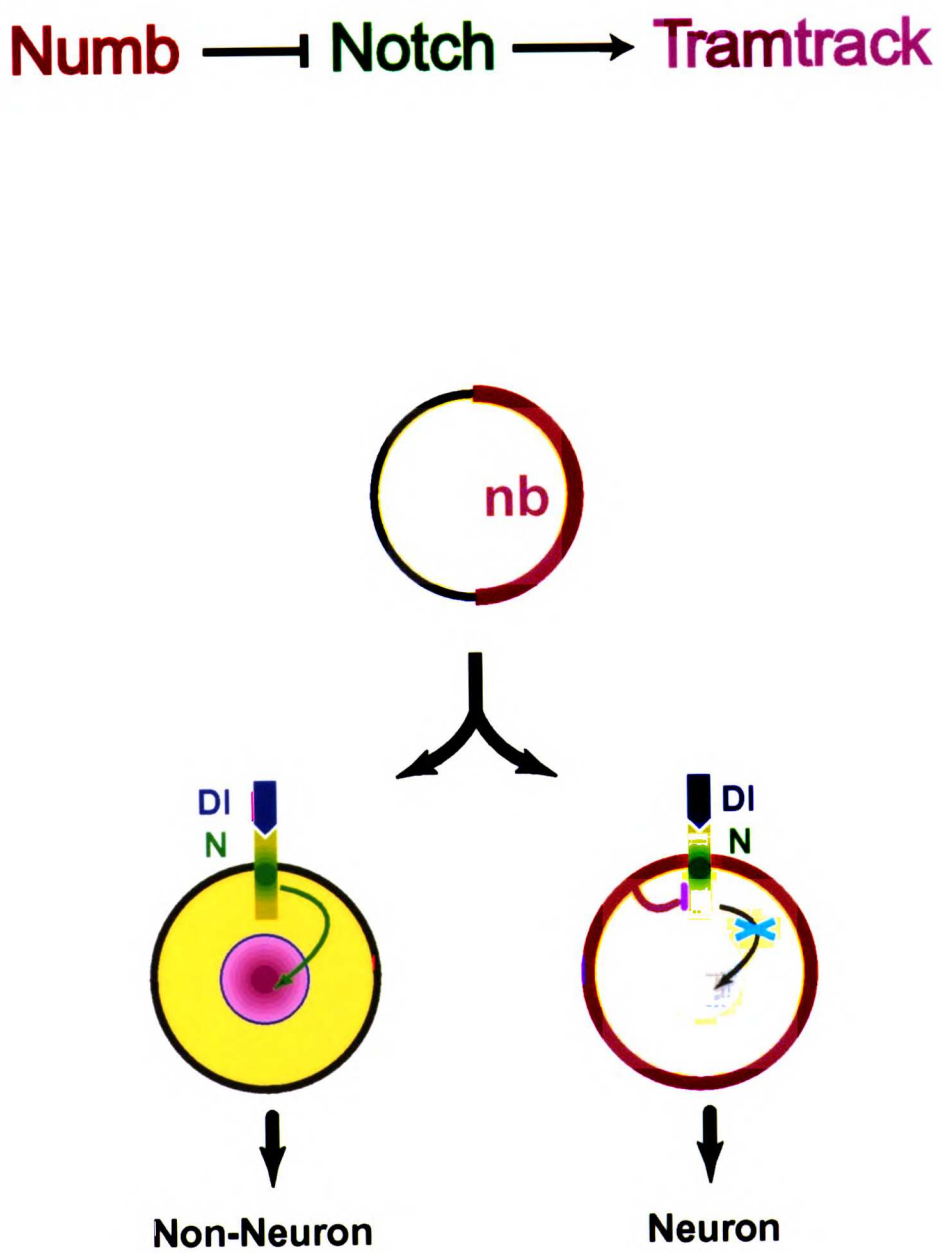


Figure 5.1

Figure 5.2. Schematic drawing of the intersection of the *N/Su(H)/E(spl)* pathway and *numb/N/ttk* pathway during SOP progeny fate specification.

During SOP selection, it has been shown that N binds Su(H) and positively regulates Su(H). Su(H) may directly activate transcription of the *E(spl)* genes. The regulatory cascade of *N/Su(H)/E(spl)* may also be utilized as a "genetic cassette" during SOP progeny fate specification. Also, during this process, Numb negatively regulates N, possibly through a direct protein-protein interaction. *N* then activates Ttk expression. The epistasis relationships between *ttk* and *Su(H)*, and between *ttk* and *E(spl)*, are not yet known. However, at least in some cell fate decisions, *ttk* may act downstream of *N* independent of *Su(H)*.

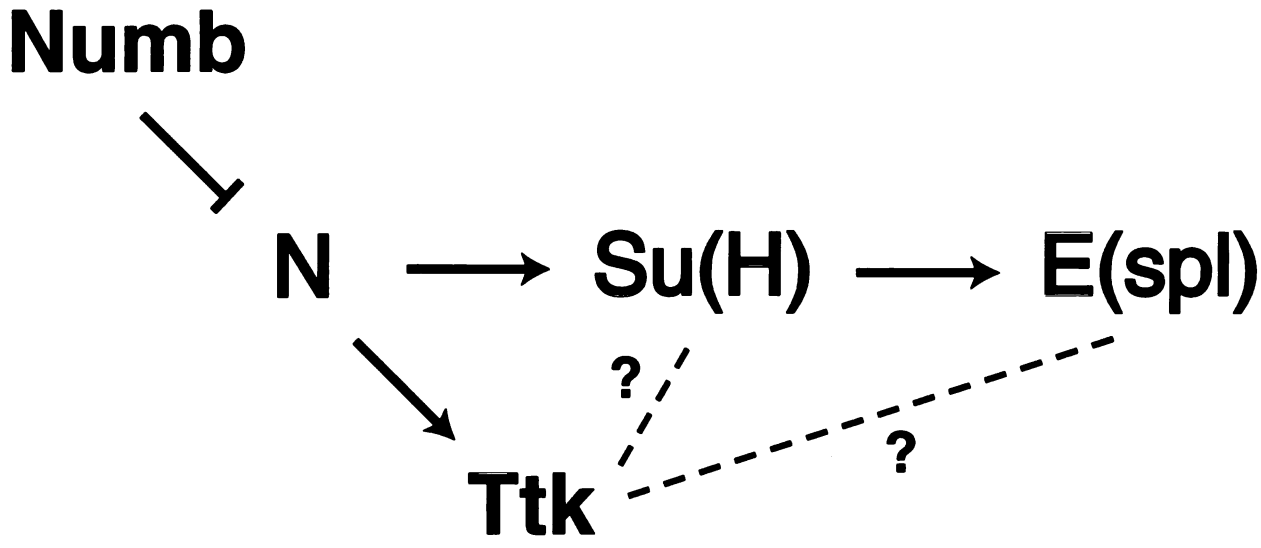


Figure 5.2

APPENDIX

The Requirement of *asense* in Bristle Development

During *Drosophila* neurogenesis, the expression of proneural genes, such as *achaete* (*ac*, T5), *scute* (*sc*, T4), and *lethal of scute* (*l'sc*, T3) of the *achaete-scute* gene (*AS-C*) complex, endow cells with the competence to become neuronal precursors including SOPs in the PNS. These genes encode proteins with bHLH motifs, each of which can form a heterodimer with Daughterless (Da), another bHLH protein expressed ubiquitously. *ac* and *sc*, but not *l'sc*, are required during adult es organ development, since removal of *ac* or *sc* function results in loss of specific subsets of bristles in the notum.

A fourth genes in the *AS*-C complex, *asense* (*ase*, T8), also encodes a protein with a bHLH motif. Ase, like other gene products of the AS-C complex, can dimerize with Da (Jarman et al., 1993). Work from M. Brand and A. Jarman in the lab shows that Ase has a distinct expression pattern. Both Ac and Sc are expressed in proneural clusters, and accumulates in SOPs and then disappears. In contrast, Ase is not expressed in proneural clusters. It first becomes detectable in SOPs, and persists in the two SOP daughter cells (Brand et al., 1993). This "pan-neural" expression pattern of Ase thus distinguishes it from other proneural genes. Studies on the requirement of *ase* in PNS development may help us understand how proneural genes and neuronal precursor genes function.

Since there are no point mutations that remove *ase* function alone, I compared phenotypes of $Df(1)sc^{B57}$ which removes a region that contains all four genes in the *AS*-C complex, with those of $Df(1)sc^{19}$ which removes *ac*, *sc*, and *l'sc*, but not *ase* (Figure 1). Three types of bristles on the anterior wing margin (AWM): stout, slender and recurved, were examined, as well as bristles on the notum. Since these deficiencies are embryonic lethal, I used the FLP-FRT induced mosaic analysis to generate homozygous mutant patches in otherwise heterozygous flies. In $Df(1)sc^{B57}$ clones, slender and recurved bristles in the AWM were missing (Figure 1, 2F, 2G, and 2H), as were bristles on the notum

(Figure 3B). Some stout bristles were also missing (Figure 2F, 2G, and 2H). The remaining stout bristles had either an appearance of brushes (numerous small, densely packed hairs) (Figure 2F and 2H), or aberrant hairs and sockets (Figure 2H), indicative of differentiation defects in hair and socket cells. In *Df(1)sc*^{19} clones on the other hand, both slender and recurved bristles were missing, but stout bristles were still present (Figure 1 and 2E). Bristles on the notum were also missing (Figure 3A). Thus, as, *sc*, and *l'sc* alone have no effect on stout bristle formation. Since the region that is removed by *Df(1)sc* ^{B57} but not *Df(1)sc*^{19} contains at least one additional lethal complementation group, *l(1)EC4* (Figure 1), I asked if the defect caused by *Df(1)sc*^{B57} is due to a lack of *ase*. To do this I tested if I could rescue the *Df(1)sc*^{B57} phenotype with an *ase* transgene. Figure 2I shows that *Df(1)sc*^{B57} clones carrying a genomic fragment of *ase* (made by A. Jarman) rescued the stout bristle phenotype (Figure 1, 2I). The bristle phenotype on the notum was not rescued(Figure 3C), consistent with the finding that *ac* and *sc* are required for the development of these bristles.

To further test for a requirement of *ase* in stout bristle development, I examined the phenotype of a small deficiency, *ase*^{1}, that eliminates *ase* and part of the regulatory region of *sc* (Figure 1). *ase*^{1} flies survived to adulthood, but stout bristles were misformed and recurved bristles were missing (data not shown). Replacing *ase* in flies that were homozygous *ase*^{1} rescued the stout bristle phenotype, consistent with the finding that *ase* only affects stout bristles. Recurved bristles were, however, still absent in these flies (Jarman et al., 1993). This is most likely due to removal of the *sc* regulatory region in *ase*^{1}, since in *sc*$^{10.1}$, a point mutation eliminating *ac* and *sc*, recurved bristles were also lost, but stout bristles were not (Figure 1, 2C and 2D).

In comparing the phenotypes of *ase*^{1} and *Df(1)sc*^{B57} in the wing margin it is clear that while in *ase*^{1}, most, if not all stout bristles were present but malformed,

while in *Df(1)sc*^{B57}, a significant number of the stout bristles were absent. Thus, removal of *ac, sc* and *l'sc* enhanced the phenotype due to removal of *ase* alone. This suggests that although *ac, sc* and *l'sc* alone do not affect stout bristles, they still play a role in stout bristle development. Since the removal of *ase* caused a phenotype in which stout bristles formed but their differentiation was abnormal, it seems that *ase* does not play a strict proneural role. The expression pattern of Ase, is present only following SOP selection, supports this hypothesis.

Garcia-Bellido and Santamaria previously reported a similar phenotype in clones of *Df(1)sc*^{19} and *Df(1)260-1* (which removes the *yellow [y]* gene in addition to *Df(1)sc*^{B57}). Based on analysis of three *Df(1)260-1* clones, they concluded that removal of genes residing in a region between the breakpoints of *Df(1)sc*^{19} and *Df(1)260-1* has no additional affect on bristle development (Garcia-Bellido and Santamaria, 1978). In contrast, we observed a striking stout bristle phenotype associated with *Df(1)sc*^{B57}, and concluded that *ase* plays a role in stout bristle formation. A role for *ase* in stout bristle formation is also supported by other studies (Dominguez and Campuzano, 1993; Jarman et al., 1993).

Since bHLH proteins of the *AS-C* complex are thought to function as heterodimers with Da, we asked if *da* clones generate a phenotype similar to that seen with *Df(1)sc*^{B57}. The *da* clone phenotypes were quite striking in that all bristles were removed in the anterior wing margin (Figure 1 and 2J) and on the notum (data not shown). Since in the *Df(1)sc*^{B57} clone some bristles remained (Figure 2F, G, and H), this results suggests that proteins, most likely bHLH proteins, other than those present in the *Df(1)sc*^{B57} region are important for stout bristle development.

I made an attempt to search for other genes which allow the formation of some stout bristles in the absence of the *AS-C* genes. I focused on a gene known as *ventral nervous system defective (vnd)*, which is located to the right of *Df(1)sc*^{B57}

(Figure 1). In *Df(1)sc*^{B57} embryos, 20-25% of the neuroblasts are absent. Loss of *vnd* function also removes 20-25% of the neuroblasts. Using a deficiency that removes a region containing both the *AS-C* complex and *vnd* results in 50% of the neuroblasts missing, indicative of additive effects of these mutations (Jimenez and Campos-Ortega, 1990). At the time my experiments were conducted (1992), the molecular nature of *vnd* was not known. In 1995, Jimenez et. al. showed that *vnd* encodes a homeodomain protein (Jimenez et al., 1995). I conducted a mosaic analysis of *vnd*, and observed no phenotypes in bristles either in the AWM or on the notum. To test if *vnd* carries a redundant function with *AS-C* genes, I generated clones using *Df(1)svr*, which removes genes in the *AS-C* complex and *vnd* (Figure 1). Unfortunately, removal of genes in *Df(1)svr* resulted in a cell-lethality and no clones can be recovered. Thus, *vnd* and *AS-C* genes functioning together may be required for survival. Alternatively, it seems more likely that other genes present in the deficiency may confer cell lethality. One way to overcome this cell lethality problem might be to generate *Df(1)svr* clones in a background of overexpressing p35. The baculovirus protein p35 is capable of preventing apoptotic cell death when overexpressed in *Drosophila* (Hay et al., 1994). By preventing cell death, it might be fruitful to examine bristle phenotypes due to removal of both *vnd* and *AS-C* genes..

MATERIALS AND METHODS

FLP-FRT induced mosaic analysis

First instar larva were heat-shocked for one hour at 37^{0}C to induce the expression of *FLP*.

For generating *Df(1)sc^{B57}* clones, males carrying *y w* p[FRT]; p[FLP]/*Cyo* were mated with females carrying *Df(1)sc^{B57} w sn p[FRT]/FM6, y w ct B*. The female progeny with non-*Bar* eyes and straight wings were collected and their phenotypes examined. According to this genetic scheme, bristles in mutant clones should be marked with *sn*, and those in the twin spots should be marked with *y*. To generate *Df(1)sc^{19}* clones, males carrying *w sn m p[FRT]; p[FLP]/Cyo* were mated with females carrying *Df(1)sc^{19}, w p[FRT]/FM6, y w ct B*. The female progeny with non-Bar eyes and straight wings were collected. Bristles in mutant clones are marked with *y* (*Df(1)sc^{19}* removes the *y* gene.) and those in twin spots are marked with *sn* . To generate *ase* genomic rescue in *Df(1)sc^{B57}* clones, males carrying *y w p[FRT]; p[FLP]/Cyo* were mated with females carrying *Df(1)scB57 w sn p[FRT]/FM6, y w ct B; p[ase$^+$] /TM3 Sb*. The female progeny with *B$^+$*, *Cyo$^+$*, and *Sb$^+$* were collected and their phenotypes examined. A control experiment in which flies carrying *y w, p[FRT]; p[FLP]/Cyo* were mated with flies carrying *w sn p[FRT]* showed that neither *sn* nor *y* generates a missing bristle phenotype. To generate *da* clones, females carrying *y w; p[FLP]; [y+] p[FRT]/Cyo* were mated with males carrying *y w da^{Kx136} p[FRT]/Cyo*. The progeny with straight wings were collected and examined. Bristles in mutant clones should be marked with *y*, instead, no bristles were observed. To generate *vnd* clones, females carrying *y vnd^6 p[FRT]/FM6, y w ct B* were mated with males carrying *w sn m p[FRT]; p[FLP]/Cyo*. The progeny with straight wings and non-*Bar* eyes were collected. Clone are marked by *y*. To generate *Df(1)svr* clones, males carrying *w sn m p[FRT]; p[FLP]/Cyo* were mated with females carrying *Df(1)svr w p[FRT]/FM6, y w*

ct B. The female progeny that are B^+, Cyo^+ were collected. Bristles in mutant clones are marked with *y* (*Df(1)svr* removes the *y* gene) and those in twin spots are marked with *sn*.

Figure 1. Schematic representation of AWM bristle phenotypes due to deficiencies and point mutations located in the X chromosome within and near the *AS-C* complex (modified from Jimenez and Campos-Ortega, 1990). +, bristle formation is not affected; -, bristle formation is affected. "[ ]" depicts a chromosomal region deleted in this deficiency. "X" depicts a point mutation in the gene. Genes that are not described in the text are: *l(1)EC5* , *silver (svr), embryonic lethal abnormal visual system (elav), amyloid precursor protein-like (Appl), and l(1)Bg.*

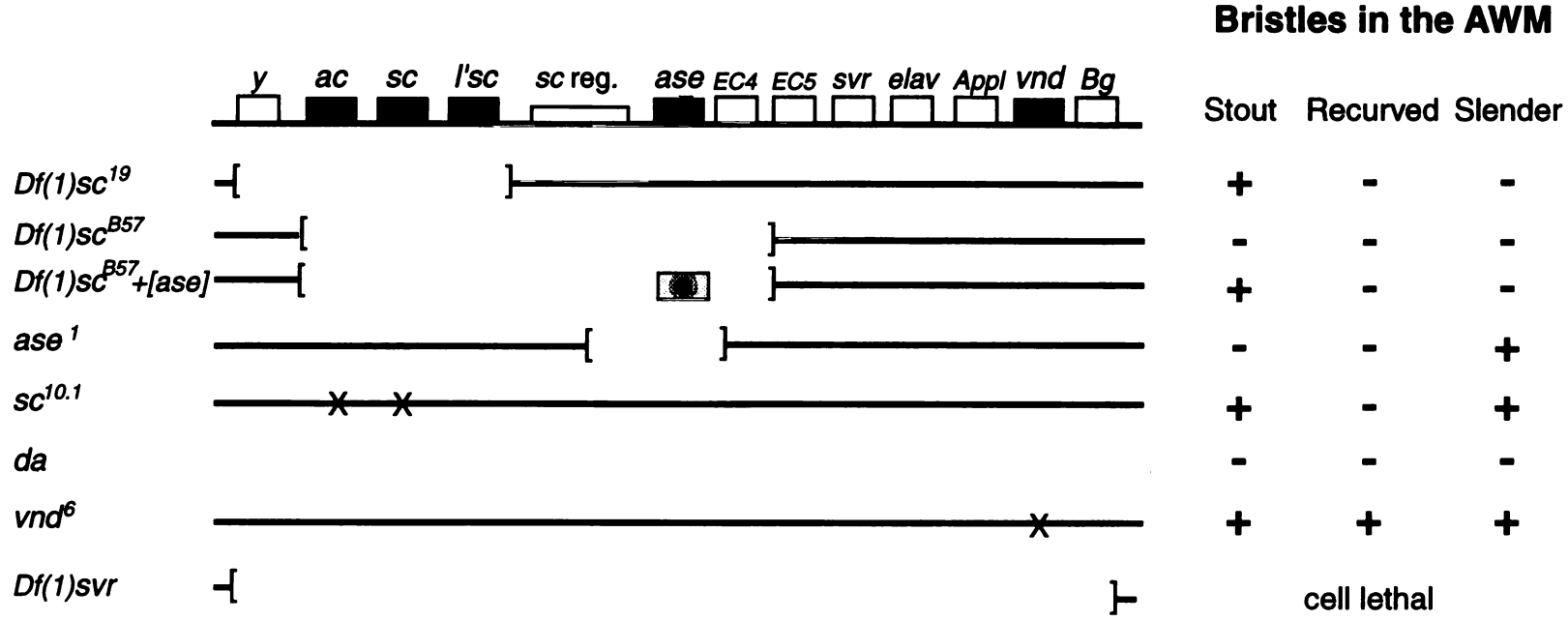

Bristles in the AWM
y
ac
sc
l'sc
sc reg.
ase
EC4
EC5
svr
elav
Appl
vnd
Bg
Stout
Recurved
Slender
Df(1)sc19
Df(1)scB57
Df(1)scB57+[ase]
ase1
sc10.1
da
vnd6
Df(1)svr
cell lethal

Figure 1

Figure 2. Bristle phenotypes in the anterior wing margin.

(A and B) wildtype. Stout bristles are shown in (A). In (B) the left arrow is pointing to a recurved bristle while the right arrow is pointing to a slender bristle.

(C and D) Two focal planes of the AWM of a *sc10.1* fly. Both stout bristle (C) and slender bristles (D) are present (indicated by arrowheads). Recurved bristles are absent from both (C) and (D).

(E) *Df(1)sc*^{19}. No effect on stout bristle development is observed in *Df(1)sc19 clones*. Bristles from the twin spot (white arrowheads) are marked with *sn. yellow* stout bristles (*Df(1)sc*^{19}) to the right of the twin spot appear normal. One wildtype bristle (heterozygous *Df(1)sc*^{19}) is marked with a black arrowhead.

(F, G and H) *Df(1)sc*^{B57} . *Df(1)scB57 shows* multiple phenotypes at the wing margin. In (F), several brushes (numerous small, densely packed bristles) are present. In (G) a mutant patch is present in the middle of the panel. All bristles are removed. In (H), a bristle with two hairs and one socket is observed (white arrowhead) in a mutant patch.

(I) One copy of *p[ase* $^{+}$*]* in a *Df(1)sc*^{B57} clone causes most of the stout bristles reappearing, but recurved or slender bristles are still missing.

(J) In *da* clones no bristles are present.

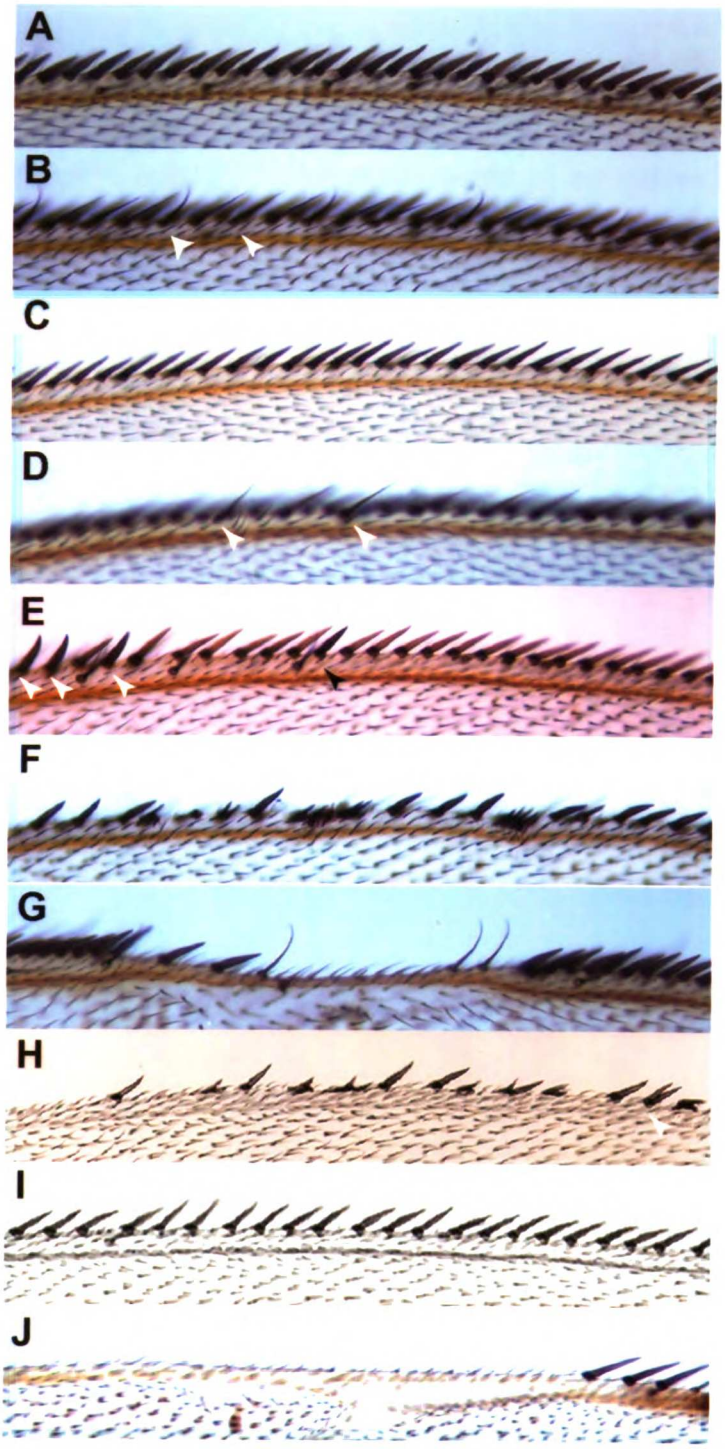


Figure 2

Figure 3. Bristle phenotypes in the notum.

(A) In a *Df(1)sc*^{19} clone, bristle balding was observed. The curved bristles are due to the twin spot marked by *sn*.

(B) In a *Df(1)sc*^{B57} clone, similar bristle balding is observed.

(C) A clone of *Df(1)sc*^{B57}, *[ase+]/+*. The replacement of *ase+* does not rescue the bald phenotype seen in *Df(1)sc*^{B57} mutant patches.

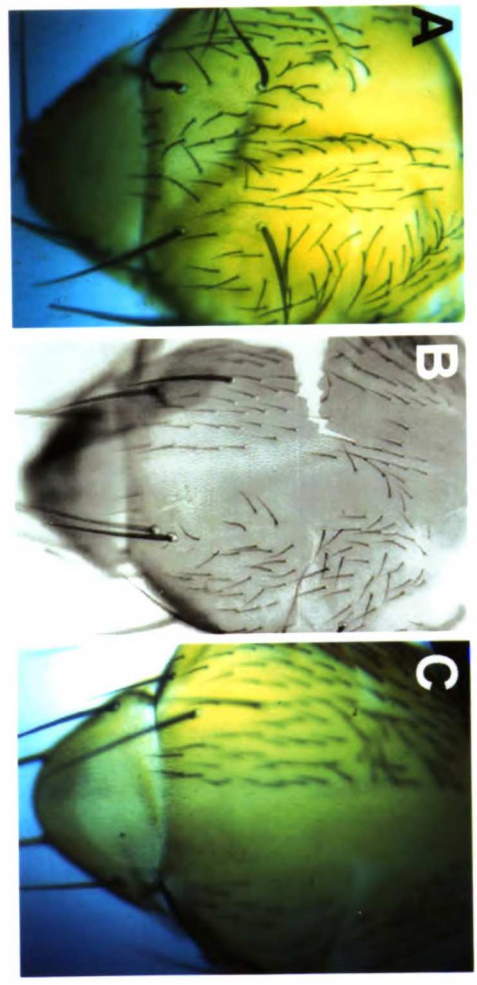


Figure 3

REFERENCES

Artavanis-Tsakonas, S., Matsuno, K., and Fortini, M. E. (1995). Notch signaling. Science *268*, 225-232.

Artavanis-Tsakonas, S., and Simpson, P. (1991). Choosing a cell fate: A view from the *Notch* locus. Trends Genet. *7*, 403-408.

Ashburner, M. (1982). The genetics of a small autosomal region of containing the structureal gene for alcohol dehydrohenase III. Hypomorphic mutations affecting the expression of *Hairless*. Genetics *101*, 447-459.

Bailey, A. M., and Posakony, J. W. (1995). Suppressor of Hairless directly activates transcription of *Enhancer of split* complex genes in response to Notch receptor activity. Genes Dev. *9*, 2609-2622.

Bang, A., Hartenstein, V., and Posakony, J. (1991). *Hairless* is required for the development of adult sensory organ precursor cells in *Drosophila*. Development *111*, 89-104.

Bang, A., and Posakony, J. (1992). The *Drosophila* gene *Hairless* encodes a novel basic protein that controls alternative cell fates in adult sensory organ development. Genes Dev. *6*, 1752-1769.

Bardwell, V., and Treisman, R. (1994). The POZ domain: A conserved protein-protein interaction motif. Genes Dev. *8*, 1664-1677.

Bartel, P., Chien, C.T., Sternglanz, R., and Fields, S. (1993). Using the two-hybrid system to detect protein-protein interactions. In *Cellular Interactions in Development: A Practical Approach*, D.A. Hartley, ed. (Oxford University Press, Oxford), pp. 153-179.

Bate, C. M. (1978). Development of sensory systems in arthropods. In Handbook of Sensory Physiology [ed. M. Jacobson], Springer-Verlag, Berlin/Heidelberg /New York. *IX*, pp. 1-53.

Bier, E., Ackerman, L., Barbel, S., Jan, L. Y., and Jan, Y. N. (1988). Identification and characterization of a neuron-specific nuclear antigen in *Drosophila*. Science *240*, 913-916.

Bodmer, R., Carretto, R., and Jan, Y. N. (1989). Neurogenesis of the peripheral nervous system in *Drosophila melanogaster* embryos: DNA replication patterns and cell lineages. Neuron *3*, 21-32.

Bork, P., and Margolis, B. (1995). A phosphotyrosine interaction domain. Cell *80*, 693-694.

Brand, M., Jarman., A. P., Jan, L. Y., and Jan, Y. N. (1993). *asense* is a *Drosophila* neural precursor gene and is capable of initiating sense organ formation. Development *119*, 1-17.

Brewster, R., and Bodmer, R. (1995). Origin and specification of type II sensory neurons in *Drosophila*. Development *121*, 2923-2936.

Brou, C., Logeat, F., Lecourtois, M., Vandekerckhove, J., Kourilsky, P., Schweisguth, F., and Israel, A. (1994). Inhibition of the DNA-binding activity of *Drosophila* Suppressor of Hairless and of its human homolog, KBF2/RBP-J kappa, by direct protein-protein interaction with *Drosophila* Hairless. Genes Dev. *8*, 2491-2503.

Brown, J. L., Sonoda, S., Ueda, H., Scott, M., and Wu, C. (1991). Repression of the *Drosophila fushi tarazu (ftz)* segmentation gene. EMBO J. *10*, 665-674.

Brown, J. L., and Wu, C. (1993). Repression of *Drosophila* pair-rule segmentation genes by ectopic expression of tramtrack. Development *113*, 45-58.

Busseau, I., Diederich, R. J., Xu, T., and Artavanis-Tsakonas, S. (1994). A member of the *Notch* group of interacting loci, *deltex* encodes a cytoplasmic basic protein. Genetics *136*, 585-596.

Cabrera, C. V., and Alonso, M. C. (1991). Transcriptional activation by heterodimers of the *achaete-scute* and *daughterless* gene products of *Drosophila*. EMBO J. *10*, 2965-2973.

Cagan, R. L., and Ready, D. F. (1989). *Notch* is required for successive cell decisions in the developing *Drosophila* retina. Genes Dev. *3*, 1099-1112.

Campos-Ortega, J. A. (1988). Cellular interactions during early neurogenesis in *Drosophila melanogaster*. Trends Neurosci. *11*, 400-405.

Campuzano, S., Carramolino, L., Cabrera, C. V., Ruiz-Gomez, Villares, R., Boronat, A., and Modolell, J. (1985). Molecular genetics of the *achaete-scute* gene complex of *D. melanogaster*. Cell *40*, 327-338.

Campuzano, S., and Modolell, J. (1992). Patterning of the *Drosophila* nervous system: the *achaete-scute* gene complex. Trends Genet. *8*, 202-208.

Carthew, R. W., and Rubin, G. M. (1990). *seven in absentia*, a gene required for specification of R7 cell fate in the *Drosophila* eye. Cell *63*, 661-577.

Caudy, M., Vässin, H., Brand, M., Tuma, R., Jan, L. Y., and Jan, Y. N. (1988). *daughterless*, a *Drosophila* gene essential for both neurogenesis and sex determination has sequence similarities to *myc* and the *achaete-scute complex*. Cell *95*, 1061-1067.

Chang, H. C., Solomon, N. M., Wassarman, D. A., Karim, F. D., Therrien, M., Rubin, G. M., and Wolff, T. (1995). *phyllopod* functions in the fate determination of a subset of photoreceptors in Drosophila. Cell *80*, 463-472.

Chen, W., Zollman, S., Couderc, J. L., and Laski, F. A. (1995). The BTB domain of bric a brac mediates dimerization *in vitro*. Mol Cell Biol *15*, 3424-3429.

Coffman, C. R., Skoglund, P., Harris, W. A., and Kintner, C. R. (1993). Expression of an extracellular deletion of *Xotch* diverts cell fate in Xenopus embryos. Cell *73*, 659-671.

Dambly-Chaudiere, C., and Ghysen, A. (1987). Independent subpatterns of sense organs require independent genes of the *achaete-scute* complex in *Drosophila* larvae. Genes Dev. *1*, 297-306.

de Celis, J. F., Garcia-Bellido, A., and Bray, S. J. (1996). Activation and function of Notch at the dorsal-ventral boundary of the wing imaginal disc. Development *122*, 359-369.

Delidakis, C., and Artavanis-Tsakonas, S. (1992). The *Enhancer of split* [*E(spl)*] locus of *Drosophila* encodes seven independent helix-loop-helix proteins. Proc. Natl. Acad. Sci. USA *89*, 8731-8735.

DiBello, P. R., Withers, D. A., Bayer, C. A., Fristrom, J. W., and Guild, G. M. (1991). The *Drosophila Broad-Complex* encodes a family of related proteins containing zinc fingers. Genetics *129(2)*, 385-397.

Dickson, B. J., Dominguez, M., van der Straten, A., and Hafen, E. (1995). Control of Drosophila photoreceptor cell fates by *phyllopod*, a novel nuclear protein acting downstream of the Raf kinase. Cell *80*, 453-462.

Diederich, R. J., Matsuno, K., Hing, H., and Artavanis-Tsakonas, S. (1994). Cytosolic interaction between deltex and Notch ankyrin repeats implicates *deltex* in the *Notch* signaling pathway. Development *120*, 473-481.

Doherty, D., Feger, G., Younger-Shepherd, S., Jan, L. Y., and Jan, Y. N. (1996). Delta is a ventral to dorsal signal complementary to Serrate, another Notch ligand, in Drosophila wing formation. Genes Dev. *10*, 421-434.

Dominguez, M., and Campuzano, S. (1993). *asense*, a member of the *Drosophila achaete-scute* complex, is a proneural and neural differentiation gene. EMBO J *12*, 2049-2060.

Driesch, H. (1892). The potency of the first two cleavage cells in echinoderm development. Experimental production of partial and double formations. *In* B.H. Willier and J. M. Oppenheimer (eds.), *Foundations of Experimental Embryology*. Hafner, New York.

Fortini, M. E., and Artavanis-Tsakonas, S. (1994). The Suppressor of Hairless protein participates in Notch receptor signaling. Cell *79*, 273-282.

Fortini, M. E., Rebay, I., Caron, L. A., and Artavanis-Tsakonas, S. (1993). An activated Notch receptor blocks cell-fate commitment in the developing Drosophila eye. Nature *365*, 555-557.

Frise, E., Knoblich, J., Younger-Shepherd, S., Jan, L., and Jan, Y. N. (1996). The *Drosophila* Numb protein inhibits signaling of the Notch receptor during cell-cell interaction in sensory organ lineage. Proc. Natl. Acad. Sci. USA *93*, 11925-11932.

Furukawa, T., Maruyama, S., Kawaichi, M., and Honjo, T. (1992). The *Drosophila* homolog of the immunoglobulin recombination signal-binding protein regulates peripheral nervous system development. Cell *69*, 1191-1197.

Garcia-Bellido, A., and Santamaria, P. (1978). Developmental analysis of the *achaete-scute* system of Drosophila melanogaster. Genetics *88*, 469-486.

Gho, M., Lecourtois, M., Geraud, G., Posakony, J. W., and Schweisguth, F. (1996). Subcellular localization of Suppressor of Hairless in *Drosophila* sense organ cells during *Notch* signalling. Development *122*, 1673-1682.

Ghysen, A., and Dambly-Chaudiere, C. (1988). From DNA to form: The *achaete-scute* complex. Genes Dev. *7*, 723-733.

Ghysen, A., and Dambly-Chaudiere, C. (1989). Genesis of the *Drosophila* peripheral nervous system. Trends Genet. *5*, 251-255.

Ghysen, A., Dambly-Chaudiere, C., Jan, L. Y., and Jan, Y. N. (1993). Cell interactions and gene interactions in peripheral neurogenesis. Genes Dev. *7*, 723-733.

Giniger, E., Jan, L. Y., and Jan, Y. N. (1993). Specifying the path of the intersegmental nerve of the *Drosophila* embryo: a role for *Delta* and *Notch*. Development *117*, 431-440.

Giniger, E., Tietje, K., Jan, L. Y., and Jan, Y. N. (1994). *lola* encodes a putative transcription factor required for axon growth and guidance in *Drosophila*. Development *120*, 1385-1398.

Godt, D., Couderc, J. L., Cramton, S. E., and Laski, F. A. (1993). Pattern formation in the limbs of *Drosophila*: bric a brac is expressed in both a gradient and a wave-like pattern and is required for specification and proper segmentation of the tarsus. Development *119*, 799-812.

Greenwald, I. (1994). Structure/function studies of lin-12/Notch proteins. Curr. Opin. Genet. Dev. *4*, 556-562.

Harrison, R. G. (1969). Organization and Development of the embryo (New Haven: Yale University Press).

Harrison, S., and Travers, A. (1990). The *tramtrack* gene encodes a *Drosophila* finger protein that interacts with the *ftz* transcriptional regulatory region and shows a novel embryonic expression pattern. EMBO J. *9*, 207-216.

Hartenstein, V., and Posakony, J. W. (1989). Development of adult sensilla on the wing and notum of *Drosophila melanogaster*. Development *107*, 389-405.

Hartenstein, V., and Posakony, J. W. (1990). A dual function of the *Notch* gene in *Drosophila* sensillum development. Dev. Biol. *142*, 13-30.

Hartenstein, A. Y., Rugendorff, A., Tepass, U., and Hartenstein, V. (1992). The function of the neurogenic genes during epithelial development in the Drosophila embryo. Development *116*, 1203-20.

Hay, B. A., Wolff, T., and Rubin, G. M. (1994). Expression of baculovirus P35 prevents cell death in *Drosophila*. Development *120*, 2121-2129.

Heitzler, P., and Simpson, P. (1991). The choice of cell fate in the epidermis of Drosophila. Cell *64(6)*., 1083-92.

Horowitz, H., and Berg, C. A. (1996). The *Drosophila pipsqueak* gene encodes a nuclear BTB-domain-containing protein required early in oogenesis. Development *122*, 1859-1871.

Horvitz, H. R., and Herskowitz, I. (1992). Mechanisms of asymmetric cell division: two Bs or not two Bs, that is the question. Cell *68*, 237-255.

Hsieh, J. J., Henkel, T., Salmon, P., Robey, E., Peterson, M. G., and Hayward, S. D. (1996). Truncated mammalian Notch1 activates CBF1/RBPJk-repressed genes by a mechanism resembling that of Epstein-Barr virus EBNA2. Mol Cell Biol *16*, 952-959.

Hu, S., Fambrough, D., Atashi, J. R., Goodman, C. S., and Crews, S. T. (1995). The *Drosophila abrupt* gene encodes a BTB-zinc finger regulatory protein that controls the specificity of neuromuscular connections. Genes Dev. *9*, 2936-2948.

Ito, H., Fujitani, K., Usui, K., Shimizu-Nishikawa, K., Tanaka, S., and Yamamoto, D. (1996). Sexual orientation in *Drosophila* is altered by the satori mutation in the sex-determination gene *fruitless* that encodes a zinc finger protein with a BTB domain. Proc. Natl. Acad. Sci. USA *93*, 9687-9692.

Jan, Y. N., and Jan, L. Y. (1993). Functional gene cassettes in development. Proc Natl Acad Sci U S A *90*, 8305-7.

Jan, Y. N., and Jan, L. Y. (1990). Genes reqired for specifying cell fates in *Drosophila* embryonic sensory nervous system. Trends Neurosci. *13*, 493-498.

Jan, Y. N., and Jan, L. Y. (1994). Genetic control of cell fate specification in *Drosophila* peripheral nervous system. Annu. Rev. Genet. *28*, 373-393.

Jarman, A., Brand, M., Jan, L., and Jan., Y. N. (1993). The regulation and function of the helix-loop-helix gene, *asense*, in *Drosophila* neuronal precursors. Development *119*, 19-29.

Jarman, A., Grau, Y., Jan, L. Y., and Jan, Y. N. (1993). *atonal* is a proneural gene that directs chordotonal organ formation in the *Drosophila* pheripheral nervous system. Cell *73*, 1307-1321.

Jarriault, S., Brou, C., Logeat, F., Schroeter, E. H., Kopan, R., and Israel, A. (1995). Signalling downstream of activated mammalian Notch. Nature *377*, 355-358.
Jimenez, F., and Campos-Ortega, J. (1990). Defective neuroblast commitment in mutants of the *achaete-scute* complex and adjacent genes of D. melanogaster. Neuron *5*, 81-89.

Jimenez, F., Martin-Morris, L. E., Velasco, L., Chu, H., Sierra, J., Rosen, D. R., and White, K. (1995). vnd, a gene required for early neurogenesis of Drosophila, encodes a homeodomain protein. EMBO J. *14*, 3487-3495.

Kauffmann, R., Li, S., Gallagher, P., Zhang, J., and Carthew, R. (1996). Ras1 signalling and transcriptional competence in the R7 cell of *Drosophila*. Genes Dev. *10*, 2167-2178.

Kavanaugh, W. M., and Williams, L. T. (1994). An alternative to SH2 domains for binding tyrosine-phosphorylated proteins. Science *266*, 1862-1865.

Kidd, S., Kelley, M. R., and Young, M. W. (1986). Sequence of the *Notch* locus of *Drosophila melanogaster*: relationship of the encoded protein to mammalian clotting and growth factors. Mol. Cell. Biol. *6*, 3094-3108.

Knoblich, J., Jan, L. Y., and Jan, Y. N. (1995). Localization of Numb and Prospero via a novel mechanism for asymmetric protein segregation. Nature *377*, 624-627.

Knust, E., Schrons, H., Grawe, F., and Campos-Ortega, J. A. (1992). Seven genes of the *Enhancer of split* complex of *Drosophila melanogaster* encode helix-loop-helix proteins. Genetics *132*, 505-518.

Kopan, R., Nye, J. S., and Weintraub, H. (1994). The intracellular domain of mouse Notch: a constitutively activated repressor of myogenesis directed at the basic helix-loop-helix region of MyoD. Development *120*, 2385-2396.

Kopczynski, C. C., Alton, A. K., Fechtel, K., Kooh, P. J., and Muskavitch, M. A. T. (1988). *Delta*, a *Drosophila* neurogenic gene, is transcriptionally complex and encodes a protein related to blood coagulation factors and epidermal growth factor of vertebrates. Genes Dev. *2*, 1723-1735.

Lai, Z. C., Harrison, S. D., Karim, F., Li, Y., and Rubin, G. M. (1996). Loss of *tramtrack* gene activity results in ectopic R7 cell formation, even in a sina mutant background. Proc. Natl. Acad. Sci. USA *93*, 5025-5030.

Lecourtois, M., and Schweisguth, F. (1995). The neurogenic Suppressor of Hairless DNA-binding protein mediates the transcriptional activation of the Enhancer of split complex genes triggered by Notch signaling. Genes Dev. *9*, 2598-2608.

Lees, A. D., and Waddington, C. H. (1942). The development of the bristles in normal and some mutant types of *Drosophila melanogaster*. Proc. Roy. Soc. Lond. *(B) 131*, 87-110.

Lehmann, R., Jimenez, F., Dietrich, U., and Campos-Ortega, J. A. (1983). On the phenotype and development of mutants of early neurogenesis in *Drosophila melanogaster*. Wilhelm Roux's Arch. Dev. Biol. *192*, 62-74.

Lieber, T., Kidd, S., Alcamo, E., Corbin, V., and Young, M. W. (1993). Antineurogenic phenotypes induced by truncated Notch proteins indicate a role in signal transduction and may point to a novel function for Notch in nuclei. Genes Dev. *7*, 1949-1965.

Maier, D., Stumm, G., Kuhn, K. and Preiss, A. (1992). *Hairless*, a *Drosophila* gene involved in neural development, encodes a novel, serine rich protein. Mech. Dev. *38*, 143-156.

Mango, S. E., Thorpe, C. J., Martin, P. R., Chamberlain, S. H., and Bowerman, B. (1994). Two maternal genes, *apx-1* and *pie-1*, are required to distinguish the fates of equivalent blastomeres in the early *Caenorhabditis elegans* embryo. Development *120*, 2305-2315.

Matsuno, K., Diederich, R. J., Go, M. J., Blaumueller, C. M., and Artavanis-Tsakonas, S. (1995). Deltex acts as a positive regulator of Notch signaling through interactions with the Notch ankyrin repeats. Development *121*, 2633-2644.

Mello, C. C., Draper, B. W., and Priess, J. R. (1994). The maternal genes apx-1 and glp-1 and establishment of dorsal-ventral polarity in the early C. elegans embryo. Cell *77*, 95-106.

Menne, T. V., and Klambt, C. (1994). The formation of commissures in the *Drosophila* CNS depends on the midline cells and on the *Notch* gene. Development *120*, 123-133.

Mickey, K. M., Mello, C. C., Montgomery, M. K., Fire, A., and Priess, J. R. (1996). An inductive interaction in 4-cell stage C. elegans embryos involves APX-1 expression in the signalling cell. Development *122*, 1791-8.

Murre, C., McCaw, P. S., Vaessin, H., Caudy, M., Jan, L. Y., and Jan, Y. N. (1989). Interactions between heterologous helix-loop-helix proteins generate complexes that bind specifically to a common DNA sequence. Cell *58*, 537-544.

Nakamura, M., Okano, H., Blendy, J. A., and Montell, C. (1994). Musashi, a neural RNA-binding protein required for Drosophila adult external sensory organ development. Neuron *13*, 67-81.

Nakao, K., and Campos-Ortega, J. (1996). Persistent expression of genes of the *Enhancer of Split* Complex suppresses neural development in Drosophila. Neuron *16*, 275-286.

Parks, A., and Muskavitch, M. A. (1993). *Delta* function is required for bristle organ determination and morphogenesis in *Drosophila*. Dev. Biol. *157*, 484-496.

Read, D., Levine, M., and Manley, J. (1992). Ectopic expression of the *Drosophila tramtrack* gene results in multiple embryonic defects, including repression of *even-skipped* and *fushi tarazu*. Mech. Dev. *38*, 183-196.

Read, D., and Manley, J. (1992). Alternatively spliced transcripts of the *Drosophila tramtrack* gene encode zinc finger proteins with distinct DNA binding specificities. EMBO J. *11*, 1035-1044.

Rebay, I., Fleming, R. J., Fehon, R. G., Cherbas, L., Cherbas, P., and Artavanis-Tsakonas, S. (1991). Specific EGF repeats of Notch mediate interactions with Delta and Serrate: implications for Notch as a multifunctional receptor. Cell *67(4)*, 687-699.

Rhyu, M., Jan, L., and Jan, Y. N. (1994). Asymmetric distribution of numb protein during division of the Sensory Organ Precursor confers distinct fates to daughter cells. Cell *76*, 477-491.

Roux, W. (1888). Contributions to the developmental mechanics of the embryo. On the artificial production of half-embryos by destruction of one of the first two blastomeres and the later development (postgeneration) of the missing half of the body. *In* B.H. Willier and J. M. Oppenheimer (eds.), *Foundations of Experimental Embryology*. Hafner, New York, pp. 2-37.

Ruohola-Baker, H., Jan, L. Y., and Jan, Y. N. (1994). The role of gene cassettes in axis formation during *Drosophila* oogenesis. Trends Genet. *10*, 89-94.

Ruohola, H., Bremer, K. A., Baker, D., Swedlow, J. R., Jan, L. Y., and Jan, Y. N. (1991). Role of neurogenic genes in establishment of follicle cell fate and oocyte polarity during oogenesis in Drosophila. Cell *66*, 433-449.

Schweisguth, F. (1995). *Suppressor of Hairless* is required for signal reception during lateral inhibition in the *Drosophila* pupal notum. Development *121*, 1875-1884.

Schweisguth, F., Nero, P., and Posakony, J. W. (1994). The sequence similarity of the *Drosophila* Suppressor of Hairless protein to the integrase domain has no functional significance *in vivo*. Dev. Biol. *166*, 812-814.

Schweisguth, F., and Posakony, J. W. (1994). Antagonistic activities of *Suppressor-of-Hairless* and *Hairless* control alternative cell fates in the *Drosophila* adult epidermis. Development *120*, 1433-1441.

Schweisguth, F., and Posakony, J. W. (1992). *Suppressor-of-Hairless*, the *Drosophila* homolog of the mouse recombination signal-binding protein gene, controls sensory organ cell fates. Cell *69*, 1199-1212.

Siegel, V., Jongens, T.A., Jan, L.Y., and Jan, Y.N. (1993). *Pipsqueak*, an early acting member of the posterior group class of genes, regulates vasa level and localization in the developing egg chamber. Development *119*, 1187-202.

Soeller, W., Oh, C. E., and Kornberg, T. B. (1993). Isolation of cDNAs encoding the *Drosophila* GAGA transcription factor. Mol. Cell. Biol. *13*, 7961-7970.

Spana, E., Kopczynski, C., Goodman, C., and Doe, C. (1995). Asymmetric localization of numb autonomously determines sibling neuron identity in the Drosophila CNS. Development *121*, 3489-3494.

Spana, E., and Doe, C. (1996). Numb antagonizes Notch signalling to specify sibling neuron cell fates. neuron *17*, 21-26.

Struhl, G., Fitzgerald, K., and Greenwald, I. (1993). Intrinsic activity of the *lin-12* and *Notch* intracellular domains *in vivo*. Cell *74*, 331-345.

Tamura, K., Taniguchi, Y., Minoguchi, S., Saka, T., Tun, T., Furukawa, T., and Honjo, T. (1995). Physical interaction between a novel domain of the receptor Notch and the transcription factor RBP-J_k/Su(H). Curr. Biol. *5*, 1416-1423.

Tata, F., and Hartley, D. A. (1995). Inhibition of cell fate in *Drosophila* by *Enhancer of split* genes. Mech. Dev. *51*, 305-315.

Uemura, T., Shepherd, S., Ackerman, L., Jan, L. Y., and Jan, Y. N. (1989). *numb*, a gene required in determination of cell fate during sensory organ formation in *Drosophila* embryos. Cell *58*, 349-360.

Uemura, T., Shiomi, K., Togashi, S., and Takeichi, M. (1993). Mutation of *twins* encoding a regulator of protein phosphatase 2A leads to pattern duplication in *Drosophila* imaginal discs. Genes Dev. *7*, 429-440.

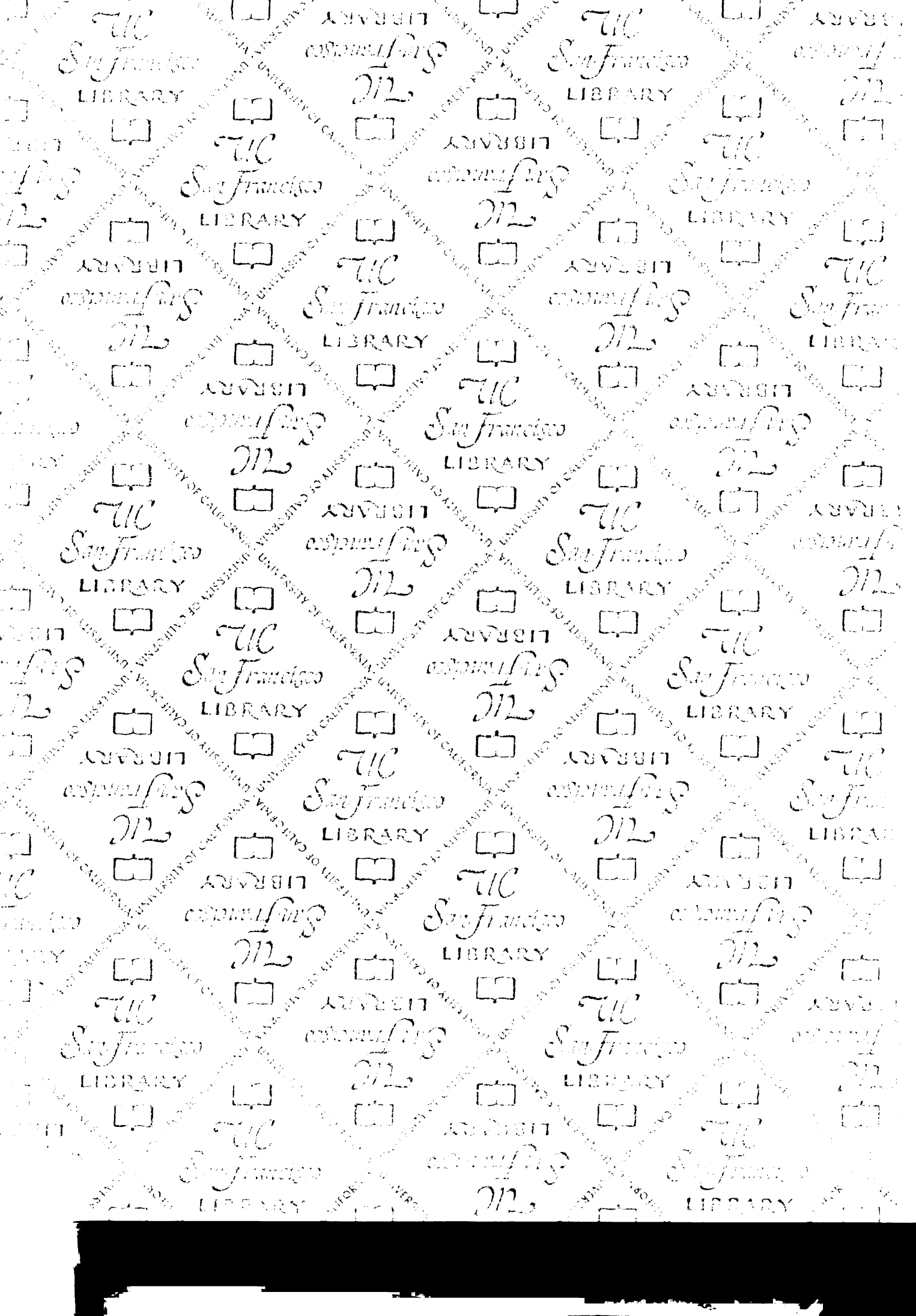
Vaessin, H., Vielmetter, J., and Campos-Ortega, J. A. (1985). Genetic interactions in early neurogenesis of *Drosophila melanogaster*. J. Neurogenet. *2*, 291-308.

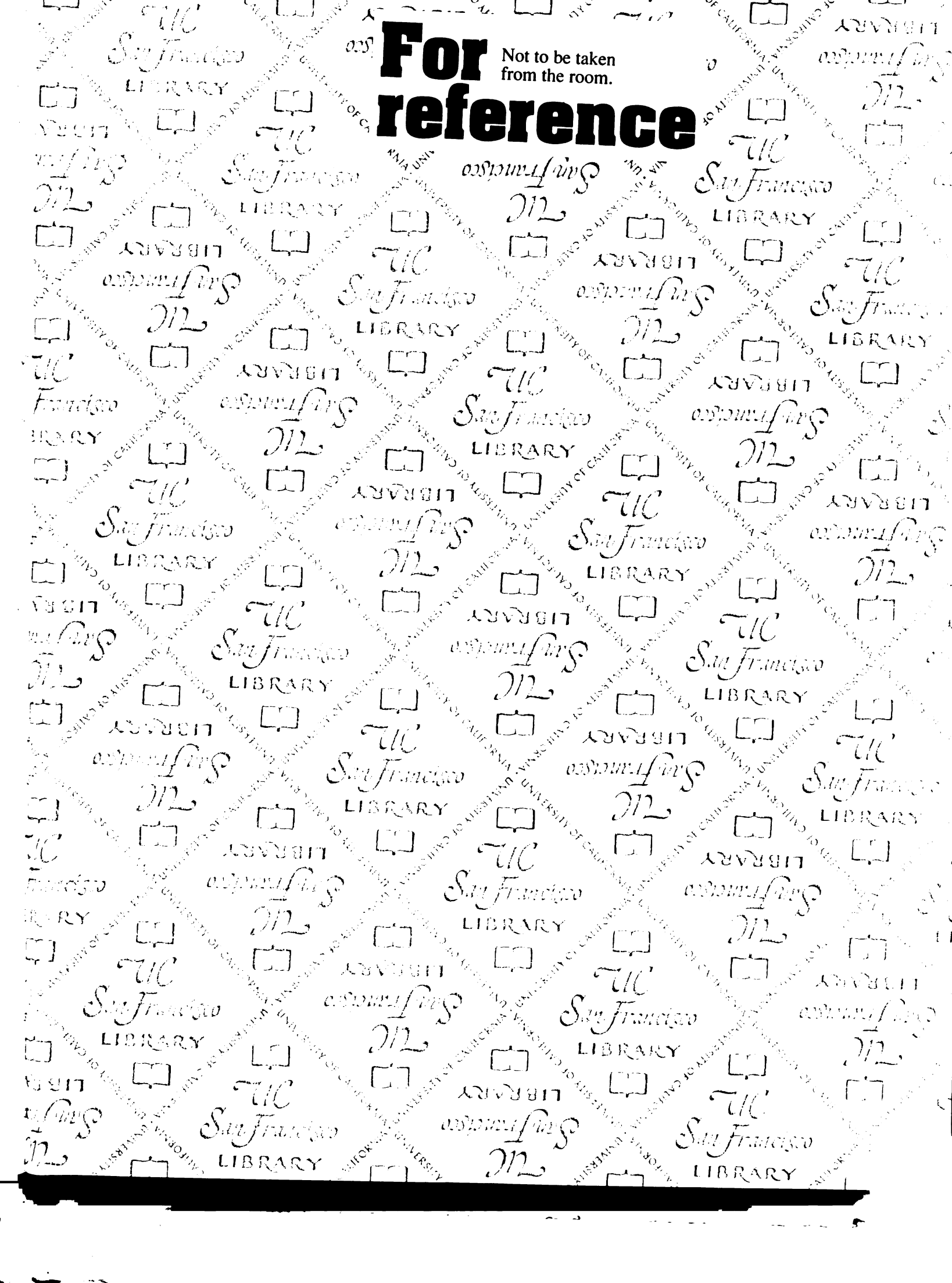
Vaessin, H., Bremer, K. A., Knust, E., and Campos-Ortega, J. (1987). The neurogenic gene *Delta* of *Drosophila melanogaster* is expressed in neurogenic territories and encodes a putative transmembrane protein with EGF-like repeats. EMBO J. *6*, 3431-3440.

Wharton, K. A., Johansen, K. M., Xu, T., and Artavanis-Tsakonas, S. (1985). Nucleotide sequence from the neurogenic locus *Notch* implies a gene product that shares homology with proteins containing EGF-like repeats. Cell *43*, 567-581.

Xu, T., and Artavanis-Tsakonas, S. (1990). *deltex*, a locus interacting with the neurogenic genes, *Notch*, *Delta* and *mastermind* in *Drosophila melanogaster*. Genetics *126*, 665-677.

Zollman, S., Godt, D., Prive, G. G., Couderc, J. L., and Laski, F. A. (1994). The BTB domain, found primarily in zinc finger proteins, defines an evolutionarily conserved family that includes several developmentally regulated genes in *Drosophila*. Proc. Natl. Acad. Sci. USA *91*, 10717-10721.

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