The Numb protein is involved in cell fate determination during Drosophila neural development. Numb has a protein domain homologous to the phosphotyrosine-binding domain (PTB) in the adaptor protein Shc. In She, this domain interacts with specific phosphotyrosine containing motifs on receptor tyrosine kinases and other signaling molecules. Residues N-terminal to the phosphotyrosine are also crucial for phosphotyrosine binding to the Shc PTB domain. Several amino acid residues in She have been implicated by site-directed mutagenesis to be critical for Shc binding to receptor tyrosine kinases. We have generated homologous mutations in Numb to test whether, in vivo, these changes affect Numb function during Drosophila sensory organ development. Two independent amino acid changes that interfere with She binding to phosphotyrosine residues do not affect Numb activity in vivo. In contrast, a mutation shown to abrogate the ability of the Shc PTB domain to bind residues upstream of the phosphotyrosine virtually eliminates Numb function. Similar results were observed in vitro by examining the binding of the Numb PTB domain to proteins from Schneider S2 cells. Our data confirm the importance of the PTB domain for Numb function but strongly suggest that the Numb PTB domain is not involved in phosphotyrosine-dependent interactions.

During the development of an organism, different cell fates can be acquired through both intrinsic or extrinsic mechanisms. In an intrinsic mechanism, a certain factor in the cell is asymmetrically distributed within the mother cell, such that when the mother cell undergoes cytokinesis, the factor is distributed differentially to the two daughter cells. Conversely, in an extrinsic mechanism, the daughter cell receives signals from neighboring cells (or its own sibling cell) to acquire a certain cell fate. The Numb protein represents a key point in linking an intrinsic and extrinsic means of assigning cell fates during both intrinsic and extrinsic means of assigning cell fates during asymmetric divisions in the Drosophila peripheral nervous system (Refs. 1 and 2; for reviews see Refs. 3–5). In Drosophila, Numb is necessary for the asymmetric division of cells in neural lineages (1, 6–8). In the case of simple external sensory organ lineages, the sensory organ precursor undergoes two sets of divisions as follows: the first set produces two daughter cells (IIa and IIb), and the second set produces a bristle and socket cell from the IIa cell and a neuron and glial cell from the IIb cell (Fig. 1A). Numb protein has been shown to be localized to one side of the sensory organ precursor prior to division (1). After cell division it is then segregated asymmetrically to only the IIb cell. In numb mutants, the IIb daughter cell is transformed to a IIa cell fate resulting in two sets of bristle and socket cells (1). In numb mosaics, occasionally four socket cells are observed suggesting that Numb is also needed during the second division (Fig. 1B). Conversely, when Numb protein is overexpressed, the IIa cell is transformed into a IIb cell, resulting in two sets of neuron and glial cells (see Fig. 1C, balding; Ref. 1). When Numb is overexpressed after the first cell division, only the second stage of the lineage is affected, resulting in a transformation of a socket to a bristle cell (see Fig. 1D, twinning) or a glia into a neuron (1). Genetic studies have indicated that numb functions to antagonize signaling by the Notch receptor (2, 9). Lack of Notch function leads to an increase in neuronal cells in the Drosophila peripheral nervous system, whereas activated Notch leads to a decrease in cells adopting the neuronal fate (2, 10). A reduction in Notch signaling partially suppresses the numb mutant phenotype leading to the generation of some neuronal cells. These data suggest that numb functions to inhibit Notch signaling and thus promotes the neuronal phenotype. Furthermore, there is evidence that Notch binds directly to the Numb PTB domain (2).

The phosphotyrosine binding (PTB) domain of Numb is critical for Numb function but not for the asymmetric localization of Numb (11). The PTB domain was first described in the protein, She, and was subsequently identified by sequence homology to be present in several other proteins including Numb (12–16). The PTB domain of Numb and She are 20% identical at the amino acid level (Fig. 2A, see Refs. 12 and 17), suggesting that the Numb PTB domain may act similarly to the Shc PTB domain in mediating signal transduction events by binding to the NPXY motif (18). In Shc, the PTB domain binds a YXNPXYP motif on receptor tyrosine kinases, where Y is a hydrophobic residue, X is any amino acid residue, N is asparagine, P is proline, and Y is phosphotyrosine. Several individual amino acid residues in Shc have been implicated by site-directed mutagenesis to be critical for Shc binding to the

---

* This research was supported by Public Health Service Grant NS29119 (to R. B.), Public Health Service National Research Service award, and Chemical and Hearing Senses training grant (to L. Y.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked ‘advertisment’ in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ Both authors contributed equally to this work and should be considered joint co-authors.

¶ Investigator of the Howard Hughes Medical Institute. To whom correspondence should be addressed: Dept. of Biology, University of Michigan, Ann Arbor, MI 48109-1048. Tel.: 734-677-3192; Fax: 734-647-0884; E-mail: rolf@umich.edu.

The abbreviations used are: PTB, phosphotyrosine-binding domain; Eve, Even skipped; GST, glutathione S-transferase; PAGE, polyacrylamide gel electrophoresis; Shc, Src homologous and collagen protein; EGF, epidermal growth factor; NGF, nerve growth factor; UAS, upstream activation sequence; w, white; pY, phosphotyrosine.
**Mutagenesis of the Numb PTB Domain**

$\Psi XNPxYP$ motif (17, 19, 20). These include residues involved in binding the phosphorytrosine as well as residues necessary for contacting the amino acids upstream of the phosphorytrosine in the $\Psi XNPxYP$ peptide. An important question is whether all or only some of the PTB domains are involved in phosphorytrosine-dependent interactions (21–26). In an attempt to address whether or not the Numb PTB domain is involved in phosphorytrosine binding, we generated point mutants in the Numb PTB domain analogous to those generated in Shc (19, 21). Two of these mutations affect conserved residues involved in the binding of the Shc PTB domain to phosphorytrosine. These are serine 148 and arginine 171 of Numb. Another mutation targets phenyl alanine 195 of Numb. In Shc, this phenyl alanine binds to the hydrophobic residue –5 and the asparagine –3 to the phosphorytrosine without directly interacting with phosphorytrosine (17). Finally, a fourth mutation, phenyl alanine to leucine 149 of Numb, which results in reduced binding affinity of the Shc PTB domain, was also tested.

We have examined how these mutations affect Numb function *in vivo* both during *Drosophila* sensory organ development and mesodermal development. The phenyl alanine to valine mutation (amino acid residue 195) is able to virtually eliminate the gain-of-function when overexpressed in transgenic flies using the UAS-GAL4 system (27). Altering the residues in Numb homologous to those involved in Shc phosphorytrosine binding has little or no effect on the lineage transformation activity of Numb overexpression *in vivo*. Another mutation, phenyl alanine to leucine (position 149), which causes Shc to bind with a reduced affinity *in vitro*, exhibits an intermediate phenotype *in vivo*. In binding studies using *Drosophila* cell culture, we detect several proteins that bind to the wild-type PTB domain and to the S148A and R171Q mutants but not to the F195V mutant of Numb. These data with site-directed mutagenesis confirm that the PTB domain is crucial for Numb function but strongly suggest that phosphorytrosine is not required for Numb PTB domain function *in vivo*.

**MATERIALS AND METHODS**

**DNA Constructs and Mutagenesis—**Wild-type Numb PTB domain GST fusion protein constructs were made by subcloning a polymerase chain reaction-generated fragment of numb DNA spanning the 966–1490-base pair region of numb cDNA into the pGStag vector (28) in the EcoRI site. PTB domain point mutants were generated by polymerase chain reaction-based site-directed mutagenesis from the wild-type pGStag-Numb PTB domain. The full-length numb cDNA (760–2800 base pairs) was subcloned into the pUAST vector in the Kpal site. Site-directed mutagenesis of full-length numb was done by using Transformer Site-directed Mutagenesis kit (CLONTECH) or U.S.E. Mutagenesis kit (Amersham Pharmacia Biotech) of these mutations affect conserved residues involved in the binding of the Shc PTB domain to phosphorytrosine. These are serine 148 and arginine 171 of Numb. Another mutation targets phenyl alanine 195 of Numb. In Shc, this phenyl alanine binds to the hydrophobic residue –5 and the asparagine –3 to the phosphorytrosine without directly interacting with phosphorytrosine (17). Finally, a fourth mutation, phenyl alanine to leucine 149 of Numb, which results in reduced binding affinity of the Shc PTB domain, was also tested.

We have examined how these mutations affect Numb function *in vivo* both during *Drosophila* sensory organ development and mesodermal development. The phenyl alanine to valine mutation (amino acid residue 195) is able to virtually eliminate the gain-of-function when overexpressed in transgenic flies using the UAS-GAL4 system (27). Altering the residues in Numb homologous to those involved in Shc phosphorytrosine binding has little or no effect on the lineage transformation activity of Numb overexpression *in vivo*. Another mutation, phenyl alanine to leucine (position 149), which causes Shc to bind with a reduced affinity *in vitro*, exhibits an intermediate phenotype *in vivo*. In binding studies using *Drosophila* cell culture, we detect several proteins that bind to the wild-type PTB domain and to the S148A and R171Q mutants but not to the F195V mutant of Numb. These data with site-directed mutagenesis confirm that the PTB domain is crucial for Numb function but strongly suggest that phosphorytrosine is not required for Numb PTB domain function *in vivo*.

**MATERIALS AND METHODS**

**DNA Constructs and Mutagenesis—**Wild-type Numb PTB domain GST fusion protein constructs were made by subcloning a polymerase chain reaction-generated fragment of numb DNA spanning the 966–1490-base pair region of numb cDNA into the pGStag vector (28) in the EcoRI site. PTB domain point mutants were generated by polymerase chain reaction-based site-directed mutagenesis from the wild-type pGStag-Numb PTB domain. The full-length numb cDNA (760–2800 base pairs) was subcloned into the pUAST vector in the Kpal site. Site-directed mutagenesis of full-length numb was done by using Transformer Site-directed Mutagenesis kit (CLONTECH) or U.S.E. Mutagenesis kit (Amersham Pharmacia Biotech) of these mutations affect conserved residues involved in the binding of the Shc PTB domain to phosphorytrosine. These are serine 148 and arginine 171 of Numb. Another mutation targets phenyl alanine 195 of Numb. In Shc, this phenyl alanine binds to the hydrophobic residue –5 and the asparagine –3 to the phosphorytrosine without directly interacting with phosphorytrosine (17). Finally, a fourth mutation, phenyl alanine to leucine 149 of Numb, which results in reduced binding affinity of the Shc PTB domain, was also tested.

We have examined how these mutations affect Numb function *in vivo* both during *Drosophila* sensory organ development and mesodermal development. The phenyl alanine to valine mutation (amino acid residue 195) is able to virtually eliminate the gain-of-function when overexpressed in transgenic flies using the UAS-GAL4 system (27). Altering the residues in Numb homologous to those involved in Shc phosphorytrosine binding has little or no effect on the lineage transformation activity of Numb overexpression *in vivo*. Another mutation, phenyl alanine to leucine (position 149), which causes Shc to bind with a reduced affinity *in vitro*, exhibits an intermediate phenotype *in vivo*. In binding studies using *Drosophila* cell culture, we detect several proteins that bind to the wild-type PTB domain and to the S148A and R171Q mutants but not to the F195V mutant of Numb. These data with site-directed mutagenesis confirm that the PTB domain is crucial for Numb function but strongly suggest that phosphorytrosine is not required for Numb PTB domain function *in vivo*.

**Western Blot of Embryo Lysates—**Wild-type and mutant UAS-Numb flies were crossed to several different GAL4 driver lines that were kindly donated by several different laboratories including that of Gerhard Technau (60-GAL4, 189-GAL4, and 281-GAL4), Elisabeth Knust (daG32-GAL4, Ref. 29), Gabrielle Boulianne (C96-GAL4, Ref. 32) and the Bloomingtom Drosophila Stock Center (32B-GAL4 and 71B-GAL4, Ref. 27). Many of the imaginal GAL4 lines used (including 60-GAL4, 189-GAL4, 281-GAL4, 32B-GAL4, and 71B-GAL4) exhibited a mix of twinning and bristle loss over much of the fly body, including the wing margin, to varying degrees (data not shown). The phenotype was restricted to the body, with an essentially wild-type wing margin, in UAS-Numb;160-GAL4 flies. Conversely, one GAL4 driver, C96-GAL4 (32), when crossed to UAS-Numb, exhibited a moderate degree of twinning of the wing margin bristles but an essentially wild-type body bristle phenotype (see Fig. 3B). We therefore examined the effect of wild-type and mutant Numb on mesodermal development, a double GAL4 line of twist-GAL4;24B-GAL4 (Refs. 27 and 33) was used (this line was constructed and kindly donated by Wendy Lockwood).

**Immunohistochemical Staining—**To examine the effects of the UAS-Numb constructs (wild-type and mutant) during embryonic development, the UAS-Numb flies were crossed to a daG32-GAL4 line (29) for assaying neuronal development and twist-GAL4;24B-GAL4 for assaying mesodermal development. The embryos from this cross were then collected, dechorionated, and fixed as described previously (34). The neurons were then visualized using the 22C10 monoclonal antibody (35) at a concentration of 1:50. Cardiac and muscle cells were visualized with an anti-Even-skipped antibody (36) used at a concentration of 1:10,000. Horseradish peroxidase-conjugated goat anti-mouse IgG or IgG (Bio-Rad Laboratories) was used as the secondary antibody at a concentration of 1:200. Imunogold discs for immunohistochemical staining were prepared and stained as described by Patel (37). Anti-Myc antibody (9E10) was used at a 1:50 dilution to visualize transgenic Numb overexpression. Horseradish peroxidase-conjugated goat anti-mouse IgG (Bio-Rad) was used as the secondary antibody at a concentration of 1:200.
Shc binding to its receptor targets. An alignment of the human PTB domain and the Drosophila Numb PTB domain is shown in Fig. 2A. The amino acids indicated by asterisks in Fig. 2A have been identified as being crucial for Shc PTB domain binding and are conserved in the Numb PTB domain. The Shc F198V mutation inhibits binding of the Shc PTB domain to a number of receptor tyrosine kinase targets (19, 38, 39). A homologous mutation was made in the Drosophila Numb PTB domain, F195V, within a full-length Numb construct in the pUAST vector (Fig. 2). Four transgenic lines were made from this construct and crossed to both embryonic and imaginal GAL4 driver lines (see “Materials and Methods”). The progeny from these crosses appeared almost indistinguishable from wild type (Fig. 3E, compare with Fig. 3A). There is strong evidence to suggest that this mutant Numb protein is being expressed at high levels. In Western blot analysis, a higher level of Numb protein is seen in the progeny of da^{G32}-GAL4 crossed to UAS-Numb relative to the amount observed in wild-type flies, suggesting that the Numb F195V mutant protein is strongly induced (Fig. 4A). Furthermore, by using immunohistochemical staining with an antibody that recognizes the Myc epitope tag on the transgenic Numb protein, strong expression of both wild-type and Numb F195V was observed in larval third instar wing discs (Fig. 4B). Finally, a very low number of duplicated bristles and/or missing bristles (3.3% macrochaetae per fly compared with 0.5% in wild type) is observed with Numb F195V overexpression (driven by certain GAL4 drivers, especially T80-GAL4, but also 1445-GAL4 and 30A-GAL4). This suggests that although the mutant Numb protein is being highly expressed, it has virtually no activity.

Two other sets of mutations, S151A and R175Q, have been shown to specifically interfere with the binding of the Shc PTB domain to phosphotyrosine residues (17, 19). Homologous mutations were made in the full-length Numb protein (S148A and R171Q), which was then subcloned into the pUAST vector for the creation of transgenic lines (Fig. 2B). When these Numb mutants are overexpressed in the UAS-GAL4 system, the overexpression phenotypes are indistinguishable from that of wild-type Numb protein, exhibiting an almost complete balding phenotype (Fig. 3, C and D; Table I).

Finally, a mutation, F149L, which is analogous to the F152L mutation in Shc, was tested. Shc PTB domains with this mutation bind less well than the wild-type PTB domain but better than the F198V mutant (19). Similarly, this mutation in Numb yielded an intermediate bristle phenotype in transgenic flies. When using 189-GAL4 to drive expression, these flies exhibited a moderate degree of bristle loss (Fig. 3F; Table I), as opposed to the very extensive bristle loss observed with wild-type and S148A and R171Q Numb overexpression (Fig. 3, B–D; Table I).

We also wanted to determine if these mutations had the same effect on Numb function during embryonic PNS development and during mesodermal development. Compared with wild-type embryos, excess neurons were observed when UAS-Numb flies were crossed to da^{G32}-GAL4 flies, which drives expression throughout the ectoderm (29). As in the adult, the S148A and R171Q mutations gave a phenotype similar to overexpression of wild-type Numb, whereas overexpression of the F195V mutant gave a wild-type phenotype (Fig. 5, A–C). Since Numb appears to influence cell fate decisions in mesoderm as well,2 we wanted to see if the mutations that we were examining in neural development have a similar effect during mesoderm development. To assay for this, the UAS-Numb wild-type and mutant lines were crossed to the twist-GAL4:24B-GAL4 line (27, 33), which drives Numb expression throughout the

---

**Table I**

| UAS-NUMB line | Phenotype
|---------------|---------|
| UAS-Numb+ (1) | Semi-lethal (+ + + + ) |
| UAS-Numb+ (2) | Semi-lethal (+ + + + ) |
| UAS-Numb+ (7) | Viable (+ + + + ) |
| UAS-Numb+ (10) | Semi-lethal (+ + + + ) |
| UAS-Numb+ (11) | Viable (+ + + + ) |
| UAS-Numb+ (12) | Semi-lethal (+ + + + ) |
| UAS-Numb (R171Q) (1) | Semi-lethal (+ + + + ) |
| UAS-Numb (R171Q) (2) | Viable (+ + + + ) |
| UAS-Numb (R171Q) (5) | Semi-lethal (+ + + + ) |
| UAS-Numb (R171Q) (7) | Semi-lethal (+ + + + ) |
| UAS-Numb (S148A) (1) | Viable (+ + + + ) |
| UAS-Numb (S148A) (3) | Viable (+ + + + ) |
| UAS-Numb (S148A) (5) | Semi-lethal (+ + + + ) |
| UAS-Numb (S148A) (6) | Viable (+ + + + ) |
| UAS-Numb (S148A) (7) | Pupal lethal (+ + + + ) |
| UAS-Numb (F149L) (1) | Viable (+ ) |
| UAS-Numb (F149L) (4) | Viable (+ + ) |
| UAS-Numb (F149L) (6) | Viable (+ + + ) |
| UAS-Numb (F149L) (8) | Viable (+ + + + ) |

*Percentage of bristle loss observed when crossed to 189-GAL4: (+) 0–25% macrochaete missing, (+ + ) 26–50% macrochaete missing, (+ + + ) 51–75% macrochaete missing, and (+ + + + ) 76–100% macrochaete missing. The transgenic mutation is followed by the line designation, both in parentheses.

---

2 M. Park, L. Yaich, and R. Bodmer, unpublished observations.
mesoderm. In the dorsal mesoderm, there are segmental clusters of Evenskipped (Eve) expressing cardiac cells adjacent to the syncytium of one dorsal muscle (Fig. 5D). When wild-type Numb protein is overexpressed only early in the lineage, the IIa cell is transformed into an IIb cell, resulting in two sets of glia and neurons. Conversely, overexpression of the F195V mutant does not give a Numb overexpression phenotype (Fig. 5F).

**Numb PTB Domain Function in Vitro**—To confirm our results in vivo, we analyzed the in vitro binding ability of the Numb PTB domain. Wild-type and mutant Numb PTB domains were generated as a GST fusion protein in bacteria. These proteins were purified on glutathione-agarose and used as an affinity matrix to bind proteins from [35S]methionine-labeled Schneider S2 cell lysate. After binding, the beads were washed with lysis buffer containing 1% Triton X-100. The bound proteins were then separated by SDS-PAGE and exposed for autoradiography. Several proteins could be seen to bind to the

---

**Fig. 1.** Numb mutant and overexpression lineages (modified from Ref. 1). A, a simple wild-type external sensory organ lineage. B, numb mutant lineage. The IIb cell is transformed into a IIa cell, which can lead to two sets of socket and hair cells. In numb mosaics, the second stage of the lineage is affected as well (as shown here), resulting in four socket cells. C, when Numb protein is overexpressed only early in the lineage, the IIa cell is transformed into an IIb cell, resulting in two sets of glia and neurons. D, when Numb is overexpressed only after the first cell division, the IIa and IIb cells form normally, but one of their progeny is transformed from a socket to a bristle and/or a glia into a neuron, respectively.

**Fig. 2.** Sequence alignment and UAS Numb constructs. A, amino acid sequence alignment of Shc and Numb PTB domains. Amino acids with asterisks represent residues mutated in the UAS and GST Numb constructs. B, schematic representation of UAS Numb constructs. The full-length wild-type numb cDNA was subcloned into the pUAST vector (27). Four independent constructs (F195V, S148A, R171Q, and F149L) were made with site-directed mutations that had been shown to affect Shc PTB domain binding activity. A Myc epitope tag was attached to the C-terminal end of the Numb coding region in each of the constructs. These constructs were then used to make transgenic lines to assay Numb activity in vivo.
Numb PTB domain but not to the beads containing GST alone. Similarly, specific proteins could be detected that bound to wild-type Numb PTB domain but not to the F195V mutant (see arrows Fig. 6A). However, the S148A and R171Q mutations of the Numb PTB domain did not affect this binding. The effect of the F195V mutation to abrogate binding of the Numb PTB domain correlated closely with the ability of this mutation to impair Numb gain-of-function in vivo. In contrast, the S148A and R171Q mutations had no effect on binding in vitro or Numb function in vivo. Although we identified the binding of the Numb PTB domain to methionine-labeled proteins, none of these proteins appeared to contain phosphotyrosine. In the Schneider S2 lysate we used for these experiments, we found that many of the proteins contained phosphotyrosine as indicated by phosphotyrosine immunoblotting. However, none of these tyrosine-phosphorylated proteins bound to GST alone or to the GST Numb PTB domain (Fig. 6B). Furthermore we tested the ability of the Numb PTB domain to bind tyrosine-phosphorylated proteins from growth factor-stimulated PC12 cells that overexpress the TrkA receptor (Fig. 6C, NGF receptor). Cells were stimulated with EGF or NGF leading to the tyrosine phosphorylation of many proteins in the total lysates. Lysates were incubated with GST alone, GST-Numb PTB domain, or GST-Shc PTB and bound proteins detected by antiphosphotyrosine immunoblotting. Although the activated growth factor receptors (EGF receptor and TrkA) bound to the Shc PTB domain, no tyrosine-phosphorylated proteins bound to the Numb PTB domain.

One of the proteins that bound to the Numb PTB domain in Fig. 6A had a molecular mass greater than 200 kDa. We wondered if this protein might be Notch as it had previously been suggested that the Numb PTB domain can bind Notch (2). We utilized S2 cells that express full-length Notch under the control of the heat shock promoter (30). After induction, S2 cells were lysed in the same lysis buffer used in the [35S]methionine experiments in Fig. 6A. However, in this experiment Notch was phosphorylated.
overexpressed and analyzed by a sensitive immunoblotting technique using anti-Notch antibodies. Notch overexpression after induction was easily detected by immunoblotting cell lysates with anti-Notch antibodies (Fig. 6D, lanes 1 and 2). To determine if the Numb PTB domain could bind Notch, we immobilized the Numb PTB domain on glutathione beads and added lysates from cells with and without induction of Notch overexpression. We used 10 times the amount of lysates used for immunoblotting in the 1st two lanes of Fig. 6D with an excess of Numb PTB domain on glutathione beads. Despite
using this large amount of lysate that contained overexpressed Notch, we were unable to detect Notch binding by the Numb PTB domain. This suggests that Notch may not be a high affinity binding partner for the Numb PTB domain.

**DISCUSSION**

The PTB domain of Shc has been shown to be critical for binding to a number of receptor tyrosine kinases. This domain is well conserved in the *Drosophila* Numb protein (12, 17), suggesting that there may also be some conservation of biochemical activity. A number of amino acid residues have been identified in Shc as being critical for its ability to bind to NPXY motifs (17, 19, 20). Our data demonstrate that the mutation F195V severely impairs the function of the Numb PTB domain in vitro and in vivo. The phenylalanine mutated in this experiment is conserved in almost all members of the PTB domain family (19). These results confirm the utility of using this mutation to probe PTB domain function. Although our data are the first to use point mutants to disrupt Numb PTB domain function, the work of others has also pointed to a crucial role for this domain in Numb function. Frise et al. (11) showed that deletion of the PTB domain impaired Numb function in vivo, and Verdi et al. (23) showed that overexpression of rat Numb PTB domain alone affected neural development in neural crest derived MONC-1 cells.

Numb has been shown to be genetically upstream of Notch and is proposed to inhibit Notch signaling (2). In addition, yeast two-hybrid studies and protein binding assays show that Notch and Numb are capable of a direct physical interaction and that this interaction is probably mediated by the PTB domain of Numb (2). However, in our studies we were not able to detect high affinity interactions between Numb and Notch under conditions where we could detect the binding of several labeled proteins to the Numb PTB domain. Further work will be necessary to determine if the genetic interaction between Numb and Notch rely on direct interactions or occurs via secondary proteins.

A central question that this study helps address is whether phosphotyrosine is required for PTB domain interactions. Original studies of the Shc and insulin receptor substrate-1 PTB domains showed that phosphotyrosine is required for PTB domain interactions. Using this large amount of lysate that contained overexpressed Notch, we were unable to detect Notch binding by the Numb PTB domain. This suggests that Notch may not be a high affinity binding partner for the Numb PTB domain.
Mann and Spyros Aravtis-Tsakonas for providing the Notch C17.9C6 monoclonal antibody.

REFERENCES

1. Rhyu, M. S., Jan, L. Y., and Jan, Y. N. (1994) Cell 76, 477–491
2. Guo, M., Jan, L. Y., and Jan, Y. N. (1996) Neuron 17, 27–41
3. Campos-Ortega, J. A. (1996) Neuron 17, 1–4
4. Hafen, E., and Brand, M. (1997) Curr. Opin. Neurobiol. 7, 29–39
5. Vervoort, M., Dambly-Chaudiere, C., and Ghysen, A. (1997) Curr. Opin. Neurobiol. 7, 21–28
6. Uemura, T., Shepherd, S., Ackerman, L., Jan, L. Y., and Jan, Y. N. (1989) Cell 58, 349–360
7. Brewster, R., and Bodmer, R. (1995) Development 121, 2923–2936
8. Spany, E. P., Kopczynski, C., Goodman, C. S., and Doe, C. Q. (1995) Development 121, 3489–3494
9. Spany, E. P., and Doe, C. Q. (1996) Neuron 17, 21–26
10. Hartenstein, V., and Posakony, J. W. (1990) Dev. Biol. 142, 13–30
11. Frise, E., Knoblich, J. A., Younger-Shepherd, S., Jan, L. Y., and Jan, N. Y. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 11925–11932
12. Bork, P., and Margolis, B. (1995) Cell 80, 693–694
13. Blakie, P., Immanuel, D., Wu, J., Li, N., Yajnik, V., and Margolis, B. (1994) Biochem. Biophys. Res. Commun. 209, 260–265
14. Kavanauagh, W. M., and Williams, L. T. (1994) Science 266, 1862–1865
15. Gustafson, T. A., He, W., Craparo, A., Schaub, C. D., and O’Neill, T. J. (1995) Mol. Cell. Biol. 15, 2509–2518
16. Margolis, B. (1996) J. Lab. Clin. Med. 128, 235–241
17. Zhou, M.-M., Ravichandran, K. S., Olejniczak, E. T., Peters, A. M., Meadows, R. P., Sattler, M., Harlan, J. E., Wade, W. S., Burakoff, S. J., and Fesik, S. W. (1995) Nature 378, 584–592
18. Dikic, I., Batzer, A. G., Blakie, P., Oubermeier, A., Ullrich, A., Schlessinger, J., and Margolis, B. (1995) J. Biol. Chem. 270, 15125–15129
19. Yajnik, V., Blakie, P., Bork, P., and Margolis, B. (1996) J. Biol. Chem. 271, 1813–1816
20. Van der Geer, P., Wiley, S., Gish, G. D., Lai, V. K., Stephens, R., White, M. F., Kaplan, D., and Pawson, T. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 963–968
21. Borg, J.-P., Ooi, J., Levy, E., and Margolis, B. (1996) Mol. Cell. Biol. 16, 6229–6241
22. Charreire, A., Wagner, J., Jacob, S., McGlade, C. J., and Tremblay, M. L. (1996) J. Biol. Chem. 271, 8424–8429
23. Verdi, J. M., Schmandt, R., Bashirullah, A., Jacob, S., Salvino, R., Craig, C. G., Angenent EST Program, Lipschitz, H. D., and McGlade, C. J. (1996) Curr. Biol. 6, 1134–1145
24. Howell, B. W., Gertler, F. B., and Cooper, J. A. (1997) EMBO J. 16, 121–132
25. Li, S.-C., Songyang, Z., Vincent, S. J. F., Zwahlen, C., Wiley, S., Cantley, L., Ray, L., Pernan-Kay, J., and Pawson, T. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 7204–7209
26. Zambrano, N., Buchbaum, J. D., Minopoli, G., Fiore, F., De Candia, P., Cheetham, J., Sudol, M., and Russe, T. (1997) J. Biol. Chem. 272, 6399–6405
27. Brand, A. H., and Perrimon, N. (1993) Development 118, 401–415
28. Ron, D., and Dressler, H. (1992) BioTechniques 13, 866–869
29. Wodarz, A., Hinz, U., Engelbert, M., and Knust, E. (1995) Cell 82, 67–76
30. Lieber, T., Kidd, S., Alcama, E., Corbin, V., and Young, M. W. (1993) Genes Dev. 7, 1949–1965
31. Karsemeyer, R. E., and Rubin, G. M. (1984) Cell 38, 135–146
32. Gustafson, R., and Boulianne, G. L. (1996) Genome 39, 174–182
33. Greig, S., and Akam, M. (1995) Nature 376, 630–635
34. Bodmer, R., and Jan, Y. N. (1987) Roux’s Arch. Dev. Biol. 196, 69–77
35. Zipursky, S. L., Venkatesh, T. R., Teplow, D. B., and Benzer, S. (1984) Cell 36, 15–26
36. Frasch, M., Hoey, T., Rushlow, C., Doyle, H., and Levine, M. (1987) EMBO J. 6, 749–759
37. Patel, N. H. (1994) Drosophila melanogaster: Practical Uses in Cell and Molecular Biology, Goldstein, L. S. B., and Fryberg, E. A. eds, pp. 445–487, Academic Press, San Diego
38. Isakoff, S. J., Yu, Y.-P., Yu, S.-C., Blakie, P., Yajnik, V., Rose, E., Weidner, K. M., Sachs, M., Margolis, B., and Skolnik, E. Y. (1996) J. Biol. Chem. 271, 3959–3962
39. Blakie, P. A., Fourrier, E., Dilworth, S. M., Birnbaum, D., Berg, J.-P., and Margolis, B. (1997) J. Biol. Chem. 272, 20671–20677
40. Wolf, G., Trub, T., Ottinger, E., Groninga, L., Lynch, A., White, M. F., Miyazaki, M., Lee, J., and Shoeless, S. E. (1995) J. Biol. Chem. 270, 27407–27410
41. Dha, S. E., Jacob, S., Wolting, C. D., French, M. B., Roehrnschneider, L. R., and McGlade, C. J. (1998) J. Biol. Chem. 273, 9179–9187

Acknowledgements—We thank Dr. Yuh Nung Jan for the Numb cDNA and Numb antibodies. We also thank the following people for providing us with GAL4 lines: Gabrielle Boulianne for providing the C96-GAL4 line; Elisabeth Knust for providing us with the daGal4-GAL4 line; and Gerhard Technau for providing 60-GAL4, 160-GAL4, 189-GAL4, 281-GAL4, 605/6-GAL4, 1407-GAL4, 1445-GAL4, and 1481-GAL4. Wendy Lockwood kindly donated the twist-GAL4:24B-GAL4 line. Other GAL4 lines were kindly provided by Kathy Matthews at the Bloomington Drosophila Stock Center. We also thank Toby Lieber and Mike Young for providing us with Notch expressing Schneider cells and Robert

3 J. McGlade, personal communication.