Development 125, 3301-3312 (1998)
Printed in Great Britain © The Company of Biologists Limited 1998
DEV8528



# The *Drosophila* EGF receptor controls the formation and specification of neuroblasts along the dorsal-ventral axis of the *Drosophila* embryo

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*Accepted 19 June; published on WWW 6 August 1998*

## SUMMARY

**The segmented portion of the *Drosophila* embryonic central nervous system develops from a bilaterally symmetrical, segmentally reiterated array of 30 unique neural stem cells, called neuroblasts. The first 15 neuroblasts form about 30-60 minutes after gastrulation in two sequential waves of neuroblast segregation and are arranged in three dorsoventral columns and four anteroposterior rows per hemisegment. Each neuroblast acquires a unique identity, based on gene expression and the unique and nearly invariant cell lineage it produces. Recent experiments indicate that the segmentation genes specify neuroblast identity along the AP axis. However, little is known as to the control of neuroblast identity along the DV axis. Here, I show that the *Drosophila* EGF receptor (encoded by the *DER* gene) promotes the formation, patterning and individual fate specification of early forming neuroblasts along the DV axis. Specifically, I use molecular markers that identify particular neuroectodermal domains, all neuroblasts or individual neuroblasts, to show that in *DER* mutant embryos (1) intermediate column neuroblasts do not form, (2) medial column neuroblasts often acquire identities inappropriate for their position, while (3) lateral neuroblasts develop normally. Furthermore, I show that active *DER* signaling occurs in the regions from which the medial and intermediate neuroblasts will later delaminate. In addition, I demonstrate that the concomitant loss of *rhomboid* and *vein* yield CNS phenotypes indistinguishable from *DER* mutant embryos, even though loss of either gene alone yields minor CNS phenotypes. These results demonstrate that *DER* plays a critical role during neuroblast formation, patterning and specification along the DV axis within the developing *Drosophila* embryonic CNS.**

Keywords: *Drosophila*, CNS, EGF receptor, neuroblast, dorsoventral patterning, *DER* gene

## INTRODUCTION

*'A fertilized egg divides into two cells, these into four and so on, until billions of cells have been generated. Why do these cells not simply form a large heap, each cell like the other and perhaps resembling the original undivided egg cell? Why, instead, do some cells become nerve cells, or bone cells or liver cells, or any other of the many differentiations of which cells are capable? Why...are they present not in haphazard disorder but in fixed, specific regions organized into a nervous system, bones, a liver, and all the other organs which themselves have fixed and specific locations?'*
Curt Stern, *American Scientist*, 1954

A central theme in developmental biology is to elucidate the mechanisms that specify particular cell types as well as those that govern where and when these cell types form during animal development. The *Drosophila* embryonic CNS provides an ideal system in which to dissect the developmental programs that create cell-type specific patterns. The segmented portion of the *Drosophila* CNS arises from a simple uniform sheet of neuroectodermal cells located in the ventrolateral region of the blastoderm embryo (reviewed in Goodman and Doe, 1993). The neuroectoderm is sub-divided along the anteroposterior (AP) and dorsoventral (DV) axes into a precise orthogonal pattern of neuroectodermal or 'proneural' clusters. Cell interactions between cells within a cluster direct one cell into the neuroblast fate. These cells enlarge and delaminate into the interior of the embryo to form an invariant pattern of 30 neuroblasts per hemisegment (a hemisegment is a bilateral half of a segment and is the developmental unit of the segmented portion of the CNS). Each neuroblast divides via a specific lineage to produce a characteristic family of neurons and/or glia. In addition, each neuroblast expresses a distinct set of molecular markers. It is clear that within a hemisegment each neuroblast acquires a unique fate based on its position and time of formation. However, the genetic regulatory mechanisms that create the stereotyped neuroblast pattern and specify unique fates to cells at characteristic positions within this pattern remain unclear.

Neuroblast formation occurs in five temporally and spatially distinct waves (S1-S5; Doe, 1992). The first 15

neuroblasts develop during the first two waves, S1 and S2, and form an orthogonal array of four AP rows (1, 3, 5, 7) and three DV columns (medial, intermediate and lateral). 15 additional neuroblasts form during later waves of neuroblast segregation. Neuroblast formation is controlled primarily by the action of two sets of genes. The proneural genes of the *achaete-scute* complex (AS-C) promote neuroblast formation and are expressed in proneural clusters (Jimenez and Campos-Ortega, 1990; Martin-Bermudo et al., 1991; Skeath and Carroll, 1992). The neurogenic genes suppress proneural gene function and limit the number of neuroblasts that form from each cluster (reviewed in Campos-Ortega, 1993). The activity of the AP- and DV-axis patterning genes are thought to establish the pattern of AS-C-expressing proneural clusters in the neuroectoderm, which in turn dictates where and when neuroblasts form (Martin-Bermudo et al., 1991; Rao et al., 1991; Skeath et al., 1992). The role the segmentation genes play along the AP axis to pattern neuroblast formation is well established; however, less is understood about the mechanisms that pattern neuroblasts along the DV axis.

Within a hemisegment each neuroblast acquires a unique fate based on where and when it forms. Recent genetic and cell transplantation analyses indicate that neuroblasts inherit their identity from the cluster of neuroectodermal cells from which they delaminate (Chu-LaGraff and Doe, 1993; Skeath et al., 1995; Udolph et al., 1995). For example, along the AP axis the *wingless* and *gooseberry* segment polarity genes function within the neuroectoderm to promote neuroblast formation and to specify the fate of neuroblasts that develop adjacent to *wingless*-expressing cells or from *gooseberry*-expressing neuroectodermal cells (Chu-LaGraff and Doe, 1993; Skeath et al., 1995). In contrast to the AP axis, little is known about the developmental mechanisms that control neuroblast identity along the DV axis.

*DER* (also referred to as *faint little ball* and *torpedo*), which encodes a receptor tyrosine kinase, is a key determinant of cell fate along the DV extent of the neuroectoderm (reviewed in Schweitzer and Shilo, 1997). In *DER* mutant embryos, lateral cell fates replace ventral cell fates and CNS development is grossly disrupted. Recent genetic, molecular and biochemical analyses indicate that *spitz* and *vein* each encode ligands that activate *DER* independently of one another (Rutledge et al., 1992; Schweitzer et al., 1995; Golembo et al., 1996; Schnepp et al., 1996) and that *DER* uses the conserved RAS signal transduction pathway to transduce signals to the nucleus (reviewed in Seger and Krebs, 1995). Localized transcription of *vein* and *rhomboid* in the ventral domains of the neuroectoderm lead to localized *DER* activation (Bier et al., 1990; Golembo et al., 1996; Schnepp et al., 1996; Gabay et al., 1997a). *rhomboid* encodes a membrane-spanning protein (Bier et al., 1990) that appears to mediate *DER* signaling by helping to process Spitz from its inactive transmembrane form to its active secreted form (Golembo et al., 1996). *vein* encodes a secreted protein (Schnepp et al., 1996).

The earliest known function for *DER* in patterning the ventral ectoderm occurs after germ-band extension at approximately stage 10 (reviewed in Schweitzer and Shilo, 1997), when midline cells appear to secrete Spitz (Golembo et al., 1996). Secreted Spitz (s-Spitz) is thought to diffuse bilaterally to pattern the adjacent ventral neuroectoderm. However, recent results indicate that *DER* signaling is active and regulates gene expression in a discrete DV domain in the neuroectoderm during gastrulation well before the extended germ-band stage (D'Alessio and Frasch, 1996; Gabay et al., 1997a; Yagi and Hayashi, 1997). This suggests that *DER* may play a role prior to the production of s-Spitz by midline cells to pattern the neuroectoderm along the DV axis. However, loss of *DER* activity at this time has not been associated with any morphological defects.

In this paper, I show that *DER* functions to establish the correct pattern, and helps to specify the individual fates, of neuroblasts. Specifically, I show that in *DER* mutant embryos (1) intermediate column neuroblasts do not form, (2) medial neuroblasts are partially mis-specified and acquire some traits characteristic of lateral neuroblasts, while (3) lateral neuroblasts develop normally. I also show that active *DER* signaling is restricted to the medial and intermediate neuroectodermal columns. Furthermore, I demonstrate that the concomitant loss of *rhomboid* and *vein* yield CNS phenotypes indistinguishable from *DER* mutant embryos, even though the loss of either *rhomboid* or *vein* produces only minor CNS defects. In addition, I show that DER is activated twice in the neuroectoderm of the embryo: once at the onset of (and during) gastrulation by a combination of Spitz group and Vein activities, the second time after germband extension, presumably by Spitz activity produced by the midline. Together these results demonstrate that DER functions along the DV axis to promote the formation, patterning, and individual fate specification of neuroblasts within the *Drosophila* CNS.

## MATERIALS AND METHODS

### Genetics

Wild-type patterns of gene expression were examined in Oregon R embryos. Mutant lines used were: DER, alleles $flb^{1K35}$, $flb^{2C82}$ and $flb^{2W74}$, provided by Alan Michelson (Clifford and Schupbach, 1994), and *l(2)03033* (Spradling et al., 1995); $vein^{dddRy}$ and $vein^{\gamma\beta}$ (Schnepp et al., 1996); $spitz^{IIA}$, $Star^{IIN}$ (Nüsslein-Volhard et al., 1984); $rhomboid^{7M}$ (Mayer and Nusslein, 1988) and $rhomboid^{del\text{-}1}$ (Bier et al., 1990); H162 is an enhancer trap line inserted into the *seven-up* gene (Mlodzik et al., 1990) and is referred to as *svp-lacZ*. The double mutant $rhomboid^{7M}$ $vein^{dddRy}$ was generated by recombination.

### Immunohistochemistry and RNA in situ analysis of whole mount embryos

Single- and double-label immunohistochemistry and RNA in situ analysis was performed as described in Skeath et al. (1992). For the active MAP kinase antibody, biotinyl tyramide (NEN Life Science Products) was used to amplify the signal following the manufacturer's protocol. The following antibodies were used at the indicated dilutions: rabbit anti-Eve (1:5000; Frasch et al., 1986); mouse anti-Eve 2B8 (1:50; Patel et al., 1994); mouse anti-Engrailed 4D9 (1:5; Patel et al., 1989); mouse anti-βgal (1:2000; Promega); rabbit anti-βgal (1:15000; Cappel); mouse anti-Pros MR1A (1:4; Spana and Doe, 1995); mouse anti-Ftz (1:1000; Kellerman et al., 1990); rabbit anti-Odd (1:5000; provided by Ellen Ward); rabbit anti-Deadpan (1:300; Bier et al., 1992); and mouse anti-Active MAP kinase (1:2000; Gabay et al., 1997a; Sigma).

## RESULTS

### Initial identification of CNS defects in *DER* mutant embryos

To identify mutations that disrupt embryonic CNS development I screened a collection of approximately 1300 second chromosomal lethal P element lines obtained through the Berkeley *Drosophila* Genome project (Spradling et al., 1995) for defects in the CNS expression pattern of the *eve* gene (Doe et al., 1988; Patel et al., 1989; Broadus et al., 1995). Among the cells that express Eve in the wild-type CNS are the progeny of two medial neuroblasts, the aCC/pCC neurons (neuroblast 1-1) and the U/CQ neurons (neuroblast 7-1), as well as the progeny of intermediate neuroblast 4-2, the RP2 and RP2 sib neurons. In the screen I uncovered one P element mutation, *l(2)03033*, that causes a loss of essentially all Eve-positive RP2/RP2 sib neurons (Fig. 1). This P element mapped to cytological position 57F1-2 in the right arm of the second chromosome and was known to be inserted within the *DER* locus (Spradling et al., 1995). To verify that lesions in *DER* result in a nearly complete loss of RP2 motoneurons I obtained three additional *DER* mutants, including the *DER* null allele, $flb^{1K35}$ (Clifford and Schupbach, 1994). Essentially all Eve-positive RP2 motoneurons are absent from embryos homozygous mutant for each *DER* allele (Fig. 1; Table 1). I used the $flb^{1K35}$ null allele for all subsequent experiments; I refer to $flb^{1K35}$ embryos as *DER* mutant embryos.

### *DER* regulates the DV limits of AS-C proneural gene expression

The absence of the Eve-positive RP2 motoneuron observed in *DER* mutant embryos could arise from a number of different developmental defects. For example, the intermediate neuroblast that produces RP2 may not form, it may not divide or it may acquire an inappropriate identity and not produce RP2. DER is expressed and is active in the neuroectoderm prior to neuroblast formation (Zak et al., 1990; Gabay et al., 1997a,b) but is not expressed in neuroblasts or their progeny prior to or during RP2 motoneuron formation (Zak et al., 1990). Given this and DER's demonstrated role in establishing DV patterning, it seemed plausible that *DER* controls early steps of CNS development by regulating the DV limits of gene expression in the neuroectoderm prior to neuroblast formation.

The initial step of CNS development involves the activation of the AS-C proneural genes in a precise pattern of proneural clusters as gastrulation commences (Cabrera et al., 1987; Martin-Bermudo et al., 1991; Skeath and Carroll, 1992). To investigate whether *DER* regulates AS-C expression in the neuroectoderm, I followed the expression patterns of the *achaete* (*ac*) and *lethal of scute* (*l'sc*) genes in *DER* mutant embryos. Loss of *DER* causes specific defects to the DV registration of *ac* and *l'sc* gene expression in the neuroectoderm; however, I observed no defects to the AP registration of *ac* or *l'sc* gene expression. In wild-type embryos during stages 8/9, *ac* is expressed in the medial and lateral, but not intermediate, clusters of rows 3 and 7 (Skeath and Carroll, 1992); *l'sc* is expressed in the medial and lateral, but not intermediate, clusters of row 7 and in the medial, intermediate and lateral clusters of rows 1 and 5 (Martin-Bermudo et al., 1991). A single neuroblast subsequently forms from each proneural cluster. In *DER* mutant embryos *ac* gene expression expands into the intermediate column in rows 3 and 7 and *l'sc* expression expands into the intermediate column in row 7; *l'sc* is expressed normally in rows 1 and 5 (Fig. 2; not shown for *l'sc*). The lateral limits of *ac* and *l'sc* gene expression in the neuroectoderm are unaltered in *DER* mutant embryos. The changes to the DV registration of *ac* and *l'sc* gene expression in *DER* mutant embryos suggest that neuroectodermal cells in the intermediate column change their fate. Both *ac* and *l'sc* are normally expressed in the medial and lateral columns in the affected rows, thus the phenotype is consistent with intermediate cells acquiring either a lateral or a medial fate. D'Alessio and Frasch (1996) recently showed that *msh-1*, which is expressed exclusively in the lateral column, expands into the intermediate column in *DER* mutant embryos. In this context, it appears that *ac* and *l'sc* expression expand from the lateral column into the intermediate column in the absence of *DER*.

### *DER* promotes the formation of intermediate column neuroblasts

The defects observed to the pattern of *ac*- and *l'sc*-expressing proneural clusters in *DER* mutant embryos suggested that

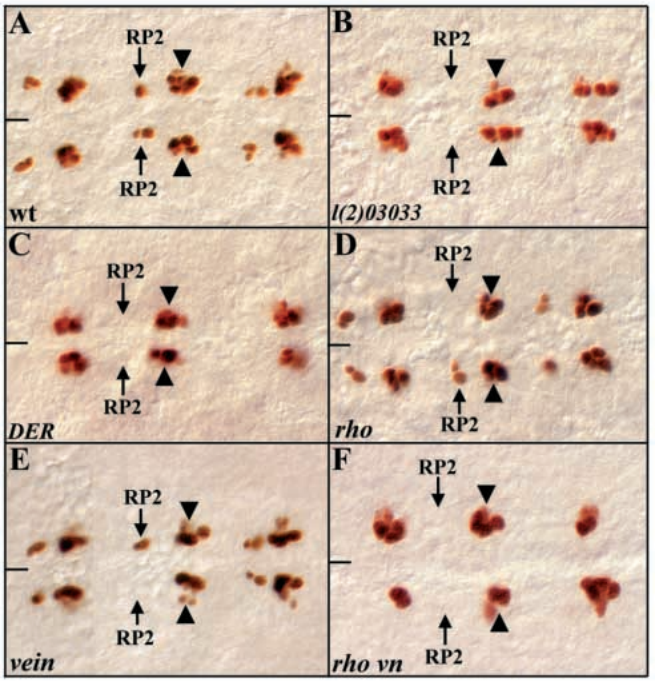


**Fig. 1.** The RP2 motoneuron and its sibling fail to form in the absence of *DER* function. High magnification views of the ventral neuroectoderm of late stage 10 (A) wild-type, (B) *l(2)03033*, (C) *DER*, (D) $rho^{del-1}$, (E) $vn^{dddRy}$ and (F) $rho^{del-1}$ $vn^{dddRy}$ embryos labeled for Eve-protein expression. (A) In wild-type embryos, the Eve-positive RP2 motoneuron and its sibling form (arrows) in every hemisegment just anterior to a cluster of Eve-positive progeny produced by neuroblasts 1-1 and 7-1 (arrowheads). In embryos that contain either (B) a lethal P element insert or (C) a null mutation in *DER*, neither RP2 nor its sibling forms (arrows). In (D) *rho* or (E) *vn* embryos these neurons develop in most hemisegments (arrows). (F) In *rho vn* mutant embryos the neurons do not develop (arrows). Anterior, left; line, ventral midline.

**Table 1. DER promotes RP2 formation and MP2 specification**

| Genotype | RP2 formation | | MP2 specification | | | | | |
|---|---|---|---|---|---|---|---|---|
| | % EVE | *n* | % PROS | *n* | % FTZ | *n* | % ODD | *n* |
| Wild type | 100 | 296 | 98.1 | 414 | 100 | 112 | 99.6 | 276 |
| *flb*$^{IK35}$ | 0.8 | 234 | 52.5 | 240 | 47.7 | 241 | 42.7 | 206 |
| *flb*$^{2W74}$ | 1.1 | 366 | 71.9 | 196 | ND | | 59.7 | 196 |
| *flb*$^{2C82}$ | 0.6 | 328 | 75 | 224 | ND | | 68.7 | 217 |
| *Star*$^{IIN}$ | 99.2 | 238 | 98.0 | 204 | ND | | 99.2 | 260 |
| *spitz*$^{IIA}$ | 100 | 196 | 99.0 | 202 | 98.2 | 219 | 99.6 | 254 |
| *rho*$^{del-1}$ | 76.7 | 348 | 95.3 | 212 | 94.1 | 238 | 96.0 | 202 |
| *rho*$^{7M}$ | 89.2 | 232 | ND | | ND | | 90.9 | 276 |
| *vein*$^{dddRy}$/*vein*$^{\gamma\beta}$ | 94.0 | 436 | 98.5 | 204 | 100 | 228 | 98.5 | 202 |
| *rho*$^{7M}$ *vein*$^{dddRY}$ | 1.4 | 220 | 51.0 | 204 | 46.1 | 206 | 40.2 | 204 |

Percentages indicate the percent formation of an EVE-positive RP2 neuron, PROS-positive, FTZ-positive or ODD-positive MP2 precursor cell. *n*, number of hemisegments scored. ND, not determined.

neuroblast formation would also be altered. I used the anti-Deadpan antibody, which labels all neuroblasts (Bier et al., 1992), to follow neuroblast formation in wild-type and *DER* mutant embryos. Normally early forming neuroblasts develop in three mediolateral columns by mid-stage 9 (Fig. 3A). However, in *DER* mutant embryos only two neuroblast columns develop. With respect to the midline these neuroblasts form in the medial and intermediate columns (Fig. 3B). However, within a hemisegment each neuroblast column contains four SI neuroblasts as per wild-type medial and lateral columns; note that only two SI intermediate column neuroblasts form in wild-type embryos (Fig. 3; Table 2). This, together with the apparent transformation of intermediate column cells towards a lateral fate (Fig. 2) and the absence of RP2/RP2 sib, which form from an intermediate neuroblast (Fig. 1; Table 1), suggest that the intermediate neuroblast column does not form in *DER* mutant embryos.

## Medial neuroblasts acquire traits of lateral neuroblasts in the absence of *DER*

To assay the fate of the neuroblasts that do form in *DER* mutant embryos, I used various molecular markers that are expressed in specific subsets of neuroblasts. In wild-type embryos the row 1 medial (1-1) and lateral (2-5) but not intermediate (3-2) S1 neuroblasts express the Odd-skipped (Odd) protein during stage 9 (Fig. 4A) (Broadus et al., 1995). In the absence of *DER* function two row 1 neuroblasts form and they both express Odd (Figs 3B, 4B) suggesting that these are neuroblasts 1-1 and 2-5. I used Eve expression to determine whether these neuroblasts acquire medial and/or lateral traits. Eve is expressed in the progeny of neuroblast 1-1 (100%; *n*=438) but not 2-5 (Broadus et al., 1995). In the absence of *DER* function, neuroblast 1-1 produces Eve-positive progeny in 88.0% of neuroblasts examined (*n*=457); I never observed Eve-positive progeny in the neuroblast 2-5 position. These results suggest that, for the most part, neuroblast 1-1 acquires its correct fate and are consistent with neuroblast 2-5 acquiring its normal lateral fate. However, 12.0% of the time (*n*=466) neuroblast 1-1 fails to produce Eve-positive progeny and thus fails to follow its normal developmental path. Neuroblast formation and division in the medial column are essentially normal in *DER* mutant embryos (Table 2; data not shown). Thus, the simplest interpretation of these data is that loss of *DER* has no effect on the development of the lateral neuroblast 2-5, causes a low but consistent mis-specification of the medial neuroblast 1-1, and removes the intermediate neuroblast 3-2.

In contrast to Odd, most neuroblasts eventually express Seven-up-lacZ (Svp-lacZ; Doe, 1992). Each neuroblast activates Svp-lacZ at a specific time and expresses Svp-lacZ at a stereotyped quantitative level. In wild-type embryos immediately after S1 neuroblast formation, only neuroblasts 5-2 and 7-4 express Svp-lacZ, and they express Svp-lacZ at high levels (Fig. 5A). In equivalently staged *DER* mutant embryos most neuroblasts in the 5-2 position (54.3%; *n*=116) do not express Svp-lacZ (Fig. 5B) while both row 7 neuroblasts express Svp-lacZ strongly (Fig. 5B). Thus, the row 5 medial neuroblast (5-2) fails to acquire one of its specific traits while the row 7 medial neuroblast (7-1) acquires a trait characteristic of the row 7 lateral neuroblast. As development proceeds in *DER* mutant embryos, most neuroblasts in the 5-2 position eventually activate Svp-lacZ, although relative to wild type they express it at reduced levels. In addition, the neuroblast in the 7-1 position does acquire traits characteristic of its position. For example, in wild-type embryos neuroblast 7-1 divides to produce multiple Eve-positive progeny (99.8%; *n*=438) (Broadus et al., 1995) and in *DER* mutant embryos the majority of neuroblasts in the 7-1 position produce Eve-positive progeny (78.4%; *n*=444). Thus, in the absence of *DER* function medial neuroblasts can acquire appropriate traits. Nonetheless, a significant fraction of medial neuroblasts still fail to activate their appropriate gene expression profile.

**Table 2. Medial neuroblast formation in wild-type, *flb*, *rho vn* and *rho* mutant embryos**

| Neuroblast | Genotype | | | |
|---|---|---|---|---|
| | Wild type | *flb*$^{IK35}$ | *rho*$^{7M}$ *vn*$^{dddRY}$ | *rho*$^{del-1}$ |
| 1-1 | 100% (176) | 98.1% (212) | 96.2% (132) | 100% (112) |
| MP2 | 97.2% (176) | 93.9% (214) | 83.8% (136) | 99.1% (112) |
| 5-2 | 100% (176) | 94.3% (209) | 89.2% (130) | 99.1% (110) |
| 7-1 | 100% (176) | 95.3% (214) | 94.4% (142) | 99.1% (112) |

Percentages indicate percent formation of a Deadpan-expressing neuroblast in the indicated position. Numbers in parentheses indicate number of hemisegments scored.

And, in at least one case, a medial neuroblast, neuroblast 7-1, acquires traits characteristic of its lateral neuroblast neighbor. Thus, loss of *DER* function appears to cause a partial mis-specification of medial column neuroblasts and may cause medial neuroblasts to acquire some traits characteristic of lateral neuroblasts.

At progressively later stages of development, Svp-lacZ can be used to follow the fate of all SI lateral neuroblasts. In wild-type embryos, neuroblast 7-4 activates Svp-lacZ first followed sequentially by the lateral neuroblasts of row 1 (neuroblast 2-5; Fig. 5C), row 3 (3-5) and row 5 (5-6; Fig. 5E). In addition, as one moves posteriorly from neuroblast 7-4 each neuroblast expresses Svp-lacZ at a quantitatively lower level (Fig. 5E). In *DER* mutant embryos lateral column neuroblasts recapitulate the wild-type expression pattern of Svp-lacZ. For example, the lateral row 7 neuroblast activates Svp-lacZ first, at the time and level diagnostic of neuroblast 7-4 (Fig. 5B). Neuroblasts in rows 1, 3 and 5 then express Svp-lacZ in temporal order and at progressively lower levels as per wild-type lateral neuroblasts (Fig. 5D, F). Thus, lateral neuroblasts appear to form and to develop normally in *DER* mutant embryos. These results using Svp-lacZ and Odd expression to follow neuroblast fates, taken together with those using Eve, Ac and L'sc expression, indicate that in the absence of *DER* medial neuroblasts exhibit defects in their specification and intermediate neuroblasts do not form, while lateral neuroblasts develop normally.

### *DER* helps specify the fate of medial column neuroblasts

To examine more closely the effect loss of *DER* has on the fate specification of medial neuroblasts I analyzed carefully the fate of MP2 in *DER* mutant embryos. I focused on MP2 because multiple molecular markers uniquely identify MP2 (Doe, 1992; Broadus et al., 1995; Parras et al., 1996; Skeath and Doe, 1996). MP2 normally localizes the Prospero protein to its nucleus and expresses the Fushi-tarazu (Ftz) and Odd proteins (Fig. 6; Table 1). At equivalent developmental stages no other neuroblasts express Ftz or Odd and all other neuroblasts localize Prospero to their cell cortex. In addition, MP2 divides nearly 1.5 hours after delamination while all other neuroblasts divide nearly immediately after delamination. In *DER* mutant embryos MP2 is often incorrectly specified. Approximately 50% of the neuroblasts in the MP2 position localize Prospero to the cell cortex (Fig. $6B_1$) or divide prematurely (Fig. $6B_2$; Table 1) and fail to activate Ftz or Odd (Fig. 6; Table 1). The remaining fraction of MP2s express Ftz and Odd and localize Prospero protein to the nucleus as per wild-type embryos (Fig. 6; Table 1). Thus, removal of *DER* causes half of all MP2s to acquire traits characteristic of other neuroblasts. Based on results detailed in the previous section, MP2 may acquire traits characteristic of neuroblast 3-5, its lateral neighbor. However, the present lack of markers specific for neuroblast 3-5 precludes a test of this hypothesis. Nonetheless, these results combined with those using Svp-lacZ demonstrate that *DER* helps to specify medial neuroblast fate and indicate that *DER*-independent mechanisms also function to specify medial neuroblast fate.

### *rhomboid* and *vein* act synergistically to activate *DER*

*spitz* and *vein* are expressed in the early embryo and appear to function in independent pathways to activate *DER* during embyrogenesis (Rutledge et al., 1992; Schnepp et al., 1996; Schweitzer and Shilo, 1997). Thus, it is possible that either Spitz or Vein or both activate *DER* to promote neuroblast formation and specification. *rhomboid* and *Star* encode for transmembrane factors (Bier et al., 1990; Kolodkin et al., 1994) that appear to promote the production of s-Spitz and to act in the same linear pathway as *spitz* (Golembo et al., 1996). To investigate the extent to which *spitz*, *rhomboid* and *Star*, as well as *vein* participate in *DER*-mediated control of early CNS development, I assayed neuroblast formation and specification in embryos singly mutant for each gene (Figs 1-3; Tables 1, 2). Early CNS development is essentially normal in embryos singly mutant for *spitz*, *Star* or *vein*. *ac* expression is restricted correctly to the medial and lateral columns (data not shown); an Eve-positive RP2 motoneuron forms at least 94% of the time (Fig. 1; Table 1) and medial neuroblast specification appears normal (Table 1). Embryos that lack *rhomboid* function exhibit more severe, yet still relatively mild, CNS defects. For example, *ac* expression expands incompletely into the intermediate column (Fig. 2C); three neuroblast columns form but there is a weak decrease in the formation of intermediate neuroblasts and their progeny (Fig. 3), and there is a weak mis-specification of medial neuroblasts (Table 1).

Although embryos singly mutant for any one *spitz* group

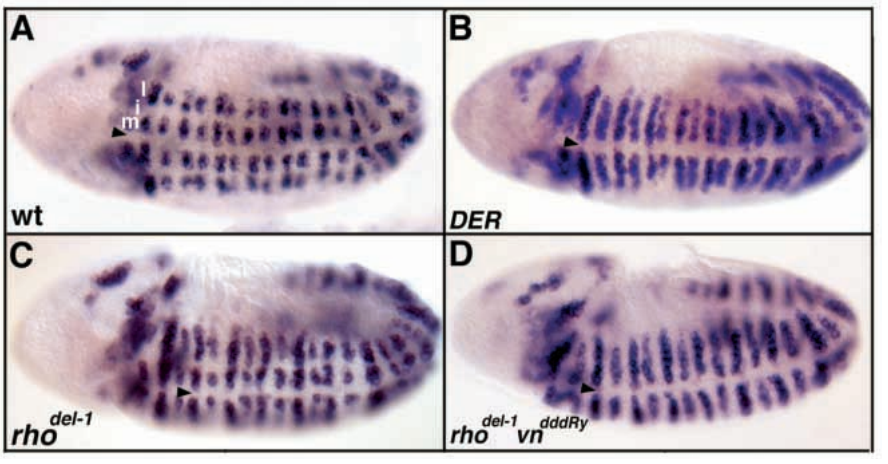


**Fig. 2.** *achaete* expression expands into the intermediate column in *DER* mutant embryos. Late stage 8 whole-mount views of (A) wild-type, (B) *DER*, (C) $rho^{del-1}$ and (D)$rho^{del-1}$ $vn^{dddRy}$ embryos labeled for *ac* transcript. (A) In wild-type embryos *ac* is expressed in proneural clusters in the medial and lateral columns of alternating rows prior to SI neuroblast formation. (B) In the absence of *DER* function or in embryos doubly mutant for *rho* and *vn* (D), *ac* transcripts are detected in the medial, intermediate and lateral columns of these rows. (C) In *rhomboid* mutant embryos, there is an incomplete expansion of *ac* expression into the intermediate column of alternating rows. Anterior, left; arrowhead, ventral midline. m, i, l, indicate positions of medial, intermediate and lateral neuroblast columns, respectively.

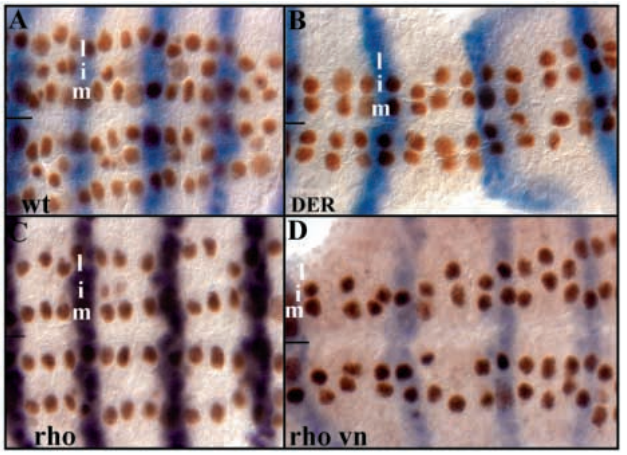


**Fig. 3.** Two rather than three neuroblast columns form in *DER* mutant embryos. High magnification views of the pattern of early forming neuroblasts (brown) in wild-type (A), *DER* (B), $rho^{del-1}$ (C) and $rho^{del-1}$ $vn^{dddRy}$ (D) stage 9 embryos. Deadpan protein is expressed in all neuroblasts and is shown in brown; Engrailed protein (blue) is expressed in the posterior compartment of each segment and indicates segmental position. (A) In wild-type embryos, three neuroblast columns form. From the midline, these are the medial (m), intermediate (i) and lateral (l) columns. (B) In embryos that lack *DER* function, two neuroblast columns develop. (C) In the absence of *rhomboid* function, three neuroblast columns form, however there is a reduction in intermediate neuroblast formation. (D) In *rho vn* double mutant embryos two neuroblast columns develop. Anterior, left; line, ventral midline.

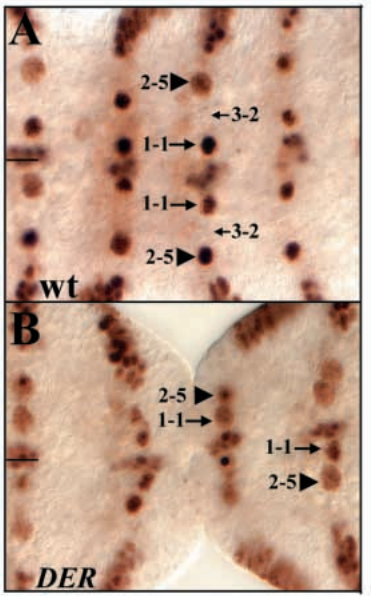


**Fig. 4.** Medial and lateral but not intermediate fate neuroblasts form in *DER* mutant embryos. High-magnification views of the developing ventral CNS in stage 9 (A) wild-type and (B) *DER* mutant embryos labeled for Odd. (A) In wild-type embryos, the medial (1-1) and lateral (2-5) but not intermediate (3-2) column neuroblasts of row 1 express Odd. (B) In *DER* mutant embryos, two Odd-positive neuroblasts form in row 1 (arrows, arrowheads). Anterior, left; line, ventral midline.

gene or *vein* do not display an appreciable early CNS phenotype, it is possible that the *spitz* group genes and *vein* act together to mediate full activation of *DER*. Removal of one group or the other may cause only mild CNS defects because the other remains to activate *DER*. Alternatively, additional factors may activate *DER* to promote early CNS development. To determine the CNS phenotype of removing both *spitz* group activity and *vein*, I constructed a fly stock doubly mutant for *rhomboid* and *vein*. I chose *rhomboid* instead of *spitz* or *Star* because loss of *rhomboid* causes more severe CNS phenotypes than loss of either *spitz* or *Star*. Removal of *rhomboid* and *vein* produces CNS defects virtually indistinguishable from those observed in *DER* mutant embryos (Table 1): *ac* expression expands completely into the intermediate column in rows 3 and 7 (Fig. 2); only two neuroblast columns form (Fig. 3); the RP2 motoneuron almost never forms (Fig. 1); and, roughly half of MP2s are mis-specified (Table 1). The severity of these phenotypes correlates with the null *DER* allele, $flb^{1K35}$, and are stronger than the moderate *DER* alleles, $flb^{2W74}$ and $flb^{2C82}$ (Table 1). The only minor difference I observe is a slightly greater decrease in the formation of the medial neuroblasts, MP2 and 5-2, in *rhomboid vein* mutant embryos relative to *DER* mutant embryos. These data suggest that the activity of the *spitz* group and *vein* are sufficient to account for all signals that activate *DER* during early CNS development.

### *DER* acts during gastrulation to control neuroblast formation and specification

DER signals through the conserved RAS pathway (reviewed in Seger and Krebs, 1995). One of the final effectors of the RAS pathway is MAP kinase. MAP kinase is activated by dual phosphorylation of threonine and tyrosine residues by MEK (Seger et al., 1992; Cobb and Goldsmith, 1995). The recent production of a monoclonal antibody that specifically recognizes the active, dual phosphorylated form of MAP kinase allows one to follow *in situ* the activation pattern of receptor tyrosine kinase pathways such as DER (Gabay et al., 1997a,b). To identify when and in which cells DER signaling is required to promote early CNS development, I assayed for the presence of active MAP kinase in wild-type, *DER*, *vein*, *spitz* and *rhomboid vein* mutant embryos (Fig. 7).

During early embryogenesis, active MAP kinase is present in two temporally and spatially distinct patterns within the neuroectoderm in wild-type embryos (Fig. 7A,D). Prior to and during gastrulation active MAP kinase is first found in two broad bilaterally paired longitudinal bands of cells that run down the length of the neuroectoderm (Fig. 7A; Gabay et al., 1997a,b). At stage 10, active MAP kinase is again expressed in the neuroectoderm in the most medial neuroectodermal cells that flank the midline (Fig. 7D). In *DER* and in *rhomboid vein* mutant embryos active MAP kinase is not present in either pattern (not shown). In embryos singly mutant for either *vein* or *spitz*, the first wave of active MAP kinase appears in its normal pattern although at reduced levels (Fig. 7B,C). However, the second wave of active MAP kinase is absent in *spitz*, but normal in *vein*, mutant embryos (Fig. 7E,F). Thus, early CNS defects correlate with the absence of the first but not the second wave of active MAP kinase as clear CNS defects occur in *DER* and *rhomboid vein* but not in *spitz* mutant embryos.

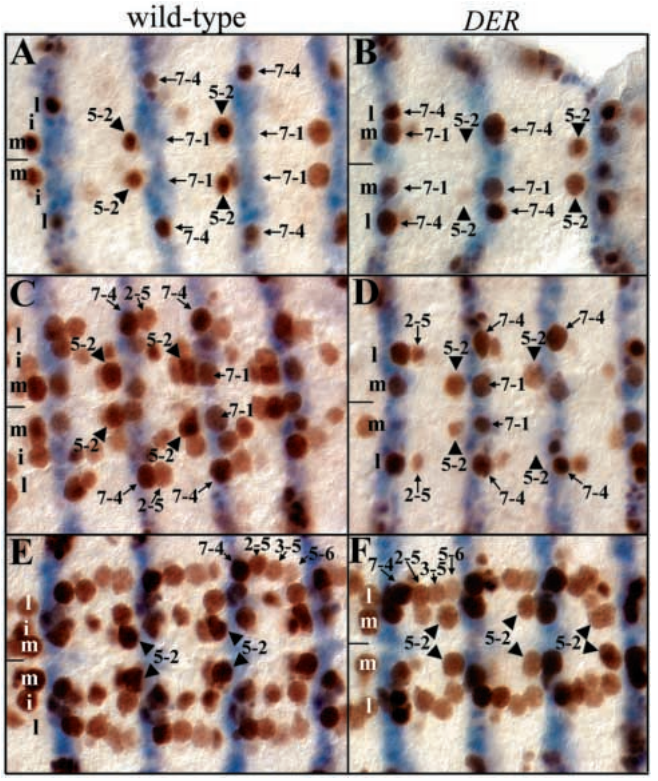


**Fig. 5.** Neuroblast specification in *DER* mutant embryos. High magnification views of the ventral neuroectoderm of (A,B) stage 9, (C,D) stage 10 and (E, F) stage 11 (A,C,E) wild-type and (B,D,F) *DER* mutant embryos labeled for Svp-lacZ expression (brown) to identify neuroblasts and Engrailed expression (blue) to mark segmental position. (A) In wild-type embryos the SI neuroblasts 5-2 and 7-4 express seven-up lacZ as they form (stage 9). (B) As neuroblasts form in *DER* mutant embryos many neuroblasts in the 5-2 position do not express Svp-lacZ, while both the medial and lateral row 7 neuroblasts express Svp-lacZ strongly. (C) During the mid-point of neuroblast formation in wild-type embryos (stage 10), additional neuroblasts located in all three neuroblast columns express Svp-lacZ. Neuroblasts 5-2 and 7-4 still express Svp-lacZ most intensely and neuroblast 2-5, which forms just posterior to neuroblast 7-4, and neuroblast 7-1 are Svp-lacZ positive. (D) At stage 10 in *DER* mutant embryos only two neuroblast columns exist. Slightly greater than half of the neuroblasts in the 5-2 position express Svp-lacZ and both row 7 neuroblasts still express Svp-lacZ strongly and the neuroblast in the 2-5 position begins to express Svp-lacZ. (E) After neuroblast formation is complete in wild-type embryos (stage 11), nearly all neuroblasts express Svp-lacZ. Note that in the lateral column as one moves posteriorly from neuroblast 7-4 to neuroblasts 2-5, 3-5 and 5-6, each neuroblast expresses progressively less Svp-lacZ. (F) In *DER* mutant embryos after neuroblast formation is complete there are two neuroblast columns. Nearly all neuroblasts in the 5-2 position express Svp-lacZ and as one progresses posteriorly from the lateral neuroblast of row 7 each neuroblast expresses less Svp-lacZ. Anterior, left; line, ventral midline; m, i, l indicate the medial, intermediate and lateral neuroblast columns, respectively.

To determine the precise limits of the initial wave of active MAP kinase in the neuroectoderm I performed double-label immunofluorescence and cell count analyses. Immnofluorescence studies using Twist protein expression as a mesodermal marker (Thisse et al., 1988) indicate that active MAP kinase is excluded from the mesoderm, although in rare cases exceptional cells can be found that express both proteins (Fig. 7G-I). The medial to lateral width of the active MAP kinase stripes at stage 7 is 9.0 cells (s.d. 0.91, $n$=29; Fig. 7J). To estimate the width of the medial, intermediate and lateral columns at the same developmental stage I counted the width of the *ac*-positive medial and lateral clusters and the *ac*-negative intermediate clusters in similarly staged embryos. Note at this stage *ac*-positive cells in the medial column also abut mesodermal cells (Skeath and Carroll, 1994). From this analysis, I estimated that the width of the medial column is 5.7 cells (s.d. 0.66, $n$=20), the width of the medial and intermediate column is 8.8 cells (s.d. 0.76, $n$=25) and the width of the neuroectoderm (medial to lateral column) is 12.6 cells (s.d. 0.84, $n$=10). These data indicate that active MAP kinase is present in the medial and intermediate but excluded from the lateral neuroectodermal column (Fig. 7J).

## DISCUSSION

*DER* signaling first becomes active prior to gastrulation in the medial and intermediate neuroectodermal columns. The results in this paper indicate that within the medial column *DER* functions to help specify the fate of medial neuroblasts. Within the intermediate column *DER* functions to promote neuroblast formation. Conversely, *DER* function is dispensable for the development of lateral column neuroblasts. Note that Udolph et al. (1998) have reached similar conclusions through the use of cell transplantation analyses.

### *DER* acts at two stages to pattern the neuroectoderm

To date research on *DER*-dependent regulation of DV patterning in the neuroectoderm has focused predominantly on the midline as the source of the signal, s-Spitz, that activates *DER* (reviewed in Schweitzer and Shilo, 1997 and references therein). The results in this paper show that *DER* acts before midline cell production of s-Spitz to pattern the neuroectoderm. (1) In *DER* mutant embryos, defects in neuroectodermal gene expression and CNS development occur before midline production of s-Spitz (this paper and D'Alessio and Frasch, 1996; Yagi and Hayashi, 1997). (2) The presence of *DER*-dependent active MAP kinase staining in the medial and intermediate columns prior to and during gastrulation correlates with the defects observed in the formation and specification of medial and intermediate neuroblasts in *DER* mutant embryos. (3) Early CNS defects correlate with the absence of the first but not second wave of active MAP kinase in the neuroectoderm: in *DER* mutant embryos both waves of active MAP kinase are absent and severe CNS defects arise, while in *spitz* mutant embryos only the second wave is absent and CNS development is grossly normal. (4) CNS development is essentially normal in *single-minded* mutant embryos in which midline cells do not form (unpublished observations). Thus, midline cell production of s-Spitz appears not to play an early role to form, pattern and specify neuroblasts along the DV axis. However, s-Spitz clearly plays a crucial role in later patterning events in the neuroectoderm (Schweitzer and Shilo, 1997 and references

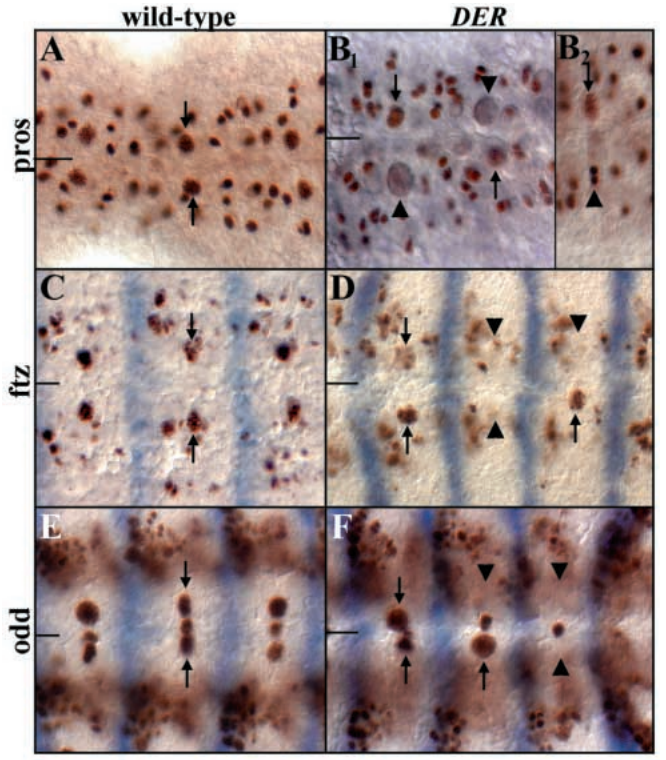


**Fig. 6.** MP2 is incorrectly specified in *DER* mutant embryos. High magnification ventral views of the developing CNS in stage 10 wild-type (A,C,E) and *DER* (B,D,F) embryos labeled for Prospero (A,B), Ftz (C,D) and Odd (E,F) proteins. In wild-type embryos, MP2 localizes Prospero protein to the nucleus (arrows, A), and expresses both the Ftz (arrows, C) and Odd (arrows, E) proteins. In *DER* mutant embryos, roughly half of the MP2s localize Prospero protein to the cell cortex (arrowheads, $B_1$) or divide prematurely (arrowheads, $B_2$) and fail to express either Ftz (arrowheads, D) or Odd (arrowheads, F). The remaining neuroblasts in the MP2 position localize Prospero protein normally to the nucleus and express Ftz and Odd (arrows, B,D,F) proteins. Anterior, left; line, ventral midline.

therein). Furthermore, as shown by Schnepp et al. (1996) and confirmed and extended in this paper, Vein acts with the *spitz* group to activate *DER* in the neuroectoderm prior to and during gastrulation. The simplest interpretation of these data is that two ligands, Vein and s-Spitz, activate *DER* prior to gastrulation within the medial and intermediate columns of the neuroectoderm. *DER* activity would then promote the formation of intermediate column neuroblasts and the fate specification of medial column neuroblasts. Thus, with respect to early DV patterning of the neuroectoderm one must now consider that *DER* acts at two separate times in two overlapping cell populations to pattern the neuroectoderm, and that two activating ligands act independently of each other to activate *DER* during the first wave of *DER* signaling.

### *DER* activity subdivides the early neuroectoderm

How do individual neuroblasts acquire unique fates? Neuroblasts form from equivalence groups called proneural clusters that arise in an invariant orthogonal pattern in the neuroectoderm (Martin-Bermudo et al., 1991; Skeath et al., 1992). Each neuroblast acquires a unique fate and neuroblasts appear to acquire their identity from the neuroectodermal cluster from which they form (reviewed in Goodman and Doe, 1993). Thus, each neuroectodermal cluster should be programmed to produce a specific neuroblast. Recent results suggest a model whereby the superimposition of the activities of the segment polarity genes along the AP axis and the activities of *DER*, *vnd* and *msh-1* along the DV axis could bestow a unique identity, in the form of differential gene expression or activity, upon each neuroectodermal cluster and thus on each neuroblast (Fig. 8).

Along the AP axis the segment polarity genes are expressed in transverse rows that divide each hemisegment into discrete AP domains. Within these domains segment polarity genes promote the formation and fate specification of neuroblasts (Patel et al., 1989; Chu-LaGraff and Doe, 1993; Zhang et al., 1994; Skeath et al., 1995; Bhat, 1996; Dunman-Scheel et al., 1997). For example, *gooseberry* enables row 5 neuroblasts to acquire fates different from neuroblasts in the adjacent anterior row (Skeath et al., 1995). Along the DV axis, the activity/expression of *DER*, *vnd* and *msh-1* divides the neuroectoderm into the three mediolateral domains from which the three SI neuroblast columns form (Fig. 8). *vnd* and *msh-1* expression is restricted, respectively, to the medial and lateral columns (Jimenez et al., 1995; Mellerick and Niremberg, 1995; D'Alessio and Frasch, 1996; Isshiki et al., 1997) and *DER* signaling is active in the medial and intermediate columns. *vnd* may enable medial neuroblasts to acquire fates different than intermediate neuroblasts, as the *vnd* phenotype is consistent with a duplication of intermediate fates at the expense of medial fates (Skeath et al., 1994; J. McDonald, C. Doe, D. Mellerick Dressler, personal communication; F. Jimenez and K. White, personal communication). *msh-1* appears to help specify lateral neuroblast fates, as loss of *msh-1* duplicates intermediate neuroblasts while lateral neuroblasts fail to develop correctly (Isshiki et al., 1997; Buescher and Chia, 1997). As shown in this paper, *DER* promotes the formation of intermediate neuroblasts and helps to specify the individual fate of medial neuroblasts. *DER* may function at or near the top of the hierarchy that controls neuroblast formation and fate along the DV axis, as *DER* activity restricts *msh-1* expression to the lateral column (D'Alessio and Frasch, 1996) and may modulate Vnd activity medially, since Vnd contains five consensus MAP kinase sites (D. Mellerick-Dressler, personal communication). Clearly additional genes likely act with and/or downstream of *DER*, *vnd* and *msh-1* to maintain this subdivision and potentially to subdivide the neuroectoderm further.

The expression patterns and phenotypes of the segment polarity genes and *DER*, *vnd* and *msh-1* suggest that these genes play similar roles to pattern, form and specify neuroblasts along the AP and DV axes, respectively. Note that the superimposition of the expression/activity of these genes subdivides the neuroectoderm into an orthogonal pattern of cell clusters (Fig. 8). This pattern of cell clusters matches very closely, and likely represents, the actual pattern of neural equivalence groups, 'proneural clusters', from which individual neuroblasts arise. Each cluster expresses a unique combination of these patterning genes and the unique gene expression profile of a cluster likely determines or, at least,

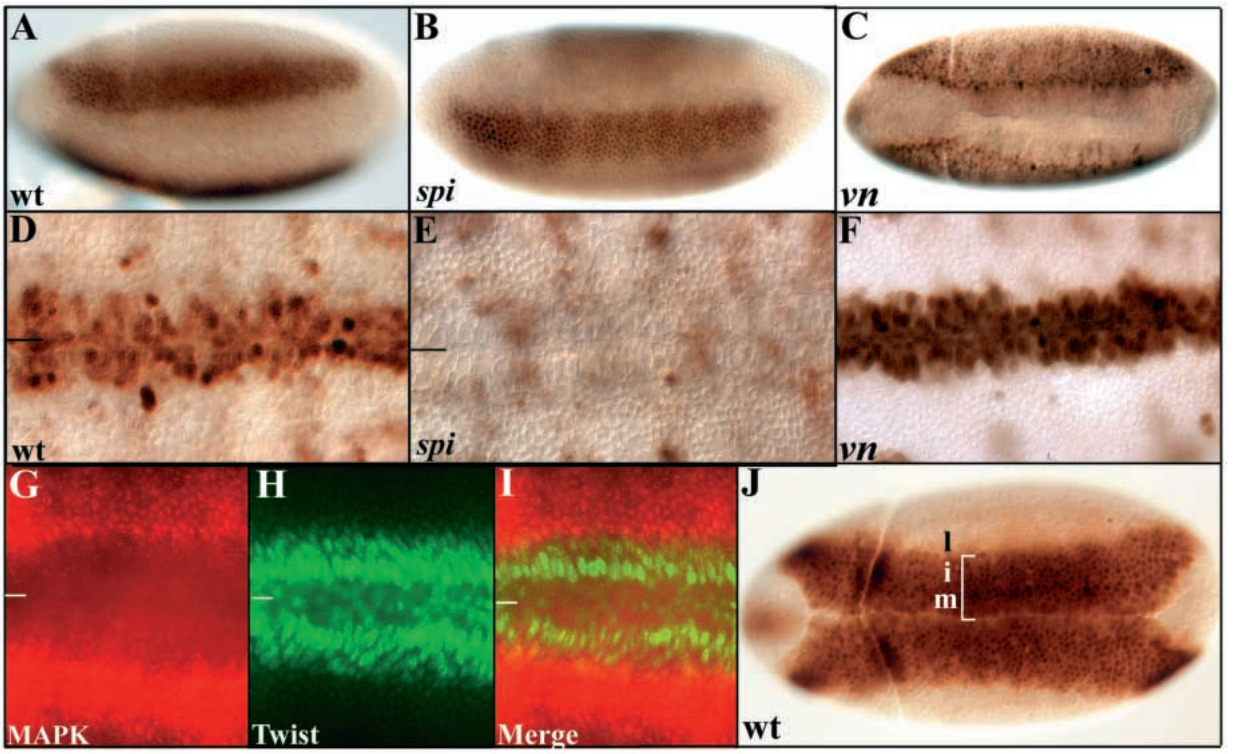


**Fig. 7.** Active *DER* signaling occurs in the neuroectoderm prior to neuroblast formation. (A-C) Whole-mount views of stage 6 and (D-F) high magnification ventral views of stage 10 wild-type (A,D), *spitz* (B,E) or *vein* (C,F) mutant embryos labeled for active MAP kinase. (G-I) High magnification ventral view of stage 6 wild-type embryo doubly labeled for active MAP kinase (G), Twist (H) or both proteins (I). (J) Wild-type stage 7 embryo labeled for active MAP kinase. (A) In wild-type embryos prior to and during gastrulation (stage 6), active MAP kinase is present in bilaterally symmetric stripes in the presumptive neuroectoderm. In stage 6 *spitz* (B) or *vein* (C) mutant embryos active MAP kinase is present at reduced levels in its normal neuroectodermal pattern. (D) In stage 10 wild-type embryos, active MAP kinase staining is detected in the most ventral neuroectodermal cells that flank the midline. (E) In stage 10 *spitz* mutant embryos no active MAP kinase staining is detected in these cells while these cells contain active MAP kinase in *vein* mutant embryos (F). (G) In stage 6 wild-type embryos active MAP kinase is found in the neuroectoderm and (H) Twist is expressed in the mesoderm. (I) Merged image of active MAP kinase and Twist illustrate that mostly non-overlapping cell populations express these two proteins. (J) Active MAP kinase is present in the medial (m) and intermediate (i) but not lateral (l) neuroectodermal columns (see text). Anterior, left; small line in D and E, ventral midline.

helps determine, the fate of each neuroblast that will later form from that cluster. The subsequent process of *Notch*-mediated lateral inhibition would then act within each cluster to produce a uniquely fated neuroblast (reviewed in Campos-Ortega, 1993).

The model can explain the early CNS defects observed in *DER* mutant embryos (Fig. 8). Loss of *DER* appears to remove the boundary between the intermediate and lateral columns and to cause intermediate column cells to acquire lateral cell fates. For example, *msh-1* and *ac* gene expression appear to expand from the lateral into the intermediate column in *DER* mutant embryos (D'Alessio and Frasch, 1996; Yagi and Hayashi, 1997; this paper). AP subdivision of the neuroectoderm is normal at this stage. Thus, in a given AP row, cells within the intermediate and lateral columns appear to express the same combination of genes (Fig. 8). In each row, one early forming neuroblast forms from this domain and it acquires a lateral fate (Fig. 8). Thus, it is likely that this region represents a lateral neural equivalence group of twice its normal width. *Notch*-mediated lateral inhibition would then act within cells of this group to choose one as the neuroblast. *vnd*, which is initially expressed normally in *DER* mutant embryos (Gabay et al., 1996), likely specifies the fate of medial column cells and thus promotes the formation and, to some extent, the proper fate specification of medial neuroblasts in the absence of *DER*. Thus, a failure in the process of *DER*-mediated subdivision of the neuroectoderm appears to result in the formation of two rather than three neuroblast columns. An alternative, but not mutually exclusive, model to *DER* dependent subdivision of the neuroectoderm, is that *DER* normally functions to oppose *Notch* pathway activity during lateral inhibition in the neuroectoderm. *DER* signaling has been shown to oppose *Notch* function in other tissues (e.g. see Miller and Cagan, 1998). Thus, loss of *DER* function might increase effective *Notch* signaling and result in an overall decrease in neuroblast formation. This model alone, however, does not readily explain the specific loss of intermediate column neuroblasts in *DER* mutant embryos.

### *DER* helps promote the specification of medial neuroblasts

*DER* helps to specify the fate of medial neuroblasts, as loss of *DER* causes a failure of medial neuroblasts to display

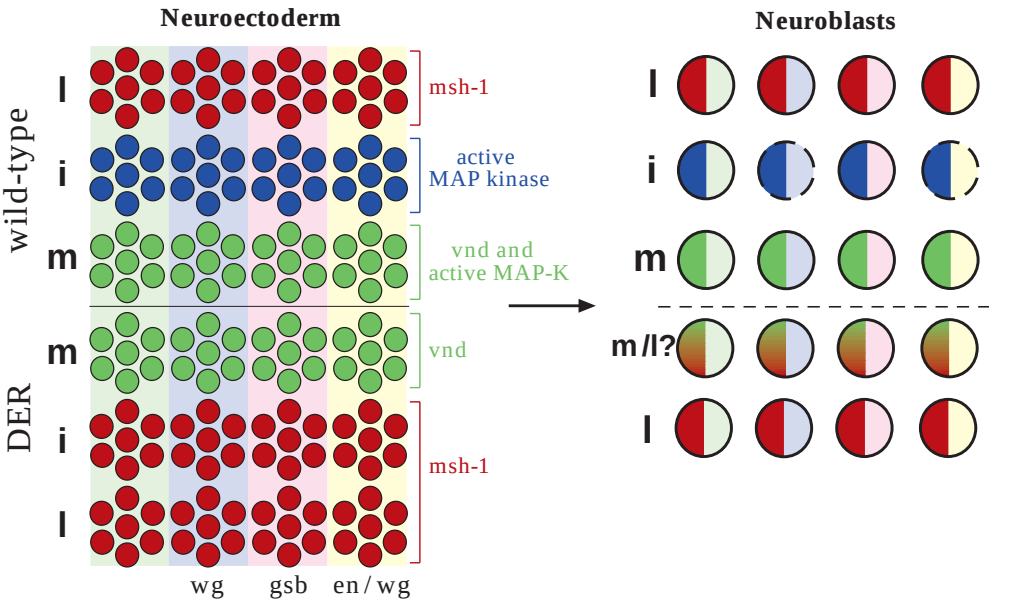


**Fig. 8.** Schematic model of the subdivision of the neuroectoderm by *DER*, *vnd*, *msh-1* and segment polarity genes (left) and the subsequent generation of uniquely fated neuroblasts (right) in wild-type (top) and *DER* (bottom) mutant embryos. (Top) The expression and/or activity of *vnd*, *DER* and *msh-1* subdivide the neuroectoderm into three medial to lateral columns, while segment polarity gene expression and/or activity subdivides the neuroectoderm along the AP axis. The expression domain of *gooseberry* (*gsb*) and *engrailed* (*en*) and the domains in which active *wingless* (*wg*) signaling occurs are indicated. The superimposition of these patterns generates an orthogonal pattern of cell clusters. Each cluster expresses a unique combination of these genes and produces a uniquely fated neuroblast. Two S2 neuroblasts are indicated by dashed lines. (Bottom) In the absence of *DER*, the neuroectoderm appears to be divided into two neuroectodermal domains as cells in the intermediate column appear to acquire lateral fates, as indicated by expanded *msh-1* expression. Subsequently, only one neuroblast column forms from the intermediate and lateral domains and these neuroblasts acquire lateral fates. Note that medial neuroblasts can acquire traits characteristic of both medial and lateral neuroblasts in *DER* mutant embryos. Anterior, left; line, ventral midline; m, i, and l mark the medial, intermediate and lateral neuroectodermal columns, respectively.

characteristic gene expression profiles and cell behaviors. Furthermore, in at least one case, loss of *DER* function causes a medial neuroblast to acquire traits characteristic of a lateral neuroblast; I never observed medial neuroblasts that acquired traits characteristic of intermediate neuroblasts. *DER* is clearly not the sole factor that promotes medial neuroblast fate as medial neuroblasts can develop normally in the absence of *DER* function. In fact, *vnd* may be the critical factor that determines medial neuroblast fates as its loss of function phenotype is consistent with a duplication of intermediate fates at the expense of medial fates (Skeath et al., 1994; J. McDonald, C. Doe, D. Mellerick Dressler, personal communication; F. Jimenez and K. White, personal communication). *DER* does not regulate the initial activation of *vnd* expression (Gabay et al., 1996), but as noted *DER* may affect medial neuroblast fate through modulation of Vnd activity.

With respect to MP2 specification, loss of *DER* produces an essentially identical phenotype to that caused by replacing the endogenous expression of *ac* and *scute* in MP2 with that of *l'sc* (Parras et al., 1996; Skeath and Doe, 1996). However, loss of *DER* function does not change the AP registration of AS-C gene expression. Thus, the activity of *DER* and the specific combination of proneural genes expressed in a neuroblast may act in parallel to specify medial neuroblast fate. If these pathways are partially redundant this might explain the observation that only a fraction of medial neuroblasts are mis-specified in the absence of *DER* function or upon mis-expression of the AS-C genes. In the future, it will be instructive to remove *DER* function in embryos in which *l'sc* gene expression replaces that of *ac* and *sc* in MP2 to determine the effect of removing both inputs on medial neuroblast fate.

Many additional factors likely contribute to neuroblast specification. For example, the initial phases of *DER* signaling and *msh-1* expression within the neuroectoderm disappear prior to the segregation of late forming neuroblasts. Thus, additional factors must act along the DV axis to pattern and to specify the fate of these neuroblasts. Also, genes other than AS-C proneural genes are expressed in neuroblasts and the proneural clusters from which they form (Duffy and Gergen, 1990; Chu-LaGraff et al., 1995; McDonald and Doe, 1997). Some of these genes, like *huckebein* and *runt*, are known to control specific aspects of the fate of individual neuroblasts, while the effect of other such genes on neuroblast specification awaits investigation. Future experiments that address these and other questions will lead to an increasingly lucid picture of the genetic regulatory mechanisms that pattern and specify the fate of neuroblasts in the *Drosophila* embryonic CNS.

The initial phase of this work was initiated in the laboratory of Chris Doe and I am grateful for his support. I also thank Todd Laverty and Gerald Rubin and the BDGP for the use of the second chromosomal lethal P element collection, and Julie Gates and Carl Thummel for their generosity in sending me the collection. I thank Manfred Frasch, Barry Condron, Siegfried Roth, Harald Vaessin, Ellen Ward, Nipam Patel, Eric Spana and Ian and Diane Duncan for antibodies. In addition, I thank Kathy Matthews and the Bloomington Stock Center for providing many stocks and Amanda Simcox, Alan Michelson and Shigeo Hayashi for sending stocks and for helpful discussions. I also thank Julie Broadus, Chris Doe, Jocelyn MacDonald, Dervla Mellerick-Dressler, Fernando Jimenez and Kalpana White for

communication of unpublished results. Finally, I thank Raphi Kopan, Ross Cagan, Susan Spencer, Chris Doe, Julie Broadus, Grace Panganiban, Teresa Orenic, Hemlata Mistry and Ellen Ward for critical comments on the manuscript. This work was supported by a grant from NINDS (RO1-NS36570). J. B. S is supported by the Cancer Research Fund of the Damon Runyon-Walter Winchell Foundation Award, DRS-9 and by a HHMI Research Resources Program for Medical Schools Junior Faculty Award #76296-538202.

## Note added in proof

Similar results have been obtained by Y. Yagi, T. Suzuki and S. Hayashi as those presented here.

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