Convergence of Dorsal, Dpp, and Egfr Signaling Pathways Subdivides the Drosophila Neuroectoderm into Three Dorsal-Ventral Columns

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An important question in neurobiology is how different cell fates are established along the dorsoventral (DV) axis of the central nervous system (CNS). Here we investigate the origins of DV patterning within the Drosophila CNS. The earliest sign of neural DV patterning is the expression of three homeobox genes in the neuroectoderm—ventral nervous system defective (vnd), intermediate neuroblasts defective (ind), and muscle segment homeobox (msh)—which are expressed in ventral, intermediate, and dorsal columns of neuroectoderm, respectively. Previous studies have shown that the Dorsal, Decapentaplegic (Dpp), and EGF receptor (Egfr) signaling pathways regulate embryonic DV patterning, as well as aspects of CNS patterning. Here we describe the earliest expression of each DV column gene (vnd, ind, and msh), the regulatory relationships between all three DV column genes, and the role of the Dorsal, Dpp, and Egfr signaling pathways in defining vnd, ind, and msh expression domains. We confirm that the vnd domain is established by Dorsal and maintained by Egfr, but unlike a previous report we show that vnd is not regulated by Dpp signaling. We show that ind expression requires both Dorsal and Egfr signaling for activation and positioning of its dorsal border, and that abnormally high Dpp can repress ind expression. Finally, we show that the msh domain is defined by repression: it occurs only where Dpp, Vnd, and Ind activity is low. We conclude that the initial diversification of cell fates along the DV axis of the CNS is coordinately established by Dorsal, Dpp, and Egfr signaling pathways. Understanding the mechanisms involved in patterning vnd, ind, and msh expression is important, because DV columnar homeobox gene expression in the neuroectoderm is an early, essential, and evolutionarily conserved step in generating neuronal diversity along the DV axis of the CNS.

INTRODUCTION

An early event in Drosophila embryogenesis is the subdivision of the embryo into specific domains along the dorsoventral (DV) axis. Three signaling pathways—Dorsal, Decapentaplegic (Dpp), and Epidermal Growth Factor Receptor (Egfr)—work in concert to subdivide the embryo into specific tissue types: mesoderm, neuroectoderm, dorsal epidermis and PNS, and amnioserosa (Fig. 1A). Each tissue is further subdivided into more precise DV domains. For example, the neuroectoderm expresses three homeobox genes in adjacent DV columns: the ventral column expresses vnd, the intermediate column expresses ind, and the dorsal column expresses msh (Mellerick and Nirenberg, 1995; Jiménez et al., 1995; D’Alessio and Frasch, 1996; Isshiki et al., 1997; Weiss et al., 1998). Here we investigate how the Dorsal, Egfr, and Dpp pathways converge to establish the three domains of homeobox gene expression along the DV axis of the CNS.

The ventral side of the embryo is patterned by maternally contributed Dorsal protein, a member of the Rel/NF-kB family, which is selectively transported into ventral nuclei in a graded fashion such that the highest levels of Dorsal protein are found in the most ventral nuclei (reviewed in Anderson, 1998). Nuclear localization of Dorsal is regulated by Cactus protein, which binds to Dorsal and prevents its nuclear localization (Whalen and Steward, 1993). In embryos lacking Cactus, high levels of Dorsal protein accumulate in both ventral and dorsal nuclei, leading to dorsal cells acquiring ventral fates. Peak levels of Dorsal in ventral regions activate the twist and snail genes resulting in mesodermal cell fates (reviewed in Rusch and Levine, 1996). Intermediate levels of Dorsal can directly or indi-
directly activate neuroectoderm-specific genes including vnd and rhomboid (rho); these genes are not expressed in the mesoderm because they are repressed by Snail (Mellerick and Nirenberg, 1995; Ip et al., 1992; Thisse et al., 1991; Jiang et al., 1991). Thus, the Dorsal gradient promotes the formation of mesoderm and ventral ectoderm, positions the boundary between them, and activates vnd expression; however, its role in promoting or repressing ind and msh expression is unknown.

The dorsal surface of the embryos is patterned by zygotically expressed Dpp, a secreted protein of the TGFβ family. dpp transcription is repressed by Dorsal, thus limiting dpp expression to the dorsal surface of the embryo. The Dpp activity gradient is hard to predict, but it is clearly high dorsally and much lower within the neuroectoderm due to expression of the Dpp pathway antagonists brinker (brk) and short gastrulation (sog) within the neuroectoderm (Biehs et al., 1996; Jazwinska et al., 1999). Embryos with reduced Dpp activity show an expansion of neuroectoderm at the expense of dorsal structures (amnioserosa and dorsal epidermis; D'Alessio and Frasch, 1996; Jazwinska et al., 1999; Irish and Gelbart, 1987; Ferguson and Anderson, 1992a,b; Wharton et al., 1993) and have been reported to show an expansion of the vnd within the neuroectoderm (Mellerick and Nirenberg, 1995). In contrast, ectopic Dpp activity leads to expansion of dorsal tissues at the expense of neuroectoderm (D'Alessio and Frasch, 1996; Jazwinska et al., 1999; Ferguson and Anderson, 1992a,b). Thus, the Dpp gradient promotes the formation of dorsal epidermal tissues and establishes the dorsal boundary of the neuroectoderm, but its role in specifying different DV domains within the neuroectoderm has not been fully explored.

**FIG. 1.** Summary of dorsal-ventral patterning in the Drosophila embryo. (A) Schematic depiction of the limits of Dpp, Egfr, and Dorsal signaling. Egfr signaling is active in the intermediate and ventral columns. Dorsal is active ventrally in tissues that give rise to mesoderm and ventral ectoderm. Dpp signaling is active dorsally, outside the neuroectoderm; dashed line indicates that the activity of Dpp is lower but uncharacterized within the neuroectoderm. (B) Summary of the results from this paper and others. See Materials and Methods for genotypes; see text for details.
The Egr signaling pathway has also been implicated in DV patterning within the CNS (Skeath, 1998; Yagi et al., 1998). Egr is ubiquitous, but its ligand Spitz is restricted to the ventral midline of the neuroectoderm. An additional activating ligand, Vein, is also expressed in the ventral neuroectoderm and is important for robust activation of the Egr pathway (Schnepf et al., 1996; Golembo et al., 1999). Consistent with these data, a reporter for active Egr signaling (diphosphorylated MAP kinase) is detected in the ventral and intermediate columns of the neuroectoderm (Skeath, 1998; Yagi et al., 1998). Embryos lacking Egr function show early defects in neuroblast formation in the intermediate column of the neuroectoderm (Skeath, 1998; Yagi et al., 1998), and late defects in gene expression within the ventral neuroectoderm (Yagi et al., 1998). However, it is not known whether Egr is involved in establishing vnd, ind, or msh expression domains, nor whether it acts by modulating Dorsal or Dpp activity.

MATERIALS AND METHODS

Fly Lines

yw flies were used as the wild-type stock. Embryos lacking Dorsal function (dorsal mothers) were derived from homozygous dorsal flies. Embryos with ectopic Dorsal function were derived from homozygous cactus mothers.

Embryos lacking Dpp function (dpp embryos) were homozygous dpp/homozygous dpp/CyOZ3 flies. Embryos with ectopic Dpp function (ectopic Dpp) embryos were either homozygous sog/embryos derived from sog/FTM lacerZ flies (Fig. 7E) or homozygous sog/embryos derived from sog/FTM lacerZ; Dp(2;2)DTD48/CyOZ3, Pdpp+ flies (Figs. 3E and 4G), or homozygous brk/Sog/embryos derived from brk/Sog/FTM lacerZ flies (Figs. 3F, 4H, and 7F). The latter two stocks were provided by Jazwinska and Roth (Jazwinska et al., 1999).

Embryos lacking Egr function (Egr embryos) were either homozygous Egr/ or homozygous rho/ embryos. Embryos expressing or rho/ embryos (Fig. 5D and 5H) were derived from Embryos expressing or rho/ embryos (obtained from Jim Skeath, Washington University, St. Louis, MO). rho/ embryos have an indistinguishable phenotype (Skeath, 1998). Ectopic activation of the Egr pathway was accomplished by overexpression of rho using a heat shock-inducible promoter (Sturtevant et al., 1993). Cyo, rho embryos, 2-3 h old, were heat shocked at 37°C for 25 min and then allowed to recover for 1 h at 25°C before fixing.

Embryos lacking both Dorsal and Dpp function (dorsal dpp embryos) were of the genotype dorsal/CyOZ3 flies originated from homozygous dorsal mothers obtained from Dp(2;1)G146dpp+ /dorsal/CyOZ3 flies (Panzer et al., 1992) crossed to dorsal/CyOZ3. Of 93 embryos scored for ind expression, 9 had a ring of ind around the head region (due to expansion of a dorsal-lateral head domain of ind expression); this phenotype is not seen in either single mutant and was observed at a ratio of 1:10, consistent with the ratio of 1:12 dorsal dpp embryos observed in a previous study (Panzer et al., 1992). The “ind head ring” was then used to identify dorsal dpp embryos in subsequent experiments.

vnd ind embryos were obtained from vnd/FTM6; ind/TM3 flies.

mRNA and Protein Detection in Embryos

Embryos were collected and fixed according to standard procedures (McDonald et al., 1998). Primary antibodies used were rabbit anti-Vnd (1:20; Mc Donald et al., 1998), rabbit anti-Msh (1:500; T. Ishii and A. Nose), rat anti-ind (1:250; Weiss et al., 1998), mouse anti-diphosphorylated MAP kinase (1:2000; Gabay et al., 1997; Sigma), mouse anti-β-galactosidase (1:500; Promega), and rabbit anti-β-galactosidase (1:5000; Cappel). Fluorescent images were collected using a Bio-Rad confocal microscope. Histochemical images were collected using a Zeiss Axioplan and a Sony DFC-5000 digital camera. Standard methods were used for RNA in situ hybridizations (Tautz and Pfeiffle, 1989). and ind and msh CDNA clones have been described (Ishii et al., 1997; Weiss et al., 1998); vnd cDNA was a gift from Dervela Mellerick (Michigan).

RESULTS

Initiation of vnd, ind, and msh Expression

Previous studies have shown that vnd, ind, and msh are expressed in adjacent domains of the neuroectoderm, from ventral to dorsal, respectively (Mellkierick and Nirenberg, 1995; Jiménez et al., 1995; D’Alessio and Frasch, 1996; Ishii et al., 1997; Weiss et al., 1998); however, the timing and spacing of their initial expression patterns have not been investigated. Using double-label in situ hybridization, we find that early stage 5 embryos express vnd in a narrow domain similar to its final width; ind and msh are not detected (Fig. 2A; staging according to Campos-Ortega and Hartenstein, 1985). By the end of stage 5, both vnd and ind are expressed with a one to two cell wide gap; again in domains similar to their final widths (Fig. 2B). The gap fills in during development resulting in the precise juxtaposition of the vnd and ind domains (Weiss et al., 1998). Expression of msh in the trunk is not detected until stage 7 (Fig. 2C). Thus, the timing of gene expression from ventral to dorsal: vnd is detected first, ind appears soon after, and msh is observed last.

Activation and Patterning of the vnd Expression Domain

To investigate the mechanisms establishing the domain of vnd expression we examined embryos lacking, or ectopically expressing, each of the three known signaling pathways active along the DV axis (Dorsal, Dpp, and Egr). We confirm that embryos lacking Dorsal function (called dorsal embryos; derived from homozygous dorsal mothers, see Materials and Methods) fail to express vnd (Mellerick and Nirenberg, 1995; Fig. 3B). Conversely, embryos where Dorsal is ectopically activated (called ectopic Dorsal embryos; derived from homozygous cactus mothers, see Materials and Methods) show a dorsal expansion of vnd expression, to a width of 7 to 9 cell diameters instead of the normal width of 5 cell diameters (Fig. 3C). Our results do not reveal whether Dorsal regulates vnd directly, or indirectly via repression of Dpp within the neuroectoderm. There is good precedent for considering the latter mechanism: dorsal

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Embryos show elevated Dpp activity in the neuroectoderm (Biehs et al., 1996; Jazwinska et al., 1999), and Dpp has been proposed to repress vnd expression (Mellerick and Nirenberg, 1995). Surprisingly, we find that embryos lacking both Dorsal and Dpp function (dorsal dpp embryos; see Materials and Methods) have a lack of detectable levels of vnd protein in the trunk region of the embryo (Fig. 4D), showing that loss of vnd expression in dorsal embryos is not due to de-repression of Dpp activity in the neuroectoderm. We conclude that Dorsal is necessary to activate vnd expression, probably directly, and that increased Dorsal levels can expand the vnd domain.

It has been proposed that Dpp signaling represses vnd expression and thus establishes the dorsal border of the vnd domain (Mellerick and Nirenberg, 1995). In contrast, we find that embryos with severely reduced Dpp activity (dpp embryos, see Materials and Methods) show no change in the pattern of vnd expression (Fig. 3D). Moreover, two different genetic backgrounds leading to ectopic Dpp activity (ectopic Dpp embryos, see Materials and Methods) show no repression of vnd expression (Figs. 3E and 3F). These results show that Dpp is not required to activate vnd expression, nor to establish the dorsal border of vnd expression (see Discussion).

Embryos lacking Egfr signaling (Egfr embryos, see Materials and Methods) have normal early vnd expression followed by premature loss of expression, indicating that Egfr signaling is not required for initiating vnd expression (Gabay et al., 1997; data not shown). To determine if Egfr signaling is sufficient to induce vnd expression, we examined vnd expression in embryos where Egfr signaling is ectopically activated (Hs-rho embryos, see Materials and Methods). Embryos with ectopic Egfr activity have a normal pattern of vnd expression, despite the expanded expression of diphosphorylated MAP kinase (data not shown), a marker for Egfr activity (Gabay et al., 1997). Thus, the timing and pattern of Egfr signaling play no role in establishing the initial domain of vnd expression, although it does have a late function in maintaining vnd levels.

**Activation and Patterning of the ind Expression Domain**

We have previously shown that Vnd represses ind expression and thus establishes the ventral border of the ind domain (Weiss et al., 1998; McDonald et al., 1998), however, the inputs that activate ind expression and set its dorsal border are unknown. In dorsal embryos, stripes of ind expression are not detected at any stage of development (Fig. SB); in contrast, ectopic Dorsal embryos show an...
expansion of the ind expression domain and a shift in the ventral border of ind expression toward a more dorsal position (Fig. 5C). Thus, elevated Dorsal activity will expand the ind expression domain, consistent with Dorsal acting as a concentration-dependent activator of ind expression. The shift of the ind ventral border in ectopic Dorsal embryos is likely due to the expansion of vnd (Fig. 3C), because vnd is a known repressor of ind expression (Weiss et al., 1998; McDonald et al., 1998).

In Egfr embryos, we do not detect ind expression (Fig. 5D); we confirm the loss of Egfr signaling in these embryos by the absence of activated MAP kinase (data not shown). Conversely, ectopic Egfr embryos show a dorsal expansion of ind expression (Fig. 5E), as well as the

FIG. 3. Establishing the vnd expression domain. Vnd protein staining in stage 8 embryos, anterior is up; arrowhead indicates ventral midline of CNS. Genotypes are as labeled and described under Materials and Methods. Width of Vnd stripe is indicated by brackets. (A) Wild-type embryo: Vnd is detected in the ventral column of neuroectoderm. (B) dorsal embryo: Vnd is not detected. (C) Embryo with ectopic Dorsal activity: Vnd is expanded dorsally. (D) dpp embryo: Vnd expression is similar to wild type. (E) Embryo with ectopic Dpp activity: Vnd expression may show a slight dorsal expansion. (F) Embryo with ectopic Dpp activity: Vnd expression is similar to wild type.
expected ubiquitous expression of activated MAP kinase (data not shown).

In order to discern the relationship between Dorsal and Egfr pathways, we performed epistasis experiments with loss- and gain-of-function mutations. In embryos with ectopic activation of both Dorsal and Egfr (see Materials and Methods), the domain of ind expression expands beyond that observed in either genetic background alone (compare Fig. 6B with Figs. 5C and 5E); we also observe the expected ubiquitous activation of MAP kinase (Fig. 6F). In embryos with ectopic Egfr but lacking Dorsal (see Materials and Methods), we observe ubiquitous activated MAP kinase (Fig. 6G), yet there is absolutely no ind expression (Fig. 6C). In the converse experiment, embryos with ectopic Dorsal but no Egfr function (see Materials and Methods) show a brief initiation of ind expression with a slight dorsal expansion (similar to embryos with ectopic Dorsal only), but the expression decays prematurely (Fig. 6D); we confirm that Egfr signaling is abolished in the neuroectoderm by lack of activated MAP kinase (Fig. 6H). Taken together, our results show that (1) both Dorsal and Egfr are required to activate ind expression; (2) ectopic expression of either Dorsal or Egfr in an otherwise wild-type embryo can expand the ind expression domain; (3) ectopic expression of both Dorsal and Egfr expands ind more than either alone; and (4) overexpression of Dorsal in the absence of Egfr can transiently activate ind expression, but not vice versa, suggesting that Dorsal is a more potent activator of ind expression. We conclude that Dorsal and Egfr normally act together to activate ind expression, and that the dorsal border of the ind domain is set by the dorsal border of Egfr signaling (see Discussion).

We assayed ind expression in dpp embryos and found it to be normal (Fig. 5F). However, ectopic Dpp embryos of two different genotypes showed significant repression of ind expression (Figs. 5G and 5H). Thus, ind expression requires that Dpp activity be kept low. Because Dpp can repress ind expression, and because dorsal embryos have high Dpp in the neuroectoderm, we assayed dl dpp embryos for rescue of ind expression. We found no ind expression in dl dpp embryos except a ring of ind expression in the head (Fig. 4E), showing that the loss of ind expression in dorsal embryos is not due to ectopic Dpp activity.

**Activation and Patterning of the msh Expression Domain**

msh is expressed in the most dorsal column of neuroectoderm beginning at stage 7. At this time of development, the msh domain may be exposed to low levels of Dpp, but both Egfr activity and Dorsal protein are not detectable. We find that dorsal embryos lack msh expression (Fig. 7B), whereas ectopic Dorsal embryos show an expansion of msh expression around the dorsal circumference of the embryo (Fig. 7C). Dorsal is unlikely to be a direct activator of msh expression, however, because dorsal dpp double mutants show widespread expression of msh throughout the embryo (Fig. 4F). We conclude that Dorsal keeps Dpp activity low within the neuroectoderm, thus allowing msh expression (see Discussion). Consistent with this conclusion, a reduction in Dpp activity expands msh expression dorsally (Fig. 7D; D’Alessio and Frasch, 1996), while a slight increase in Dpp activity in the neuroectoderm (sog embryos, see Materials and Methods) leads to a partial reduction in Msh expression (Fig 7E; D’Alessio and Frasch, 1996), and high level ectopic Dpp in the neuroectoderm (brk sog embryos, see Materials and Methods) represses msh expression (Fig. 7F). Thus, msh is expressed only where Dpp activity is low.

The entire neuroectoderm has low Dpp activity, due to expression of the Dpp inhibitors brk and sog. What keeps msh expression off in the ventral and intermediate columns of the neuroectoderm? Previously, we reported that in ind mutant embryos, msh expression shows a slight ventral expansion (Weiss et al., 1998; Fig. 7H). Here we show that in vnd ind double mutant embryos (see Materials and Methods), msh expression is detected throughout the neu-
roectoderm (Fig. 7I). Thus, msh has the potential to be expressed in the entire neuroectoderm, but is normally restricted to the dorsal column due to repression by Vnd and Ind.

Egfr signaling also modulates msh expression. In embryos lacking Egfr signaling, msh expands slightly into the intermediate column (D’Alessio and Frasch, 1996); this is likely an indirect effect caused by the loss of ind expression (Fig. 5D), because ind mutant embryos show an identical phenotype (Weiss et al., 1998). Ectopic Egfr leads to a loss of msh expression (data not shown) and an expansion of ind expression (Fig. 5E); again, the msh phenotype is likely caused by the expansion of ind, since misexpression of ind can repress msh (data not shown).

DISCUSSION

Expression of vnd, ind, and msh follows a ventral to dorsal progression: vnd is expressed first, followed by ind and lastly msh. There is a gap between the initial vnd and ind domains, suggesting that each gene is independently activated at a precise DV position. Subsequently, ind can be expressed in the ventral domain, but this is normally

FIG. 5. Establishing the ind expression domain. ind mRNA expression in stage 7 embryos, anterior is up, ventral to left; ventral midline of CNS, arrowhead. Genotypes are as labeled and described under Materials and Methods. Width of ind stripe indicated by brackets. (A) Wild-type embryo: ind is expressed in the intermediate column of neuroectoderm. (B) dorsal embryo: ind expression is not detected. (C) Embryo with ectopic Dorsal activity: ind expression is expanded slightly dorsally. (D) Egfr embryo: ind is not expressed. (E) Embryo with ectopic Egfr activity: ind expression is expanded slightly dorsally. (F) dpp embryo: ind expression is the same as in wild type. (G) Embryo with ectopic Dpp activity (sog 4xdpp): ind expression is repressed in the trunk. (H) Embryo with ectopic Dpp activity (brk sog): ind is not expressed in the trunk.
prevented by Vnd-mediated repression (Weiss et al., 1998; McDonald et al., 1998). Because ind is capable of repressing vnd expression (Weiss et al., 1998), if ind were to be expressed first in both the ventral and intermediate columns, it might fully inhibit the expression of vnd. Thus, the temporal pattern of vnd and ind expression is likely to be important for establishing their final spatial pattern of gene expression.

The activation and borders of vnd expression appear to be wholly dependent on the Dorsal morphogen gradient. High levels of Dorsal in the mesoderm/mesectoderm anlage can activate twist, snail, and vnd (Thisse et al., 1991; Ip et al., 1992), but Snail activity represses vnd expression (Mellerick and Nirenberg, 1995). Intermediate levels of Dorsal are sufficient to activate vnd, but not snail, thus establishing the ventral column of neuroectoderm. It is unclear how the dorsal border of Vnd is positioned, but it may be dependent on the concentration of nuclear Dorsal, because if Dorsal levels are increased in dorsal cells, there is a corresponding expansion of the vnd domain. It is unclear how the dorsal border of Vnd is positioned, but it may be dependent on the concentration of nuclear Dorsal, because if Dorsal levels are increased in dorsal cells, there is a corresponding expansion of the vnd domain. In contrast to a previous report (Mellerick and Nirenberg, 1995), we find no evidence that Dpp signaling establishes the dorsal border of the vnd domain. We observe no change in the width of the vnd domain in dpp embryos, and we fail to observe repression of vnd in ectopic Dpp embryos. In fact, elevated Dpp activity in the neuroectoderm (in sog 4xdpp embryos) gives a slight

FIG. 6. Regulation of ind expression: epistasis between dorsal and egfr. (A–D) ind mRNA expression and (E–H) activated MAP kinase expression in stage 7 embryos, anterior is up, ventral to left; ventral midline of CNS, arrowhead. Genotypes are as labeled and described under Materials and Methods. Width of ind or activated MAP kinase domains indicated by brackets. (A, E) Wild-type embryos: (A) ind is expressed in the intermediate column of neuroectoderm; (E) activated MAP kinase is expressed in the ventral and intermediate columns of neuroectoderm. (B, F) Embryos with ectopic Dorsal and Egfr: (B) ind expression expands dorsally to cover the dorsal surface of the embryo; (F) activated MAP kinase is detected throughout the DV axis of the embryo. (C, G) Embryos with ectopic Egfr activity in the absence of Dorsal function: (C) ind expression is not detected; (G) activated MAPK is detected throughout the DV axis of the embryo. (D, H) Ectopic Dorsal activity in the absence of Egfr function: (D) ind expression is expanded slightly dorsally but is not maintained past stage 7. (H) activated MAPK is not detected in neuroectoderm, although it is still detected in the mesoderm (out of focus).
expansion of the vnd domain (Fig. 3E), and even higher levels of Dpp (in brk sog embryos) still fail to repress vnd expression (Fig. 3F), despite eliminating much of the remaining CNS (Jazwinska et al., 1999). The reason the vnd domain is expanded in sog 4xdpp embryos remains unclear; however, we feel that our combined results clearly demonstrate that dpp signaling does not repress vnd and therefore cannot position the dorsal border of vnd. All existing data are consistent with Dorsal acting as a direct, concentration-dependent activator of vnd expression. In contrast, the Egfr and Dpp signaling pathways have no role in establishing the correct vnd expression pattern, although Egfr is required to maintain vnd expression later in embryogenesis (Gabay et al., 1996).

Initiation and maintenance of ind expression require both Dorsal and Egfr signaling pathways, but not Dpp activity. The ventral border of ind expression is established by dorsal limit of vnd expression (Weiss et al., 1998). The dorsal border of ind expression has more complex regulation. Dpp repression does not establish the dorsal border of ind, since the ind domain is normal in dpp embryos. In contrast, both Dorsal and Egfr are required to activate ind and set its dorsal border. In wild-type embryos, the domains of ind and activated Egfr have identical dorsal borders. When Egfr activity is increased throughout the embryo, ind expression shows a partial dorsal expansion, showing that the dorsal border of Egfr activity sets the precise dorsal border of ind expression. Ectopic Dorsal activity can also expand the ind domain (without affecting the Egfr activation domain), showing that sufficiently high levels of nuclear Dorsal protein can independently activate ind expression. As expected, when Egfr activity and nuclear Dorsal levels are simultaneously increased there is a complete dorsal expansion of the ind domain. The data presented here suggest that ind expression is activated by both Dorsal and Egfr pathways, limited ventrally by Vnd, and limited dorsally by lack of Dorsal and Egfr activity. Our data do not distinguish between a linear pathway in which Egfr signaling activates or potentiates Dorsal to allow ind transcription and a parallel pathway in which Dorsal and Egfr signaling act independently to activate ind expression.

Although Dpp is not required for any aspect of ind expression in wild type embryos, ectopic Dpp signaling in the neuroectoderm can repress ind expression. This shows that Dpp signaling must be kept low in the intermediate column to allow ind transcription and raises the possibility with high level ectopic Dpp activity (brk sog): Msh expression is completely repressed. (G) Wild-type embryo: Msh expression is detected in the dorsal column of neuroectoderm. (H) ind mutant embryo: Msh expression expands ventrally into the ind domain. (I) vnd ind double mutant embryo: Msh expression expands to the ventral midline.
that the loss of ind expression seen in dorsal embryos is an indirect effect, due to the de-repression of Dpp activity within the neuroectoderm. dorsal dpp double mutants fail to express ind, however, proving that loss of ind expression in dorsal mutants is not due to de-repression of Dpp within the neuroectoderm. We propose that Dorsal must both activate ind expression and repress Dpp signaling to allow ind expression.

msh is expressed in a DV domain that has low Vnd, Ind, and Dpp activity. Overexpression of any of these genes will repress msh expression, and dorsal dpp embryos that lack all vnd, ind, and dpp expression show ectopic msh expression around the DV axis. Thus, the borders of the msh domain are defined by repression: Vnd and Ind ventrally, and Dpp dorsally. What activates msh expression? msh expression could be activated by “basal” transcription factors present uniformly in the early embryo. Alternatively, msh expression may be induced by a low level of ubiquitous TGFβ activity, similar to the observed activation of zebrafish msh homologs (reviewed in Mayor et al., 1999). The screw gene encodes a TGFβ-like protein expressed at low levels throughout the embryo, and although it has no striking CNS phenotype (Arora et al., 1994), it would be interesting to see if screw dpp embryos lose dorsal msh expression, or whether screw dorsal dpp embryos lose global msh expression.

The patterned expression of vnd, ind, and msh within the neuroectoderm appears to have been evolutionarily conserved between insects and vertebrates. Murine, zebrafish, and chick embryos express homologous genes in DV columns of the developing CNS: several Nkx genes (similar to vnd) are expressed ventrally, Gsh1/2 genes (similar to ind) are expressed in intermediate columns, and Msx genes (similar to msh) are expressed in the dorsal CNS (reviewed in Arendt and Nübler-Jung, 1999; Cornell and Von Ohlen, 2000). Although the vnd/Nkx, ind/Gsh, msh/Msx patterns appear to be conserved between insects and vertebrates, the regulatory inputs that establish these patterns appear different. In vertebrates, Sonic hedgehog signaling patterns the ventral CNS and induces expression of Nkx family members (Ericson et al., 1995; Qiu et al., 1998; Barth and Wilson, 1995; Ericson et al., 1997). In Drosophila, the ventral CNS is patterned by Dorsal and Egr, which induce expression of vnd and ind genes, whereas hedgehog mutants show normal initiation of vnd and ind gene expression (S. Cheeseman, T. Von Ohlen, and C. Q. Doe, unpublished observations). In vertebrates, the Dpp-related BMP2/4 proteins activate msh expression (Suzuki et al., 1997), whereas Dpp represses msh expression in Drosophila. Clearly further analysis, including examination of DV pattern formation in different phyla, will be necessary to understand how the conserved vnd/Nkx, ind/Gsh, msh/Msx expression patterns are generated by such diverse regulatory inputs and how they direct diverse cell fate decisions.

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