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Localized repressors delineate the neurogenic ectoderm in the early *Drosophila* embryo

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Abstract

The Dorsal gradient produces sequential patterns of gene expression across the dorsoventral axis of early embryos, thereby establishing the presumptive mesoderm, neuroectoderm, and dorsal ectoderm. Spatially localized repressors such as Snail and Vnd exclude the expression of neurogenic genes in the mesoderm and ventral neuroectoderm, respectively. However, no repressors have been identified that establish the dorsal limits of neurogenic gene expression. To investigate this issue, we have conducted an analysis of the *ind* gene, which is selectively expressed in lateral regions of the presumptive nerve cord. A novel silencer element was identified within the *ind* enhancer that is essential for eliminating expression in the dorsal ectoderm. Evidence is presented that the associated repressor can function over long distances to silence neighboring enhancers. The *ind* enhancer also contains a variety of known activator and repressor elements. We propose a model whereby Dorsal and EGF signaling, together with the localized Schnurri repressor, define a broad domain of *ind* expression throughout the entire presumptive neuroectoderm. The ventral limits of gene expression are defined by the Snail and Vnd repressors, while the dorsal border is established by the newly defined silencer element.
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Keywords: Dorsoventral patterning; *Drosophila*; Repressor; Neurogenic ectoderm; Dorsal; Ind; Vnd; Schnurri

Introduction

The Dorsal gradient initiates dorsal–ventral patterning by regulating the expression of ~30–50 target genes in a concentration-dependent fashion (Stathopoulos and Levine, 2002). Previous studies have identified enhancers for 16 of the genes, and these direct diverse patterns of expression across the dorsoventral axis of early embryos (Markstein et al., 2002, 2004; Stathopoulos and Levine, 2004; Stathopoulos et al., 2002). It is possible to subdivide these enhancers into three general groups: type 1, type 2, and type 3 enhancers are regulated by high, intermediate, and low levels of the Dorsal gradient in the mesoderm, neurogenic ectoderm, and dorsal ectoderm, respectively. The staining patterns produced by many of the enhancers display sharp dorsal boundaries. For example, the dorsal limits of the type 1 *snail* expression pattern define the boundary separating the future mesoderm and neurogenic ectoderm (Ip et al., 1992; Kosman et al., 1991). Similarly, the dorsal border of the type 2 *ventral nervous system defective* (*vnd*) pattern coincides with the boundary separating ventral and lateral regions of the future nerve cord (Chu et al., 1998; Jimenez et al., 1995; McDonald et al., 1998; Mellerick and Nirenberg, 1995).

It has been suggested that spatially localized repressors might establish the dorsal limits of gene expression (Huang et al., 1997). Perhaps repressors localized within the neurogenic ectoderm restrict the *snail* expression pattern to the presumptive mesoderm and thereby define the mesoderm/neuroectoderm boundary. Such repressors would be comparable to the gap repressors that delineate the borders of pair-rule stripes during segmentation along the anterior–posterior axis. However, there is currently no

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definitive evidence for such localized repressors. To investigate this issue, we have conducted a regulatory analysis of the neurogenic gene, *intermediate neuroblasts defective* (*ind*).

Three evolutionarily conserved homeobox genes control the dorsal–ventral patterning of the ventral nerve cord in *Drosophila*: *vnd*, *ind*, and *muscle segment homeobox* (*msh*) (review in Arendt and Nubler-Jung, 1999; Cornell and Ohlen, 2000). Their sequential expression in the neurogenic ectoderm of gastrulating embryos is the earliest manifestation of the dorsal–ventral patterning of the future ventral nerve cord (Skeath, 1998; Udolph et al., 1998; Yagi et al., 1998). Orthologous genes (*nkx*, *gsh*, and *msx*) display analogous patterns of expression in the vertebrate neural tube. Previous studies suggest that a combination of Dorsal, EGF, and Dpp (TGF-β) is responsible for the localized *ind* expression pattern, which consists of sharp lateral stripes on either side of the embryo (von Ohlen and Doe, 2000). The proposed model is that low levels of the Dorsal gradient work in concert with transcriptional activators induced by EGF signaling to define a broad domain where *ind* can be expressed. *ind* is kept off in ventral regions of the neurogenic ectoderm by the spatially localized Vnd repressor since the *ind* expression pattern expands ventrally in *vnd* mutant embryos (Weiss et al., 1998). It is unknown how the dorsal border of *ind* is established. Absence of Dpp signaling (from the dorsal-most region of the embryo) or of *msh* (from the dorsal column) has no effect on the *ind* dorsal border or its expression (D'Alessio and Frasch, 1996; von Ohlen and Doe, 2000; Weiss et al., 1998). It is currently unclear whether the dorsal borders of the *ind* stripes are delineated by spatially localized repressors or by limiting amounts of the Dorsal gradient or EGF activators.

Here we characterize a 1.4-kb enhancer located downstream of the *ind* gene in order to dissect molecularly the signaling inputs that regulate *ind* expression. This enhancer is composed of 3 interdependent modules of ~500 bp apiece. The distal-most module (A) mediates broad activation throughout the neurogenic ectoderm and contains an optimal Dorsal binding site along with potential EGF activator elements (ETS motifs). The central module (B) mediates repression in the ventral mesoderm, the ventral neurogenic ectoderm, the dorsal neurogenic ectoderm, and the dorsal ectoderm. Finally, the proximal module (C) mediates repression in the ventral mesoderm and ventral neurogenic ectoderm. These results indicate that the *ind* expression pattern is produced from the combined action of multiple spatially localized repressors. The dorsal repressors that interact with module B mediate dominant silencing of a linked *even-skipped* (*eve*) stripe 3 enhancer, thereby providing strong evidence that the dorsal border of the *ind* expression pattern is not formed by limiting amounts of Dorsal or EGF activators but instead depends on localized repressors. We suggest that localized repressors–possibly restricted to dorsal regions of the embryo–are generally important for the global patterning of the dorsoventral axis.

Materials and methods

Fly strains

Wild-type embryos correspond to the *yw Drosophila melanogaster* fly stock. P-element-mediated transformation was performed using standard methods (Spralding and Rubin, 1982). *CtBP*-germline mosaic females were obtained using the FRT P1590 fly stock and used to obtain *CtBP*-embryos as described previously (Nibu et al., 1998a). Virgin *CtBP*-mosaic females were crossed to transgenic *yw* males containing the *eve.3-ind* chimeric enhancer P-element (AMS301).

In situ hybridization

Embryos were collected, fixed, and then hybridized with dioxygenin-UTP or biotin-UTP labeled antisense probes as previously described (Jiang et al., 1991; Kosman et al., 2004). The *ind* cDNA used to make antisense riboprobes was obtained from Weiss et al. (1998). Templates for the *vnd* and *shn* probes were obtained using oligonucleotide primers in PCR on genomic DNA obtained from *yw* DNA. The *lacZ* probe has been previously described (Jiang et al., 1991). Fluorescent in situ hybridization (FISH) images were obtained using a Leica LS confocal microscope.

Plasmid construction

PCR products were TA-cloned into the PGEM-T Easy vector (Promega). *Not*I fragments containing these PCR products were isolated and cloned into an eve_p-lacZ-Casper P-element injection vector which was modified to contain a unique *Not*I site upstream of the eve promoter (eve_p) sequence (Not-eve_p-lacZ-Casper), in place of the *Eco*RI site originally present in this vector (Small et al., 1992). eve stripe 3/7(eve.3)-ind chimeric enhancers were created by first inserting either an *Xba*I or *Xba*I–*Spe*I eve.3 fragment into the unique *Spe*I sites of the pGEMT-Easy *ind* full-length or partial enhancer clones. Subsequently, *Not*I fragments containing the chimeric enhancers were inserted into Not-eve_p-lacZ-Casper.

The eve.3 enhancer sequence was obtained by using primers AS377 (5-GC<u>TCTAGA</u>GGATCCTCGAAATCGAGAGCGACC-3′) and AS378 (5-GC<u>TCTAGA</u>GAGCTCGTAAAAACGTGAATGCCATCG-3′) or AS439 (5-GC<u>ACTAGT</u>GAGCTCGTAAAAACGTGAATGCCATCG-3′) to PCR a 0.5-kb fragment from *yw* genomic DNA.

The genomic sequence corresponding to the *ind* full-length enhancer region (1–1429) was obtained using primers AS247 (5-GCTTCAAAGCTCCGGGAAACG-3′) and AS248 (5-TCTGGGCCTTCGGTCCGAAAATG-3′). This sequence was inserted into Not-eve_p-lacZ-Casper and transgenic flies obtained using either this DNA alone (AMS255) or the chimeric enhancer with eve.3 (AMS301). *ind*

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