A novel homeobox gene mediates the Dpp signal to establish functional specificity within target cells

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Morphogen gradients of secreted molecules play critical roles in the establishment of the spatial pattern of gene expression. During midgut development in *Drosophila*, secreted molecules of Decapentaplegic (Dpp) and Wingless (Wg) establish unique transcriptional regulation within target cells to specify the resultant cell types. Here we report the identification of a novel homeobox gene, *defective proventriculus* (*dve*), which is required for the midgut specification under the control of Dpp and Wg. In *dve* mutants, two distinct parts of the midgut, the proventriculus and middle midgut, are abnormally organized. The Wg signal regulates *dve* expression during proventriculus development. On the other hand, *dve* is a downstream target of Dpp in the middle midgut and defines the functional specificity of copper cells along with another Dpp target gene, *labial*. Thus, the *dve* gene acts under the two distinct extracellular signals at distant parts of the midgut primordia.

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During animal development, a mass of homogeneous cells have distinct developmental fates depending on their positions, and are organized in a stereotyped manner into a variety of functional tissues. Positional information mediated by extracellular signals plays major roles in giving rise to such a diversity of cell types within the uniform cell mass. The transforming growth factor- β (TGF- β) and Wnt superfamilies (*Drosophila* Dpp and Wg, respectively) have been well studied among secreted molecules that transmit such extracellular signals, and regulate a wide variety of cellular responses including differentiation, proliferation, adhesion, and migration (for review, see Nusse and Varmus 1992; Kingsley 1994). Signaling by the TGF- β superfamily is elicited by way of two types of receptor serine-threonine kinase, type-I (*thick veins* and *saxophone* in *Drosophila*) and type-II (*punt*) (for review, see Massagué et al. 1994). Intracellular molecules essential for the signal transduction by the TGF- β family include Smads family molecules [*Mothers against dpp*] (for review, see Massagué et al. 1997), and nuclear factors such as *Xenopus* FAST1 (Chen et al. 1996) and *Drosophila schnurri* (Arora et al. 1995; Grieder et al. 1995). Signaling by the Wnt superfamily is medi-

ated by the Frizzled receptor family, Dishevelled, Glycogen synthase kinase-3 β (*shaggy/zeste-white 3*), β -Catenin (*armadillo*), and nuclear factors such as TCF/LEF-1 (*pangolin*) (for review, see Cadigan and Nusse 1997; Cavallo et al. 1997). Studies of *Drosophila* have provided valuable insights into the roles of these highly conserved signaling pathways in morphogenesis such as wing patterning (Blair 1995) and midgut specification (Brenz 1994; Graba et al. 1997).

The gut epithelium of *Drosophila* is derived from the anterior and posterior primordia at both ends of the blastoderm embryo. These primordia are initially nonsegmental and fused into a single continuum. Secreted molecules, such as Dpp and Wg, induce subsequent morphogenetic events that ultimately compartmentalize the primordia into morphologically distinct sectors. During this process, these signals also act for cells to take distinct developmental paths to establish the functional organization of the midgut.

The proventriculus develops at the junction of the foregut and the midgut, and functions as a valve regulating the passage of food into the midgut. It is composed of three layers; the outer layer is derived from the anterior-most region of the midgut, the middle layer is derived from the foregut of mesoderm-free keyhole structure, and the inner layer is derived from the esophagus (for review, see Skaer 1993). The late steps of proventriculus

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morphogenesis are attributable to migration of cells, and are controlled by at least Hedgehog (Hh) and Wg, which are expressed in the mesoderm-free keyhole structure (Pankratz and Hoch 1995; Fig. 1E). Little is known so far about downstream targets that respond to Hh/Wg signals during proventriculus development.

The midgut consists of two germ layers, the visceral mesoderm and the endoderm. The middle midgut cells derived from the endoderm differentiate into four distinct types of cells: copper, interstitial, large flat, and iron cells. These endodermal cell types are specified by Dpp and Wg, which are expressed in the adhering visceral mesoderm of the parasegments (PS) 7 and 8, respectively. Copper cells exhibit a unique morphology with banana shapes and exhibit UV light-induced fluorescence after copper feeding. These characteristics are specified by a homeotic gene, *labial* (*lab*), which is activated by the Dpp signal in the midgut. Two different thresholds of Wg define copper and large flat cells (Hoppler and Bienz 1995). However, it remains unclear how Lab confers the transcriptional regulation to specify copper cells.

Here, we have identified a new gene, *defective proventriculus* (*dve*), which encodes a homeodomain protein. *dve*¹ homozygous mutants are defective in proventricu-

lus morphogenesis and in the arrangement of middle midgut cells. The *dve* gene responds differentially to the Wg or Dpp signal in the anterior-most or middle midgut, respectively. In the anterior-most midgut, *dve* activity is required to maintain the three-layered structure indispensable for a functional proventriculus. In the middle midgut, the *dve* gene is expressed in all precursors of four distinct cell types, subsequently it is repressed only in copper cells. This repression is mediated by two Dpp target genes, *lab* and *dve* itself, and is also essential for the functional specification of copper cells. Thus, *dve* is involved in different developmental aspects of the midgut under the control of the different extracellular signals. We discuss the roles of *dve* in the context of the network of inductive signals that organize midgut development.

Results

Identification of the *dve* locus

We have identified the *dve* locus by two enhancer-trap insertions at 58D1-2 on the second chromosome; one is a viable allele, *dve*^{SH255}, and the other is a homozygous lethal allele, *dve*¹ (Fig. 2A). Immediately after hatching, *dve*¹ homozygous larvae exhibit normal locomotion behavior; however, develop into small larvae and die within a day. The lethality of the *dve*¹ allele is attributable to the P-element insertion, because the P-element excision recovered homozygous viable adults (15 of 28). Embryos bearing *dve*¹ in trans with *Df(2R)X58-3*, which uncovers the *dve* locus, hatch normally into first instar larvae but die, suggesting that animals of this genotype have a lethal stage similar to that of *dve*¹ homozygotes. In addition, *dve*¹ homozygous embryos express no detectable *dve* transcript (data not shown) or Dve protein (see Fig. 6B, below) until stage 14; faint staining with the anti-Dve serum was detectable at early stage 17 (Fig. 3H). These observations indicate that *dve*¹ is a strong hypomorphic allele. We term this locus *defective proventriculus* based on the defects in the proventriculus formation as described below.

The reduced body sizes of *dve*¹ homozygous larvae suggest that the feeding is affected by the *dve*¹ mutation. The colored yeast fed to heterozygous larvae stained their guts red throughout their length (see Fig. 1A). On the other hand, it accumulated in the proventriculus in *dve*¹ mutant larvae (arrow in Fig. 1B). Consistent with this observation, *dve*¹ larvae fail to form the proventriculus correctly (Fig. 1, cf. C and D). In the wild type, cell movement leads to formation of the internal portion of the proventriculus during embryonic stages 16–17; cells of the foregut epithelium invaginate into the anterior-most midgut that normally expresses *dve* (Fig. 1E). In *dve*¹ embryos, the cell migration was greatly delayed and the internalization was only temporary (data not shown). As a result, *dve*¹ larvae cannot form the three-layered structure of the proventriculus (Fig. 1D) as observed in *hh* or *wg* mutant embryos (Pankratz and Hoch 1995). As the *dve*-expressing anterior-most midgut constitutes the

Figure 1. Proventriculus phenotypes of *dve* mutants. (A,B) First instar larvae of the wild type (A) or *dve*¹ mutant (B) were fed for 5 hr with a colored yeast. In *dve* mutants, the colored yeast is accumulated in the proventriculus (arrow in B). The magnification of B is twice that of A. (C,D) The morphology of the proventriculus in dissected first instar larvae of the wild type (C) or *dve*¹ mutant (D). Arrows indicate the proventriculus. (E) Schematic representation of the proventriculus morphogenesis. The mesoderm-free keyhole structure (blue) of the foregut is most evident at stage 14. This region expresses *hh* and *wg*, whose activities are essential for the subsequent migration into the anterior-most midgut (black). The anterior-most midgut expresses *dve* and constitutes the outer layer of the proventriculus after stage 16. The internalized foregut epithelium of the esophagus (red) and the keyhole structure constitute the inner and middle layers of the proventriculus, respectively.

Figure 2. *dve* gene structure and homology alignment. (A) Genomic organization of the *dve* locus. Triangles *dve*^l and *dve*^{SH255} indicate the P-element insertions. Closed boxes represent the six exons of the *dve* gene. (E) *Eco*RI; (S) *Sal*I. (B) Predicted amino acid sequence of the Dve protein. The region homologous to the homeodomain is indicated in yellow and the three helices are underlined. The putative transcriptional activation domain of an acidic amino acid cluster is indicated in red. (C) Homology alignment with known homeodomain proteins. Identical amino acids are indicated in yellow. Dashes indicate gaps introduced to maximize similarity. The positions of α -helices are indicated below: (#) Invariant amino acids; (*) highly conserved amino acids in homeodomains. The ninth amino acid of the recognition helix (helix 3) is indicated by a red arrowhead. The amino acids (Q, Y, and R) marked by green are highly conserved in homeodomains except for POU homeodomains. Invariant amino acids in POU homeodomains (Herr and Cleary 1995) are indicated above by plus signs (+), and the blue amino acids (V, C, and Q) are conserved in POU homeodomains but not in other types of homeodomains. The homeodomain sequences are from the following sources: m-Pit1 (Bodner et al. 1988; Ingraham et al. 1988), Cfla (Johnson and Hirsh 1990), m-Brn1 (Hara et al. 1992), h-Brn2 (Oct3N; He et al. 1989; Schreiber et al. 1993), m-Oct6 (Meijer et al. 1990; Suzuki et al. 1990), h-Rdc1 (Collum et al. 1992), Pdm-2 (Billin et al. 1991; Lloyd and Sakonju 1991), h-Otx1, m-Otx2 (Simeone et al. 1993), Otd (Finkelstein et al. 1990), x-Gsc (Cho et al. 1991), Bagpipe (d-NK3), d-NK2 (Kim and Nirenberg 1989), h-Msx1 (Hox-7; Hewitt et al. 1991), Rough (Kimmel et al. 1990), and Ems (Dalton et al. 1989).

outer layer of the proventriculus (Fig. 1E), these *dve*^l phenotypes suggest that *dve* activity is required for the functional development of outer layer cells to retain the internal portion of the proventriculus.

Molecular cloning of the *dve* gene

To isolate the *dve* gene, we used genomic DNAs flanking the *dve*^{SH255} insertion and identified the full-length cDNA of a 4.9-kb transcript. Analysis of the genomic DNA of the *dve* gene revealed that the transcript consists of six exons and that the P-element of *dve*^{SH255} is inserted at 167 bp upstream from the transcription start site. The P-element insertion of the lethal *dve*^l allele is located within the second intron of the *dve* gene (Fig. 2A).

The predicted open reading frame of Dve is 1019 amino acids long. Homology with the homeodomain is found near its carboxy-terminal region (yellow in Fig. 2B). The Dve homeodomain contains all four invariant amino acids located within helix 3 (sharps in Fig. 2C), and matches well with other highly conserved residues (asterisks in Fig. 2C; Gehring 1992). However, the Dve homeodomain is unusual, as it has a 10-amino-acid insertion between helices 2 and 3 (Bürglin 1997). The ninth amino acid of helix 3 confers the recognition specificity for its binding sequences (red arrowhead in Fig. 2C; Hanes and Brent 1989; Treisman et al. 1989), and the recognition helix of Dve is closest to the *orthodenticle*

(*otd*) class homeodomain. In contrast, helices 1 and 2 of Dve exhibit homology with POU homeodomains rather than *otd* class homeodomains (Fig. 2C). Therefore, the Dve homeodomain seems to be a novel class of homeodomain that is intermediate between POU and *otd* class homeodomains. The Dve staining observed in the nucleus is consistent with its homology with the homeodomain and suggests that Dve is a putative transcription factor.

Expression pattern of *dve* in the embryonic midgut

The expression of the Dve protein exhibits a pleiotropic pattern including three separate domains of the midgut: anterior-most, middle, and posterior-most regions. Because defects in the lethal *dve*^l allele are evidently observed in the gut, we focus on *dve* expression during gut development. At stage 10, *dve* expression is first observed in the invaginated stomodeum (Fig. 3A), where *hh* and *wg* are expressed under the control of the winged-helix transcription factor Fork head (Hoch and Pankratz 1996). We compared anti-Dve staining with *wg* gene expression that was monitored with *wg-lacZ*. The expression of *dve* and *wg* overlaps initially in the stomodeum, and is then segregated into nonoverlapping but adjacent regions; the mesoderm-free keyhole structure expresses *wg*, whereas *dve* is solely expressed in the anterior-most region of the midgut (Fig. 4A,E), which becomes outer layer of the proventriculus later at stage 16 (Fig. 4H).

Figure 3. Dve expression in the embryonic midgut. Lateral view of wild-type (A–D) or *dve'* mutant embryos (G,H) stained with anti-Dve serum (A–D; anterior is left and dorsal is up). The morphology of the gut was visualized by double staining with BP102 (E–H; brown signal). In E–H, the right side of embryos is visualized (anterior is right) so as to better show the overall morphology of the gut. (A–C) Wild-type embryos at stages 11, 12, and 13, respectively. (D) A stage-15 embryo. The arrow indicates the position of the middle constriction. (E,G) Stage-16 embryos of the wild type (E) and *dve'* mutant (G), respectively. (F,H) Stage-17 embryos of the wild type (F) and *dve'* mutant (H), respectively.

From stage 13 onward, *dve* is also expressed in two other parts of the endodermal midgut; one is the middle region where the anterior and posterior midgut primordia are fused, and the other is the posterior-most region (see Fig. 3C). The middle region expressing *dve* corresponds to both the anterior and posterior sides of the middle constriction (arrow in Fig. 3D). At stage 16, this region becomes the second and third gut lobes (Fig. 3E). Dve-expressing cells in this region include copper cell precursors that coexpress *lab* (see Fig. 7A,D, below).

wg signal regulates *dve* expression during proventriculus development

As the *dve*-expressing domain in the presumptive proventriculus initially overlaps with the *wg*- and *hh*-expressing domain (Fig. 4A), we examined the possibility that Wg or Hh regulates the expression of the *dve* gene. In *wg^{CX4}* null embryos, *dve* expression is abolished in the stomodeum, whereas epidermal expression is normally detected (Fig. 4B). In *hh^{AC}* mutant embryos, *dve* is normally expressed (Fig. 4C). Thus, Wg rather than Hh is required to activate the *dve* gene in the presumptive proventriculus.

The keyhole structure also expresses *cubitus interruptus* (*ci*). In *ci^D* mutants, the expression of *wg* and *hh* is not maintained, leading to the absence of a keyhole structure. The maintenance of *wg* expression also requires *hh* activity (Pankratz and Hoch 1995). In *ci^D* mutants, *dve* expression is never detected in the presumptive proventriculus until stage 13 (Fig. 4D), whereas it begins to be detectable at stage 14 despite the absence of a keyhole structure (Fig. 4F). In addition, *dve* expression expands ectopically into the presumptive first gut lobe after stage 15 (Fig. 4G). Similar ectopic expression of *dve*

Figure 4. Wg signal regulates *dve* expression in the proventriculus. Embryos were stained with anti-Dve serum (black) and anti- β -gal (brown in A,D–F). (A–C) Dve expression in the invaginated stomodeum overlapped with *wg-lacZ* expression (brown in A) at stage 11, and was abolished in *w^{CX4}*, but not in *hh^{AC}* mutants (arrows in B,C, respectively). Arrowheads indicate the stomodeum invagination. (D) In *cubitus interruptus* (*ci^D*) mutants, the expression of *wg* and *hh* is not maintained and *dve* expression is also undetectable at stage 12 (arrow). (E) The mesoderm-free keyhole structure of the wild type (stage 14) expresses *wg-lacZ* (black arrow), and the adjacent region expresses *dve* (white arrow). (F) In *ci^D* mutants, the keyhole structure is not formed (black arrow); however, *dve* expression begins to be detectable in the anterior-most region of the stage-14 midgut (white arrow). (G) In *ci^D* mutants at stage 15, *dve* expression expands posteriorly (white arrow) in contrast to the great reduction in the posterior-most region of the midgut (black arrow). (H,I) Dve expression in the proventriculus (PV) of a wild-type (stage 16) and a *hh^{U35}* mutant embryo (stage 17) is indicated (white arrow in H and I, respectively). In *hh^{U35}* mutants, *dve* expression appears to expand posteriorly (black arrow). (A–G) Lateral view; (H,I) ventral view. Anterior is left in all panels.

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is observed in *hh^{U35}* or *wg^{IL114}* mutant embryos (Fig. 4I; data not shown). The ectopic *dve* expression in these mutants suggests that the Wg signal is also required to define the posterior border of late *dve* expression in the presumptive proventriculus, in addition to the initial activation of the *dve* gene.

Dpp signal regulates *dve* expression in the middle midgut

In the middle midgut, Dpp and Wg signals from the visceral mesoderm control gene expression on both the anterior and posterior sides of the middle constriction. Hence we examined whether *dve* expression depends on the Dpp or Wg signals in this region. In *thick veins* (*tkv*) mutants that lack the functional type I receptor for Dpp, *dve* expression is completely absent in the middle midgut, whereas the expression in other parts of the midgut is not affected (Fig. 5, cf. A with C). This indicates that *dve* expression in the middle midgut requires the Dpp signal. On the other hand, the ubiquitous expression of *dpp* throughout the visceral mesoderm, using the combination of UAS-*dpp* and 24B-Gal4 driver, induced *dve* expression throughout the underlying endoderm (Fig. 5, cf. A with E). This dependence of *dve* expression on Dpp is quite similar to that of *lab* expression (Staehling-Hampton and Hoffmann 1994).

We used *abdominal-A* (*abd-A*) mutants to examine the dependence of *dve* expression on Wg. In *abd-A* mutants, the expression of *Ultrabithorax* (*Ubx*) and *dpp* in the visceral mesoderm expands throughout the posterior midgut and endogenous *wg* is not activated (Immerglück et al. 1990; Panganiban et al. 1990; Reuter et al. 1990). In *abd-A* mutants, *dve* expression also expands posteriorly (Fig. 5D). These three observations are consistent with the notion that Dpp is sufficient to induce *dve* expression without Wg in the midgut. To confirm this, we examined the effect of the ubiquitous *wg* expression in the visceral mesoderm on *dve* expression. The ubiquitous activation of the Wg signal using *shaggy* null mutations induces the expression of *Ubx* and *dpp* only anteriorly, but not posteriorly, in the midgut (Yu et al. 1996). The ubiquitous *wg* expression in the whole visceral mesoderm induced ectopic *dve* expression only anteriorly (Fig. 5F), indicating that this induction appears to be indirect and mediated by ectopic *dpp* activation. It is noteworthy that the ectopic *dve* induction is especially strong in the anterior-most region that is normally responsive to Wg (Fig. 5F). Taken together, these results indicate that *dve* expression in the middle midgut does not depend on Wg but on Dpp, which is in contrast to *dve* expression during proventriculus development.

Two distinct transcription factors, *schnurri* (*shn*) and *Mad*, have been reported to mediate the Dpp signal (Arora et al. 1995; Grieder et al. 1995; Kim et al. 1997). The expression of Dve protein in the middle midgut is absent in *shn* mutants (Fig. 5G; data not shown). In addition, the ubiquitous mesodermal expression of *dpp* in the *shn* mutant background fails to induce endodermal *dve* expression (Fig. 5G), indicating that *dve* induction

Figure 5. Dpp signal regulates *dve* expression in the middle midgut. Lateral (A–G) or ventral (H) view of embryos stained with anti-Dve serum. Anterior is left in all panels. (A) A wild-type embryo (stage 14). The arrow indicates the middle midgut expression. (B) *dve¹* homozygous embryos at stage 14 are not stained at all. (C) Dve expression in the middle midgut (arrow) is absent in *thick veins* (*tkv⁸*) mutant embryos. (D) Dve expression is expanded throughout the posterior midgut (arrow) in *abdominal-A* (*abd-A^{M1}*) mutants. (E,F) Ectopic expression of *dpp* and *wg* was induced in the whole visceral mesoderm on the crossing of UAS-*dpp* (E) and UAS-*wg* (F), respectively, with 24B-Gal4. Note the absence of *dve* induction in the posterior midgut (arrow in F), and the strong posterior expansion in the anterior-most region. (G) In *schnurri* (*shn¹*) mutants, *dpp* was induced ubiquitously in all the visceral mesoderm, whereas *dve* expression was absent in the middle midgut. (H) In *Mothers against dpp* (*Mad¹²*) mutants, *dve* is normally expressed in the middle midgut, although the middle constriction does not occur at stage 16 (indicated by an arrow).

requires *shn* activity in the midgut endoderm. In contrast, *dve* expression is not affected in *Mad* mutants, whereas *lab* expression is completely absent in the same mutant background (Fig. 5H; see Newfeld et al. 1996 and also Discussion).

Phenotypes of *dve* mutants in the larval middle midgut

Although the *dve* gene is strongly expressed in the embryonic middle midgut, morphological defects are not evident in this region in *dve¹* mutants; gut constriction normally occurs, and the arrangement of the stage-17 midgut appears to be normal (see Fig. 3E–H). Cells that express the *dve* gene at embryonic stages develop into four types of larval midgut cells: copper, interstitial,

Figure 6. Phenotypes of *dve* mutants in the larval midgut. The expression of *lacZ* from the *dve*¹ allele (*dve*¹-*lacZ*) or an interstitial cell marker (C5-2-7) was examined by X-gal staining. (A) Interstitial, large flat (lfc), and iron cells (irc) are stained in the midgut prepared from heterozygous *dve*¹/*CyO* larvae. The copper and interstitial cell region (cop/int) is boxed. (B,C) High magnification views of copper and interstitial cell regions from *dve*¹/*CyO* (B) and *dve*¹/*Df(2R)X58-3* mutant larvae (C), respectively. Arrowheads indicate the positions of copper cells. (D,E) The expression of an interstitial cell marker, C5-2-7, was examined in C5-2-7, *dve*^{E38}/*CyO* (D) and C5-2-7, *dve*^{E38}/*Df(2R)X58-3* mutant larvae (E), respectively. Arrowheads indicate the positions of copper cells. (F,G) Rescue experiments on a mutant phenotype of ectopic *dve*¹-*lacZ* in copper cells. The exogenous *dve* transgene was induced at embryonic stage 17 under the control of heat shock promoter-Gal4 (hs-Gal4) in the *dve*¹ mutant background. (F) The abnormal morphology of the proventriculus in a *dve*¹ homozygous mutant was visualized by colored yeast feeding. (G) High magnification view of the boxed region in F shows that the ectopic *dve*¹-*lacZ* expression is partially suppressed in copper cells (arrows). Anterior is left in all panels.

large flat, and iron cells. The expression of the *dve* gene in these larval cells was monitored as the expression of the *lacZ* gene that is located in the *dve*¹ enhancer-trap insertion (*dve*¹-*lacZ*). β -Galactosidase activity attributable to *dve*¹-*lacZ* is observed in interstitial, large flat, and iron cells, but not in copper cells in heterozygous larvae (Fig. 6A,B). In *dve* mutant larvae, we found the ectopic *dve*¹-*lacZ* expression in copper cells, in addition to highly disorganized arrangement of these cells (Fig. 6C). These observations suggest that the *dve* activity is required to repress its own expression in copper cells. To determine whether the *dve* gene could rescue this mutant phenotype of ectopic *dve*¹-*lacZ* expression, the *dve* gene was induced ubiquitously in the *dve*¹ mutant background under the control of Gal4-UAS system (Brand and Perrimon 1993). Weak *dve* induction in stage-17 embryos suppressed the ectopic *dve*¹-*lacZ* expression in larval copper cells (Fig. 6C, F,G), indicating that a mutant

phenotype is exactly caused by the lack of the *dve* gene product.

To monitor cell types in the larval midgut, the C5-2-7 enhancer-trap insertion, which marks specifically interstitial cells, was introduced into the *dve*^{E38} background (see Materials and Methods). In *dve*^{E38} mutants, C5-2-7 expression remained unaffected although the arrangement of copper cells was highly disorganized (Fig. 6D,E), suggesting that interstitial cells are not strongly affected by the *dve* mutation.

In the larval midgut, the *dve* gene is thus expressed in a cell type-specific manner that requires its own activity. As described below, the repression of the *dve* gene in copper cells depends on the function of both Lab and Dve itself, and is essential for the functional specification of these cells.

Copper cells are specified by the cross-regulation between dve and lab

In the middle midgut, *lab* is expressed under the control of Dpp as is *dve*, whereas *lab* is regulated negatively by the Wg signal to generate a sharp posterior border (Hoppler and Bienz 1995). We compared the expression of *lab* and *dve* using *lab* upstream 6.3 kb-*lacZ* (*lab*-6.3*lacZ*), which mimics endogenous *lab* expression (Tremml and Bienz 1992). The expression of *lab* is observed in the endoderm just beneath the *dpp*-expressing visceral mesoderm of PS 7, but not in the inner endodermal cells as described (Reuter et al. 1990; Fig. 7B). In contrast, *dve* is expressed more broadly throughout the inner endodermal layers including presumptive interstitial cell precursors (Fig. 7A-C). Another difference in *lab* and *dve* expression is that *dve* expression is repressed subsequently in *lab*-expressing cells that become copper cells (see Fig. 6B). We examined the possibility that Lab might be involved in the repression of *dve* in copper cells. In *lab* mutants, *dve*^{E38}-*lacZ* expression is not repressed in presumptive copper cells as observed in *dve* mutants (Fig. 7E-G). This pattern of gene expression is similar to that of neighboring interstitial cells, which express *dve* continuously without *lab* expression in the wild type. Hence the inability of *dve* repression in the presumptive copper cells of *lab* mutants could be attributable to a change in cell fate—copper cells to interstitial cells. In this case, the expression of an interstitial cell marker C5-2-7 should expand in this region. However, it turned out that its expression is abolished in *lab* mutants (Fig. 7H,I), suggesting that interstitial cells are also affected by the *lab* mutation. Thus, it is unlikely that the *lab* mutation causes the transformation of copper cells into interstitial cells. Taken together, the repression of *dve* requires the activities of both Lab and Dve itself.

To determine whether the *dve* repression in copper cells is essential for establishment of their correct identity, *dve* was overexpressed ubiquitously at stage 17. Strong heat shock-induced *dve* expression resulted in an abnormal morphology of copper cells. The typical banana shape of copper cells is frequently lost, the cells becoming circular (Fig. 7J), suggesting an abnormal cyto-

Figure 7. Functional specification of copper cells by Dve and Lab. (A–C) Confocal images of *lab-6.3lacZ* (red in B) and *dve* (green in C) expression at stage 14 (dorsal view). A merged image is shown in A. Arrows indicate a Dve-positive cell located in the inner layer of the endoderm. (D) Coexpression of *dve* (black) and *lab-6.3lacZ* (brown) in copper cell precursors (black arrow) at stage 16 (ventral view). Interstitial cell precursors (Dve single-positive; white arrow) and copper cell precursors are intermingled. (E–G) The expression of *dve^{E8}-lacZ* in *lab^{vd1}* heterozygous larvae (E) and homozygous mutants (F, G), respectively. The copper and interstitial cell regions are boxed. (G) High magnification view of the boxed region in F. (H, I) The *lacZ* expression of interstitial cell marker C5-2-7 in *lab^{vd1}* heterozygous larvae (H) and homozygous mutants (I), respectively. The *lab* mutation abolishes C5-2-7 expression in the midgut (boxed region); however, it does not affect the expression in the ventral nerve cord (arrows). (J, K) X-gal staining of guts from *dve^{1/+}; hs-GAL4/UAS-dve-9B2* larvae heat shocked at embryonic stage 17 (J, 37°C for 60 min; K, 37°C for 30 min). (L–N) UV-induced fluorescence after copper feeding. Guts from *dve^{1/+}; hs-GAL4/+* (L) and *dve^{1/+}; hs-GAL4/UAS-dve-9B2* larvae (M, N) heat shocked at embryonic stage 17 (37°C for 30 min) were excited with UV light. The copper and interstitial cell regions are boxed. Control guts exhibit strong fluorescence of copper cells (L). Ectopic *dve* expression greatly reduces the fluorescence of copper cells (M, N). Note the presence of posterior fluorescence corresponding to the iron cell regions (arrows). Anterior is left in all panels.

skeletal organization in these cells. To determine the effect of ectopic *dve* on the copper cell function, ubiquitous *dve* expression was induced alternatively by a milder heat shock. Under this condition, the copper cells appear to retain their normal morphology (Fig. 7K), however, the typical character of copper cells, UV light-induced orange fluorescence on copper feeding, is greatly reduced in these guts (Fig. 7L–N). This mild heat shock does not affect the posterior fluorescence attributable to iron cells, suggesting that the function of copper cells is

specifically impaired by this treatment. Taken together with the results for *dve* mutants described above, both the loss of function and ectopic expression of the *dve* gene affect the morphology of copper cells, and ectopic *dve* expression impairs the function of copper cells without affecting their morphology. Unfortunately, the function of copper cells in *dve* mutants could not be examined because of the feeding defect in the proventriculus; however, the morphological abnormality observed in *dve* mutants suggests that *dve* activity is also required for the functional development of copper cell precursors. Our results indicate that temporally restricted *dve* repression is essential for this functional specification in addition to the *lab* gene, which is indispensable for copper cell development (Hoppler and Bienz 1994). This repression depends on Lab and Dve itself. Thus, the cross-regulation of the two Dpp target genes *lab* and *dve* specifies the functional identity of copper cells.

Discussion

Dve expression is regulated differentially by Dpp and Wg

Accumulating evidence has shown that signal inputs from Dpp and Wg exert cooperative or antagonistic effects to define the border of target gene expression. The development of the leg imaginal disc has provided a typical example for the antagonistic and cooperative functions of these two factors. *dpp* and *wg* are expressed adjacently on the anterior side of the anterior-posterior compartment boundary of the disc; *wg* is expressed ventrally, whereas *dpp* is expressed at high levels dorsally. The convergence of both signals is required to induce *Distal-less* and *aristaless* at the center of the disc (Campbell et al. 1993; Cohen et al. 1993). On the other hand, the expression of a ventral marker, H15, is activated by Wg and repressed by Dpp. Thus, the antagonistic effects of these two factors restrict the H15 expression ventrally (Brook and Cohen 1996). Another example of the complex gene regulation by Dpp and Wg has been found in midgut morphogenesis, where both *dpp* and *wg* are expressed in adjacent regions of the visceral mesoderm of PS 7 and 8, respectively. Dpp and Wg act independently but synergistically on the visceral mesoderm enhancer of *Ubx*; however, Wg-dependent transcriptional stimulation requires the Dpp signal (Riese et al. 1997). In contrast, the *lab* expression in the endoderm is activated by Dpp and repressed by Wg to confer the discrete boundary between copper and large flat cells (Hoppler and Bienz 1995).

The roles of Dpp and Wg in *dve* expression are quite different from those reported previously. Dve-expressing domains are exposed to both Wg and Dpp; however, *dve* expression responds differentially to either of them in distinct parts of the midgut. In the middle midgut, *dve* expression depends mainly on Dpp, although a possible contribution of the Wg signal cannot be excluded, as observed for *Ubx* expression. In contrast, the *dve* expression in the anterior-most midgut depends on Wg, but not

Dve specifies the midgut function

on Dpp. In this region, the posterior border of *dve* expression appears to be defined by the Wg signal.

How does the *dve* gene sort the Wg and Dpp signals depending on the domains of the midgut? Enhancer elements responding to the two signals in the midgut have been well studied for the *Ubx* gene. In the case of the *Ubx* gene, the two signal inputs are mediated by the B enhancer, which includes the Dpp response sequence (DRS) and the Wg response sequence (WRS). These two response sequences are closely located so that signal inputs from Dpp and Wg coordinately induce the expression in a pattern spanning both sides of the middle constriction in the visceral mesoderm (Riese et al. 1997). This expression pattern is similar to that of the *dve* gene, although the B enhancer of the *Ubx* gene drives the expression mainly in the visceral mesoderm. Interestingly, four copies of this enhancer derivative (L-CRE) drive Wg-dependent expression in the anterior-most endoderm of the midgut where the *dve* gene is activated. The similarity in the spatial pattern between *dve* expression and the *Ubx* enhancer activation raises the possibility that Dpp and Wg act on similar regulatory elements that are present in the *dve* gene to induce the broad expression in the middle midgut. We do not know anything about the regulatory mechanisms that activate *dve* gene depending on Wg but not on Dpp in the anterior-most midgut. Characterization of the regulatory elements of the *dve* gene would provide useful clues for spatial regulation during Dpp- and Wg-dependent pattern formation.

Early gradient of Dpp defines the cell identity in the midgut

In the middle midgut, both the *lab* and *dve* genes are activated by the Dpp signal, but they do not respond equally to Dpp. *lab* expression is restricted strictly to the endodermal cells that are in contact with the *dpp*-expressing visceral mesoderm (Reuter et al. 1990). In contrast, *dve* is widely activated even in endodermal cells that are remote from the Dpp source (Fig. 7A–C). Assuming a concentration gradient of Dpp in the endoderm as well as wing disc (Lecuit et al. 1996; Nellen et al. 1996), these observations suggest that *lab* expression requires a higher concentration of Dpp than *dve* expression. Therefore, *dve* can be activated by low levels of Dpp that are insufficient to induce *lab*. Furthermore, we found a difference between *lab* and *dve* gene activation in the requirement for *Mad*, which transduces the Dpp signal. Mutant embryos for *Mad* fail to express *lab* but show normal activation of *dve* (Newfeld et al. 1996; Fig. 5H). If the maternal supply of *Mad* transcripts is sufficient to induce *dve*, but not *lab* expression, the difference in *Mad* dependence might be explained by the different thresholds for the Dpp signal between *lab* and *dve* activation.

The early gradient of Dpp in the endoderm thus gives rise to two distinct cells in terms of gene expression; one is *lab*[−] and *dve*⁺, and the other is *lab*⁺ and *dve*⁺ (Fig. 8). If this situation is disrupted by ubiquitous *lab* induction during 7–9 hr of embryonic development, interstitial cells are transformed into copper cell-like cells that ex-

Figure 8. Model for the functional specification of copper cells by Dve and Lab. Schematic representation of gene expression during midgut development. High levels of Dpp can induce the expression of *lab* (red) and *dve* (green) in the endoderm (END) just beneath the PS 7 visceral mesoderm (VM). These cells indicated by yellow nucleus become to copper cells (copper cell precursors; *lab*⁺*dve*⁺). More inner and posterior endodermal cells, which are exposed to low levels of Dpp, can express *dve* but not *lab* (green nucleus; *lab*[−]*dve*⁺), and become to interstitial, large flat (lfc) and iron cells (irc). Copper cell precursors coexpress *dve* and *lab*, and subsequently repress *dve* expression (red nucleus; *lab*⁺*dve*[−]) in a Lab- and Dve-dependent manner. Two alternative mechanisms of *dve* repression (cell autonomous and cell nonautonomous) are boxed in the middle. After stage 17, the Lab function is cell nonautonomously required for the expression of interstitial cell marker C5-2-7. The *dve* repression mediated by Lab and Dve itself is critical for acquisition of the functional specificity in larval copper cells (see text for details).

hibit orange fluorescence on copper feeding (Hoppler and Bienz 1994). At later stages, these two types of cells intermingle, and interstitial cell precursors are also exposed to high Dpp (Fig. 7D; Reuter et al. 1990). Nonetheless, these cells never express *lab* at later stages, indicating that the cell identity once established by the early Dpp gradient seems to be maintained. Mechanisms involved in this maintenance of cell identity remain unclear. One of the simplest explanations would be that Dve continues to suppress *lab* in interstitial cell precursors. However, this is unlikely because ectopic expression of *lab*-6.3*lacZ* is not detectable in *dve* mutants (data not shown). Another possibility is that the negative autoregulation of *lab* is involved in the continuous *lab* suppression in interstitial cell precursors. In fact, heat shock induction of the *lab* transgene between 9 and 11 hr of development frequently results in severe repression of copper cell development, suggesting that heat-induced Lab during a critical period suppresses the endogenous *lab* gene (Hoppler and Bienz 1994). The time window for this negative *lab* autoregulation coincides well with when interstitial cell precursors are exposed to high Dpp. It is also an intriguing possibility that Dpp-induced Smad molecules, which inhibit the TGF- β /Dpp signal,

participate in these processes as a negative feedback loop (Hayashi et al. 1997; Nakao et al. 1997; Tsuneizumi et al. 1997).

Dve function in copper cell specification

Dve is expressed in all precursors of four distinct types of middle midgut cells, and is repressed subsequently only in copper cells. This repression is mediated by Lab and Dve itself, and turns out to be essential for the functional specification of copper cells, as judged by the ability of copper uptake. This does not necessarily mean that *dve* activity is required for the cell fate decision of copper cells. In fact, the expression of several cell-type markers for middle midgut cells is unaffected in *dve* mutants (Fig. 6E; data not shown). *dve* activity seems to be required to refine the functional specificity at later stages of development after the cell fate decision has been completed.

It is very important to understand the mechanism of *dve* repression that is critical for the acquisition of functional identity in copper cells. There are two possibilities that explain how the repression of the *dve* gene occurs in larval copper cells (Fig. 8). One is a cell nonautonomous mechanism; interstitial cells might send a signal to adjacent copper cells to repress *dve* gene expression. For instance, an interstitial cell marker, C5-2-7, is abolished in *lab* mutants (Fig. 7H,I), suggesting a cell nonautonomous interaction between interstitial cells and copper cells. The other is a cell autonomous mechanism by which the *dve* gene is autoregulated negatively in copper cells. In copper cell precursors, Lab and Dve are coexpressed during embryogenesis, and are able to act coordinately as transcriptional regulators. Thus, the latter possibility is more plausible, and Dve might function as a transcriptional cofactor for Lab to specify the functional identity of copper cells. Further characterization of the *dve* gene would clarify the molecular mechanism conferring the functional specificity to each of the four distinct types of middle midgut cells.

Materials and methods

Fly strains

The following mutant strains were used: *Df(2R)X58-3* (Kerbrock et al. 1995) uncover the *dve* locus; *wg^{CX4}*, *wg^{IL114}*, *hh^{AC}*, *hh^{U35}*, *ci^D*, *tkv⁸*, *abd-A^{M1}*, *shn¹*, *Mad¹²*, and *lab^{vd1}* (FLYBASE; <http://morgan.harvard.edu/>). *dpp* or *wg* was induced ectopically in the embryonic mesoderm by using 24B-Gal4, UAS-*dpp* (Staehling-Hampton and Hoffmann 1994) and UAS-*wg* (Lawrence et al. 1995). To monitor the cell type-specific expression in the midgut, copper cell marker *lab-6.3lacZ* (Tremml and Bienz 1992), interstitial cell marker C5-2-7, and large flat cell markers A3-2-66 and B1-2-49 (Hartenstein and Jan 1992; Hopper and Bienz 1995) were used. The following *lacZ* balancers were used to distinguish homozygous embryos: *en¹¹CyO*, *Cy-Oftz-lacZ*, *TM3ftz-lacZ*, and *TM3Ubx-lacZ*. The *ci^D* homozygous embryos were identified as to the expression pattern of *wg-lacZ* derived from *en¹¹CyO*. *dve^{SH255}* and *dve¹* are enhancer-trap lines of FZ and PZ, respectively; *dve¹* is identical to *l(2)01738⁰¹⁷³⁸* generated by the Berkeley Drosophila Genome Project (Karpen and Spradling 1992; Mlodzik and Hiromi 1992). By the P-element excision of *dve¹*, an imprecise excision allele *dve^{E38}* was recovered that has the internal deletion of both the

rosy marker and the *lacZ* gene. *dve^{E38}* mutants showed essentially the same Dve protein expression and phenotype as the *dve¹* allele. To examine roles in cell-type specification, each of the midgut cell type markers was introduced into the *dve^{E38}* background by recombination. *dve^{E8}* is a hypomorphic allele that lacks the *rosy* marker but retains the *lacZ* gene, and this allele was used to monitor *dve* expression in the *lab* mutant background.

A 4.5-kb *EcoRI* fragment of *dve* cDNA was inserted into the pUAST vector (Brand and Perrimon 1993) to construct pUAS-*dve*, and several independent transformants were obtained. Of these lines, UAS-*dve-9B2* (homozygous viable on the third chromosome) was used in this study. For the rescue experiments, *yw; dve¹/CyO[y⁺]; hs-GAL4* flies were crossed with *y w; dve¹/CyO[y⁺]; UAS-dve-9B2*, and stage-17 embryos were heat shocked at 35°C for 30 min. Homozygous first instar larvae were identified from the absence of the yellow (*y*) marker. For ectopic expression, *yw; dve¹/CyO[y⁺]; UAS-dve-9B2* flies were crossed with *hs-Gal4*, and *yw; dve¹/CyO[y⁺]; hs-GAL4* flies were crossed with Oregon-R as a control, and stage-17 embryos were heat shocked at 37°C. Subsequently, the gut morphology and copper fluorescence were examined.

Isolation of genomic and cDNA clones

The *dve* gene was identified originally from an enhancer trap line that shows an abnormal object fixation response (*dve^{SH255}*; H. Nakagoshi and F. Matsuzaki, unpubl.). A 4.5-kb *SalI* genomic fragment flanked to *dve^{SH255}* insertion was used for screening Oregon-R adult head cDNA library. A nearly full-length *dve* cDNA clone (W12) was isolated, and its 5' end was determined by 5' RACE. Genomic clones covering the *dve* locus were isolated from Oregon-R genomic DNA library, and the exon-intron boundaries were determined. DNA sequencing was carried out using an ABI 373 DNA sequencer (Applied Biosystems).

Antibody staining

A 2.3-kb *EcoRI* fragment of *dve* cDNA was inserted into the pTrcHisC vector (Invitrogen Xpress System). As a result, the carboxy-terminal 368 amino acid fragment was fused to the amino-terminal histidine tag. The resultant fusion protein (HE26) was purified and immunized to a rabbit. The HE26 antiserum was used for further immunostaining experiments. The stages of embryonic development are according to Campos-Ortega and Hartenstein (1985). For whole-mount staining, embryos were dechorionized and then fixed in a 4% formaldehyde/phosphate-buffered saline (PBS): heptane = 1:1 solution for 30 min, devitellinized with methanol and then washed with PBS-0.2% Tween 20. Embryos were incubated with HE26 antiserum (1:1000 dilution) in the blocking solution (5% skim milk/PBS-0.2% Tween 20), and then washed with PBS-0.2% Tween 20. For the detection, a secondary antibody (biotin-conjugated anti-rabbit IgG, diluted 1:1000; Vector Labs) and a horseradish peroxidase (HRP)-conjugated avidin-biotin complex solution (ABC Elite; Vector Labs) were used. To detect *lacZ* expression, an anti-β-galactosidase monoclonal antibody (diluted 1:1000; Promega) was used.

For confocal microscopic observations with a Bio-Rad MRC-1024, Cy-3 conjugated anti-mouse IgG and Cy-5 conjugated anti-rabbit IgG (Jackson) were used as secondary antibodies.

Feeding assay and X-gal staining of larval guts

For the feeding assay, larvae were allowed to grow on sucrose-supplied agar plates of 100-mm dish with a red-colored yeast (dyed with carmine; Sigma). Several hours feeding was enough

to visualize gut motility. To analyze the copper cell function, larvae were fed with the yeast containing a high concentration (2–3 mg/ml) of CuSO₄ for at least 5 hr to visualize posterior fluorescence attributable to iron cells. The guts of dissected larvae were immediately mounted in 80% glycerol and excited with UV light using a DAPI filter. The procedure was basically according to Hoppler and Bienz (1994).

For X-gal staining, first instar larvae were dissected in a fixing solution (1% glutaraldehyde/PBS), and subsequently their guts were washed with PBS and stained in 0.1% 5-bromo-4-chloro-3-indolyl- β -galactopyranoside (X-gal) for β -galactosidase activity. The stained guts were washed with PBS–0.2% Tween 20 and then mounted in 80% glycerol.

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Note

The nucleotide sequence data reported in this paper will appear in the DDBJ, EMBL, and GenBank nucleotide sequence databases under the following accession number AB010299.

References

- Arora, K., H. Dai, S.G. Kazuko, J. Jamal, M.B. O'Connor, A. Letsou, and R. Warrior. 1995. The *Drosophila schnurri* gene acts in the Dpp/TGF β signaling pathway and encodes a transcription factor homologous to the human MBP family. *Cell* **81**: 781–790.
- Bienz, M. 1994. Homeotic genes and positional signaling in the *Drosophila* viscera. *Trends Genet.* **10**: 22–26.
- Billin, A.N., K.A. Cockerill, and S.J. Poole. 1991. Isolation of a family of *Drosophila* POU domain genes expressed in early development. *Mech. Dev.* **34**: 75–84.
- Blair, S.S. 1995. Compartments and appendage development in *Drosophila*. *BioEssays* **17**: 299–309.
- Bodner, M., J.-L. Castrillo, L.E. Theill, T. Deerinck, M. Ellisman, and M. Karin. 1988. The pituitary-specific transcription factor GHF-1 is a homeobox-containing protein. *Cell* **55**: 505–518.
- Brand, A.H. and N. Perrimon. 1993. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118**: 401–415.
- Brook, W.J. and S.M. Cohen. 1996. Antagonistic interactions between *wingless* and *decapentaplegic* responsible for dorsal-ventral pattern in the *Drosophila* leg. *Science* **273**: 1373–1377.
- Bürglin, T.R. 1997. Analysis of TALE superclass homeobox genes (MEIS, PBC, KNOX, Iroquois, TGIF) reveals a novel domain conserved between plants and animals. *Nucleic Acids Res.* **25**: 4173–4180.
- Cadigan, K.M. and R. Nusse. 1997. Wnt signaling: A common theme in animal development. *Genes & Dev.* **11**: 3286–3305.
- Campbell, G., T. Weaver, and A. Tomlinson. 1993. Axis specification in the developing *Drosophila* appendage: The role of *wingless*, *decapentaplegic*, and the homeobox gene *aristal-less*. *Cell* **74**: 1113–1123.
- Campos-Ortega, J.A. and V. Hartenstein. 1985. *The embryonic development of Drosophila melanogaster*. Springer-Verlag, Berlin, Germany.
- Cavallo, R., D. Rubenstein, and M. Peifer. 1997. Armadillo and dTCF: A marriage made in the nucleus. *Curr. Opin. Genet. Dev.* **7**: 459–466.
- Chen, X., M.J. Rubock, and M. Whitman. 1996. A transcriptional partner for MAD proteins in TGF- β signalling. *Nature* **383**: 691–696.
- Cho, K.W., B. Blumberg, H. Steinbeisser, and E.M. De Robertis. 1991. Molecular nature of Spemann's organizer: The role of the *Xenopus* homeobox gene *goosecoid*. *Cell* **67**: 1111–1120.
- Cohen, B., A.A. Simcox, and S.M. Cohen. 1993. Allocation of the thoracic imaginal primordia in the *Drosophila* embryo. *Development* **117**: 597–608.
- Collum, R.G., P.E. Fisher, M. Datta, S. Mellis, C. Thiele, K. Huebner, C.M. Croce, M.A. Israel, T. Theil, T. Moroy, R. DePinho, and F.W. Alt. 1992. A novel POU homeodomain gene specifically expressed in cells of the developing mammalian nervous system. *Nucleic Acids Res.* **20**: 4919–4925.
- Dalton, D., R. Chadwick, and W. McGinnis. 1989. Expression and embryonic function of *empty spiracles*: A *Drosophila* homeo box gene with two patterning functions on the anterior–posterior axis of the embryo. *Genes & Dev.* **3**: 1940–1956.
- Finkelstein, R., D. Smouse, T.M. Capaci, A.C. Spradling, and N. Perrimon. 1990. The *orthodenticle* gene encodes a novel homeo domain protein involved in the development of the *Drosophila* nervous system and ocular visual structures. *Genes & Dev.* **4**: 1516–1527.
- Gehring, W.J. 1992. The homeobox in perspective. *Trends Biochem. Sci.* **17**: 277–280.
- Graba, Y., D. Aragnol, and J. Pradel. 1997. *Drosophila* Hox complex downstream targets and the function of homeotic genes. *BioEssays* **19**: 379–388.
- Grieder, N.C., D. Nellen, R. Burke, K. Basler, and M. Affolter. 1995. Schnurri is required for *Drosophila* Dpp signaling and encodes a zinc finger protein similar to the mammalian transcription factor PRDII-BF1. *Cell* **81**: 791–800.
- Hanes, S.D. and R. Brent. 1989. DNA specificity of the *bicoid* activator protein is determined by homeodomain recognition helix residue 9. *Cell* **57**: 1275–1283.
- Hara, Y., A.C. Rovescalli, Y. Kim, and M. Nirenberg. 1992. Structure and evolution of four POU domain genes expressed in mouse brain. *Proc. Natl. Acad. Sci.* **89**: 3280–3284.
- Hartenstein, V. and Y.N. Jan. 1992. Studying *Drosophila* embryogenesis with P-lacZ enhancer trap lines. *Wilhelm Roux's Arch. Dev. Biol.* **201**: 194–220.
- Hayashi, H., S. Abdollah, Y. Qiu, J. Cai, Y.-Y. Xu, B.W. Grinnell, M.A. Richardson, J.N. Topper, M.A. Gimbrone Jr., J.L. Wrana, and D. Falb. 1997. The MAD-related protein Smad7 associates with the TGF β receptor and functions as an antagonist of TGF β signaling. *Cell* **89**: 1165–1173.
- He, X., M.N. Treacy, D.M. Simmons, H.A. Ingraham, L.W. Swanson, and M.G. Rosenfeld. 1989. Expression of a large family of POU-domain regulatory genes in mammalian brain development. *Nature* **340**: 35–41.

- Herr, W. and M.A. Cleary. 1995. The POU domain: Versatility in transcriptional regulation by a flexible two-in-one DNA-binding domain. *Genes & Dev.* **9**: 1679–1693.
- Hewitt, J.E., L.N. Clark, A. Ivens, and R. Williamson. 1991. Structure and sequence of the human homeobox gene HOX7. *Genomics* **11**: 670–678.
- Hoch, M. and M.J. Pankratz. 1996. Control of gut development by *fork head* and cell signaling molecules in *Drosophila*. *Mech. Dev.* **58**: 3–14.
- Hoppler, S. and M. Bienz. 1994. Specification of a single cell type by a *Drosophila* homeotic gene. *Cell* **76**: 689–702.
- . 1995. Two different thresholds of *wingless* signalling with distinct developmental consequences in the *Drosophila* midgut. *EMBO J.* **14**: 5016–5026.
- Immerglück, K., P.A. Lawrence, and M. Bienz. 1990. Induction across germ layers in *Drosophila* mediated by a genetic cascade. *Cell* **62**: 261–268.
- Ingraham, H.A., R. Chen, H.J. Mangalam, H.P. Elsholtz, S.E. Flynn, C.R. Lin, D.M. Simmons, L. Swanson, and M.G. Rosenfeld. 1988. A tissue-specific transcription factor containing a homeodomain specifies a pituitary phenotype. *Cell* **55**: 519–529.
- Johnson, W.A. and J. Hirsh. 1990. Binding of a *Drosophila* POU-domain protein to a sequence element regulating gene expression in specific dopaminergic neurons. *Nature* **343**: 467–470.
- Karpen, G.H. and A.C. Spradling. 1992. Analysis of subtelomeric heterochromatin in the *Drosophila* minichromosome Dp1187 by single P element insertional mutagenesis. *Genetics* **132**: 737–753.
- Kerrebrock, A.W., D.P. Moore, J.S. Wu, and T.L. Orr-Weaver. 1995. Mei-S332, a *Drosophila* protein required for sister-chromatid cohesion, can localize to meiotic centromere regions. *Cell* **83**: 247–256.
- Kim, J., K. Johnson, H.J. Chen, S. Carroll, and A. Laughon. 1997. *Drosophila* Mad binds to DNA and directly mediates activation of vestigial by Decapentaplegic. *Nature* **388**: 304–308.
- Kim, Y. and M. Nirenberg. 1989. *Drosophila* NK-homeobox genes. *Proc. Natl. Acad. Sci.* **86**: 7716–7720.
- Kimmel, B.E., U. Heberlein, and G.M. Rubin. 1990. The homeodomain protein *rough* is expressed in a subset of cells in the developing *Drosophila* eye where it can specify photoreceptor cell subtype. *Genes & Dev.* **4**: 712–727.
- Kingsley, D.M. 1994. The TGF- β superfamily: New members, new receptors, and new genetic tests of function in different organisms. *Genes Dev.* **8**: 133–146.
- Lawrence, P.A., R. Bodmer, and J.-P. Vincent. 1995. Segmental patterning of heart precursors in *Drosophila*. *Development* **121**: 4303–4308.
- Lecuit, T., W.J. Brook, M. Ng, M. Calleja, H. Sun, and S.M. Cohen. 1996. Two distinct mechanisms for long-range patterning by Decapentaplegic in the *Drosophila* wing. *Nature* **381**: 387–393.
- Lloyd, A. and S. Sakonju. 1991. Characterization of two *Drosophila* POU domain genes, related to oct-1 and oct-2, and the regulation of their expression patterns. *Mech. Dev.* **36**: 87–102.
- Massagué, J., L. Attisano, and J.L. Wrana. 1994. The TGF- β family and its composite receptors. *Trends Cell Biol.* **4**: 172–178.
- Massagué, J., A. Hata, and F. Liu. 1997. TGF- β signaling through the Smad pathway. *Trends Cell Biol.* **7**: 187–192.
- Meijer, D., A. Graus, R. Kraay, A. Langeveld, M.P. Mulder, and G. Grosveld. 1990. The octamer binding factor Oct6: cDNA cloning and expression in early embryonic cells. *Nucleic Acids Res.* **18**: 7357–7365.
- Mlodzik, M. and Y. Hiromi. 1992. Enhancer trap method in *Drosophila*: Its application to neurobiology. *Methods Neurosci.* **9**: 397–414.
- Nakao, A., M. Afrakhte, A. Morén, T. Nakayama, J.L. Christian, R. Heuchel, S. Itoh, M. Kawabata, N.E. Heldin, C.H. Heldin, and P. ten Dijke. 1997. Identification of Smad7, a TGF β -inducible antagonist of TGF- β signalling. *Nature* **389**: 631–635.
- Nellen, D., R. Burke, G. Struhl, and K. Basler. 1996. Direct and long-range action of a DPP morphogen gradient. *Cell* **85**: 357–368.
- Newfeld, S.J., E.H. Chartoff, J.M. Graff, D.A. Melton, and W.M. Gelbart. 1996. *Mothers against dpp* encodes a conserved cytoplasmic protein required in DPP/TGF- β responsive cells. *Development* **122**: 2099–2108.
- Nusse, R. and H.E. Varmus. 1992. Wnt genes. *Cell* **69**: 1073–1087.
- Panganiban, G.E., R. Reuter, M.P. Scott, and F.M. Hoffmann. 1990. A *Drosophila* growth factor homolog, *decapentaplegic*, regulates homeotic gene expression within and across germ layers during midgut morphogenesis. *Development* **110**: 1041–1050.
- Pankratz, M.J. and M. Hoch. 1995. Control of epithelial morphogenesis by cell signaling and integrin molecules in the *Drosophila* foregut. *Development* **121**: 1885–1898.
- Reuter, R., G.E. Panganiban, F.M. Hoffmann, and M.P. Scott. 1990. Homeotic genes regulate the spatial expression of putative growth factors in the visceral mesoderm of *Drosophila* embryos. *Development* **110**: 1031–1040.
- Riese, J., X. Yu, A. Munnerlyn, S. Eresh, S.-C. Hsu, R. Grosschedl, and M. Bienz. 1997. LEF-1, a nuclear factor coordinating signaling inputs from *wingless* and *decapentaplegic*. *Cell* **88**: 777–787.
- Schreiber, E., A. Tobler, U. Malipiero, W. Schaffner, and A. Fontana. 1993. cDNA cloning of human N-Oct3, a nervous-system specific POU domain transcription factor binding to the octamer DNA motif. *Nucleic Acids Res.* **21**: 253–258.
- Simeone, A., D. Acampora, A. Mallamaci, A. Stornaiuolo, M.R. D'Apice, V. Nigro, and E. Boncinelli. 1993. A vertebrate gene related to *orthodenticle* contains a homeodomain of the *bicoid* class and demarcates anterior neuroectoderm in the gastrulating mouse embryo. *EMBO J.* **12**: 2735–2747.
- Skaer, H. 1993. The alimentary canal. In *The development of Drosophila melanogaster* (ed. M. Bate, and A. Martinez Arias), pp. 941–1012. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Staehling-Hampton, K. and F.M. Hoffmann. 1994. Ectopic decapentaplegic in the *Drosophila* midgut alters the expression of five homeotic genes, *dpp*, and *wingless*, causing specific morphological defects. *Dev. Biol.* **164**: 502–512.
- Suzuki, N., H. Rohdewohld, T. Neuman, P. Gruss, and H.R. Schöler. 1990. Oct-6: A POU transcription factor expressed in embryonal stem cells and in the developing brain. *EMBO J.* **9**: 3723–3732.
- Treisman, J., P. Gönczy, M. Vashishtha, E. Harris, and C. Desplan. 1989. A single amino acid can determine the DNA binding specificity of homeodomain proteins. *Cell* **59**: 553–562.
- Tremml, G. and M. Bienz. 1992. Induction of *labial* expression in the *Drosophila* endoderm: Response elements for *dpp* signalling and for autoregulation. *Development* **116**: 447–456.
- Tsuneizumi, K., T. Nakayama, Y. Kamoshida, T.B. Kornberg, J.L. Christian, and T. Tabata. 1997. *Daughters against dpp* modulates *dpp* organizing activity in *Drosophila* wing development. *Nature* **389**: 627–631.
- Yu, X., S. Hoppler, S. Eresh, and M. Bienz. 1996. *decapentaplegic*, a target gene of the *wingless* signalling pathway in the *Drosophila* midgut. *Development* **122**: 849–858.

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