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Jelly belly: A *Drosophila* LDL Receptor Repeat-Containing Signal Required for Mesoderm Migration and Differentiation

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Summary

Inductive interactions subdivide the *Drosophila* mesoderm into visceral, somatic, and heart muscle precursors. The muscle precursors form organs by executing tissue-specific migrations and cell fusions. We identified a novel gene, *jelly belly (jeb)*, which is required for visceral mesoderm development. *jeb* encodes a secreted protein that contains an LDL receptor repeat. In *jeb* mutants, visceral mesoderm precursors form, but they fail to migrate or differentiate normally; no visceral muscles develop. Jeb protein is produced in somatic muscle precursors and taken up by visceral muscle precursors. *jeb* reveals a signaling process in which somatic muscle precursors support the proper migration and differentiation of visceral muscle cells. Later in embryogenesis, *jeb* is transcribed in neurons and Jeb protein is found in axons.

Introduction

The earliest patterning events of *Drosophila* development have been the objects of intense and fruitful research. Much is known about the establishment of the anterior-posterior and dorsal-ventral axes, as well the genes that are required to translate this early positional information into various germ tissues like ectoderm and mesoderm. Our understanding of the developmental events that follow this early patterning is notably less complete. How do organs form and what genes are required for the complex processes of migration and differentiation that give rise to physiologically functional tissues? To approach these questions, we performed a screen to identify direct targets of transcriptional regulation by the homeodomain protein Tinman (Tin), an essential regulator of cardiac and visceral mesoderm development.

Tin, a member of the NK family of homeodomain proteins (Kim and Nirenberg, 1989), is required for organogenesis of the embryonic heart and visceral mesoderm (Azpiazu and Frasch, 1993; Bodmer, 1993). It is one of a number of transcription factors whose functions in mesoderm development are conserved from insects to mammals (Evans, 1999; Gajewski et al., 1999; Gossett et al., 1989; Griffin et al., 2000; Lebestky et al., 2000; Lilly et al., 1994; Nguyen et al., 1994). How these transcription factors contribute to the development of diverse mesoderm-derived tissues is an important and largely unsolved question in cell type determination and organogenesis.

Inductive interactions subdivide the early mesoderm into groups of cells that will give rise to the heart, somatic muscle, visceral muscle, fat body, hemocytes, and gonads. The patterning genes *decapentaplegic (dpp)* and *hedgehog (hh)* encode two of the signals that mediate these inductions. *dpp* signaling from the dorsal ectoderm induces heart and visceral mesoderm (Frasch, 1995; Staehling-Hampton et al., 1994). *Hh* acts in combination with *dpp* to activate *bagpipe (bap)*, a gene encoding an NK class homeodomain protein, and induce the formation of visceral mesoderm (Azpiazu et al., 1996).

Following the early subdivision of the mesoderm, cells specified to contribute to distinct tissues perform coordinated migrations to form organs. The *Drosophila* visceral mesoderm is composed of two sets of muscles, an inner layer of circular muscles derived from cells along the trunk of the embryo, and an outer layer of longitudinal muscles derived from the posterior end of the embryo (Campos-Ortega and Hartenstein, 1997). The 12 clusters on opposite sides migrate longitudinally to form two parallel bands along most of the length of the embryo, then ventrally and dorsally to form a closed tube lined by endoderm (Nguyen and Xu, 1998). Longitudinal visceral muscle precursors migrate over the circular muscle cells.

We describe here a gene, *jelly belly (jeb)*, that is required for visceral mesoderm migration and differentiation. *jeb* was identified in a screen for genes whose transcription is directly regulated by Tin. *jeb* is required for a signal to be transduced from somatic mesoderm to visceral mesoderm. We show that the protein encoded by *jeb* is secreted from somatic muscle precursors and taken up by visceral muscle precursors. Ectopic expression of *jeb* alters visceral mesoderm migration and differentiation. Based on these findings, we propose that *jeb* encodes a signal necessary for visceral mesoderm migration and differentiation.

Results

A Screen for Genes Downstream of Tin

We performed a screen to identify genes that are transcriptionally regulated by the homeodomain protein Tin. The method (Figure 1A) relies on genetic selection in yeast for a protein-DNA interaction (Liu et al., 1993; Mastick et al., 1995; Weiss et al., 1998; Wilson et al., 1991). We screened a library that represents 15% of the

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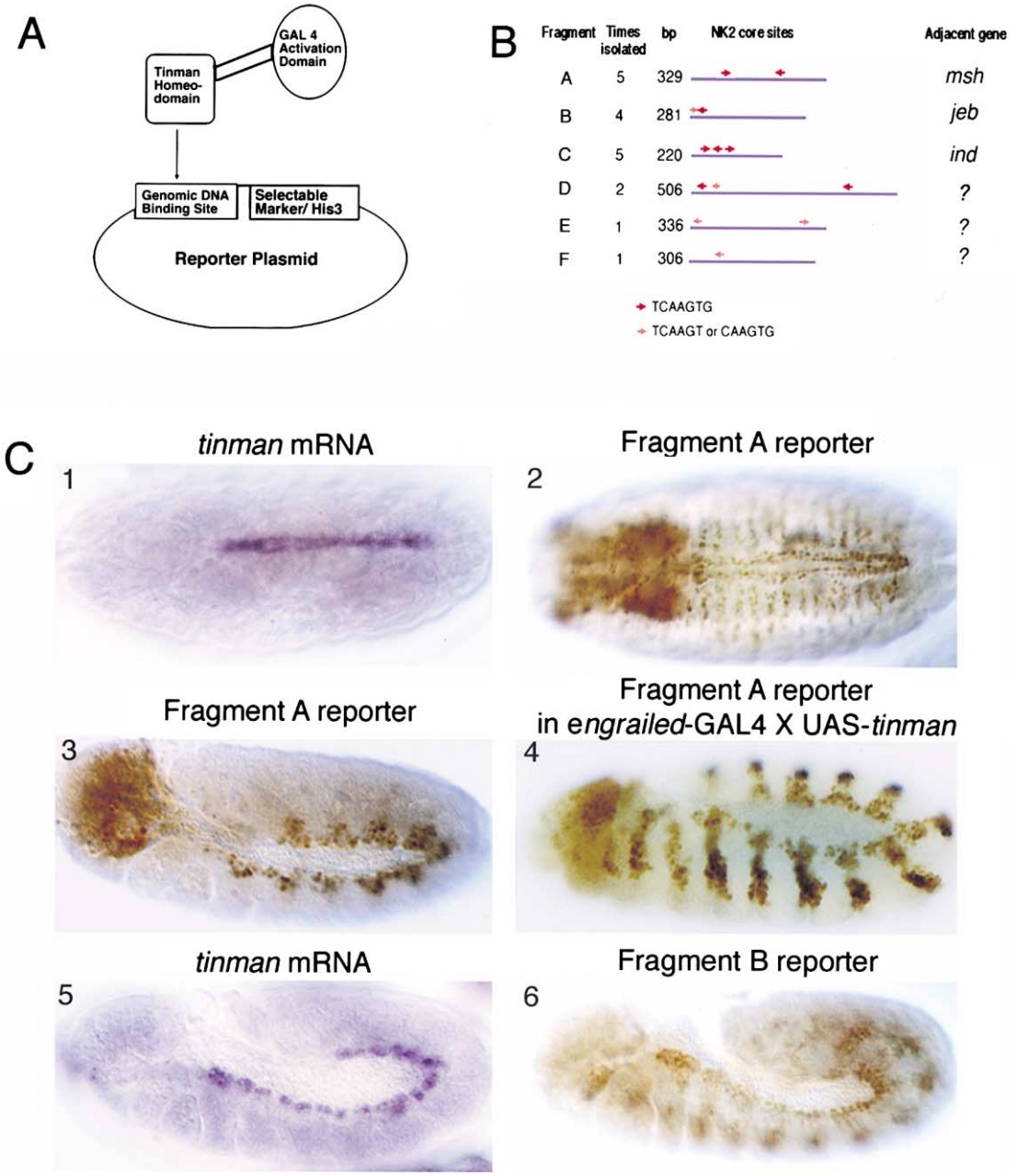


Figure 1. A Screen for Genes Downstream of Tin Identifies Tin Response Elements

(A) Strategy for identifying Tin-regulated genes. Genetic selection in yeast identified fragments of genomic DNA that are recognized by the Tin homeodomain. When a hybrid protein that contains the homeodomain of Tin fused to a yeast GAL4 activation domain binds to a fragment of *Drosophila* genomic DNA, the selectable marker *HiS3* is transcribed.

(B) The results of a pilot screen that covered 15% of the *Drosophila* genome. Six fragments were identified, most of them repeatedly, as recognition sites for the Tin-GAL4 hybrid protein. All the fragments contain a core recognition sequence for NK2 class homeodomains. Three of the fragments lie adjacent to known or newly characterized genes. *jeb* and *ind* were first identified in this screen. *msh* is a homeobox gene expressed in dorsal somatic mesoderm, a tissue that develops under Tin control.

(C) Reporter constructs containing the fragments identified in the yeast screen drive expression of *lacZ* in the mesoderm. (1) Dorsal view of a stage 16 embryo showing *tin* mRNA expression; and (5) lateral view of *tin* mRNA expression in a stage 11 embryo. At these stages, *tin* expression is restricted to precardiac mesoderm (5) and a subset of heart cells (1). (2) Dorsal view of a stage 16 transgenic embryo transformed with the fragment A reporter construct, showing nuclear β-galactosidase detected with anti-β-gal staining. *lacZ* is expressed in dorsal somatic mesoderm and the heart, Tin-dependent tissues. Fragment A maps next to *msh*, which is also expressed in dorsal somatic mesoderm at this stage. (3) A lateral view of a stage 11 transgenic embryo with the fragment A reporter construct. *lacZ* is expressed in dorsal precardiac and somatic mesoderm (compare with [5]). (4) Ectopic expression of Tin in the pattern of *engrailed* activates ectopic expression of the reporter construct. A stage 11 transgenic embryo transformed with a fragment A reporter construct. Ectopic expression of Tin activates the fragment A reporter construct in the pattern of *engrailed*. (6) Lateral view of a stage 11 transgenic embryo showing expression of the fragment B construct in all of the muscle derivatives of the mesoderm. Fragment B lies adjacent to *jeb*, which is expressed just in ventral somatic muscle.

Drosophila genomic DNA and obtained six DNA fragments that satisfied genetic criteria in yeast for Tin binding sites (Figure 1B). Most of the genomic DNA fragments were isolated multiple times. Sequence analysis confirmed the presence of core recognition sites for NK class homeodomains in all of the fragments (Figure 1B) (Chen and Schwartz, 1995). To show that these fragments function as Tin-responsive enhancers in vivo, we asked if they could drive expression of a reporter gene in patterns consistent with Tin regulation.

The screen is surprisingly specific for genes regulated by Tinman (or closely related genes), as demonstrated both by the reporter-construct results and the genes that are located adjacent to the Tinman binding sites. Four fragments identified in the screen (A–D in Figure 1B) were inserted upstream of a *lacZ* reporter. Three of the four reporter constructs, tested as transgenes, are active in patterns consistent with Tin regulation (Figure 1C). The fragment A transgene is expressed in dorsal somatic and cardiac mesoderm (Figure 1C2). This fragment maps near *msh*, a gene that is also expressed in dorsal somatic mesoderm (Lord et al., 1995). The fragment A construct responds to Tinman when it is misexpressed in the striped pattern of *engrailed* (Figure 1, C3 versus C4). A construct containing fragment B is expressed throughout the mesoderm similar to the earliest expression of Tin. This fragment lies adjacent to *jelly belly (jeb)*, a gene expressed in ventral, early mesoderm. The expression pattern, structure, and function of *jeb* are described below. Fragment C, the one of the four that did not drive *lacZ* expression, lies adjacent to *ind*. *ind* was identified in this screen and is a target of negative regulation by Vnd/NK2, a protein structurally similar to Tin (Weiss et al., 1998).

Identification of the *jelly belly* Gene and Transcription of the *jeb* Locus

The Tin binding site that led us to *jeb* (fragment B in Figure 1B) contains two Tin/NK2 class homeodomain recognition sites oriented as an imperfect inverted repeat. This genomic fragment was mapped to interval 48E9 of polytene chromosome 2R by in situ hybridization and based on the *Drosophila* genome sequence. The Tin binding sites lie adjacent to a P element insertion within a large intron of the *jeb* gene (Berkeley *Drosophila* Genome Project: http://www.fruitfly.org/blast/, Figure 2A).

Multiple cDNAs were isolated by hybridization to the *jeb* genomic DNA. The cDNA sequences and developmental RNA blots (Figure 2B) demonstrate two size classes of transcripts derived from the *jeb* locus during early to mid embryogenesis. Later in embryogenesis, a third, larger, transcript is detected. The two early embryonic transcripts contain the same open reading frame. They differ only in 5′ and 3′ untranslated regions. The predicted protein product of the *jeb* locus contains a secretory signal sequence and a single LDL receptor repeat motif (Figures 2C and 2D). In the region of the LDL receptor repeat, Jeb is most similar to two bovine proteins, Sco-spondin and enterokinase (Figure 2D).

The pattern of *jeb* transcription was determined by whole-mount in situ hybridization to embryos. *jeb* mRNA is first detectable at stage 8 in repeated, segmental clusters of ventral mesoderm cells (Figure 3). These cells are precursors of somatic muscle (Azpiazu et al., 1996; Riechmann et al., 1997). Subsequently, *jeb* is transcribed in two roughly parallel, continuous bands in the ventral mesoderm. At stage 12, *jeb* mRNA is no longer detectable in the mesoderm.

Tin Is Sufficient but Not Necessary for *jeb* Expression in the Mesoderm

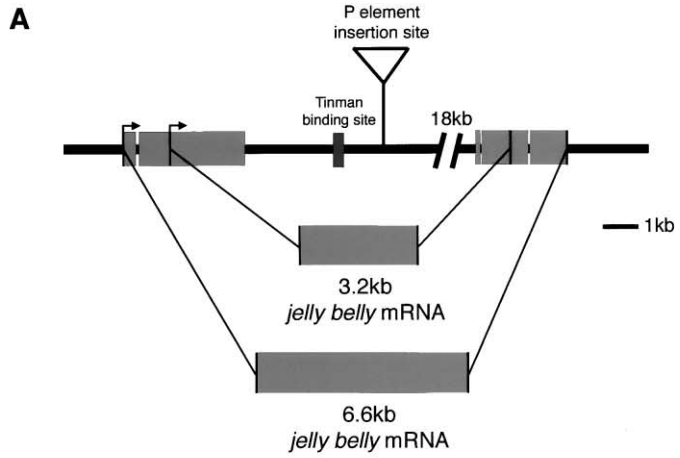
jeb was identified as a putative Tin target gene. *jeb* expression in *tin* mutant embryos is scarcely different from wild-type, though it may be somewhat reduced (data not shown). Tin activation of *jeb* transcription is likely to be redundant with other regulators of mesoderm development. To test the sufficiency of Tin for activating *jeb*, embryos in which *tin* was ectopically expressed were assessed for ectopic *jeb* expression. Misexpression of *tin* in the ectoderm with an *engrailed* GAL4 driver did not alter *jeb* expression (data not shown). Misexpression of *tin* throughout the mesoderm is sufficient to activate *jeb* expression at a late time (stage 12) when it is not expressed in wild-type embryos (Figure 3F), and in cells where *jeb* is not normally expressed. A cofactor in the mesoderm may be required for Tin-mediated activation of *jeb* transcription. The expression domains of *tin* and *jeb* imply that Tin's role in the regulation of *jeb* is restricted to the earliest stages of *jeb* expression, since at late stage 10, Tin is only in dorsal mesoderm and Jeb is in ventral mesoderm.

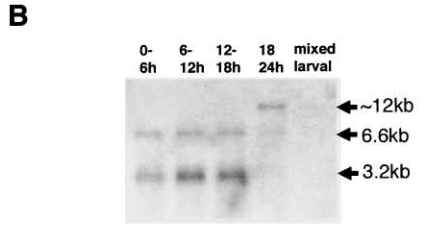
jeb Is Required for Visceral Mesoderm Development

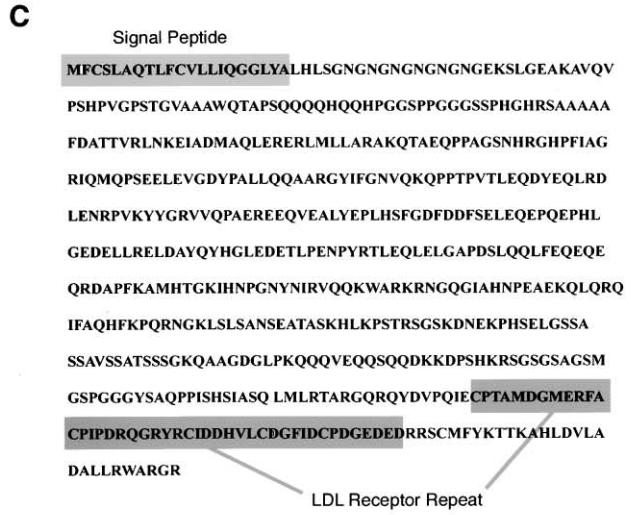
Two alleles of *jeb* have been isolated: the P element transposon insertion that interrupts the *jeb* transcription unit (Figure 2A) and a P element excision derivative of it that results in loss of detectable *jeb* mRNA and Jeb protein. Both mutations cause the recessive lethal phenotype described below. The phenotypes of the two alleles are indistinguishable from each other and from a heterozygote with the excision allele in *trans* to the original P element allele (data not shown). The mutant phenotype can be rescued by driving expression of a *jeb* cDNA transgene in mutant embryos (Figures 4I–4K). These results demonstrate that the phenotype is attributable solely to loss of Jeb function.

jeb function is required specifically for visceral mesoderm development (Figure 4). Anti-myosin heavy chain antibody staining shows the thin layer of mesoderm overlying the yolk in the gut of wild-type embryos (Figure 4A, arrows). In *jeb* mutants, no differentiated visceral mesoderm is detectable (Figure 4B). Other muscular components of the mesoderm, the somatic muscles and dorsal vessel or heart, and other mesoderm tissues, fat body and hemocytes, develop normally in *jeb* mutants (not shown).

Only differentiated muscle contains myosin heavy chain, so the *jeb* mutation could affect differentiation or a prior step in visceral mesoderm development. To look at earlier stages, *jeb* mutant embryos were stained with an antibody against D-Mef2 (Lilly et al., 1994; Nguyen et al., 1994). D-Mef2 is produced early in all muscle lineages of the *Drosophila* embryo. Early *D-mef2* expression is normal in the mesoderm of *jeb* mutants. Later D-Mef2 staining in visceral mesoderm is absent







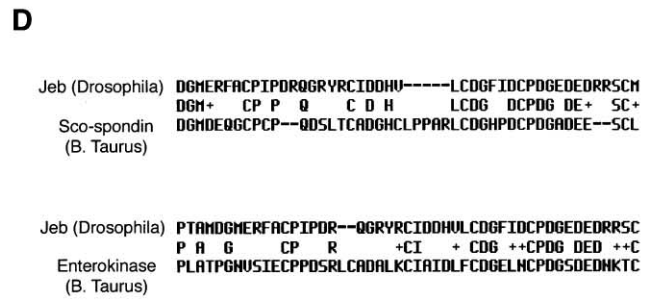


Figure 2. Map of the *jeb* Locus with the Transcript Structure and Predicted Protein Product

(A) The *jeb* locus, ~30 kb of genomic DNA, produces two transcripts in early embryos (B), both with the same open reading frame. Later in embryogenesis, a larger transcript of about 12 kb is detectable. In situ hybridization indicates that the larger transcript is restricted to the central nervous system (Figure 8).

(B) RNA blot showing the size and time of *jeb* mRNA accumulation. Each lane was loaded with 2 mg of poly-A^+ RNA. During the stages when *jeb* is first required, two sizes of transcript are detected, 3.2 kb and 6.6 kb.

(C) The predicted Jeb protein product is shown. The first 20 amino acids are hydrophobic and predicted to be a secretory signal. Close to the carboxy terminus of the protein is a type A LDL receptor repeat, encoded in a single exon.

(D) Alignment of the type A LDL receptor repeat of Jelly belly with the two most closely related proteins, both from *B. taurus*. The first, Sco-Spondin, encodes a secreted protein found in the central nervous system; the second, Enterokinase, is a secreted protease localized to the digestive system.

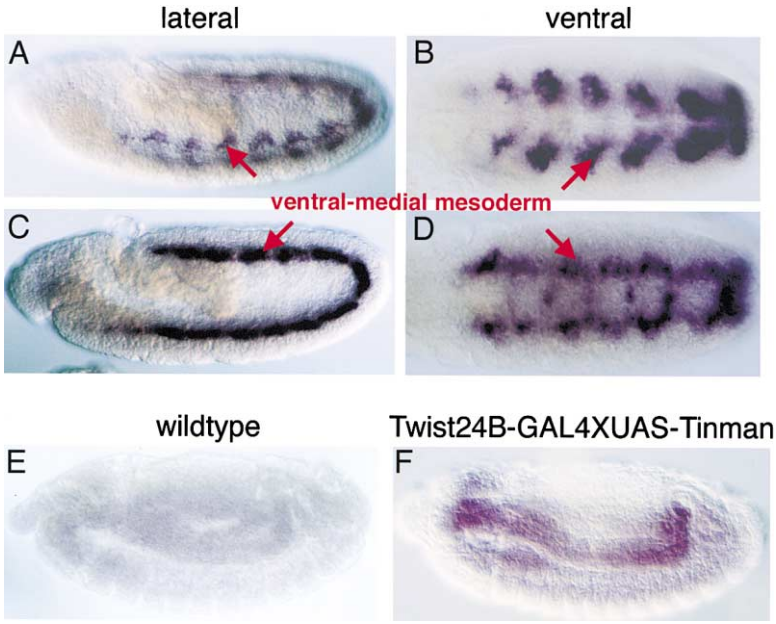


Figure 3. In Situ Hybridization with Antisense *jeb* Probe Shows Early Expression in the Mesoderm

(A and C) Lateral views of early stage 9 embryos stage-matched with ventral views of embryos in (B) and (D). In all panels, anterior is to the left. *jeb* expression is first observed in segmentally repeated clusters of ventral mesodermal cells.

(C and D) Persistence of *jeb* mRNA expression in stage 10 embryos. Segmental variation in level of expression and the dorsal-ventral position of *jeb*-expressing cells is evident.

(E and F) Ectopic expression of *tin* activates *jeb* transcription. Lateral views of stage 12 embryos hybridized with an antisense probe to *jelly belly*. In the embryo shown in (F), *tin* mRNA is ectopically expressed throughout the mesoderm when it would normally be restricted to precursors of the heart. Misexpression of Tin throughout the mesoderm is sufficient to activate ectopic *jeb* expression. *jeb* mRNA is not normally detectable at this stage of development (E).

(Figure 4C versus 4D), though the somatic mesoderm makes D-Mef2 normally.

Endoderm development in *jeb* mutants is not primarily or severely affected. Antibodies against Hindsight protein, a marker of midgut endoderm (Figure 4E), were used to follow endoderm development in *jeb* mutants. Despite the absence of visceral mesoderm, the endoderm is specified normally and migrates to form two longitudinal bands (Figure 4F). Subsequent dorsal and ventral endoderm migration is abnormal in *jeb* mutants, presumably because dorsal and ventral migration depends on the visceral mesoderm (Azpiazu and Frasch, 1993; Reuter et al., 1993).

Visceral Mesoderm Precursors Are Specified in *jeb* Mutants but They Fail to Migrate Normally

Specification of visceral mesoderm requires Dpp and Hh, produced by the overlying ectoderm, to induce Bap, a homeodomain protein related to Tin, in the precursors. Bap protein accumulates normally in *jeb* mutants (Figure 5A versus 5B). In wild-type embryos during stage 11, *bap*-expressing cells, initially specified as segmentally repeated, discrete clusters, commence midgut morphogenesis by migrating longitudinally to form two parallel continuous bands. In *jeb* mutants, *bap*-expressing cells fail to migrate normally to form these two continuous bands. Instead, the cells persist as discrete clusters through the end of stage 11(Figure 5C versus 5D and 5E versus 5F). Shortly after the longitudinal migration of the *bap*-expressing cells, Bap protein decays, and Fas3, a mid-stage marker of visceral mesoderm, is produced. Fas3, a structural protein, is at this stage made only in the visceral part of the mesoderm, and is a useful marker of early differentiation. In *jeb* mutants, Fas3 is weakly and transiently produced (Figure 5G versus 5H and 5I versus 5J). At the germ band retraction stage, when Fas3 production is robust in wild-type embryos, it is absent in *jeb* mutants (Figure 5I versus 5J).

Visceral Mesoderm Cell Fate in *jeb* Mutants

jeb is transcribed in somatic mesoderm cells, yet Bap staining shows that visceral mesoderm precursors form but fail to migrate normally in the absence of *jeb* function. There is no evidence of visceral mesoderm in *jeb* mutants after stage 11, so what becomes of the *bap*-expressing cells? They could undergo programmed cell death. Transcription patterns of three genes that serve as markers of apoptosis, *grim*, *hid*, and *reaper* (Chen et al., 1996; Grether et al., 1995; White et al., 1994), are the same in *jeb* mutants as in wild-type embryos (data not shown). TUNEL staining confirmed the result; no evidence of increased programmed cell death was found (not shown).

Since the cells in question do not express known markers of visceral mesoderm, it is difficult to follow their fates in *jeb* mutants. D-Mef2 stains of *jeb* mutant embryos show increased numbers of nuclei in positions consistent with an increase in somatic muscle precursors (Figure 4G versus 4H). Anti-myosin staining of *jeb* mutants shows that no major disruption of somatic muscle patterning occurs in *jeb* mutants. In *jeb* mutants, the visceral mesoderm precursor cells may default to a somatic mesoderm fate and become incorporated into the normal somatic muscle pattern, as in *bap* mutants (Azpiazu and Frasch, 1993).

jeb Is Expressed in Somatic Mesoderm but Required for Visceral Mesoderm Development

jeb is required for visceral mesoderm development, but not for somatic muscle, fat body, or hemocyte development. To understand how Jeb might function biochemically, we determined where, within the mesoderm, *jeb* is expressed in relation to early visceral mesoderm. *jeb* is clearly expressed in ventral and medial mesoderm immediately adjacent to the visceral mesoderm cells that depend on Jeb function (Figures 6A–6C). The cells that express *jeb* are somatic muscle precursors (Azpiazu

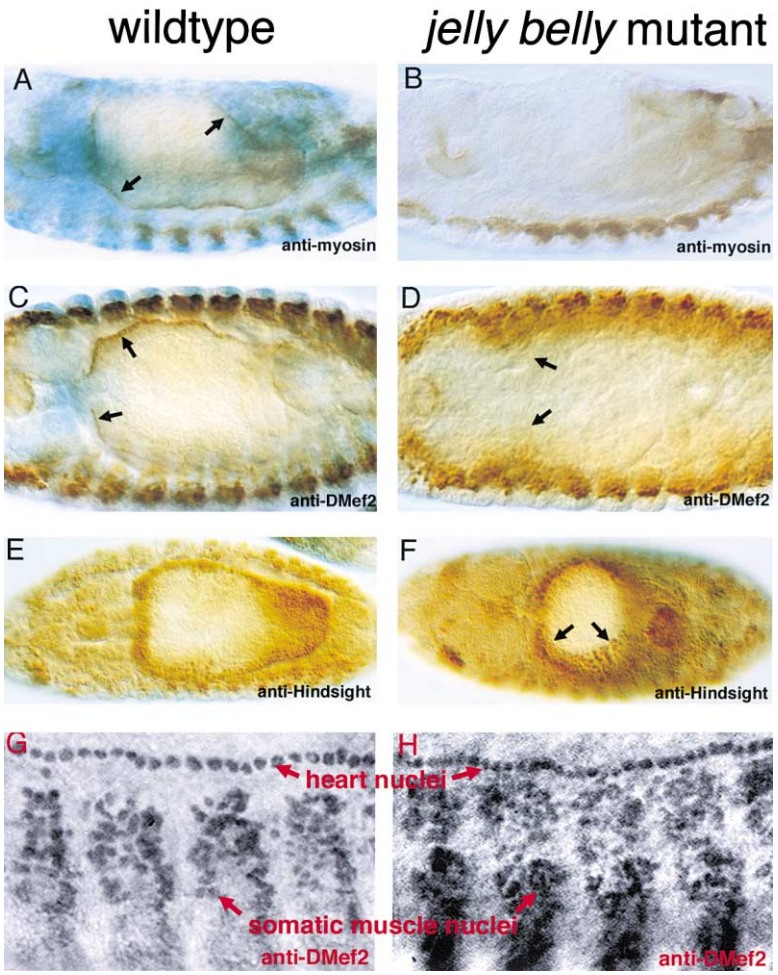


Figure 4. Jeb Is Required for the Development of Midgut Muscles

(A and B) Stage 14 embryos stained with anti-myosin heavy chain antibody. In (A), the midgut musculature of a wild-type embryo is indicated by arrows. In (B), a *jeb* mutant, no midgut musculature is detected with anti-myosin antibody.

(C and D) Dorsal views of stage 14 embryos stained with an antibody against the myogenic transcription factor Dmef2. In (C), the wild-type embryo has normal anti-Dmef2 staining of visceral muscles marked by arrows. The *jeb* mutant (D) shows that Jeb is required for visceral muscle development and that the defect in *jeb* mutants precedes differentiation.

(E and F) Endoderm specification and longitudinal migration are normal in *jeb* mutants, but dorsal and ventral endoderm migrations are defective. (E) Endoderm of a wild-type embryo stained with an anti-Hindsight antibody. In the *jeb* mutant embryo (F), endoderm precursors have migrated in the anterior-posterior axis to reach the middle of the embryo, but they fail to migrate dorsally, which produces a hole (arrows) in the endoderm.

(G and H) Increased numbers of somatic muscle precursors are found in *jeb* mutants. Anti-Dmef2 staining of wild-type (G) and *jeb* mutant (H) embryos. In *jeb* mutants, more nuclei are observed in the positions of somatic muscle precursors.

et al., 1996; Riechmann et al., 1997). *jeb* mRNA is initially produced in clusters of cells ventral to clusters of *bap*-expressing cells (Figure 6A). At stage 10, *jeb*-expressing cells surround the visceral mesoderm and fill in the gaps between the clusters of *bap* expression. By mid stage 11, *jeb*- and *bap*-expressing cells lie in juxtaposed layers (Figure 6C).

Jeb Protein Is Secreted from Somatic Mesoderm and Taken up by Visceral Mesoderm Cells

The signal sequence and LDL receptor repeat predicted in Jeb protein imply that Jeb is secreted from somatic mesoderm precursor cells and acts in the extracellular compartment. Specific Jeb antisera (Figures 6D–6G) were used to monitor a possible Jeb signal from somatic to visceral mesoderm precursors. The antisera do not stain embryos homozygous for the P element excision allele (see Supplemental Figure S1, available online at http://www.cell.com/cgi/content/full/107/3/387/DC1). *bap*-expressing visceral mesoderm precursor cells that are dependent on *jeb* function, but do not transcribe *jeb*, clearly contain Jeb protein (Figures 6F and 6G).

Jeb protein is secreted from tissue culture cells (Figure 6H). Extracts of *Drosophila* tissue culture cells producing Jeb were compared to protein found in their medium. The bulk of the Jeb protein was found outside the cells. The secreted protein migrates as a broad band. Thus, Jeb protein is clearly detectable in the culture medium, evidently in a posttranslationally modified form.

The P element that is integrated into the *jeb* locus interrupts the transcription unit in a large intron (Figure 2A). Transcription of *jeb* upstream of the integration site should produce a protein of about 50 kDa. In mutant embryos, affinity-purified sera detect a truncated Jeb protein with an apparent molecular weight of 45 kDa (not shown). The predicted mutant protein would contain the secretory signal sequence but not the type A LDL receptor repeat. Antibody stains of *jeb* mutant embryos reveal two notable differences with respect to wild-type protein distribution. First, the truncated, mutant protein accumulates to lower levels than wild-type protein. Second, visceral mesoderm precursors do not take up the truncated protein (Figure 7D versus 7E). The only detectable protein in mutant embryos is in or adjacent to the cells that make it. The type A LDL receptor repeat, missing from the mutant protein, thus appears to be necessary for Jeb function.

Uptake of Jeb Protein by Visceral Mesoderm Is Dependent on Endocytosis

The pattern of Jeb protein staining in the visceral mesoderm is qualitatively different from the staining observed

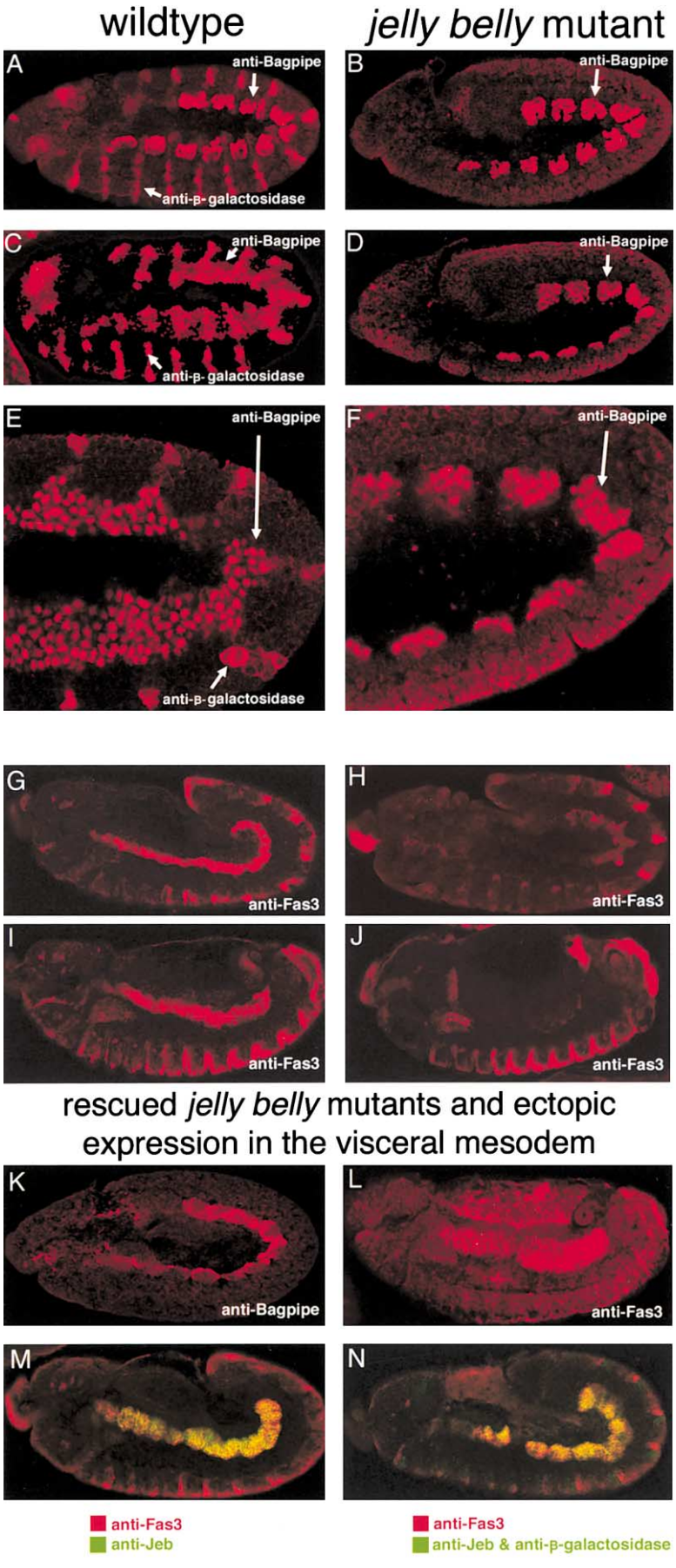


Figure 5. Visceral Mesoderm Is Specified but Fails to Migrate Normally in *jeb* Mutants

Visceral mesoderm differentiation is disrupted in *jeb* mutants. Both migration and differentiation of visceral mesoderm can be rescued by expression of a *jeb* cDNA transgene.

(A and B) Comparison of stage 10 wild-type and *jeb* mutant embryos both stained with anti-Bap antibody to mark early visceral mesoderm. Wild-type embryos in (A), (C), and (E) were identified using anti-β-galactosidase antibody; *jeb* mutant embryos ([B], [D], and [F]) lack detectable β-gal protein. Jelly belly is not required for the specification of visceral mesoderm precursors; Bap expression is normal in the mutants. By mid stage 11, the Bap-expressing cells have migrated to form continuous sheets (C); also shown at higher magnification (E). This contrasts with the *jeb* mutant embryo (D and F).

(G–J) Fas3, an early marker of visceral mesoderm differentiation, is not expressed in *jeb* mutants. Stage 12 and 13 embryos are shown in lateral views stained with an antibody against Fas3. In wild-type stage 12 (G) and stage 13 (H) embryos, Fas3 is robustly expressed in visceral mesoderm. In a *jeb* mutant stage 12 (H) and stage 13 (J) embryos, little or no Fas3 protein is detectable.

(K–M) Migration and differentiation of the visceral mesoderm are rescued by a *jeb* cDNA. Expression of a *jeb* cDNA throughout the early mesoderm rescues visceral mesoderm migration as shown by anti-Bap staining of a stage 11 embryo (K). Differentiation of visceral mesoderm is also rescued as demonstrated by Fas3 staining of a stage 13 embryo (L). Expression of *jeb* in the visceral mesoderm is also sufficient to rescue migration and differentiation in a *jeb* mutant background (M). Staining of a stage 12 mutant embryo with antibodies against Fas3 (red) and Jeb (green) show near normal migration and differentiation of the visceral mesoderm.

(N) Ectopic expression of *jeb* in the visceral mesoderm of *jeb* heterozygotes results in frequent gaps in the visceral mesoderm. A stage 12, embryo stained with anti-Jeb (green) anti-β-galactosidase (green) and anti-Fas3 (red) shows abnormal migration of the visceral mesoderm in a *jeb* heterozygote.

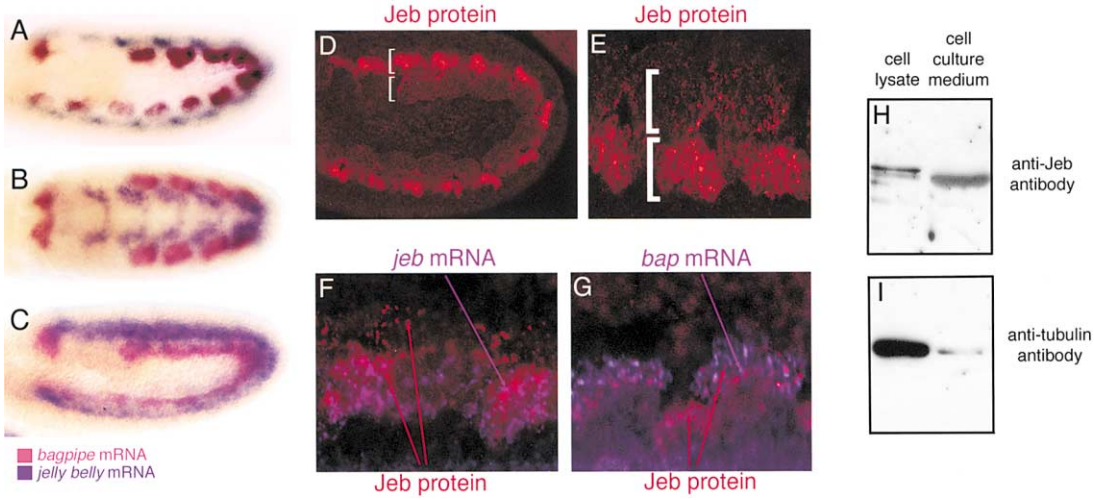


Figure 6. *jeb* mRNA Is Expressed in Ventral Somatic Mesoderm Adjacent to Dorsal, Visceral Mesoderm Precursors

Jeb protein is secreted from cells that synthesize it and taken up by cells that require it.

(A–C) In situ hybridization with antisense probes to *bap* (in red) and *jeb* (in blue) in wild-type embryos. (A) and (B) are lateral and ventral views of stage 10 embryos. *jeb* mRNA is produced in clusters of cells that interdigitate with the *bap*-expressing cells. (C) A lateral view of a stage 11 embryo. At this stage, both the *jeb*- and *bap*-expressing cells form continuous stripes of cells with the *bap*-expressing cells lying immediately adjacent and dorsal to the *jeb*-expressing cells.

(D and E) An antibody against Jeb protein shows two patterns of staining in stage 10 embryos. In ventral mesoderm, cells diffuse and punctate cytoplasmic staining is apparent. In dorsal mesoderm, only punctate staining is observed.

(F and G) Staining with anti-Jeb antibody and in situ hybridization with antisense probes for *jeb* (F) and *bap* (G) mRNAs. Simultaneous staining for Jeb protein and *jeb* mRNA (F) demonstrates that diffuse anti-Jeb staining marks ventral mesoderm cells that express *jeb* mRNA and synthesize Jeb protein. Punctate Jeb staining occurs in dorsal mesoderm cells that do not express detectable *jeb* mRNA (F). The dorsal mesoderm cells that accumulate Jeb protein in a punctate pattern are visceral mesoderm precursors that express *bap* mRNA (G).

(H) Jeb protein is secreted from *Drosophila* tissue culture cells into the culture medium. Jeb protein is detectably more abundant in the culture medium than in the intracellular fraction shown next to it.

(I) Anti-tubulin staining of the same fractions is shown as a control for cell lysis and release of intracellular proteins into the culture medium. Controls for the specificity of the *jeb* cDNA probe and antibody are available online in Supplemental Figures S1 and S2 (http://www.cell.com/cgi/content/full/107/3/387/DC1).

in the Jeb-producing cells. It is exclusively punctate, in contrast to the diffuse staining observed in Jeb secreting cells. The punctate staining pattern suggests receptor-mediated endocytosis as a mechanism for Jeb accumulation in visceral mesoderm cells. To test this hypothesis, we employed a temperature-sensitive allele of the gene *shibire*. *shibire* encodes a dynamin-related GTPase that is required for microtubule-mediated endocytosis (van der Bliek and Meyerowitz, 1991). In *shibire* temperature-sensitive mutant embryos, raised at the nonpermissive temperature during the period of Jeb secretion and uptake, we find reduced or absent association of Jeb with

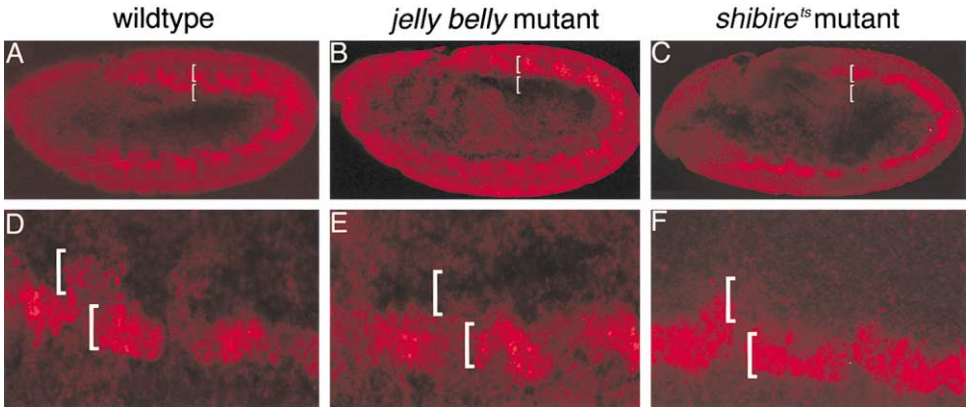


Figure 7. Visceral Mesoderm Cells Do Not Take up Jeb Protein in the P Element-Induced *jeb* Mutant and in a *shibire*^{ts} Mutant

(A–C) Comparison of wild-type, *jeb* mutant, and *shibire*^{ts} stage 10 embryos in lateral views, stained with anti-Jeb antisera. In the P element-induced *jeb* mutant and the *shibire*^{ts} mutant, anti-Jeb staining is restricted to ventral mesoderm and is observed in only one, diffuse pattern. (D–F) The same embryos at higher magnification. (D) The two patterns of anti-Jeb staining are apparent. (E and F) The ventral, punctate anti-Jeb staining associated with *bap*-expressing cells is absent in the two mutants.

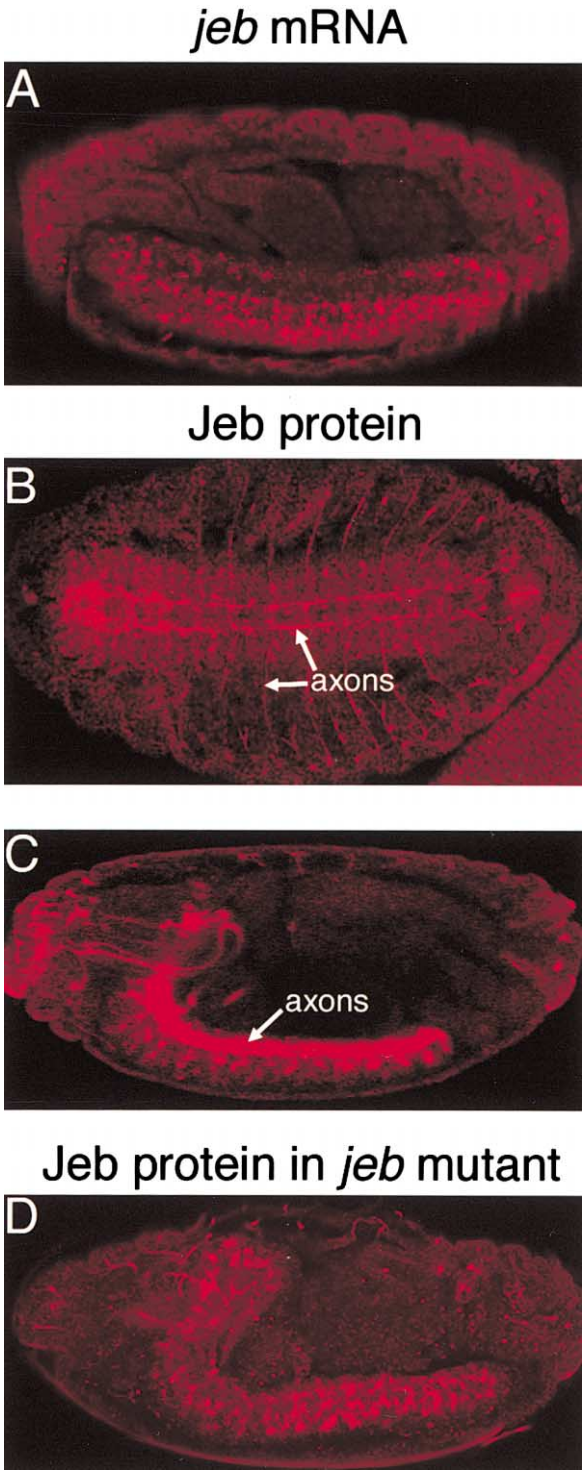


Figure 8. *jeb* mRNA Is Expressed in the Embryonic CNS and Jeb Protein Is Transported along Axons

(A) A ventro-lateral view of a stage 16 embryo hybridized with an antisense probe to *jeb* mRNA, visualized with a fluorescent chromophore. *jeb* mRNA is present in scattered cells throughout the central nervous system.

(B and C) Ventral and lateral views of stage 17 embryos stained with anti-Jeb antibodies. Jeb protein is associated with axons that form two longitudinal tracts and axons that extend laterally into the peripheral nervous system.

the visceral mesoderm (Figures 7C and 7F). This demonstrates that Shibire-mediated endocytosis is required for Jeb to accumulate in visceral mesoderm. It also suggests that a specific Jeb receptor may be required for uptake by the visceral mesoderm.

Expression of Jeb in the Visceral Mesoderm Locally Rescues Visceral Mesoderm Development

Though Jeb protein is secreted from somatic muscle precursors and taken up by visceral muscle precursors, Jeb might act in somatic muscle precursors to produce a signal that is not Jeb. We ruled out this possibility by expressing Jeb in visceral muscle precursors in a *jeb* mutant background. Production of Jeb in the visceral mesoderm of mutants rescues early visceral mesoderm development. Robust Fas3 staining is restored in the visceral mesoderm of these rescued, mutant embryos (Figure 5M). Despite the restoration of Fas3 production, subsequent visceral mesoderm migration is frequently abnormal. Longitudinal migration to form continuous bands is incomplete, resulting in gaps in the pattern of Fas3. Expression of Jeb in the visceral mesoderm is sufficient to rescue the differentiation and, to a lesser extent, migration, of visceral mesoderm precursors.

The migration defect observed in the rescue experiment could mean that the normal location of the Jeb source conveys positional information to visceral muscle precursors. Consistent with this hypothesis, misexpression of *jeb* in the visceral mesoderm of *jeb* heterozygotes produces visceral mesoderm defects. Fas3 expression in these embryos is frequently discontinuous, in contrast to the linear expression in *jeb* heterzygotes in the absence of Jeb misexpression (Figure 5N). These results show that *jeb* misexpression is sufficient to perturb the migration of visceral muscle precursors and support our model of Jeb functioning as a signal.

A Possible Role for Jeb Signaling in CNS Development or Function

Developmental signals often play multiple roles. *jeb* appears to function as a novel signal and, like other signals, is likely to be employed in multiple contexts. At stage 16, *jeb* mRNA is detected in a subset of embryonic neurons that are distributed throughout the ventral nerve cord (Figure 8). Jeb protein appears in a small set of longitudinal axons of the CNS as well as some lateral axons that exit to the PNS. Jeb signaling in the CNS and PNS may be used for communication among a restricted group of neurons.

In the P element-induced *jeb* mutant, the protein distribution is strikingly different from wild-type (Figure 8C versus 8D). In the *jeb* mutants, the protein distribution resembles the pattern of mRNA expression (Figure 8A). By extrapolation from the mesoderm results, the altered protein distribution in *jeb* mutants implies that the axonal staining observed in wild-type embryos represents transport of the protein in neurons that have taken up

(D) Lateral view of a stage 17 *jeb* mutant embryo stained with anti-Jeb antibodies. The protein distribution in *jeb* mutants does not track axons as in (C), but instead resembles the mRNA distribution as in (A).

the protein, as opposed to Jeb secreting cells. This signal transport resembles that observed for Hh in the developing eye (Huang and Kunes, 1996).

Discussion

jeb Uncovers a Novel Signaling System

jeb is required for visceral mesoderm migration and differentiation. Mutants that lack *jeb* form visceral mesoderm precursors, but these cells do not migrate normally or form visceral mesoderm. Our model is that Jeb protein acts as an extracellular signaling molecule. In support of this model, we present several lines of evidence. First, *jeb* is not transcribed in visceral mesoderm precursors, the cells affected by *jeb* mutations, but is transcribed in adjacent cells that are somatic muscle precursors. Second, Jeb protein, in contrast, is detectable both in the cells that synthesize it and in the cells that respond to it. Visceral mesoderm cells, as opposed to other equally proximate cells, specifically take up Jeb protein. This protein distribution suggests that a tissue-specific receptor may be required for uptake. Third, ectopic production of Jeb perturbs visceral mesoderm migration. The data support the hypothesis that *jeb* is a component of a novel signaling system. The simplest hypothesis is that Jeb is a positive migratory or differentiation signal for visceral mesoderm precursors.

Regulation of *jeb* Transcription

jeb was identified in a screen for genes that are downstream targets of transcriptional regulation by the NK homeodomain protein Tin. *tin* mutants have no detectable heart or visceral mesoderm, and have severely disrupted dorsal somatic muscles. *jeb* responds to Tin transcriptional activation in the mesoderm but Tin is not necessary for *jeb* transcription. The ability of Tin to activate *jeb* transcription ectopically in the mesoderm implies that Tin plays an early and redundant function in the regulation of *jeb*. Other regulators that may play roles in the regulation of *jeb* include the bHLH protein Twist and the Pax domain protein Pox Meso (M. Frasch, personal communication).

Jeb Is Required for Visceral Mesoderm Differentiation or Migration

Our data are consistent with Jeb functioning primarily in visceral mesoderm migration, but it may also be required for visceral mesoderm differentiation. When we rescue *jeb* mutants by producing Jeb in discrete clusters of visceral mesoderm cells, we observe local rescue of differentiation and subsequent gaps in the normally continuous, longitudinal bands of Fas3 expression, which is presumably a defect in migration. This is consistent with ectopic *jeb* in discrete clusters of visceral mesoderm cells in a nonmutant embryo causing longitudinal gaps in the visceral mesoderm. The result is most readily explained if Jeb acts as a positive, positional cue for visceral mesoderm migration. An alternative would be that Jeb provides a permissive differentiation function necessary for migration.

Jeb and Other LDL Repeat-Containing Proteins

Jeb has a single LDL receptor repeat. LDL receptor repeats are found in several functional classes of proteins. One large class consists of a group of receptors and coreceptors (reviewed in Cooper and Howell, 1999; Tamai et al., 2000; Wehrli et al., 2000). All these proteins, many of which function cell autonomously in signaling systems, have transmembrane and intracellular domains. The absence of a transmembrane domain from Jeb, its non-cell-autonomous phenotype, and its translocation from synthesizing to responding cells argue against a similar receptor function for Jeb.

Some secreted proteases and protease inhibitors contain LDL receptor repeats. The *Drosophila* protein Nudel (Hong and Hashimoto, 1995), a secreted protease that carries out one step of a localized, signaling, protease cascade, contains an LDL receptor repeat that is highly related to the one in Jeb. Though Jeb has no apparent similarity to known proteases or protease inhibitors other than the type A LDL receptor repeat, it is possible that Jeb acts through a second, unknown signaling protein or protease.

A mammalian protein, the 8D6 antigen, structurally resembles Jeb in that it is secreted and contains two LDL receptor repeats. 8D6 is synthesized in follicular dendritic cells of the immune system and stimulates germinal center B cell proliferation. 8D6 may function as a signal from follicular dendritic cells to B cells in immune responses (Li et al., 2000).

One other well-characterized LDL receptor repeat-containing protein may be functionally related to Jeb: the product of the *C. elegans* gene *Mig-13* which, like Jeb, contains a single LDL receptor repeat (Sym et al., 1999). Structurally, Mig-13 differs from Jeb in that it contains both a CUB and a transmembrane domain not found in Jeb. Mig-13 function, however, resembles Jeb in two notable ways. First, Mig-13 is required non-cell-autonomously, like Jeb. Second, Mig-13 is a positive migratory factor necessary for anterior migration of developing neurons in *C. elegans*, a function similar to Jeb's. Mig-13 is produced locally along the anterior-posterior body axis under the control of specific Hox genes, and appears to guide migrations in a concentration-dependent manner (Sym et al., 1999).

Is the Jeb Signaling System Used More Than Once in Development and Conserved in Evolution?

Signaling systems are often used repeatedly in a variety of developmental contexts. Jeb is no exception to this rule. *jeb* is expressed in the ventral nerve cord during embryogenesis and in the developing visual system and central nervous system late in larval development.

Whether Jeb signaling is conserved in evolution is not as simple to determine. Outside the LDL receptor repeat, a motif shared by a number of extracellular proteins, no unambiguous vertebrate Jeb homologs can be identified in the public databases. Either the LDL receptor repeat is the essential functional, and therefore conserved, portion of Jeb, or Jeb signaling is not widespread in the animal kingdom. We favor the former hypothesis because every known signaling system in *Drosophila* has also been found in vertebrates. The sequence of Sco-Spondin, a secreted protein, is significantly similar to Jeb (Gobron et al., 1996). Jeb signaling may therefore be an evolutionarily conserved process, a possibility we are now investigating using the mouse *Sco-spondin* gene.

Experimental Procedures

Yeast Screen for Genomic DNA Bound by Tinman and Gene Discovery

The screen for Tinman binding sites in *Drosophila* genomic DNA was performed as described in Weiss et al., 1998.

Antibody Production

A bacterial GST-fusion protein containing amino acids 41 through 355 of Jeb was constructed in the expression vector pGEX-4T-1 (Pharmacia) and grown in BL21 cells (Stratagene). The cells, grown at 26°C, were lysed in MTPBS (150 mM NaCl, 4 mM NaH_2PO_4, and 16 mM Na_2HPO_4) with 0.2 mg/ml of lysozyme and protease inhibitors (Protease inhibitor cocktail, Roche). Recombinant protein was partially purified by chromatography on glutathione-Sepharose 4B (Amersham Pharmacia) and further purified by SDS-PAGE. Rabbits (Josman Laboratories) were inoculated, and 161 days later sacrificed to obtain serum. Serum was affinity-purified by chromatography on an AminoLink (Pierce) agarose column to which purified, bacterially expressed GST-fusion protein had been attached.

Immunohistochemistry and In Situ Hybridization

Antibody staining with anti-β-gal, anti-myosin, anti-DMef2, anti-Hindsight, anti-Fas3, anti-Bap, and anti-Jeb was performed according to Patel, 1994. Anti-β-gal antibody was used at a titer of 1:1000 (Cappel), anti-rabbit HRP-conjugated secondary antibody at 1:400 (Jackson), and staining visualized by diaminobenzadine (DAB) reaction (Figure 1), or Cy5 Renaissance Tyramide Signal amplification (NEN) (Figure 5). Mouse anti-myosin monoclonal antibody (kindly provided by D. Kiehart) was used at 1:5, anti-mouse biotin-conjugated secondary antibody at 1:400 (Jackson). Anti-DMef2 antibody (kindly provided by B. Patterson) was used at a titer of 1:1000, anti-rabbit biotin-conjugated secondary at 1:400 (Jackson). The mouse monoclonal anti-Hindsight antibody (kindly provided by H. Lipshitz) was used at a titer of 1:10, anti-mouse biotin-conjugated secondary at 1:400 (Jackson). Signal amplification employed the ABC Reagent (Vectastain) and DAB reaction. Mouse monoclonal anti-Fas3 antibody (Developmental Studies Hybridoma Bank) was at a titer of 1:5, biotin-conjugated anti-mouse secondary at 1:400 (Jackson), anti-Bap staining with rabbit antiserum (kindly provided by M. Frasch) was at a titer of 1:100, and biotin-conjugated anti-rabbit secondary at 1:400 (Jackson), all with signal amplification by incubation with ABC reagent (Vectastain) and Cy5 Renaissance Tyramide Signal Amplification (NEN). Anti-Jeb anti-serum was used at 1:1000, and staining visualized with TRITC-conjugated anti-rabbit antibody at 1:400.

Whole-mount in situ hybridization to *Drosophila* embryos was according to Lehmann and Tautz, 1994. Proteinase K digestion was omitted. Two-color in situ hybridizations and in situ hybridization and antibody labeling were as in Weiss et al., 1998 and Knirr et al., 1999. In situ hybridizations to mRNA were visualized with Cy3 Renaissance Tyramide Signal Amplification (NEN) in Figure 6 or Cy5 Renaissance Tyramide Signal Amplification (NEN) in Figure 8. Antibody stains used TRITC-conjugated secondary antibodies (Jackson).

Tissue Culture Secretion Assay and Protein Blot

S2 *Drosophila* tissue culture cells grown in Schneider's Medium (Gibco) with 10% heat-inactivated fetal calf serum and gentamycin express Jeb protein. 10^6 cells plated in 35 mm plates were grown in 2 ml of medium for 72 hr. The medium was removed and suspended cells removed from the medium by centrifugation. The tissue culture cells remaining on the plate were lysed in 2 ml of insect lysis buffer (10 mM Tris [pH 7.5], 130 mM NaCl, 1% V/V Triton X-100, 10 mM NaF, 10 mM $Na_2H_2PO_4$, and 10 mM $Na_4P_2O_7$) with a protease inhibitor cocktail (Roche) on ice for 45 min. The cell lysate was cleared by centrifugation at 40,000 × g for 45 min. Immunoblotting was according to Harlow and Lane, 1999.

Drosophila Methods

Flies with a P element in the *jeb* locus were obtained from the Bloomington *Drosophila* Stock Center. Excision of the P element to revert the mutation and to generate new *jeb* alleles was performed according to Hamilton and Zinn, 1994. Reporter constructs created by insertion of genomic DNA fragments into C4pLZ (Wharton and Crews, 1993) were used to construct transgenic *Drosophila* lines (Rubin and Spradling, 1982). Ectopic expression of Tinman was performed using the GAL4-UAS system (Brand and Perrimon, 1993). The *tinman* cDNA was inserted into pUAST and transformed into embryos. Transgenic lines were crossed to *engrailed*-GAL4 or *twist*-24B-GAL4 drivers obtained from the Bloomington Stock Center. Rescue of the *jeb* mutation used the GAL4-UAS system. *jeb* cDNA inserted into pUAST was transformed into embryos to generate transgenic lines. Rescue of *jeb* mutants was accomplished by intercrossing two lines both carrying *jeb* mutations over a balancer that expresses *lacZ* in the embryo. One line was homozygous for UAS-*jeb* on the third chromosome, the other was homozygous for mesodermal GAL4 drivers on the third chromosome. Mutants were identified by the absence of embryonic *lacZ* expression. One rescue experiment was performed with a pan-mesodermal driver, *twist*-24BGAL4. Another rescue experiment was performed with a visceral mesoderm-specific driver, bap-GAL4 (H.-H. L. and M. Frasch, unpublished results).

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References

Azpiazu, N., and Frasch, M. (1993). tinman and bagpipe: two homeo box genes that determine cell fates in the dorsal mesoderm of *Drosophila*. Genes Dev. *7*, 1325–1340.

Azpiazu, N., Lawrence, P.A., Vincent, J.P., and Frasch, M. (1996). Segmentation and specification of the *Drosophila* mesoderm. Genes Dev. *10*, 3183–3194.

Bodmer, R. (1993). The gene tinman is required for specification of the heart and visceral muscles in *Drosophila*. Development *118*, 719–729.

Brand, A.H., and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development *118*, 401–415.

Campos-Ortega, J.A., and Hartenstein, V. (1997). The Embryonic Development of *Drosophila melanogaster,* Second Edition (Berlin: Springer Verlag).

Chen, C.Y., and Schwartz, R.J. (1995). Identification of novel DNA binding targets and regulatory domains of a murine tinman homeodomain factor, nkx-2.5. J. Biol. Chem. *270*, 15628–15633.

Chen, P., Nordstrom, W., Gish, B., and Abrams, J.M. (1996). grim, a novel cell death gene in *Drosophila*. Genes Dev. *10*, 1773–1782.

Cooper, J.A., and Howell, B.W. (1999). Lipoprotein receptors: signaling functions in the brain? Cell *97*, 671–674.

Evans, S.M. (1999). Vertebrate tinman homologues and cardiac differentiation. Semin. Cell Dev. Biol. *10*, 73–83.

Frasch, M. (1995). Induction of visceral and cardiac mesoderm by ectodermal Dpp in the early *Drosophila* embryo. Nature *374*, 464–467.

Gajewski, K., Fossett, N., Molkentin, J.D., and Schulz, R.A. (1999). The zinc finger proteins Pannier and GATA4 function as cardiogenic factors in *Drosophila*. Development *126*, 5679–5688.

Gobron, S., Monnerie, H., Meiniel, R., Creveaux, I., Lehmann, W.,

Lamalle, D., Dastugue, B., and Meiniel, A. (1996). SCO-spondin: a new member of the thrombospondin family secreted by the subcommissural organ is a candidate in the modulation of neuronal aggregation. J. Cell Sci. *109*, 1053–1061.

Gossett, L.A., Kelvin, D.J., Sternberg, E.A., and Olson, E.N. (1989). A new myocyte-specific enhancer-binding factor that recognizes a conserved element associated with multiple muscle-specific genes. Mol. Cell. Biol. *9*, 5022–5033.

Grether, M.E., Abrams, J.M., Agapite, J., White, K., and Steller, H. (1995). The head involution defective gene of *Drosophila* melanogaster functions in programmed cell death. Genes Dev. *9*, 1694–1708.

Griffin, K.J., Stoller, J., Gibson, M., Chen, S., Yelon, D., Stainier, D.Y., and Kimelman, D. (2000). A conserved role for H15-related T-box transcription factors in zebrafish and *Drosophila* heart formation. Dev. Biol. *218*, 235–247.

Hamilton, B.A., and Zinn, K. (1994). From clone to mutant gene. In *Drosophila melanogaster*: Practical Uses in Cell and Molecular Biology, L.S.B. Goldstein and E. A. Fyrberg, eds. (San Diego: Academic Press), pp. 81–97.

Harlow, E., and Lane, D. (1999). Using Antibodies: A Laboratory Manual. (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press).

Hong, C.C., and Hashimoto, C. (1995). An unusual mosaic protein with a protease domain, encoded by the nudel gene, is involved in defining embryonic dorsoventral polarity in *Drosophila*. Cell *82*, 785–794.

Huang, Z., and Kunes, S. (1996). Hedgehog, transmitted along retinal axons, triggers neurogenesis in the developing visual centers of the *Drosophila* brain. Cell *86*, 411–422.

Kim, Y., and Nirenberg, M. (1989). *Drosophila* NK-homeobox genes. Proc. Natl. Acad. Sci. USA *86*, 7716–7720.

Knirr, S., Azpiazu, N., and Frasch, M. (1999). The role of the NK-homeobox gene slouch (S59) in somatic muscle patterning. Development *126*, 4525–4535.

Lebestky, T., Chang, T., Hartenstein, V., and Banerjee, U. (2000). Specification of *Drosophila* hematopoietic lineage by conserved transcription factors. Science *288*, 146–149.

Li, L., Zhang, X., Kovacic, S., Long, A.J., Bourque, K., Wood, C.R., and Choi, Y.S. (2000). Identification of a human follicular dendritic cell molecule that stimulates germinal center B cell growth. J. Exp. Med. *191*, 1077–1084.

Lilly, B., Galewsky, S., Firulli, A.B., Schulz, R.A., and Olson, E.N. (1994). D-MEF2: a MADS box transcription factor expressed in differentiating mesoderm and muscle cell lineages during *Drosophila* embryogenesis. Proc. Natl. Acad. Sci. USA *91*, 5662–5666.

Liu, J., Wilson, T.E., Milbrandt, J., and Johnston, M. (1993). Identifying DNA-binding sites and analyzing DNA-binding domains using a yeast selection system. Methods. Companion Methods Enzymol. *5*, 127–137.

Lord, P.C., Lin, M.H., Hales, K.H., and Storti, R.V. (1995). Normal expression and the effects of ectopic expression of the *Drosophila* muscle segment homeobox (msh) gene suggest a role in differentiation and patterning of embryonic muscles. Dev. Biol. *171*, 627–640.

Mastick, G.S., McKay, R., Oligino, T., Donovan, K., and Lopez, A.J. (1995). Identification of target genes regulated by homeotic proteins in *Drosophila melanogaster* through genetic selection of Ultrabithorax protein-binding sites in yeast. Genetics *139*, 349–363.

Nguyen, H.T., and Xu, X. (1998). *Drosophila* mef2 expression during mesoderm development is controlled by a complex array of cis-acting regulatory modules. Dev. Biol. *204*, 550–566.

Nguyen, H.T., Bodmer, R., Abmayr, S.M., McDermott, J.C., and Spoerel, N.A. (1994). D-mef2: a *Drosophila* mesoderm-specific MADS box-containing gene with a biphasic expression profile during embryogenesis. Proc. Natl. Acad. Sci. USA *91*, 7520–7524.

Patel, N.H. (1994). Imagining neuronal subsets and other cell types in whole-mount *Drosophila* embryos and larvae using antibody probes. In *Drosophila melanogaster*: Practical Uses in Cell and Molecular Biology, L.S.B. Goldstein and E.A. Fyrberg, eds. (San Diego: Academic Press), pp. 445–487.

Reuter, R., Grunewald, B., and Leptin, M. (1993). A role for the mesoderm in endodermal migration and morphogenesis in *Drosophila*. Development *119*, 1135–1145.

Riechmann, V., Irion, U., Wilson, R., Grosskortenhaus, R., and Leptin, M. (1997). Control of cell fates and segmentation in the *Drosophila* mesoderm. Development *124*, 2915–2922.

Rubin, G.M., and Spradling, A.C. (1982). Genetic transformation of *Drosophila* with transposable element vectors. Science *218*, 348–353.

Staehling-Hampton, K., Hoffmann, F.M., Baylies, M.K., Rushton, E., and Bate, M. (1994). dpp induces mesodermal gene expression in *Drosophila*. Nature *372*, 783–786.

Sym, M., Robinson, N., and Kenyon, C. (1999). MIG-13 positions migrating cells along the anteroposterior body axis of *C. elegans*. Cell *98*, 25–36.

Tamai, K., Semenov, M., Kato, Y., Spokony, R., Liu, C., Katsuyama, Y., Hess, F., Saint-Jeannet, J.P., and He, X. (2000). LDL-receptor-related proteins in Wnt signal transduction. Nature *407*, 530–535.

van der Bliek, A.M., and Meyerowitz, E.M. (1991). Dynamin-like protein encoded by the *Drosophila* shibire gene associated with vesicular traffic. Nature *351*, 411–414.

Wehrli, M., Dougan, S.T., Caldwell, K., O'Keefe, L., Schwartz, S., Vaizel-Ohayon, D., Schejter, E., Tomlinson, A., and DiNardo, S. (2000). arrow encodes an LDL-receptor-related protein essential for Wingless signalling. Nature *407*, 527–530.

Weiss, J.B., Von Ohlen, T., Mellerick, D.M., Dressler, G., Doe, C.Q., and Scott, M.P. (1998). Dorsoventral patterning in the *Drosophila* central nervous system: the intermediate neuroblasts defective homeobox gene specifies intermediate column identity. Genes Dev. *12*, 3591–3602.

Wharton, K.J., and Crews, S.T. (1993). CNS midline enhancers of the *Drosophila* slit and Toll genes. Mech. Dev. *40*, 141–154.

White, K., Grether, M.E., Abrams, J.M., Young, L., Farrell, K., and Steller, H. (1994). Genetic control of programmed cell death in *Drosophila*. Science *264*, 677–683.

Wilson, T.E., Fahrner, T.J., Johnston, M., and Milbrandt, J. (1991). Identification of the DNA binding site for NGFI-B by genetic selection in yeast. Science *252*, 1296–1300.

Accession Numbers

Nucleic acid and amino acid sequences of *jeb* mRNA and Jeb protein have been deposited in GenBank with accession numbers AF425733 for the 3.2 kb transcript and AF425734 for the 6.5 kb transcript.