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Ruberte, E; Marty, T; Nellen, D; Affolter, M; Basler, K
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Abstract

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An Absolute Requirement for Both the Type II and Type I Receptors, Punt and Thick Veins, for Dpp Signaling In Vivo

Esther Ruberte,* Thomas Marty,† Denise Nellen,† Markus Affolter,* and Konrad Basler†

*Biozentrum
Universität Basel
CH-4056 Basel
Switzerland

†Zoologisches Institut
Universität Zürich
CH-8057 Zürich
Switzerland

Summary

TGFβ elicits diverse cellular responses by signaling through receptor complexes formed by two distantly related transmembrane serine/threonine kinases called type II and type I receptors. Previous studies have indicated that the product of the Drosophila thick veins (tkv) gene is a type I receptor for decapentaplegic (dpp). Here, we show that the Drosophila gene punt encodes a homolog of a vertebrate type II receptor, and we demonstrate that punt, like tkv, is essential in vivo for dpp-dependent patterning processes. Because no dpp-related signaling is apparent in the absence of either the punt or tkv receptor, we infer that both receptors act in concert to transduce the dpp signal and that their functions cannot be replaced by the other extant type II and I receptors.

Introduction

The development of multicellular organisms depends critically on the ability of cells to communicate. Communication between cells involves two primary components: a signaling molecule generated by one type of cell and a receptive system that is able to recognize and interpret this signal in responding cells. The identification and characterization of signals and their receptors is therefore a fundamental goal of developmental biology.

Much attention has been devoted to signaling molecules that belong molecularly to the transforming growth factor β (TGFβ) superfamily of secreted proteins. These signaling proteins are capable of eliciting a wide array of responses in cells of all animal species examined thus far (reviewed by Roberts and Sporn, 1993; Wall and Hogan, 1994). Recently, receptors have been identified that are able to bind ligands of this superfamily and that appear to transduce the signal across cell membranes (reviewed by Massagué, 1992; Kingsley, 1994). For TGFβ, biochemical studies have shown that its receptor complex is composed of two distantly related transmembrane serine/threonine kinases (STKs) called type I and type II receptors (reviewed by Massagué et al., 1994; Miyazono et al., 1994). TGFβ binds directly to the type II receptor, which then, as a consequence of this binding, recruits the type I receptor and modifies it by phosphorylation. This in turn allows the type I receptor to propagate the signal to as yet unidentified downstream components (Wrana et al., 1994a).

The discovery of multiple type I and type II receptors has raised the possibility that different responses could be triggered by a particular ligand depending on the complement of receptors present on the cell surface (Bassing et al., 1994a; Kingsley, 1994). It is also possible that, in the absence of a particular type I or type II receptor, other receptor STKs could functionally substitute for the missing receptor component (ten Dijke et al., 1994a; Bassing et al., 1994b). Moreover, since recent binding studies in tissue culture cells revealed that bone morphogenetic proteins (BMPs) can bind to type I receptors with high affinity without coexpression of a type II receptor, it has also been suggested that these ligands may signal solely through a type I receptor (Koenig et al., 1994; Penton et al., 1994; Graff et al., 1994; ten Dijke et al., 1994b). However, biochemical approaches are limited in their ability to determine whether such possibilities are functionally relevant in vivo. One approach taken to study the in vivo role of receptor STKs has been to express truncated forms of a receptor to block the function of wild-type proteins (Hematani-Brivanlou and Melton, 1992, 1994; Schulte-Merker et al., 1994; Graff et al., 1994). However, this strategy suffers from the complication that overexpressed dominant negative proteins may interact promiscuously with other signaling components that may not be physiological counterparts (Harland, 1994).

We have sought to identify type I and type II receptors for the TGFβ superfamily member decapentaplegic (dpp) in Drosophila to analyze their function and relation by genetic means. We and others have previously reported the identification of two type I receptors, encoded by the genes thick veins (tkv) and saxophone (sax) (Nellen et al., 1994; Brummel et al., 1994; Penton et al., 1994; Xie et al., 1994). Both are required for the control of the embryonic body pattern by dpp (Nellen et al., 1994; Xie et al., 1994). Whereas sax appears to be required exclusively for interpreting peak levels of dpp, tkv has been shown to be critically required for all cell fates specified by dpp in the early embryo (Nellen et al., 1994). Here, we demonstrate that the type II receptor for dpp is encoded by the gene punt and is identical to the Drosophila activin type II receptor homolog (Childs et al., 1993). We show that mutations that remove punt activity cause phenotypes indistinguishable from loss of dpp and loss of tkv activity. Furthermore, by expressing dpp ectopically and at elevated levels, we provide evidence that both the tkv (type I) and punt (type II) products are both essential for embryonic dpp signaling. This indicates that there is no functional redundancy between dpp type I and type II receptors in vivo. Moreover, we argue that despite the presence of multiple type I and type II receptor STKs during Drosophila development, neither tkv nor punt function can be substituted for by other receptors.
To identify the type II receptor for dpp, we used two strategies simultaneously: we screened the Drosophila genome exhaustively for genes encoding transmembrane STK receptors, and we analyzed genes required for dorsal closure, a process that is blocked by mutations in tkv, which encodes a type I receptor for dpp. The two approaches converged in our discovery that punt, a gene identified by its dorsal open mutant phenotype (Jürgens et al., 1984), encodes STK-C, a receptor STK with type II characteristics (Figure 1B). STK-C is identical to the previously described Drosophila activin type II receptor protein Atr-II (Childs et al., 1993) and is strikingly similar to the mouse activin type II receptor (Figure 1C). Consistent with its homology to vertebrate activin receptors, Atr-II has been shown to bind activin A weakly if overexpressed in COS cells (Childs et al., 1993).

The following lines of evidence indicate that punt encodes STK-C/Atr-II: we identified a punt allele (designated punt") in the collection of P element-induced lethals generated by Karpen and Spradling (1992) (see Experimental Procedures). Cloning of sequences flanking the single P element located at cytological position 88C revealed that the P[acrZ, rosy"] transposon is inserted at position 40 of our longest STK-C CDNA, 399 bp upstream of the ATG initiation codon (Figure 1A). The phenotypes and the embryonic lethality of punt" mutants can be reverted to wild type by mobilization of the P element located at 88C. Further evidence that punt corresponds to the gene encoding STK-C/Atr-II was obtained by sequencing the only available ethyl methanesulfonate-induced allele of punt (Jürgens et al., 1984). We found a single point mutation in punt" that changes the highly conserved Ala-376 to a threonine residue within the kinase domain of the STK-C open reading frame (Figure 1B). Finally, we could demonstrate that a transgene with the STK-C/Atr-II open reading frame under the control of the hsp70 heat shock promoter is able to rescue all embryonic phenotypes associated with mutations in punt (Figure 2E).

Taken together, these results show that the previously identified gene punt encodes a protein of the type II receptor STK family closely related to the vertebrate activin receptor.

Indistinguishable Ventralization Phenotypes Caused by Absence of punt, tkv, and dpp Gene Activity Both mutations in punt, punt" and punt" behave as recessive mutations that cause embryonic lethality. As described below, homozygous punt mutant embryos derived from heterozygous (punt+/+) females display a number of defects, the most obvious being the failure to fully close the dorsal epidermis, resulting in a hole in the dorsal cuticle (Figure 2B). However, all aspects of early dorsoventral patterning appear to occur normally in these embryos. For example, the expression pattern of Krüppel, a marker of determination and differentiation of the dorsalmost pattern element, the amnioserosa (Ray et al., 1991), is not affected (Figure 2C). Likewise, the cuticle of punt mutant larvae exhibits all identifiable dorsal and dorsolateral features (Figure 2D). In all these respects, the recessive zygotic phenotype caused by punt mutations closely resembles
that caused by mutations that reduce or eliminate activity of the type I dpp receptor, tkv (Affolter et al., 1994; see below), but differs from that caused by the absence of dpp gene activity, which also causes a complete ventralization of the embryonic epidermis (Irish and Gelbart, 1987).

Because punt gene expression in heterozygous females could supply mutant zygotes with maternally derived punt mRNA or protein and could thereby ameliorate the early consequences of lacking zygotic punt gene activity, we generated mutant embryos from females carrying mutant germ cells (see Experimental Procedures). As shown in Figure 2F, these embryos differentiate rings of ventral denticles around the entire dorsoventral axis. No dorsal or dorsolateral hairs are present. Similarly, dorsolateral as well as lateral pattern elements of the cephalic cuticle are missing. Hence, these embryos appear indistinguishable from homozygous dpp mutant embryos (Irish and Gelbart, 1987) as well as from embryos that lack both maternally and zygotically derived tkv activity (Nellen et al., 1994).

The simplest interpretation of this result is that the type I and type II receptors encoded by tkv and punt are individually essential for mediating the early response to dpp signaling, which organizes dorsoventral patterning in the epidermis. There are, however, at least two alternative interpretations: punt may play a role in generating the early, dorsal-specific expression of dpp, and punt may encode a receptor for a Drosophila activin homolog that, like dpp, is critically required for organizing cell fates along the dorsoventral axis of the early embryo.

To test the first of these alternative possibilities, that lack of maternal punct product alters the early transcription of dpp, we generated early embryos lacking maternal and zygotic punct activity (see Experimental Procedures) and scored them for the expression of dpp by in situ hybridization. dpp is expressed at normal levels in the dorsal 40% of such embryos (data not shown), as it is in wild-type embryos (St. Johnston and Gelbart, 1987), thereby ruling out this explanation. To assess the second possibility, that punt may transduce signaling by an independent activin-like molecule (which is also required for dorsoventral patterning), we have compared the later functions of dpp, tkv, and punt, as described below.

**Similar Requirements for punct and tkv in Other dpp-Related Signaling Processes**

After gastrulation, the spatially restricted activity of dpp is required for midgut morphogenesis, presumably to control the expression of several genes in the visceral mesoderm and the underlying endoderm (Bienz, 1994). Genes whose expression has been shown to depend on dpp activity include the homeotic genes Ultrabithorax (Panganiban et al., 1990) and labial (lab) (Immergluck et al., 1990; Reuter et al., 1990) as well as the dpp gene itself (Hursh et al., 1993). punt mutant embryos lack Ultrabithorax and dpp expression in the visceral mesoderm and fail to induce

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*Figure 2.* Maternal, but not Zygotic, punt Product Is Critically Required for the Embryonic Dorsoventral Pattern Specified by dpp

(A and B) Cuticular phenotype of a wild-type embryo and an embryo homozygous for punt<sup>135</sup>. Severe head defects and a hole in the dorsal hypoderm are visible. In all panels, dorsal is up, and anterior is to the left.

(C) Krüppel expression in a stage 13 punt<sup>135</sup> mutant embryo. In punt<sup>135</sup> and punt<sup>61</sup> mutants, the amnioserosa develops properly as shown by the normal accumulation of Krüppel protein in the large and flattened nuclei covering the dorsal side of the embryo.

(D) Higher magnification of a punt mutant cuticle, indicating that dorsal and dorsolateral cuticle markers are present in zygotically punt mutant larvae. In the trunk region, dorsal hairs, Keilin's organs, and both the ventral and lateral (T2,3) or dorsal (T1) black dots, respectively, are present. In the head, mouth hooks, cirri, and both the antennal and maxillary sense organs (arrow) are formed. The cephalopharyngeal skeleton is severely disrupted or absent, and its remains are forced out of the body cavity. At the posterior end, all dorsally derived structures, such as the Filzkörper (see Figure 2B), the spiracular hairs, as well as the anal plates, are retained in punt zygotic mutants.

(E) Zygotically punt mutant embryos are rescued to viability by a hs-punt transgene. Shown is an embryo derived from parents heterozygous for a punt<sup>1</sup> hs-punt chromosome. All larvae of such a cross are viable and appear wild type. The hs-punt transgene rescues homozygous punt mutants to pharate adults without heat induction.

(F) Embryo from a punt<sup>135</sup>/punt<sup>61</sup> germ line fertilized with punt<sup>61</sup> mutant sperm. This embryo is lacking both maternal and zygotic punct activity and is completely ventralized. Denticle belts extend over the entire circumference of such embryos. They have no dorsal structures, and Filzkörper as well as head skeleton are missing. This phenotype is indistinguishable from that found in embryos lacking maternal and zygotic tkv product and from embryos homozygous for a dpp<sup>−/−</sup> allele (Nellen et al., 1994).
Figure 3. punt Is Required for the Same Developmental Processes as Is tkv

Wild-type (A, C, E, G) and punt\textsuperscript{120} mutant (B, D, F, H) embryos were analyzed for midgut and tracheal defects. In the developing midgut, neither Ultrabithorax protein (normally present in the visceral mesoderm (arrows in A and B), lab (C and D), nor dpp (E and F) transcripts are detectable in the central portion of the midgut of punt mutant embryos. Thus, three dpp-responsive genes (Immerglück et al., 1990; Panganiban et al., 1990; Hursh et al., 1993) fail to be activated in the absence of punt activity, wingless and pdml expression patterns change in punt mutants in the same manner as they do in tkv (Affolter et al., 1994; data not shown) and dpp\textsuperscript{mut} mutants (Immerglück et al., 1990; Affolter et al., 1993). Moreover, we observed that loss of punt activity causes a homeotic transformation of parasegment 7 to parasegment 6. This is the exact same phenotype we previously observed in tkv mutants (Affolter et al., 1994). Visualization of the developing tracheal system in stage 14 embryos using the anti-crumbs monoclonal antibody revealed that punt mutants specifically fail to develop all dorsal branches (arrow in H). All other aspects of tracheal development are normal. Note that, in addition to the lack of dorsal branches, tkv mutants also display defects in the lateral trunk and the ganglionic branches (Affolter et al., 1994). For comparison, the developing tracheal system of a wild-type embryo is shown in (G). In addition to the defects illustrated, tinman expression in cardioblasts and even skipped expression in pericardial cells (Azpiazu and Frasch, 1993; Bodmer, 1993) are strongly reduced in punt and tkv mutant stage 14 embryos (data not shown). This suggests that both receptors are also involved in the proper specification of the dorsal vessel.

lab in the adjacent endodermal cells (Figures 3A–3F). Strikingly, the phenotypes observed in the midgut of punt mutant embryos are indistinguishable from the defects we previously observed in mutants lacking tkv gene function (Affolter et al., 1994; see Figure 3 legend), and they resemble the defects found in dpp short vein mutants (Immerglück et al., 1990; Panganiban et al., 1990; Hursh et al., 1993). These findings suggest that the punt and tkv receptors both participate in dpp signaling in the developing midgut.

Additional defects were observed in the developing tracheal system of punt mutants. During germband retraction, the complex branching pattern of the tracheal network is established via cell migration and cell extension (Hartenstein and Jan, 1992; Manning and Krasnow, 1993). During this process, 5–6 cells migrate out from the tracheal placode towards the dorsal side of the embryo and form the dorsal branch of the trachea, which targets the dorsal vessel and the adjacent epidermis. punt mutant embryos completely lack dorsal branches in all the tracheal metamers, whereas the remaining tracheal system is established normally (Figures 3G and 3H). In this respect, they also resemble embryos mutant for tkv (Affolter et al., 1994).

**punt and tkv Act in Parallel, Rather Than in Sequence**

The similarities between the punt and tkv mutant phenotypes could indicate that punt and tkv act together, or in parallel, in the same dpp-dependent developmental processes. Alternatively, they might reflect a transcriptional dependence of 1 of the 2 genes on the activity of the other (i.e., tkv expression might simply be lacking in punt mutant embryos). To test the latter possibility, we expressed the tkv receptor in punt mutant embryos as well as the punt receptor in tkv mutant embryos using the heterologous promoter of the hsp70 gene (see Experimental Procedures). We note that the hs–tkv transgene does not rescue punt phenotypes nor does the hs–punt construct suppress tkv mutant defects (data not shown). Nevertheless, both the tkv and punt transgenes are functional as indicated by their ability to rescue fully the embryonic phenotypes.
of tkv and punt mutant embryos, respectively (see Figure 2E; Experimental Procedures).

Thus, punt and tkv appear to be required independently for several distinct developmental processes, including the establishment of dorsoventral polarity in the early embryo, the closure of the dorsal epidermis, and the correct formation of the visceral mesoderm and the tracheal system (for additional common requirements, see Figure 3 legend). All of these processes also appear to depend on dpp signaling, strengthening the argument that the punt and tkv receptors are both essential for mediating dpp signaling during development. We also note that punt is expressed ubiquitously in the embryo (data not shown) (see Childs et al., 1993), and its product is, therefore, present in all cells that express tkv and appear competent to respond to dpp during development.

**punt and tkv Are Both Essential for Mediating the Response to Ectopic dpp Expression**

From the above evidence, we still could not exclude the possibility that punt might serve as a receptor for another TGFβ-like ligand that is expressed and required as is dpp. To assess this possibility, we performed the following experiment to test whether punt and tkv products are essential to transduce and interpret dpp activity outside the normal domains of dpp expression. dpp was expressed ubiquitously at high levels under the indirect control of a heat shock promoter (see Experimental Procedures) in wild-type, punt, or tkv mutant embryos, and expression of the lab gene was scored 4 hr after dpp induction. Transcription of the homeotic gene lab is normally limited to the central portion of the midgut endoderm, which underlies the dpp secreting visceral mesoderm (Figure 4C) (Immerglück et al., 1990; Panganiban et al., 1990; Reuter et al., 1990). Ubiquitous expression of dpp in wild-type embryos leads to an expansion of the lab domain in the midgut such that virtually all endodermal cells accumulate high levels of lab protein (Figures 4B and 4D). This effect of ubiquitous dpp expression was completely abolished when either wild-type punt or tkv gene product was removed (Figures 4E and 4F). Furthermore, defects in tracheal cell migration caused by ectopic dpp expression in early embryonic stages are totally suppressed in punt and tkv mutants (M. A., unpublished data). Thus, punt and tkv appear to function downstream of dpp, even when dpp is expressed out of context. Moreover, we cannot detect any biological response to ectopic dpp in the absence of either the punt or tkv receptor, even though dpp is expressed at elevated levels throughout the embryo (Figure 4A). Thus, punt and tkv appear to encode type II and type I dpp receptors, which act in concert to mediate all known embryonic signaling processes that depend on dpp.

**Discussion**

Biochemical studies have indicated that the receptor complex for TGFβ is composed of type I and type II receptors, both of which are transmembrane STKs (Massagué et al., 1994; Miyazono et al., 1994). Genetic and molecular analyses in Drosophila have already identified two genes encoding type I STKs that appear to function as receptors for the TGFβ homolog dpp (Neien et al., 1994; Brummel et
al., 1994; Penton et al., 1994; Xie et al., 1994). Here, we report genetic and molecular evidence that the previously identified gene punt encodes a type II receptor for dpp.

The conclusion that punt functions as a type II dpp receptor is based on the following lines of evidence. First, the receptor encoded by punt appears to be the Drosophila homolog of the mouse activin type II receptor (Mathews and Vale, 1991; Childs et al., 1993) and appears to have all the structural features that distinguish type II from type I receptor STKs. Second, the absence of punt activity causes a phenotype that is indistinguishable from that resulting from the absence of dpp activity, namely complete ventralization of the embryonic epidermis, while partial loss of punt gene function generates a series of other phenotypes that resemble those caused by partial loss of dpp gene function (T. M. and K. B., unpublished data). Third, early dpp expression is not altered in embryos lacking punt gene activity, indicating that the early ventralization phenotype reflects the altered ability of cells to respond to dpp rather than to express dpp. Fourth, punt gene activity is essential for embryonic cells to respond to high levels of dpp expressed both within and outside the normal realm of dpp action. While these findings do not rule out the possibility that punt is a receptor for a ligand, other than that encoded by dpp, that is required to render cells competent to respond to dpp, together they provide a strong argument that punt encodes a type II dpp receptor that is essential for mediating most, if not all, of the responses to dpp.

Both the maternal and the diverse zygotic phenotypes caused by punt mutations appear indistinguishable from the defects caused by mutations of the tkv gene, which encodes a type I dpp receptor. Because many of these defects can be mimicked by available partial or complete loss-of-function mutations in dpp, we conclude that both receptors must be present to allow a cell to respond to dpp. Moreover, our results indicate that the expression described to date. However, we note that relatively low levels of tkv expression, in the current levels of detection by in situ hybridization techniques, suffice to provide wild-type gene function (see Experimental Procedures). Hence, the domain of tkv gene function may be more widespread than the restricted patterns of tkv expression described to date.

The observation that tkv is expressed in a spatially heterogeneous fashion, while punt is expressed ubiquitously, would be consistent with the notion that the punt receptor can act in some developmental decisions without the tkv gene product, possibly together with other type I receptors. However, our findings that the tkv and punt mutations cause apparently indistinguishable phenotypes and are equally capable of blocking the ability of cells to respond to high levels of ubiquitous dpp expression argue against this possibility. Hence, we infer that the spatial and temporal specificity with which dpp determines embryonic cell fates is controlled primarily, if not exclusively, by the restricted presentation of the ligand. This inference raises the question of why tkv is expressed in a spatially restricted fashion, especially as this would seem to render some cells inherently insensitive to dpp. We cannot answer this question at present. However, we note that relatively low levels of tkv expression, below the current levels of detection by in situ hybridization techniques, suffice to provide wild-type gene function (see Experimental Procedures). Hence, the domain of tkv gene function may be more widespread than the restricted patterns of tkv gene expression described to date.

Punt shares extensive sequence similarities in both its extracellular and intracellular domains with the mouse activin type II receptor. Our finding that punt acts in Drosophila as a type II receptor for dpp raises the possibility that certain vertebrate activin receptors might also function as receptors for BMPs, one of which, BMP4, is functionally interchangeable with dpp in Drosophila (Padgett et al., 1993; Sampath et al., 1993). Consistent with this idea, it has been found that OPI1/BMP7 can bind to and signal through the mouse activin type II receptor in concert with appropriate type I receptors (P. ten Dijke, personal communication). Further, Schulte-Merker et al. (1994) have
observed that injections of RNA encoding truncated activin receptors abolish signaling by the BMP-related molecule Vg-1 in Xenopus embryos. Because homologs of BMPs exist in Caenorhabditis elegans and Drosophila as well as in vertebrates, whereas activins have only been found to date in vertebrates, we think it is possible that BMP signaling might have been the ancestral function of the dpp/BMP/activin subclass of type II receptors.

Although the proposed model for TGFβ signaling in tissue culture cells (Wrama et al., 1994a) would predict that the absence of either type I or type II receptor for this prototype ligand would have similar consequences in vivo, there is considerable controversy over the relative contribution of type I and type II receptors to different TGFβ responses. In particular, Chen et al. (1993) proposed that a separate TGFβ signaling pathway is associated with type I receptors alone. This interpretation is based on their observation that inhibition of type II responsiveness abrogates TGFl3-induced antiproliferative effect in mammalian cells, without affecting the TGFβ-stimulated production of the extracellular matrix. Furthermore, Chen and Derynck (1994) recently reported the existence of homodimeric TGFβ type II receptor complexes and proposed that they may function independently of type II–type I heterodimers. Likewise, the finding that tkv (Penton et al., 1994) and other type I receptors (Graff et al., 1994; Koenig et al., 1994) can bind BMP ligands in the absence of cotransfected type II receptors raised the possibility that some type I receptor functions could be carried out without type II receptors. In contrast with these studies, our findings indicate that there is an absolute requirement for the type I and type II receptors encoded respectively by tkv and punt in all known signaling events mediated by the BMP homolog dpp in Drosophila embryos.

Experimental Procedures

Cloning of punt and Identification of Mutant Alleles

STK-C was sequenced using the same strategy and materials as described previously for tkv and sax (Nielsen et al., 1994). Multiple cDNAs were identified, and the longest one, STK-C.7, was sequenced. In situ hybridization to polytene chromosomes revealed that STK-C maps to 88C. Three genomic λ phages were isolated from a LLDASHII library (provided by M. Noll), spanning approximately 15 kb of the STK-C locus (11 kb of this region are depicted in Figure 1A). Intron–exon boundaries were determined by PCR analysis, followed by partial sequence determination of cloned genomic fragments.

The P element line I(3)10480 (Karpen and Spradling, 1992) does not complement the EMS allele punt of Jürgens et al. (1984) and was therefore designated as punt. Both alleles are embryonic lethal. Cuticles of homozygous punt mutant embryos are indistinguishable from those of punt heterozygotes (506–85C), indicating that punt behaves as a null allele.

To determine the molecular lesion of the punt allele, homozygous punt mutant embryos were hand selected based on their dorsal open phenotype and were used to prepare DNA for PCR. The entire STK-C-coding region was amplified, subcloned, and sequenced.

Transgenes

The hs–punt and hs–tkv transgenes are based on the P element plasmid sev (Basler et al., 1991). The hs–G418 construct is based on Carnegie20, but it also contains two copies of the sev enhancer in front of the hsp70 promoter. The UAS-dpp transgene was introduced as a hobo element in which the transcriptional initiation sequences are separated from the dpp-coding region by a minimal P element marked with a yellow gene. To restore the desired UAS–dpp configuration, the hobo transformants were exposed P element transposase, and descendants were selected that had lost the yellow marker gene. Details about the construction of all transgenes can be obtained from K. B. upon request.

Germline Clones

To generate maternally and zygotically mat-punt mutant embryos, we selected for homozygous mutant germline clones by using an autosomal ovo insertion P(ovoI) (Chou et al., 1993) on the FRT82B chromosome (provided by N. Perrimon and T. B. Chou) and a recombinant punt FRT82B chromosome. FLP-induced germline clone-bearing females were fertilized with punt/TM3[hs-acci] males, enabling us to identify 0–3 hr embryos that were maternally and zygotically mutant for punt.

A partial loss of embryonic punt gene function was obtained by fertilizing mutant germline clones with wild-type sperm, thereby removing the entire maternal and half the zygotic gene product. This results in embryos with cuticle phenotypes resembling those caused by partial loss of dpp gene function (e.g., homozygous for the dpp mutant mutation).

Identification of Mutant Embryos

Mutant embryos were identified with the aid of balanced chromosomes carrying β-galactosidase-expressing P elements. To analyze the effect of ectopic dpp expression in a punt mutant background, embryos homozygous for punt were identified based on their characteristic tracheal phenotype, which was visualized with the crumbs antibody (Tepass et al., 1990). dpp was induced relatively late during embryogenesis (stage 12) to avoid interference of residual maternal punt gene product; at this stage, dpp induction has no effect on tracheal cell development (M. A., unpublished data).

Rescue of punt and tkv Mutants with hs–punt and hs–tkv Transgenes

To assay the rescue activity of the hs–punt construct, we prepared recombinant chromosomes mutant for punt and simultaneously carrying a hs–punt transposon. Similarly, we generated recombinants carrying the tkv mutant and a second chromosomal hs–tkv transgene.

Embryos homozygous for the punt, hs–punt chromosomes or homozygous for the tkv, hs–tkv chromosomes were analyzed. The mutant embryonic phenotypes were fully rescued, even without heat induction; the structure of the tracheal system and the endodermal expression of lab was normal. Cuticle preparations showed no head defects and no dorsal open phenotype. In situ hybridization to hs–tkv embryos revealed that the constitutive levels produced by the noninduced hs–tkv transgene are below the level of detection. This observation could also explain why tkv is required for midgut patterning, yet it is apparently not expressed in the endoderm at this stage of development (Affolter et al., 1994; Penton et al., 1994); low level ubiquitous expression of the endogenous gene, undetectable by in situ hybridization, may suffice to provide endodermal cells with a functional amount of tkv gene product.

To assay the rescue activity of the hs–tkv and hs–punt constructs in punt and tkv mutants, respectively, embryos from hs–tkv; punt mutant and hs–punt transgene lines were analyzed. Of the scored embryos, 25% showed the characteristic hs–tkv mutant phenotype, respectively (dorsal open, head defects, lack of second midgut constriction). The hs–punt construct fails to rescue tkv mutant phenotypes and vice versa.

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References


GenBank Accession Number

The accession number for the sequence derived from our longest punt cDNA clone STK-C.7 is L38485.