The relationship of decapentaplegic and engrailed expression in Drosophila imaginal disks: do these genes mark the anterior–posterior compartment boundary?

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Summary

Imaginal disks, the primordia of the adult appendages in Drosophila, are divided into anterior and posterior compartments. However, the developmental role of such compartments remains unclear. The expression of decapentaplegic (dpp), a pattern formation gene required for imaginal disk development, has the intriguing property of being expressed in a line at or near the boundary between these compartments. Here, we compare the distribution of dpp-driven reporter gene expression to the pattern of expression of the engrailed (en) gene, known to be required for the maintenance of the compartment boundary. Using confocal microscopy to obtain single cell resolution, we have determined that the majority of the en+ imaginal disk cells expressing the dpp-driven reporter genes abut those cells expressing en, while a small percentage of dpp reporter gene expressing cells also express en. In posterior regions of en^1 mutant disks, where compartmentalization is abnormal, we observe ectopic expression of the dpp-driven reporter genes. We conclude that the pattern of dpp expression in imaginal disks is delimited in part through the direct or indirect repression by engrailed. Our results lead us to question the widely held assumption that the anterior edge of en expression demarcates the A/P compartment boundary.

Key words: compartments, decapentaplegic, engrailed, pattern formation, imaginal disks, TGF-β family.

Introduction

Pattern formation involves numerous spatial and temporal decisions, including the global definition of position early in embryogenesis, followed by the segregation and elaboration of more specialized structures and organ systems within the developing animal. The pattern formation system underlying appendage development in Drosophila and other insects presumably involves the interplay of many of the same classes of genetic functions as those that participate in the initial partitioning of the embryo. One important event that has been defined in imaginal disk ontogeny is the formation of the anterior–posterior (A/P) compartment boundary (Garcia-Bellido et al. 1973; Crick and Lawrence, 1975; Steiner, 1976; Struhl, 1977; Morata and Lawrence, 1979; Brower et al. 1981; Lawrence and Struhl, 1982).

The A/P compartment boundary, which is likely to be equivalent to the parasegmental boundary of the embryo (Martínez-Arias and Lawrence, 1985), is a lineage restriction, which arises early in development separating the imaginal disk into two different cell populations (anterior and posterior). While normally, proper disk development requires fixed contributions of both the anterior and posterior cell populations, under certain experimental circumstances cells of just one compartment (the anterior) can regenerate an entire appendage (Szabad et al. 1979). However, even in these cases, the anterior cells recapitulate the formation of anterior and posterior compartments during regeneration. Thus, there is strong suggestive evidence that the presence of these compartments is required for proper appendage development. Even so, the role of these compartments in appendage development is still unclear. One intriguing possibility was raised by Meinhardt (1982), who proposed that A/P compartment formation was necessary to create a boundary that would serve as a reference line for the expression of gene products participating in the elaboration of positional information in developing imaginal disks.

Recently, we have accumulated evidence that the
decapentaplegic (dpp) gene is a strong candidate for just the sort of gene predicted by Meinhardt (1982). The product of the dpp gene is a signalling molecule of the TGF-β family of secreted proteins (Padgett et al. 1987) which, from phenotypic considerations, is likely to play an important role in the elaboration or maintenance of positional information in imaginal disk development (Spencer et al. 1982). Clonal analysis demonstrated that dpp is required solely in the region of the disk just anterior to the A/P compartment boundary for development of the entire disk (Posakony et al. 1991). Whole-mount RNA in situ hybridization and reporter gene studies indicate that the dpp product is localized to a strip of cells bisecting each of the disks (Masucci et al. 1990; Blackman et al. 1991). This pattern of expression approximates the position of the A/P compartment boundary, but morphological landmarks within the disks are insufficient to be certain of the position of the dpp expression domain.

This report addresses the relationship of the dpp expression domain in imaginal disks to the process of A/P compartmentalization. For this purpose, we use confocal microscopy to examine dpp expression in the context of the expression of the engrailed gene. From phenotypic and clonal analyses, the engrailed (en) gene is known to be required for posterior compartment identity (Garcia-Bellido and Santamaria, 1972; Morata and Lawrence, 1975; Lawrence and Morata, 1976; Kornberg, 1981). Further, from studies of its expression pattern, Kornberg et al. (1985) and Brower (1986) have suggested that en is expressed in all posterior compartment cells. We investigate the alignments of dpp and en expression domains in wild-type (en+) imaginal disks, and in disks from individuals homozygous for en1, an allele of engrailed in which many cells within the posterior region of the disk appear to adopt an anterior fate (Garcia-Bellido and Santamaria, 1972). We conclude that the expression patterns of dpp and en are closely aligned, and that appropriate en+ activity is required for proper restriction of the dpp expression domain, as well as being required for maintenance of the posterior compartment fate. We also re-evaluate the assumption that the anterior edge of en expression demarcates the A/P compartment boundary.

Materials and methods

Generation of en+ and en1 larvae marked with dpp reporter genes

The dpp reporter gene constructs BS1.1 and BS3.0 have been used in this work. Wild-type (en+) larvae were produced from the mating of two strains, one homozygous for P[en+, lacZ] BS1.1/+; a third chromosome insertion of the reporter gene construct BS1.1 and the other, P[en, lacZ] BS3.0/H1–1/CyO Q. Homozygous offspring carrying the reporter gene constructs were produced in a two generation mating protocol. First, en+/CyO Q Q were mated to homozygous P[en+, lacZ] BS1.1/H1–1/+ sons were then mated to en+/lacZ BS3.0/H1–1/CyO Q. Late third instar larvae with pales yellow Malpighian tubules (en+ P[en+, lacZ] BS3.0/H1–1/en+ ) were chosen for dissection. Half of these larvae also carried P[en+, lacZ] BS1.1–1, confirmed by the presence of the expected BS1.1-specific β-galactosidase expression (Blackman et al. 1991). Larvae were cultured as for wild type.

Whole-mount immunohistochemical staining

Mouse monoclonal antibody 4D9 was used to detect the pattern of β-galactosidase activity. The rabbit polyclonal anti-β-galactosidase antiserum was prepared in the laboratory of Bob Holmgren. The mAb4D9 cell culture supernatant and anti-β-galactosidase antibody were used at dilutions of 1:2 and 1:2000, respectively. Rhodamine-labelled goat anti-rabbit immunoglobulins (Tago Inc.) and fluorescein-labelled goat anti-mouse IgG (Jackson Immuno-research Laboratories, Inc.) were used at dilutions 1:250 and 1:100, respectively. Dissections and antibody staining were carried out as described by Glicksman and Brower (1988), except that inverted larval tissues were fixed in 30 mM Pipes pH 7.4, 160 mM KCl, 40 mM NaCl, 4 mM Na2 EGTA, 1 mM spermidine HCl, 0.4 mM spermine HCl, 0.2% mercaptoethanol, 0.1% Triton X-100, 3.4% formaldehyde (EM grade, Polysciences). Dissected disks were mounted in 90% glycerol, 0.1 M Tris pH 8.5, 0.5% propyl gallate.

For each genotype (homozygous en+ and homozygous en1), at least 20 wing and mesothoracic leg disks were examined. For the other disks (eye-antennal, haltere, prothoracic leg and metathoracic leg), at least 5 samples were examined.

Confocal microscopy

Confocal microscopy was performed on a Biorad MRC 600 system. Fluorescein and rhodamine images were collected individually, using filters designed for double-stained specimens. The image was intensified using the scale, base and, occasionally, crisp routines provided with the Biorad MRC 600 software. The amount of fluorescein bleed-through into the rhodamine channel was calculated from a region of the wing disk, and the rhodamine image was corrected to eliminate this bleed-through. Double fluorescein and rhodamine images were created using the merge routine. Color intensities were manipulated on the computer to enhance contrast in images printed by a Sony Mavigraph video printer.

Results

To study the relationship between dpp and en expression in the imaginal disks, we needed a means to identify the cells that express each of the genes. To detect en expression, we have used the mouse monoclonal antibody mAb4D9, which is specific for an epitope common to the proteins encoded by the closely related en and inverted (inv) genes (Patel et al. 1989). The spatial distributions of the two gene products are thought to be coincident (Coleman et al. 1987); there are, however, no data addressing their coincidence at the single cell level. We will refer to the antigens detected by mAb4D9 as en and inv.

In the case of dpp, an anti-dpp antibody would not be appropriate because of the possibility that the dpp polypeptide is secreted and diffuses from its site of
synthesis (Padgett et al. 1987; Panganiban et al. 1990a,b). Instead we have utilized two dpp-driven lacZ reporter gene constructs, BS1.1 and BS3.0. Comparisons of the patterns of expression of these constructs with whole-mount RNA in situ hybridization patterns (Masucci et al. 1990; Blackman et al. 1991; unpublished data) indicate that these constructs together recapitulate the wild-type pattern of dpp RNA accumulation near the A/P compartment boundary in all the appendage disks. Rabbit polyclonal anti-β-galactosidase antibodies were used to detect the spatial distribution of dpp-directed lacZ expression.

Imaginal disks from larvae containing both dpp-lacZ constructs were incubated with the above antibodies. Fluorescein- and rhodamine-labelled secondary antibodies were used to visualize the nuclear en/inv and the cytoplasmic dpp-driven reporter β-galactosidase antigens, respectively. The stained specimens were examined by confocal microscopy to obtain single cell resolution.

dpp reporter gene and en/inv expression in en+ imaginal disks

As predicted from previous clonal analysis and RNA in situ hybridization patterns (Posakony et al. 1991; Blackman et al. 1991), dpp-driven lacZ expression generally abuts the domain of en/inv expression in all of the appendage disks: wing, haltere, leg and antenna (diagrams of disks in Fig. 1; confocal micrographs in Fig. 2). In each of these disks, most cells expressing β-galactosidase are just anterior to the en/inv domain. Not all β-galactosidase-expressing cells, however, are so distributed. Frequently, one row of cells contains both antigens, and in a few sites, a broader patch of doubly stained cells is noted.

In the wing disk (Fig. 2, top row), the en/inv pattern of staining observed in the posterior compartment with mAb4D9 is the same as that observed with a polyclonal anti-en antibody (Brower, 1986). We also routinely detect faint anterior wing pouch staining (Fig. 3A); en or inv staining in this region has not been reported previously. Throughout the presumptive wing pouch and dorsal hinge regions of the wing disk, the dpp-driven lacZ expression line varies in width from about 2 cells wide at the center of the wing pouch to about 8 cells wide at the periphery of the wing pouch. There is a discontinuity of one cell diameter at the presumptive wing margin (Fig. 3B).

In the wing/dorsal hinge region, the dpp reporter gene expression partially overlaps with the anterior edge of the major en/inv domain (Fig. 3B,C). Cells expressing both antigens were defined as having a fluorescein-labelled nucleus surrounded by rhodamine-labelled cytoplasm. In general, the overlap region is 1–2 cells wide. The most extensive region of overlap is at the dorsal edge of the wing pouch, where the overlap region is 3–4 cells wide and involves cells that stain intensely for both antigens (Fig. 3C). β-galactosidase expression also abuts the anterior edge of the en/inv

Fig. 1. Line diagrams of the imaginal disks depicted in Fig. 2, denoting the fate map positions of several structures referred to in the text. (A) Wing disk. The presumptive notum, dorsal hinge and wing pouch regions are indicated. The position of the presumptive wing margin is indicated by a dashed line bisecting the wing pouch. (B) Mesothoracic (second thoracic segment) leg disk. The location of the endknob, the primordium of the adult tarsus, is indicated. (C) Eye-antennal disk. The presumptive eye and antennal regions of the disk are noted.
domain in the presumptive notum, but there appears to be no overlap with en/inv in this region. In one patch within the notum, 1–2 rows of cells expressing neither antigen separate the β-galactosidase expressing cells from those expressing en/inv (most clearly seen in Fig. 4, top panels). The haltere disk has β-galactosidase and en/inv staining patterns similar to those of the wing disk (data not shown).

The bulk of dpp-driven lacZ expression in leg disks abuts the en/inv domain (exemplified in Fig. 2, middle row, for a mesothoracic leg disk). The en/inv expression that we observe is consistent with previous studies (Brower, 1986; Condie and Brower, 1989). The β-galactosidase patterns of all three types of leg disks are analogous, except that the mesothoracic leg disk contains two additional small patches of expression adjacent to the endknob region. The more posterior of these two patches is within the en/inv domain, but these lacZ-expressing cells do not express en/inv (Fig. 2 and data not shown).

In the presumptive antennal region of the eye–antennal disk, again the general dpp-driven lacZ expression abuts the anterior edge of en expression (Fig. 2, bottom row). Within the central antennal region, fated to become the arista and third antennal segment, there are patches of cells expressing both antigens. Again, the en/inv staining is consistent with that previously reported (Brower, 1986).

**dpp reporter gene and en expression in en1 imaginal disks**

The en gene is required for maintenance of the A/P compartment boundary in imaginal disks (Garcia-Bellido, 1975; Morata and Lawrence, 1975; Lawrence and Morata, 1976). We have examined if an en mutation, en1, that is defective in maintaining the A/P boundary also affects the regulation of our dpp-driven reporter gene constructs.

en1 is an adult viable allele which, in homozygotes, variably transforms posterior into anterior wing blade structures (Garcia-Bellido and Santamaria, 1972). It also engenders defects in the other adult appendage derivatives, many or all of which can be viewed as posterior-to-anterior transformations (Lawrence and Struhl, 1982; Morata et al., 1983). en1 is not a null allele (Garcia-Bellido and Santamaria, 1972); in en1 homozygotes the extent and/or level of en protein expression is reduced in the imaginal disks (Brower, 1986; Condie and Brower, 1989). Using a polyclonal antiserum that is specific for en alone (but which is no longer available), Brower and his colleagues have shown that there is a variable loss of en protein expression in the posterior wing pouch of the wing disk. The pattern of en expression in the prothoracic leg disk is not obviously altered, although Condie and Brower (1989) believe that en may be expressed at a lower level. The en mutation is spontaneous in origin and is associated with the insertion of a repetitive element upstream of the en transcription unit (Kuner et al., 1985; Hama et al., 1990); however, it has not been resolved if the insertion is a polymorphism or if it is the lesion responsible for the mutant phenotype.

Like Brower (1986), we observe variable loss of en/inv staining in the posterior wing pouch of en1 mutant disks (Fig. 4). Generally, cells at the A/P compartment boundary still express en/inv. In contrast to the posterior wing pouch, en/inv expression in the presumptive hinge and notum regions is indistinguishable from wild type. Further, the patterns of en/inv expression in other en1 appendage disks appear unaltered in our experiments. Our image-processing techniques do not permit a comparison of the levels of staining in different samples.

In homozygous en1 imaginal disks, our dpp reporter gene constructs are clearly misregulated. The line of β-galactosidase expression on the anterior side of the en/inv domain remains, but in addition ectopic expression of these dpp-driven lacZ constructs is observed within the posterior en/inv domains of all thoracic disks and in the antennal region of the eye–antennal disk (Figs 4, 5, 6, and data not shown). We never observe ectopic β-galactosidase in the anterior region of any mutant disk, nor do we observe ectopic expression anywhere in the eye region of the eye–antennal disk. Not all antennal regions or haltere disks show ectopic expression, consistent with the observation that en1 phenotypes are weak or undetectable in these appendages (Lawrence and Struhl, 1982; Morata et al., 1983).

Every wing and leg disk examined show ectopic expression of the reporter genes, consistent with the strong en1 phenotypes associated with these appendages (Brasted, 1941; Garcia-Bellido and Santamaria, 1972). In en1 wing disks, novel β-galactosidase expression is consistently found in posterior regions of the wing pouch, although there is some variability in the precise position of ectopic expression within that compartment (Figs 4 and 6). In general, regions of the wing disk in which en/inv expression is altered also show altered β-galactosidase expression, whereas regions in which the en/inv pattern of expression is relatively unperturbed show normal dpp reporter gene regulation. No ectopic expression is observed in the presumptive notum, nor in the more proximal hinge region folds (Fig. 4). In contrast, there is considerable ectopic expression of dpp-driven β-galactosidase (Fig. 5), even though en/inv expression in the leg disk is not spatially altered. Again, this ectopic expression in leg disks is restricted to the posterior region.

A close examination of β-galactosidase and en/inv expression patterns in the posterior region of the en1 wing pouch (Fig. 6) suggests that there is no simple relationship between the patterns of expression of these two genes. Ectopic β-galactosidase expression is not observed at all posterior locations of the wing pouch that lack en/inv expression. Furthermore, in the wing and the leg disks (Figs 4, 5, and 6; and in the antenna and haltere, data not shown) cells expressing both antigens are observed.
Fig. 2. Confocal micrographs of the expression patterns of the dpp-driven lacZ reporter genes and en/inv in late third instar imaginal disks. Top row: wing disk. Middle row: mesothoracic leg disk. Bottom row: eye-antennal disk. Note that in the presumptive eye region, the limited and variable en/inv staining precludes any useful comparison with dpp’s expression in the morphogenetic furrow (Masucci et al. 1990; Blackman et al. 1991). β-galactosidase protein due to expression of the dpp-driven lacZ reporter genes is visualized in red, and is detected by indirect immunofluorescence using rabbit polyclonal anti-β-galactosidase primary antibodies and rhodamine-conjugated secondary antibodies (righthand column). en/inv protein is visualized in green, and is detected using a primary mouse monoclonal antibody (mAb4D9) and a fluorescein-conjugated secondary antisera (lefthand column). Regions with both dpp reporter gene and en/inv expression appear yellow in the double image (middle column); however, because the pixel size of the printer is larger than the diameters of the disk cells at this low magnification, the extents of the yellow staining regions are greater than the true overlap of the expression patterns.
Fig. 3. Confocal micrographs of a wing disk showing the overlap of the dpp and en/inv expression domains. (A) Low magnification view of the presumptive wing disk shown in Fig. 2. Throughout the presumptive wing pouch and dorsal hinge regions of the wing disk, the red dpp-driven lacZ expression line varies in width from about 2 cells wide at the center of the wing pouch to about 8 cells wide at the periphery of the wing pouch. It is narrower in the vicinity of the presumptive wing margin and there is a discontinuity of one cell diameter at the wing margin itself. (B) A 6x enlargement of a portion of the wing pouch shown in large brackets in A. An overlap of 1 cell diameter between the staining of the two antigens is seen throughout most of the presumptive wing pouch. (C) A 12x enlargement of a portion of the wing pouch shown in small brackets in A. The largest amount of overlap (1-3 cell diameters) is in the dorsal wing pouch near the presumptive dorsal wing hinge region. Cells expressing both antigens can be unambiguously identified because the dpp-driven β-galactosidase staining is cytoplasmic while the en/inv staining is nuclear. At the enlarged magnifications in B and C, all cells that appear yellow, as well as those combining green nuclei and red cytoplasm, indicate true overlap of expression (in contrast to the case for low magnification, as in panel A).

Fig. 6. Confocal micrographs exemplifying the altered dpp and en/inv expression patterns in the wing pouch of a homozygous en' wing disk (stained and visualized as in Fig. 2). Regions expressing both antigens appear yellow in the double image (middle panel). The patterns in these en' mutant disks should be compared with the close-up of the wing pouch in Fig. 3B which is magnified 3x relative these micrographs. Note the ectopic dpp-driven β-galactosidase expression (red) in the posterior en' wing pouch in addition to the normal pattern of expression observed along the edge of the en expressing cells (bottom panel). Also note the absence of en/inv signal (green) in portions of the posterior wing pouch (top panel). A frequent feature of the ectopic dpp reporter gene expression pattern is noted here, in which a strip of β-galactosidase-expressing cells extends into the posterior compartment along the dorsal side of the presumptive wing margin (bottom panel).
Fig. 4. Confocal micrographs comparing dpp and en expression in wild-type and en' wing disks. An en' wing disk (top row) and homozygous en' wing disk (bottom row) were stained and visualized as in Fig. 2. Regions expressing both antigens appear yellow in the double-stained image (middle column). Note the absence of homogeneous en/inv staining (green) in the posterior region of the en' disk (compare the lefthand panels of the top and bottom rows). Also note the ectopic expression of the dpp reporter genes (red) in the posterior region of the en' disk (compare the righthand panels of the top and bottom rows).
Fig. 5. Confocal micrographs comparing dpp and en expression in wild-type and en' mesothoracic leg disks. An en' leg disk (top row) and homozygous en' leg disk (bottom row) were stained and visualized as in Fig. 2. Regions expressing both antigens appear yellow in the double-stained image (middle column). Note the ectopic expression of the dpp reporter genes (red) in the posterior region of the en' disk (compare the righthand panels of the top and bottom rows).
Discussion

The alignment of dpp and en expression in wild-type disks

We anticipated that the posterior edge of dpp expression and the anterior edge of en expression in imaginal disks would exactly abut. While the two patterns are more or less adjacent, the results obtained deviate in detail from this expectation.

We demonstrate here that dpp regulatory sequences contained within constructs BS1.1 and BS3.0 direct lacZ expression that generally abuts the domain of en/inv expression, a molecular landmark that is widely considered to define the posterior compartment. However, there is overlap of 1–2 cells in many regions, and even more overlap in a few areas of the disks. The observation of overlap between these antigens can be rationalized in four ways, some of which are trivial, while others are intriguing for their implications for compartment formation.

(1) The dpp reporter gene expression is not congruent with true dpp expression. We can say that the reporter gene staining pattern is very similar to the RNA in situ hybridization pattern (Masucci et al. 1990; Blackman et al. 1991; unpublished data), but a more rigorous comparison must await appropriate double RNA in situ methodologies.

(2) The dpp expression domain does extend into the posterior compartment. This possibility assumes that the dpp reporter gene exactly mirrors the authentic pattern of dpp expression in the imaginal disks, and that the en/inv antigen is an accurate marker of posterior compartment cells. These assumptions lead to the conclusion that, while most dpp expression is confined to the anterior compartment, there is also some dpp expression in posterior compartment cells adjacent to the A/P compartment boundary. Further, we would infer that the posterior compartment expression of dpp has little functional importance. This inference would be necessary to explain why posterior dpp− clones abutting the A/P boundary are recovered in phenotypically normal wings. Additionally, as anterior dpp− clones abutting the A/P compartment boundary can be associated with fully mutant wings (Posakony et al. 1991), the postulated posterior compartment expression must be insufficient by itself to provide any amelioration of dpp-related disk phenotypes.

(3) The expression patterns of en and inv are not absolutely identical. The en and inv genes are thought to be expressed in coincident spatial patterns (Coleman et al. 1987), but their relative expression patterns in the imaginal disks have not been examined in the same detail as the work presented here. Thus it is possible that the anterior edge of en and inv expression do not exactly align and the overlap that we detect is due to the expression of inv alone.

(4) The en expression domain overlaps the A/P compartment boundary to include some cells of the anterior compartment. This possibility contradicts prevailing dogma, but like possibilities (1) through (3), it is entirely consistent with all available data. Two points have been clearly established. (a) The edge of the en expression domain in imaginal disks approximates the A/P compartment boundary. (b) en+ activity is required to maintain the A/P compartment boundary. However, these two observations do not rule out the possibility that en+ is expressed in a small set of anterior cells abutting the A/P boundary. The position of the A/P boundary need not be set precisely at the anterior limit of the en expression domain.

In support of this interpretation, in pupal wings the en/inv antigens detected by mAb4D9 extend several cell rows anterior to the A/P compartment boundary, which is delineated by marked clones midway between the third and fourth wing veins (Seth Blair, personal communication). Thus, we favor point (3) or (4) as an explanation of the discrepancy between our results and the results of dpp− clonal analyses.

Ectopic dpp expression in en+ mutant imaginal disks

Our dpp reporter genes are inappropriately expressed in the posterior compartment of en+ mutant imaginal disks. This leads us to view en gene function as an important determinant of the dpp expression pattern in imaginal disks. Cells expressing both antigens are detected in both wild-type and en+ mutant disks. We propose that other factors participate along with en in the repression of dpp posterior to its normal domain of expression. We can envision two types of mechanisms: (1) en, as a homeodomain DNA-binding protein (Desplan et al. 1985), could bind dpp disk region cis-regulatory sequences directly as a necessary, but not sufficient, repressor of dpp transcription, or (2) en could indirectly repress dpp by influencing the expression pattern of a direct regulator. At present, we cannot distinguish between these possibilities. As yet there are no candidate genes that might regulate the anterior edge of dpp expression.

The only other gene known to be expressed along the A/P compartment boundary is patched (ptc) (Phillips et al. 1990). The strongest region of ptc expression in the wing disk is a line that must overlap, at least in part, with dpp expression, since both abut en-expressing cells. How dpp and ptc might functionally relate is unclear, since unlike dpp, clones of ptc mutant cells along the A/P compartment boundary can be recovered in structurally intact wings.

We are particularly intrigued by the fact that dpp is needed for proper proximal–distal outgrowth of the adult appendages and yet is required and expressed along the anterior–posterior compartment boundary. It has been previously suggested (Gelbart, 1989) that the dpp appendage phenotypes could be related to the dpp expression pattern by a series of patterning events similar to the models proposed by Meinhardt (1982, 1986). These models propose that the intersection of compartment boundaries defines a unique position within a developing field, which could provide a source for a radial system of positional information. It is apparent from the imaginal disk fate maps that the proximal–distal axis of the appendages corresponds to a
radial axis in the disks (Bryant, 1978). Thus, dpp's expression along the A/P compartment boundary may provide one of the signals for the elaboration of proximal–distal (radial) positional information (Gelbart, 1989). We have shown here that dpp has the capacity to respond to an anterior–posterior regulatory signal that is necessary to establish and maintain the posterior compartment. Thus, the spatial expression and regulation of dpp make it a candidate for serving as a reference line in such a patterning system.

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