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Decapentaplegic transcripts are localized along the dorsal–ventral axis of the *Drosophila* embryo

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The decapentaplegic gene of *Drosophila melanogaster* (*dpp*) is related to the TGF-β family of mammalian growth factors. In order to investigate the role of *dpp* during early development we examined the spatial pattern of expression of *dpp* transcripts in embryos. Transcription is first detected along the dorsal side of early syncytial blastoderm embryos. During germ band elongation, the gene is expressed in the region which will give rise to the dorsal epidermis. Since dpp^{Hin-} embryos lack all derivatives of the dorsal epidermis, this suggests that *dpp* is required early in development for the determination of dorsal positional values. Later in embryogenesis, expression in the ectoderm changes to give two thin stripes running anterior–posteriorly along the length of the embryo. This pattern of expression may be involved in further subdivisions along the dorsal–ventral axis. At this stage, transcription can also be detected in discrete parts of the visceral mesoderm, foregut and hindgut.

Key words: Drosophila/dpp/embryonic expression

Introduction

Several genes implicated in pattern determination in invertebrates contain protein sequence homologies to mammalian growth factors or their receptors. *Notch* and *Delta*, which are both required for determination of ventral epidermal cells in *Drosophila* embryos, and *lin-12*, which affects the fate of daughter cells in a number of lineages in *Caenorhabditis elegans*, are all related to epidermal growth factor (EGF) (Wharton *et al.*, 1985; Knust *et al.*, 1987; Greenwald, 1985). Another gene, *sevenless*, which is required for the establishment of a specific photoreceptor cell in each facet of the compound eye of *Drosophila*, possesses tyrosine kinase domains similar to those of several vertebrate hormone receptors (Hafen *et al.*, 1987). Finally, *decapentaplegic*, which is involved in dorsal–ventral determination in embryos (V.F.Irish and W.M.Gelbart, submitted), larval development (Segal and Gelbart, 1985) and proximal–distal development of adult appendages (Spencer *et al.*, 1982) in *Drosophila* is related to the β-type transforming growth factor (TGF-β) family of secreted proteins [including TGF-β, Müllerian inhibiting substance (MIS) and inhibin] (Padgett *et al.*, 1987).

Our laboratory has been investigating the developmental role of the *decapentaplegic* (*dpp*) gene. The *dpp* phenotypes are highly pleiotropic and affect several important developmental events. Based on the TGF-β homology of the *dpp* polypeptide, we have postulated that the apparent determinative and morphogenetic events controlled by *dpp* are mediated through the action of the *dpp* protein as a secreted growth factor-like molecule. In this report, as a first step towards evaluating this model, we have determined the temporal and spatial pattern of *dpp* expression in embryos. Transcripts are expressed dorsally in early embryos, in a pattern consistent with the gene's role in the development of dorsal ectodermal derivatives. Later in embryonic development, transcripts become localized in a novel pattern in the ectoderm and in specific regions of the gut and visceral mesoderm, suggesting that the *dpp* product is utilized for other developmental events later in embryogenesis.

Results

The genetic and molecular organization of *dpp* is shown in Figure 1 (see the revisions to decapentaplegic nomenclature in Materials and methods). From Northern analysis and cDNA cloning and mapping (Gelbart *et al.*, 1985; St.Johnston and Gelbart, in preparation) it appears that a number of overlapping mRNAs are transcribed from the *shv-Hin* region, from left to right. All of these transcripts share two exons located in the *Hin* region which contain the open reading frame which is homologous to polypep-

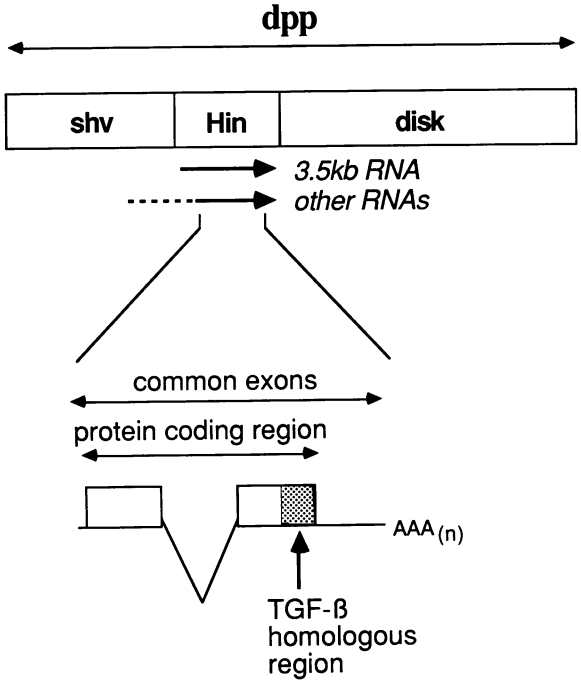

Fig. 1. A genetic and molecular map of *dpp*. The rectangular boxes at the top of the figure indicate the extent of the three genetic regions within dpp [shortvein (**shv**), the Haplo-insufficient region (**Hin**) and the imaginal disk-specific region (**disk**)]. Beneath them are shown the transcription units which give rise to the 3.5-kb RNA and the other RNAs which are initiated within the **shv** region. In the lower half of the figure, the two 3′ exons, which are present in all transcripts and which lie within the **Hin** region, are diagrammed. These two exons were used as the probes in all the experiments described in this paper. The horizontal line represents untranslated sequences; the boxes represent the protein coding region; the stippled region indicates the part of the polypeptide which is homologous to the TGF-β gene family; and the darker lines show the splice between these two exons.

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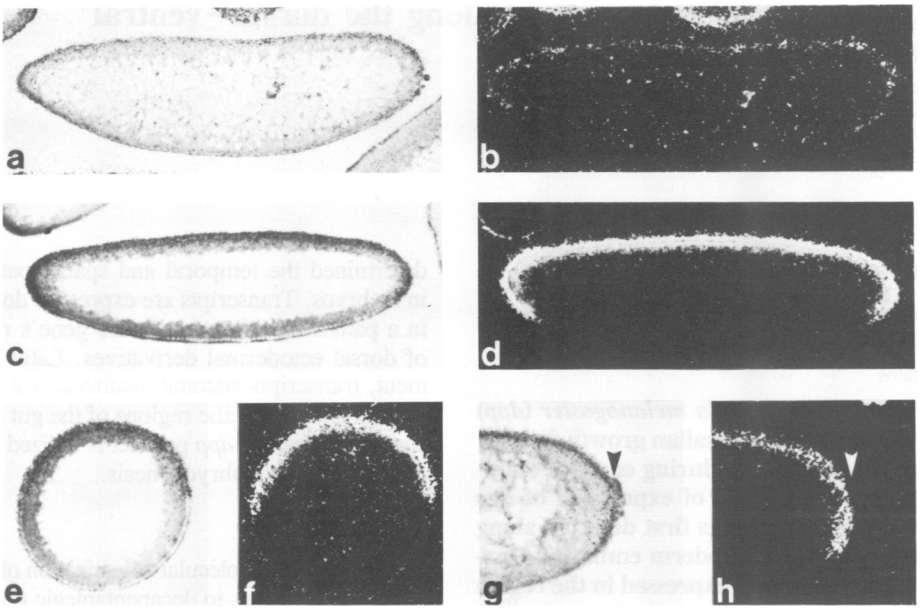

Fig. 2. Blastoderm expression of *dpp* transcripts. Matched brightfield and darkfield micrographs of embryos during blastoderm stages. **(a** and **b)** A sagittal section through an embryo after the 12th nuclear division. In this and all subsequent micrographs of longitudinal sections, anterior is to the left and the dorsal surface is uppermost. **(c** and **d)** A sagittal section through an embryo at the start of cellularization. Note that in this and in (a) and (b), expression is restricted to the dorsal surface except at the two poles of the embryo. **(e** and **f)** A transverse section through an embryo at the same stage as that in (c). In this and all other micrographs of transverse sections, dorsal is uppermost. Note that ~40% of the circumference of the blastoderm is labelled. **(g** and **h)** A sagittal section through the posterior end of an embryo similar to that in (c). In (g) the pole cells, which lie outside of the blastoderm layer at the posterior pole of the embryo are indicated by a black arrowhead. In (h) the white arrowhead also indicates the position of the pole cells, which do not show any labelling. Sections (a), (c) and (e) were all hybridized with [^{35}S]DNA probes and were exposed for 10 days prior to developing and staining. Section (g) was hybridized with a [^{3}H]DNA probe and exposed for 7 weeks.

tides of the TGF-β family (Padgett *et al.*, 1987). The probes used in these experiments were all derived from a cDNA clone containing these two common exons. Tissue *in situ* hybridizations were performed on paraffin sections of embryos of a strain of flies isogenic for a wild-type *dpp* allele. Both nick-translated DNA probes and SP6 derived riboprobes, labelled with either ^{3}H or ^{35}S, were used.

Expression of dpp transcripts during blastoderm stages

The newly fertilized *Drosophila* embryo undergoes a series of rapid nuclear divisions. After the ninth division the majority of the cleavage nuclei reach the egg cortex. This event marks the beginning of the syncytial blastoderm stage, during which a further four divisions occur prior to the onset of cellularization (Foe and Alberts, 1983). No hybridization is observed to sections of embryos which have not completed the 11th cleavage division. Transcripts from *dpp* begin to accumulate after the 11th nuclear division and localized signal is clearly visible after the 12th division (Figure 2a,b). This signal increases in intensity during the last nuclear division to reach a peak during early cellularization. Throughout this period, transcripts are localized to the dorsal side of the embryo. Figure 2c,d shows a sagittal section through an embryo which is just beginning the process of cellularization. In the darkfield micrograph it is clear that accumulation of *dpp* transcripts is restricted to the flattened dorsal side of the embryo (distinguishable from the more convex ventral surface) along most of its length. However, the signal does extend around both the anterior and posterior poles to label more ventral cells at both ends of the embryo. A transverse section through an embryo at a similar stage is presented in Figure 2e and f. Again the signal is clearly dorsal, with ~40% of the circumference of the blastoderm being labelled. Although the somatic cells at the posterior pole of the embryo are labelled, the presumptive germ-line, the pole cells, which reside outside of the unicellular blastoderm layer at the posterior pole, do not express the gene (Figure 2g,h).

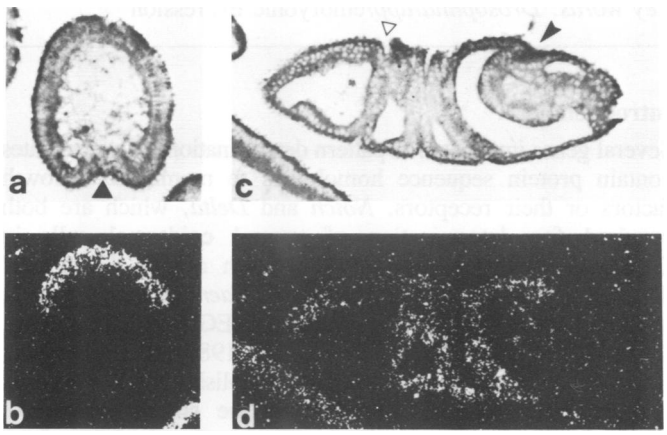

Fig. 3. *dpp* expression during gastrulation and early germ band elongation. **(a** and **b)** A transverse section through a gastrulating embryo showing the ventral furrow (black triangle). The hybridization is localized on the dorsal surface, opposite the ventral furrow. **(c** and **d)** An oblique parasagittal section through an embryo at an early stage of germ band elongation. On the dorsal surface of the embryo, the signal is greatly reduced anterior to the cephalic furrow (open triangle), except for a small region at the anterior pole. Posteriorly, the cells in the amnioproctodeal invagination (black arrowhead) also show reduced labelling. Ventrally, the section passes through the lateral margin of the germ band, which is in the process of forming. Cells in this region, which will form the dorsal epidermal anlagen of the germ band, show higher levels of transcript than do the presumptive amnioserosa cells. [^{3}H]DNA probes, 7 week exposure.

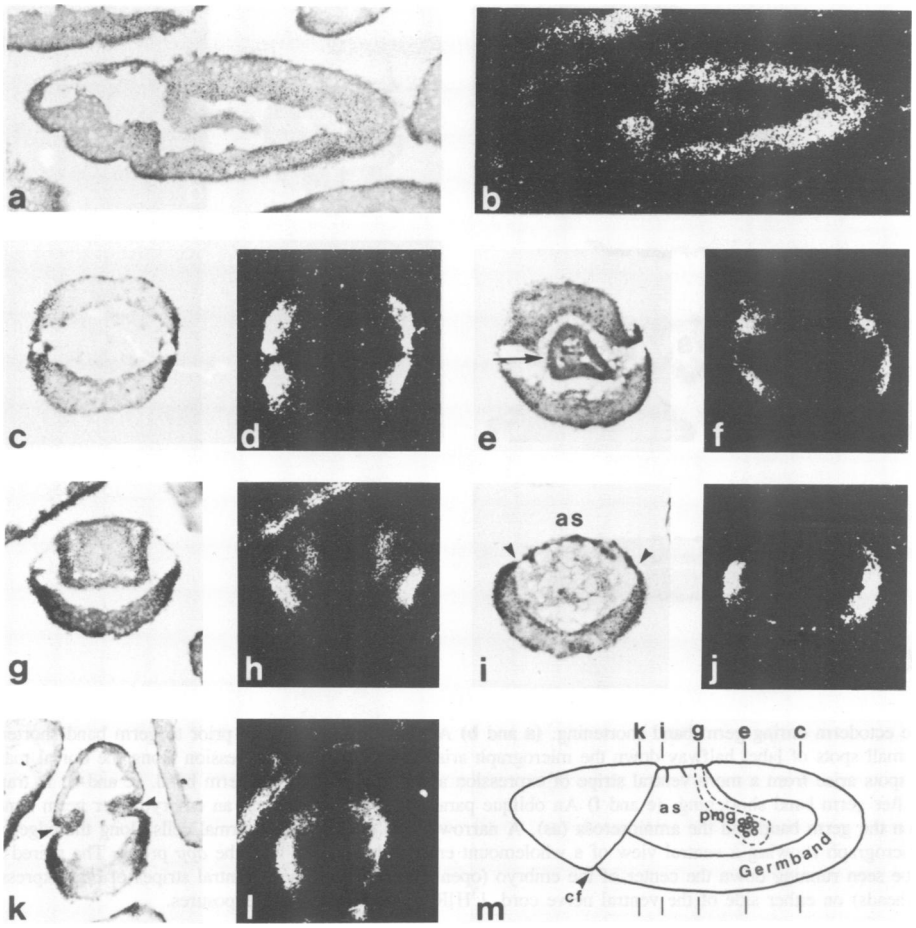

Fig. 4. *dpp* expression during germ band elongation. **(a** and **b)** A grazing parasagittal section through an embryo at full germ band extension. The germ band, which has now extended most of the way along the dorsal surface, is labelled along its lateral margin. Weak signal is also seen in the anterior–dorsal head region. **(c** and **d)** A posterior transverse section passing through the germ band on both the dorsal and ventral sides of the embryo. Only the lateral regions of the germ band (the dorsal epidermal anlagen) are labelled. **(e** and **f)** A more anterior section than (c) passing through the posterior midgut (arrow), containing the pole cells. **(g** and **h)** A transverse section through the opening of the amnioproctodeal invagination. Note the weak labelling of the lateral walls of the hindgut. **(i** and **j)** A transverse section anterior to the amnioproctodeal invagination, passing through the germ band ventrally and the amnioserosa (as) dorsally. The arrowheads mark the extent of the amnioserosa, which is not labelled. **(k** and **l)** A transverse section through the cephalic furrow (black arrowheads). The anterior end of the germ band is labelled ventrally, while a thin stripe of weak signal is seen dorsally. **(m)** A schematic drawing of an embryo during germ band elongation, showing the positions of the transverse sections presented in this figure. **cf**, cephalic furrow. **as**, amnioserosa. **pmg,** posterior midgut. [^{35}S]RNA probes, 6 day exposures.

Expression during gastrulation and germ band elongation

Immediately after cellularization is complete, about 3 h into development, the process of gastrulation begins. Cells invaginate along the ventral midline to form the ventral furrow. Figure 3a and b shows a transverse section through an embryo that has just begun gastrulation. In brightfield, the ventral furrow unambiguously defines the ventral side of the embryo. In darkfield it is clear that *dpp* expression is located on the dorsal side of the embryo, opposite the ventral furrow. Comparison with adjacent sections of younger embryos indicates that the intensity of the signal wanes during the process of gastrulation. By the time gastrulation is complete, a band of cells along the dorsal midline (the presumptive amnioserosa) contains much lower levels of transcript than the neighboring dorsal epidermal anlagen (data not shown).

While the ventral furrow is forming, cells at the posterior pole form a flat dorsal plate to which the pole cells adhere. This plate moves anteriorly on the dorsal surface and forms a depression containing the pole cells (the amnioproctodeal invagination). At the same time, the germ band forms along most of the length of the embryo as cells move ventrally to generate a U-shaped band of ectoderm, with the mesoderm inside it. The germ band proceeds to elongate along the anterior–posterior axis, and moves around the posterior pole of the embryo and along the dorsal surface, led by the amnioproctodeal invagination. The dorsal space left as cells migrate ventrally to form the germ band is filled by the stretching amnioserosa cells, and by the advancing germ band at the posterior end of the embryo.

During germ band elongation the pattern of *dpp* expression begins to change. A parasagittal section through an embryo in the early stages of germ band elongation is shown in Figure 3c and d. The cells forming the amnioproctodeal invagination (which will give rise to the posterior midgut, the hindgut and the proctodeum), which at blastoderm lay at the posterior pole, already show greatly reduced levels of *dpp* transcripts. Anterior to the posterior midgut invagination, the hybridization is stronger ventro-laterally than dorsally, as cells move ventrally to form the germ band. This section does not pass through the most ventral aspect of the embryo, the ventral neurogenic region, which still does not express the gene. The anterior end of the germ band

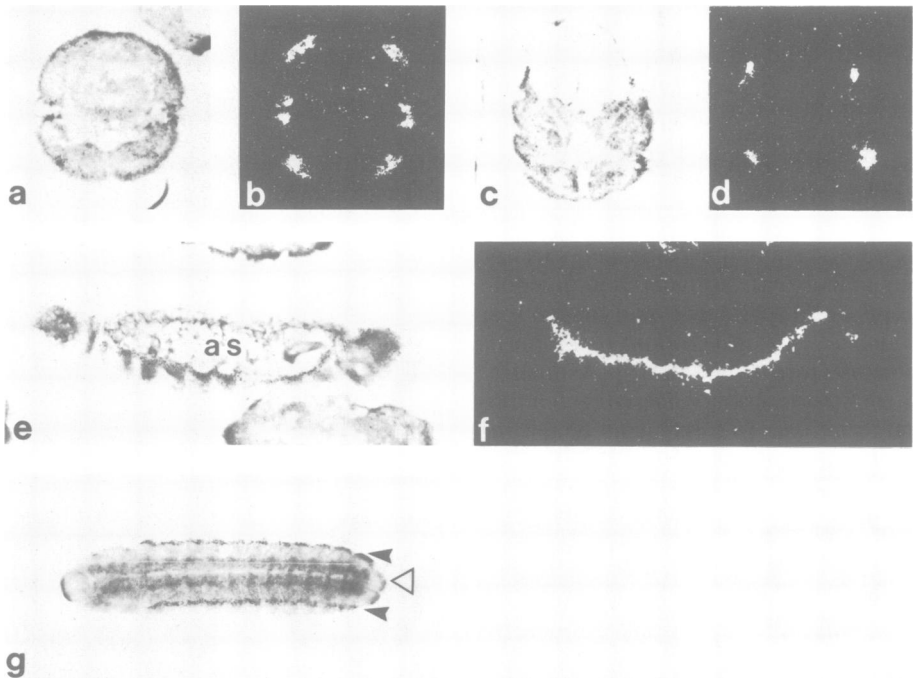

Fig. 5. *dpp* expression in the ectoderm during germ band shortening. **(a** and **b)** A transverse section just prior to germ band shortening, passing through the germ band twice. The four small spots of label halfway down the micrograph arise from a stripe of expression along the dorsal margin on each side of the germ band. The four larger spots arise from a more ventral stripe of expression along each side of the germ band. **(c** and **d)** A transverse section showing the positions of the two stripes after germ band shortening. **(e** and **f)** An oblique parasagittal section through an embryo after germ band shortening, passing through the boundary between the germ band and the amnioserosa (as). A narrow stripe of dorsal ectodermal cells along the edge of the germ band express the *dpp*. **(g)** A brightfield micrograph showing a ventral view of a wholemount embryo hybridized with the *dpp* probe. The paired segmental ganglia of the ventral nervous system can be seen running down the center of the embryo (open triangle). The more ventral stripes of *dpp* expression are visible as two lines of silver grains (black arrowheads) on either side of the ventral nerve cord. [^{3}H]RNA probes, 8 week exposures.

is marked by the cephalic furrow. Anterior to this, in the cephalic region, expression also disappears during the first stages of germ band extension, except for a thin dorsal stripe near the front of the embryo.

Rapid germ band elongation lasts ~30 min; by the end of it the germ band has extended about two-thirds of the way along the dorsal surface. During the next 2 h, it will extend anteriorly a further 15% of the egg length. By the completion of rapid germ band elongation, *dpp* expression is restricted to the lateral regions of the germ band, i.e. the presumptive dorsal epidermis. These cells derive from the dorsal 40% of the blastoderm and are therefore the progeny of a subset of cells that first expressed the gene. In a grazing parasagittal section (Figure 4a,b) one sees a broad band of expression running along the side of the germ band, around the posterior pole.

Figure 4m outlines the structure of an extended germ band embryo, indicating the positions of the transverse sections shown in the rest of Figure 4. Posterior transverse sections pass through the germ band twice (Figure 4c,d). By this stage the mesoderm covers the whole of the interior surface of the ectoderm. Only the dorsal ectodermal cells (the dorsal epidermal anlagen) accumulate transcripts from the gene. A more anterior section also passes through the posterior midgut, containing the pole cells, but no labelling of the endoderm or germ-line is observed (Figure 4e,f). However, a section through the most anterior portion of the amnioproctodeal invagination shows that the band of expression in the dorsal ectoderm extends to the lateral walls of the hindgut (Figure 4g,h). Transverse sections anterior to the amnioproctodeal invagination pass through the amnioserosa dorsally and the germ band ventrally. By this stage the amnioserosa does not express *dpp* transcripts (Figure 4i,j). In the cephalic region, a thin strip of cells spanning the dorsal midline transcribe the gene while the expression in the germ band extends up to the cephalic furrow ventrally (Figure 4k,l).

Expression in the ectoderm during and after germ band shortening

The pattern of expression described above persists throughout the slow phase of germ band elongation. However, at ~7 h, at or immediately prior to the start of germ band shortening, the transcription in the ectoderm undergoes a dramatic change. The one broad band of labelling over the dorsal ectoderm transforms into two thin parallel stripes on each side of the embryo. The dorsal stripe runs along the anterior–posterior axis along the dorsalmost margin of the germ band, forming a boundary with the amnioserosa. The more ventral stripe runs close to the fate map position of the boundary between the dorsal epidermis and the ventral neurogenic region, aligning with the ventral margin of the tracheal pits. In transverse sections that pass through the germ band twice, eight spots of hybridization are visible (Figure 5a,b), which correspond to two stripes along each side of the germ band. This pattern persists during germ band shortening. Once the germ band no longer extends along the dorsal side of the embryo, four regions of hybridization are seen (Figure 5c,d). The dorsal stripe labels the cells which lead the movement of the dorsal epidermis over the amnioserosa to achieve dorsal closure (Figure 5e,f). The location of the more ventral stripe can be seen in the ventral view of a wholemount embryo (Figure 5g). The dorsal stripe is at most a few cells wide, while the more ventral one is somewhat broader. By the time that dorsal closure is complete, these two stripes of expression have disappeared.

Internal expression during and after germ band shortening

At the same time that the ectodermal pattern is changing, two

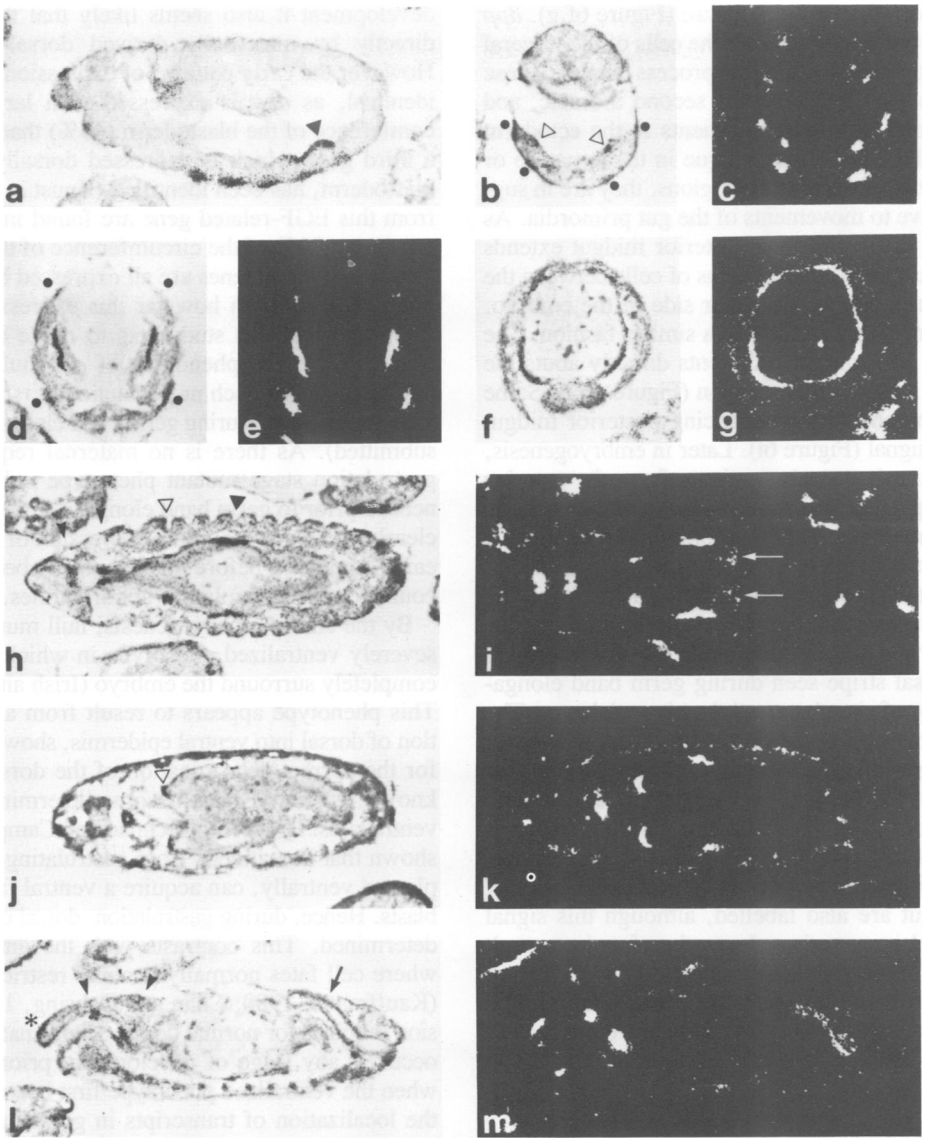

Fig. 6. Mesodermal and gut expression of *dpp*. **(a)** A parasagittal section through an embryo just prior to germ band shortening. The black triangle indicates the one and a half parasegments of the abdominal mesoderm which are labelled. **(b** and **c)** A transverse section through an embryo at a similar stage, showing the mesodermal labelling in the 2nd thoracic segment (open triangles). In this and the next micrograph, the black circles mark the positions of the ectodermal stripes of expression. **(d** and **e)** A transverse section through the abdominal region of the embryo after germ band shortening. The two vertical bands of signal label the visceral mesoderm. Cells from the midgut rudiments lie between the mesoderm and the yolk. **(f** and **g)** A transverse section through a similar region after dorsal closure. Note that the visceral mesoderm has expanded to surround the gut. **(h** and **i)** A horizontal section through an embryo after germ band shortening. The black arrowheads point to the two regions of the foregut which show signal. The open triangle and the black triangle point to the two parts of the visceral mesoderm, which are labelled. These lie in the 2nd thoracic segment and the 1st and 2nd abdominal segments. Note the anterior and posterior midgut rudiments, which lie internal to the band of visceral mesoderm on each side of the embryo. These rudiments are about to fuse at a position adjacent to the abdominal visceral mesodermal signal. In **(i)** the two white arrows indicate the weak labelling at the ends of the two arms of the posterior midgut rudiment. **(j** and **k)** A horizontal section through a late embryo, showing the formation of the gastric caeca. The open triangle indicates an evaginating gastric caecum which is surrounded by visceral mesoderm expressing *dpp* transcripts. **(l** and **m)** A sagittal section through a late embryo. The asterisk indicates the labelling of the clypeolabrum. The two black arrowheads point to the signal in the pharynx and the oesophagus of the foregut. Note that only the dorsal side of the pharynx is labelled. The black arrow points to the labelling of the hindgut. [^{3}H]RNA probes, 7 week exposures.

new sites of transcript accumulation appear in the mesoderm. After germ band shortening it is apparent that these sites correspond to two discrete regions of the visceral mesoderm, one in the thorax and one in the abdomen. Prior to germ band shortening, the mesoderm shows transient periodic indentations which demarcate parasegmental units (Martinez-Arias and Lawrence, 1985). In Figure 6a, a parasagittal section through an embryo at this stage, the abdominal region of expression can be seen to extend over one and a half parasegments. Part of the ventral ectodermal stripe is also visible in this section. The thoracic site of expression extends over a smaller region (data not shown). In a transverse section through the thoracic site of expression, it is clear that only a dorsal part of the mesoderm (the presumptive visceral mesoderm) is labelled (Figure 6b,c). After germ band shortening, the visceral mesoderm has separated from the somatic mesoderm and forms a band of cells running along the anterior–posterior axis of the embryo, between the somatic mesoderm and the gut (or the yolk). All of the visceral mesoderm follows the same developmental fate. It expands with the gut as it envelops the yolk (Figure 6d,e) and eventually surrounds the

digestive system to form the gut musculature (Figure 6f,g). *dpp* transcripts continue to be expressed in all the cells of the visceral mesoderm in these two regions while this process occurs. These two regions of expression align with the second thoracic, and with the first and second abdominal segments in the ectoderm (Figure 6h,i). While there is nothing unique in the structure or developmental fate of these two discrete regions, they are in suggestive positions relative to movements of the gut primordia. As germ band shortening takes place, the anterior midgut extends posteriorly, growing as two lateral columns of cells between the yolk and the visceral mesoderm, on either side of the embryo. The posterior midgut moves anteriorly in a similar fashion. The point of fusion of the two midgut rudiments directly abuts the A1/A2 site of visceral mesoderm expression (Figure 6h,i). Some endodermal cells at the end of the advancing posterior midgut arms also show weak signal (Figure 6i). Later in embryogenesis, four blind sacs (the gastric caeca) evaginate from the anterior end of the anterior midgut. Around the end of each of these gastric caeca lies a small group of visceral mesoderm cells from the T2 region expressing *dpp* transcripts (Figure 6j,k).

At the same time that *dpp* expression is being activated in the mesoderm and the two longitudinal stripes are forming in the ectoderm, *dpp* transcripts are also accumulating elsewhere. In the head, the thin dorsal stripe seen during germ band elongation evolves into a ring of signal around the clypeolabrum. The most ventral part of this ring is the dorsal lip of the stomodeal invagination. During germ band shortening, transcription is also seen in two regions of the foregut; in the pharynx and around the oesophagus (Figure 6l,m). Hybridization in the pharynx is confined to the dorsal side. From this region, cells invaginate to give rise to the stomatogastric nervous system. Posteriorly, stretches of the hindgut are also labelled, although this signal only appears late in embryogenesis and may therefore be a result of nonspecific binding to newly secreted cuticle. The pattern of transcript accumulation seen after germ band shortening in the mesoderm and the gut is still present in the oldest embryos examined in this study, which had started to secrete cuticle.

Discussion

The results presented in this report illuminate several aspects of *dpp* expression and regulation. The first detectable transcription of the gene occurs during syncytial blastoderm after the 11th nuclear division. At this stage, the first expression of a number of other zygotic genes which affect pattern formation is observed (Doyle *et al.*, 1986; Hafen *et al.*, 1984; Knipple *et al.*, 1985). Thus *dpp* is among the earliest zygotic genes to be transcribed. The absence of any signal prior to this stage supports the conclusion of Irish and Gelbart (1987) that there is no maternal germline component to the *dpp* embryonic phenotype.

The early expression of the gene is restricted to a dorsal band, which encompasses 40% of the circumference of the blastoderm, along most of the length of the embryo. However, expression is also seen at the two poles of the embryo. According to the blastoderm fate map (Hartenstein *et al.*, 1985), cells in this dorsal band will give rise to the dorsal epidermis and the amnioserosa, while cells at the poles will contribute to the gut. The early expression of *dpp* is similar to that of zerknült (*zen*), another zygotic gene which affects development along the dorsal–ventral axis (Doyle *et al.*, 1986; Wakimoto *et al.*, 1984). Like *dpp*, *zen* expression extends around both poles of the embryo. These similarities between the early transcription patterns of *dpp* and *zen* suggest that both genes may be activated in response to similar positional signals. Since they both initiate expression early in development it also seems likely that they are both regulated directly by maternally derived dorsal–ventral information. However the early patterns of expression of *dpp* and *zen* are not identical, as *dpp* is expressed in a larger sector of the circumference of the blastoderm (40%) than *zen* (30%). Recently, a third gene which is expressed dorsally and at both poles at blastoderm, has been identified (Knust *et al.*, 1987). Transcripts from this EGF-related gene are found in a dorsal band encompassing ~80% of the circumference of the blastoderm embryo. Thus these three genes are all expressed both dorsally and at the poles, but differ in how far this expression extends ventrally.

One goal of this study was to relate the distribution of *dpp* transcripts to the phenotype of *dpp* null mutations. Embryos homozygous for such null mutations first appear abnormal soon after gastrulation, during germ band elongation (Irish and Gelbart, submitted). As there is no maternal requirement for *dpp*, the gastrulation stage mutant phenotype indicates that the gene is needed prior to germ band elongation; the results presented here clearly show that the initial expression of the gene is sufficiently early (about 2 h before this phenotype becomes apparent) to account for the gastrulation abnormalities.

By the end of embryogenesis, null mutant embryos display a severely ventralized phenotype in which the ventral setal belts completely surround the embryo (Irish and Gelbart, submitted). This phenotype appears to result from a homeotic transformation of dorsal into ventral epidermis, showing that *dpp* is required for the correct determination of the dorsal epidermis. It is not known when cell fates become determined along the dorsal–ventral axis. However, Technau and Campos-Ortega (1986) have shown that dorsal cells from gastrulating embryos, when transplanted ventrally, can acquire a ventral fate and become neuroblasts. Hence, during gastrulation, dorsal cells are not irreversibly determined. This contrasts with the anterior–posterior axis, where cell fates normally become restricted during blastoderm (Kauffmann, 1980; Chan and Gehring, 1971). The *dpp* expression required for normal dorsal determination could, in principle, occur at any stage of development prior to cuticle formation, when the ventralized phenotype first becomes visible. However, the localization of transcripts in germ band extended embryos seems to correspond well with the positions of the dorsal epidermal anlagen, which at this stage reside on either side of the germ band at its lateral margins. The expression of *dpp* in the presumptive dorsal epidermis immediately after gastrulation may coincide with the time when these cells first become committed to a dorsal epidermal fate. The domain of *dpp* expression at blastoderm also includes the dorsal epidermal anlagen and it is possible that this earlier transcription is involved in *dpp*'s role in dorsal–ventral determination. However, at this stage, cell fate does not appear to have been restricted. Furthermore, *dpp* expression at blastoderm is not confined to just the dorsal epidermal anlagen.

Prior to germ band shortening, the distribution of *dpp* transcripts undergoes a marked transformation. Since null mutant embryos deviate from normal development so early, it is difficult to assess what the phenotypic consequences of loss of these later expression patterns might be. In the ectoderm, a transition to two thin stripes running the length of the germ band is seen. Perhaps this expression represents the subdivision of the dorsal–ventral axis into two fields, with *dpp* expression along the dorsal margin of each. Only the most dorsal epidermal cells are included in the dorsal stripe, and the ventral line of expression seems to lie close to the boundary between the dorsal and the ventral epidermis, just ventral to the tracheal pits (Hartenstein *et al.*, 1985).

It is difficult to determine the position of the more ventral stripe with respect to the previous broad band of expression seen in germ band extended embryos. It may include the cells which formed the ventral margin of this earlier band or it may arise from cells which previously did not express the gene. Since these lines of expression are uninterrupted along the length of the germ band, they are unlikely to be directly responsible for the differentiation of particular structures; rather they are more likely to be part of a general pattern forming process, such as the assignment of positions within smaller regions of the ectoderm. Such a subdivision of expression is also seen with the *paired* gene. *Paired* is initially expressed along the anterior–posterior axis of the embryo with a two segment periodicity. Later, its distribution changes to give a stripe of expression in every segment (Kilchherr *et al.*, 1986). This probably reflects the progressive subdivision of the embryo into double segments and then single segment repeats. In a similar fashion, the two stripes of *dpp* expression might reflect later subdivisions along the dorsal–ventral axis.

At the stage of germ band shortening, the *dpp* gene is also expressed in parts of the mesoderm and the gut. Thus, like several other early pattern formation genes, the *dpp* product appears to fulfill new roles later in development (Carroll and Scott, 1985; Hiromi *et al.*, 1985). The posterior mesodermal signal abuts the point at which the anterior and posterior midgut primordia fuse. The sites of expression in the more anterior visceral mesoderm surround the ends of the evaginating gastric caeca. Thus it is tempting to speculate that the visceral mesoderm expression might be involved in aspects of gut morphogenesis.

The *dpp* gene produces several transcripts which appear to differ only in their 5′ untranslated regions (Padgett *et al.*, 1987, and unpublished results). Transcript-specific probes may reveal spatial differences in their distributions. It is also possible that the translation of the *dpp* transcripts is regulated. Furthermore, since the gene product is likely to be a diffusible factor, its distribution may be broader than that of the transcripts. For example, the early dorsal expression of the gene might lead to a gradient of the product along the dorsal–ventral axis. For these reasons it will be important to compare protein and transcript distributions.

In this report we have demonstrated that transcripts from *dpp* are expressed early in development in the dorsal region of the embryo, and that a more complex pattern of expression evolves later in embryogenesis. Early dorsal localization strongly supports the conclusion that the gene is required for the determination of the dorsal epidermis. Although *dpp*'s role in this process is difficult to assess, the predicted protein sequence suggests that it is likely to encode a secreted TGF-β-like factor and act at the level of intercellular communication (Padgett *et al.*, 1987). Based on the diverse sites of expression, this growth factor-like molecule is clearly utilized at various points in early development. One might predict that similar factors, in both vertebrates and invertebrates, will also be used for many processes in early development. Finally, it is probable that such a secreted factor acts by binding to a cell surface receptor. To understand the *dpp* contribution to development, it will be essential to identify such receptors and characterize their modes of action.

Materials and methods

Revised dpp nomenclature

We have recently simplified our nomenclature for decapentaplegic (Irish and Gelbart, submitted). In our new system, all decapentaplegic mutations are referred to as *dpp* alleles. The dpp gene is divided into three regions, which have been renamed, *shv*, *Hin* and *disk* (formerly *shv*, *Hin-d* and *dpp*, respectively).

Techniques

Flies which were isogenic for a second chromosome bearing dpp^+ *dp cn bw* were raised in a population cage and embryos were collected on grape plates. Embryos were aged at 25°C until the desired developmental stages were reached. Fixation, paraffin embedding and sectioning were performed as described by Ingham *et al.* (1985) except that 5% DMSO was added to the fixation solution for older embryos (K.Harding and M.Levine, personal communication). Sections were hybridized with DNA probes following the protocol of Hafen *et al.* (1983). This procedure was modified as described by Knipple *et al.* (1985) when RNA probes were used.

The probes derived from a 3.0-kb cDNA fragment containing the two common exons within the *Hin* region. This fragment was gel-purified for nick translations and subcloned into SP64, a vector containing the SP6 promoter, to make the RNA probes (Melton *et al.*, 1984). [^{35}S]RNA probes were 1.2×10^8 d.p.m./μg; [^{35}S]DNA probes were 1.0×10^9 d.p.m./μg; [^{3}H]RNA probes were 1.8×10^8 d.p.m./μg; and [^{3}H]DNA probes were 1.0×10^8 d.p.m./μg.

The wholemount hybridizations were performed in essentially the same manner as the others. However, the embryos were rehydrated after fixation with 1 × PBS, 0.01% deoxycholate, 0.01% Triton X-100; and the treatment with 4 × SSC at 70°C during the pretreatment was replaced by two washes with 1 × PBS. After hybridization and washing, the embryos were dried onto poly-lysine coated slides and dipped in emulsion.

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