Development 117, 1211-1222 (1993)
Printed in Great Britain © The Company of Biologists Limited 1993

Cross regulation of *decapentaplegic* and *Ultrabithorax* transcription in the embryonic visceral mesoderm of *Drosophila*

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SUMMARY

The *Drosophila decapentaplegic* gene (*dpp*) encodes a TGF-β family member involved in signal transduction during embryonic midgut formation. The shortvein (*shv*) class of *cis*-regulatory *dpp* mutants disrupt expression in parasegments 4 and 7 (ps4 and ps7) of the embryonic visceral mesoderm (VM) surrounding the gut and cause abnormalities in gut morphogenesis. We demonstrate that *cis*-regulatory elements directing expression in ps4 and ps7 are separable and identify DNA fragments that generate ps4 and ps7 expression patterns using reporter gene constructs. *dpp* reporter gene expression in both ps4 and ps7 is autoregulated as it requires endogenous dpp^+ activity. Reporter gene ps7 expression requires the wild-type action of *Ultrabithorax* (*Ubx*), and *abdominal-A*. Furthermore, the expression of certain *Ubx* reporter genes is coincident with *dpp* in the VM. Both the mis-expression of *Ubx* reporter genes in the developing gastric caecae at ps4 and its normal expression in ps7 are dependent upon endogenous dpp^+ activity. We conclude that *dpp* both responds to and regulates *Ubx* in ps7 of the visceral mesoderm and that *Ubx* autoregulation within this tissue may be indirect as it requires more components than have previously been thought.

Key words: *Drosophila*, *decapentaplegic*, *Ultrabithorax*, visceral mesoderm, *cis*-regulation, TGF-β superfamily

INTRODUCTION

Intercellular communication plays a major role in elaborating developmental decisions. The *decapentaplegic* (*dpp*) gene coordinates such cell-cell signalling in a number of patterning events during *Drosophila* development. DPP is a member of the transforming growth factor-β (TGF-β) family of secreted signalling proteins (Padgett et al., 1987). Its expression pattern throughout development is temporally and spatially complex, and is regulated by a variety of spatially restricted transcription factors. *dpp*'s earliest spatial restriction is to the dorsal 40% of the early embryo (St. Johnston and Gelbart, 1987). This restriction is essential to its role in establishing dorsal ectoderm (Irish and Gelbart, 1987; Ray et al., 1991) and occurs through negative regulation in ventral regions by the *dorsal* protein, a transcription factor with homology to the mammalian transcription factor NF-κB (Steward, 1987; Kieran et al., 1990; Ghosh et al., 1990). The expression of *dpp* at or near the anterior/posterior compartment boundary in imaginal disks is necessary for the proximal-distal growth of all adult appendages (Spencer et al., 1982) in a pathway negatively regulated by the homeodomain gene *engrailed* (Raftery et al., 1991).

In the development of the embryonic midgut, *dpp* is involved in passing determinative information between tissue layers (Immerglück et al., 1990; Reuter et al., 1990; Panganiban et al., 1990). The embryonic midgut comprises endoderm surrounded by a thin layer of mesoderm called the splanchnopleura or visceral mesoderm (VM). During early development, the midgut is an open tube, and the VM appears as two broad bands running laterally along this tube. As embryogenesis proceeds, this tube closes and the VM becomes a thin sheath enclosing the underlying endoderm. At this point, cell shape changes produce three constrictions that divide the midgut into compartments (Campos-Ortega and Hartenstein, 1985; Reuter and Scott, 1990). Two areas of the VM show strong *dpp* expression (St. Johnston and Gelbart, 1987). These sites roughly underlie parasegments 4 and 7 (ps4, ps7) of the ectoderm, at the sites in the anterior midgut where the gastric caecae evaginate and the secondary midgut constriction forms, respectively. (Although these patches of expression may not actually be parasegmental in nature, we shall use parasegmental markers to simplify the description of their positions.) Rearrangement breakpoints in the proximal 5′ nonprotein-coding region of the *dpp* gene disrupt this VM expression and cause abnormalities in gut morphogenesis (Immerglück

et al., 1990; Reuter et al., 1990; Panganiban et al., 1990). Specifically, the gastric caecae fail to evaginate and the secondary midgut constriction does not form.

The products of many homeotic genes are expressed in the developing midgut. *Sex combs reduced (Scr)* is normally expressed in the VM directly posterior to the ps4 domain of *dpp*. Loss of *Scr* expression results in a failure to form gastric caecea (Reuter and Scott, 1990). In addition, in *dpp* genotypes lacking *dpp* expression in ps4, the *Scr* domain expands into the *dpp* ps4 domain. Thus *dpp* represses *Scr* expression in ps4 (Panganiban et al., 1990).

In ps7 of the VM, *dpp* expression is coincident with that of the *Ubx* gene (White and Wilcox, 1985; Bienz et al., 1988; Immerglück et al., 1990). Genotypes that result in loss-of-function for either *Ubx* or *dpp* in ps7 result in indistinguishable mutant ps7 midgut phenotypes (Immerglück et al., 1990; Panganiban et al., 1990). In *Ubx* loss-of-function alleles, *dpp* product does not accumulate to wild-type levels in ps7 (Immerglück et al., 1990; Reuter et al., 1990). Thus *dpp*'s expression in ps7 is dependent on *Ubx* although a direct interaction has not been demonstrated. However, conflicting results have been presented for *dpp*'s effect on *Ubx* protein levels. Panganiban et al. (1990) found a reduction in the amount of *Ubx* protein in a *shv* mutant background, while Immerglück et al. (1990) reported that the level of *Ubx* protein in ps7 is unaffected in a *shv* mutant background.

The homeotic gene, *labial (lab*), is expressed in the endoderm underlying ps7 of the VM. In embryos homozygous for *Ubx* loss-of-function mutations or *shv* mutations, *lab* is not expressed in the endoderm, indicating that *dpp* and *Ubx* are required for *lab* expression (Immerglück et al., 1990; Reuter et al., 1990). Immunohistochemical studies demonstrate that the *dpp* protein is transferred from its site of synthesis in the VM to putative target cells of the underlying endoderm (Panganiban et al., 1990). Expansion of the *Ubx* domain either by its derepression in loss-of-function *abdominal-A* (*abd-A*) or *Polycomb (Pc)* backgrounds, or by its ectopic expression in a heat shock *Ubx* construct, shows that *Ubx* expression is sufficient to expand both the *dpp* and *lab* domains in the midgut (Immerglück et al., 1990; Reuter et al., 1990). Thus in ps7, the passage of a *dpp* signal integrates gene expression across germ layers.

The activities of *dpp* in the midgut represent a system where the action of a signal transduction pathway causes readily identifiable morphogenetic change: the cell shape changes resulting in either evagination or constriction of tissues. We are interested in understanding how transcription factors and signal transduction molecules collaborate to effect morphogenesis. We ultimately hope to determine if the interaction between *dpp* and *Ubx* represents a direct regulation of *dpp* transcription by *Ubx*, and what other factors are involved in this regulation. As a first step in such an analysis, we have investigated the regulation of *dpp*'s transcription in the VM.

In this report we localize the *cis*-regulatory sites in *dpp* controlling its expression in ps4 and ps7 in the VM and demonstrate for the first time that ps4 and ps7 regulatory elements map to separate regions within the 5′ noncoding region. We identify DNA fragments that generate bona fide ps4 and ps7 *dpp* expression patterns in reporter gene constructs. Using these reporter genes we demonstrate autoregulation of *dpp* expression in both ps4 and ps7. Furthermore, we provide evidence that the previously identified autoregulation of *Ubx* in ps7 requires *dpp* expression and may be an indirect and complex feed-back pathway.

MATERIALS AND METHODS

Plasmid constructions

P20: A 20 kb *Sal*I fragment (*dpp* coordinates 71.2-92) derived from *dp cn cl bw* was subcloned from a cosmid into Carnegie 20 (Rubin and Spradling, 1983).

P15: A 14.4 kb *Xho*I-*Sal*I fragment (*dpp* coordinates 77.5-92) derived from *dp cn cl bw* was subcloned from a cosmid into Carnegie 20.

RD1: A 6.3 kb *Bam*HI fragment (*dpp* coordinates 75.1-81.4) was cloned from P20 into the *Bam*H1 site of Casper-β-galactosidase-AUG (Thummel et al., 1988).

RD2: An 8.9 kb *Eco*RI fragment (*dpp* coordinates 67-75.9) was subcloned from the 68R plasmid derived from *Canton-S* into pHSF6 (provided by Kevin Jones). The resultant plasmid was digested with *Not*I to release a fragment from *Not*I sites in the plasmid flanking the *Drosophila* DNA. This fragment was subcloned into the *Not*I site of HZ50PL (Hiromi and Gehring, 1987).

Germ-line transformation

P-element transformation was carried out using standard protocols (Spradling, 1986). The above constructs (at 400 μg/ml) and pπ25.7wc DNA (at 100 μg/ml; Karess and Rubin, 1984) were coinjected into *cn; ry*^{506} or *y w* embryos. Each insertion was mapped to a chromosome and made homozygous. In most cases, additional inserts were generated by mobilization of a primary transformant using the Δ2-3 (99B) genomic source of P transposase (Robertson et al., 1988).

Drosophila strains and constructions

dpp alleles are described elsewhere (Segal and Gelbart, 1985; St. Johnston et al., 1990). The alleles of homeotic mutations were as follows: *Ubx*$^{9.22}$, *Ubx*$^{6.28}$ (Kerridge and Morata, 1982); *Ubx*^{109} (Lewis, 1978) and *abd-A*$^{mx-2}$ (Sanchez-Herrero et al., 1985). The *Ubx* reporter gene, 15-1 wt*Ubx*, contains the region from −3.1 to +900 of the *Ubx* gene, fused to a nuclear β-galactosidase gene and was provided by Philip Beachy.

Drosophila embryo analysis

The phenotypes of mutant embryos were inferred from examining large numbers of progeny and looking for consistent defects in expression observable in roughly 1/4 (the expected frequency of mutant homozygotes or double heterozygotes) of the embryos of the appropriate stages. With later embryos, *dpp* or *Ubx* derived gut abnormalities could be used to confirm genotypes. Embryos were staged according to Campos-Ortega and Hartenstein (1985). The effect of homozygous *dpp*^{shv} mutations on *dpp* reporter gene constructs was assessed in *dpp*^{shv} mutant stocks balanced with a *CyO* chromosome bearing a *wingless*-β-galactosidase reporter gene (provided by Norbert Perrimon), and homozygous for insertions of either the RD1 or RD2 construct. Similarly marked balanced strains were used to examine RD1 in *Ubx* and *abd-A* mutant backgrounds. A *TM3* balancer, bearing a *fushi tarazu*-β-galactosidase reporter gene (Hiromi and Gehring, 1987) was used to distinguish critical genotypes. The P20 and P15 rescue constructs were assayed in *dpp* null backgrounds. Embryos homozygous for a *dpp* null allele *dpp*^{H46} and homozygous for P20 or P15 were generated from progeny of a balanced stock of the following geno-

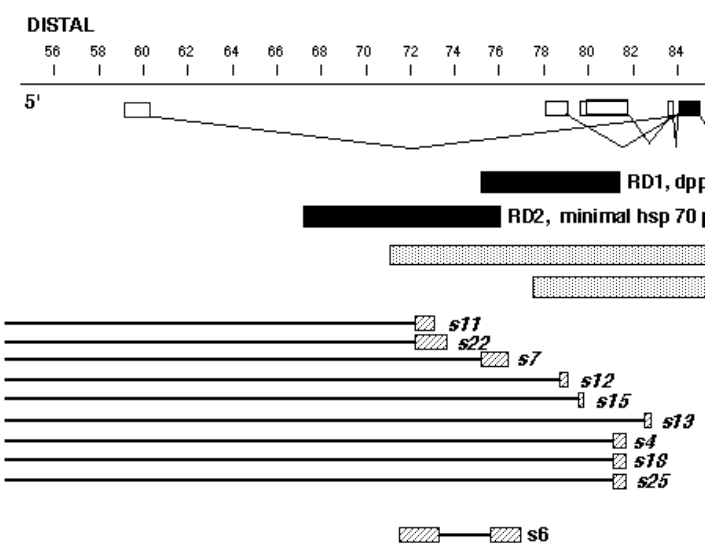

Fig. 1. Schematic diagram of the *dpp* gene. The coordinates of the gene (St. Johnston et al., 1990) are indicated across the top. The location of the exons for the *dpp* transcripts are immediately below. Black boxes represent protein coding exons and stippling indicates the 3′ untranslated region. White boxes represent five alternative 5′ noncoding exons that are spliced into the first coding exon at exactly the same site. The 5′ ends of the 2 most proximal noncoding exons have been determined by S1 analysis (St. Johnston, 1988); the others are inferred by the positions of unique cDNAs. The *shv* region begins at molecular coordinate 83, as defined by the *s13* breakpoint. Its 5′ limit is unclear. The extents of the RD1 and RD2 reporter gene constructs are indicated by black bars, and the extents of the P20 and P15 rescue constructs by stippled bars. Mapped positions of *shv* breakpoints (St. Johnston et al., 1990) are at bottom. With the exception of *s6*, all the *shv* mutations are chromosomal rearrangements. Black lines represent extent of DNA removed from the *dpp* transcription unit by a given mutation, with cross-hatched boxes representing the uncertainty of location of the endpoint of each mutation.

type: dpp^{H46}/ *CyO,Dp* dpp^{+} ; *P–/P–* . One quarter of the embryos from this strain are null for the endogenous *dpp* gene and homozygous for either the P20 or P15 rescue construct. For *Ubx* expression studies, segregants were identified by simultaneously monitoring expression of *Scr* in the VM. In the absence of *dpp*, the *Scr* domain is increased in size. For *lab* expression, mutant phenotypes were inferred on the basis of expected Mendelian ratios.

Histochemical β-galactosidase staining

Embryos were dechorionated by standard methods and fixed in 4% formaldehyde in PBS (340 mM NaCl, 6.6 mM KCl, 3.7 mM KH_2HPO_4, pH 7):n-heptane, 1:1. Embryos were washed in PBS and stained for β-galactosidase activity according to Blackman et al. (1991). Embryos were mounted in PBS or glycerol and photographed with Nomarski optics.

Antibody staining of embryos

Rabbit polyclonal anti-β-galactosidase antibody was provided by Robert Holmgren. Monoclonal antibody recognizing Ubx, FP3.38 (White and Wilcox, 1984) was provided by Danny Brower, and anti-Scr monoclonal antibody was provided by James Mahaffey. Embryos were dechorionated by standard methods and fixed according to Raftery et al. (1991). Endogenous peroxidases were inactivated by a 15 minute treatment in a 9:1 mixture of MeOH (100%) and H_2O_2 (30%). Blocking was accomplished with two 30 minute washes in PBS with 0.1% Triton X-100 (PBT) and 10% normal goat serum (NGS). Embryos were incubated overnight at 4°C with antibodies diluted in PBT and 5% NGS (1:1000 for FP3.38, 1:2000 for *Scr*, 1:1000 for anti-β-galactosidase) and washed in PBT. They were then incubated for 2 hours at room temperature with secondary antibodies directly conjugated to horseradish peroxidase and diluted 1:2000 in PBT, washed in PBT and stained with 3,3′ diaminobenzidine tetrahydrochloride for up to 30 minutes. Embryos were mounted in methyl salicylate and photographed with Nomarski optics.

Whole-mount in situ hybridizations

Digoxigenin whole-mount in situ hybridizations were done according to the protocol of Tautz and Pfeifle (1989). Digoxigenin-labeled fragments were prepared as described in Ray et al. (1991). Plasmids used as probes were pBEh1, a 4.5 kb *dpp* cDNA in pNB40 (St. Johnston et al.,1990) and a 1.7 kb *Eco*R1 fragment of a *labial* cDNA inserted into Bluescript (provided by Thomas Kaufman). Embryos were mounted in glycerol and photographed using bright-field or Nomarski optics.

RESULTS

Mutations in the shortvein region of *dpp* selectively remove aspects of embryonic visceral mesoderm expression of *dpp*

The shortvein (*shv*) region of *dpp* comprises the area 5′ to the *dpp* coding exons (Fig. 1). This area contains all but one of the promoters that drive the transcripts encoding the single *dpp* polypeptide (St. Johnston et al., 1990). Most dpp^{shv} homozygous or *trans*-heterozygous mutant combinations are larval lethals (Segal and Gelbart, 1985; Hursh and Ray, unpublished observations). The majority of dpp^{shv} mutants are chromosomal rearrangements that sever portions of the 5′ region from the transcription units they control. These mutations effectively act as deletions of all material 5′ to their *shv* breakpoints. Mutants that delete most of the *shv* region from the *dpp* gene disrupt *dpp* expression in the visceral mesoderm and exhibit gut defects (Immerglück et al., 1990; Panganiban et al., 1990). Previous reports examined only a few of the available *shv* mutations. We wished to analyse the entire region in more detail to localize potential *cis*-regulatory information.

Transcript localization by whole-mount digoxigenin in situ hybridization was carried out on both homozygous and *trans*-heterozygous combinations of the dpp^{shv} alleles indicated in Fig. 1 (for ease of presentation, *shv* mutations will be indicated only by their unique superscripts, i.e., dpp^{s4} will be called *s4*). Transcripts are detected in the clypeolabrum, pharynx, esophagus and sites of the evaginating gastric caecae (ps4) and the secondary midgut constriction (ps7) of wild-type animals (Fig. 2A,B). Some preparations also show expression at the site of the future third midgut

constriction (Fig. 2I). Breakpoints that remove DNA sequences distal to molecular coordinate 82.7 (*s13*, *s4*, *s18*, *s25*, see Fig. 1) eliminate all aspects of internal *dpp* expression (pharynx, esophagus, ps4, ps7; Fig. 2C) without alteration of the complex ectodermal expression pattern. Late embryos fail to evaginate gastric caecae or form secondary midgut constrictions and the primary midgut constriction is shifted posteriorly (Fig. 2D, also see Panganiban et al.,

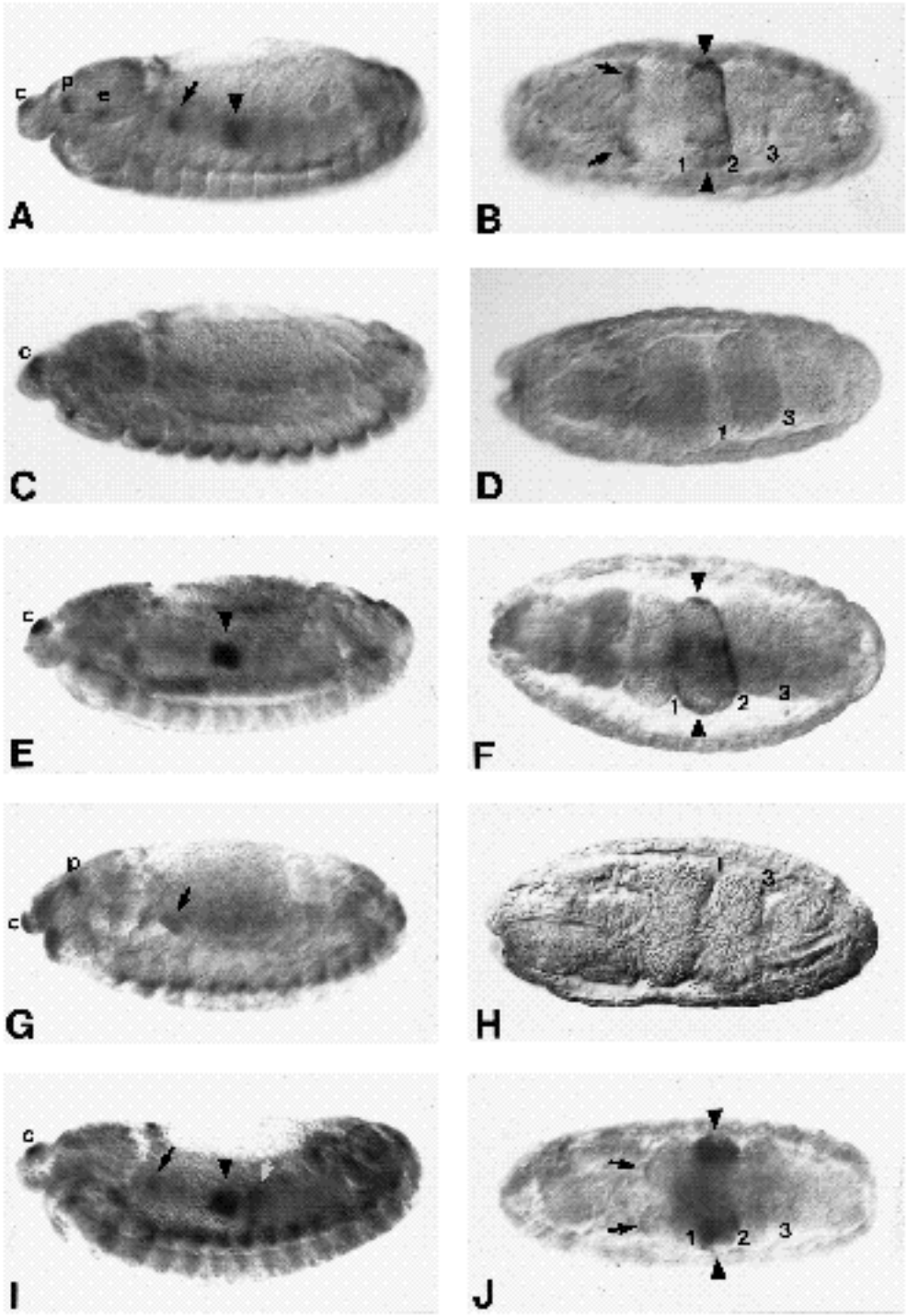

Fig. 2. Expression of *dpp* transcripts in wild-type and homozygous *shv* mutant embryos detected by whole-mount in situ hybridization. In this and in subsequent figures, anterior is to the left. A,C,E,G,I are lateral views; B,D,F,H,J are horizontal views. In later embryos, midgut constrictions are indicated by numbers. Dark staining on the periphery of embryos represents the ectodermal expression of *dpp*, that is unaltered in these genotypes. (A) Stage 13, wild-type embryo. Expression is seen in the clypeolabrum (c), pharynx (p), esophagus (e), and ps4 (arrow) and ps7 (arrowhead) of the VM. (B) Stage 16 wild-type embryo. Transcripts are detected at the sites of the evaginating gastric caecae (arrows) and surrounding the secondary compartment of the midgut (arrowheads). (C) Stage 13 of an *s4* homozygote. Clypeolabrum expression is detected (c), but all other internal expression of *dpp* is absent. (D) Stage 16, of an *s4* homozygote. No *dpp* expression is observed. The gastric caecae are absent and the midgut is missing a compartment. (E) Stage 13 of an *s22* homozygote. Staining in the clypeolabrum (c) and at the ps7 position of the VM (arrowhead) is observed. (F) Stage 16 of an *s22* homozygote. Expression in the VM at the anterior portion of the midgut is absent, as are the gastric caecae. Expression in the VM surrounding the second gut compartment is normal (arrowheads). (G) Late stage 13 of an *s6* homozygote. Staining is observed in the clypeolabrum (c), pharynx (p), and ps4 region of the VM (arrow), although this latter staining appears reduced as compared to wild type. No expression is seen at ps7. Esophageal expression can be seen in dark preparations of this genotype. (H) Late stage 16 *s6* homozygote. Transcript is undetectable at this stage, but gastric caecal evaginations are observed. Note abnormal gut morphology. (I) Stage 13 *s11* homozygote. Clypeolabrum expression is observed (c). No expression is detected in the pharynx or esophagus. Expression is visible in the ps4 region, albeit at a lower level than in wild type (compare ps4 and ps7 expression in (A) and in (I). ps7 VM expression is normal (arrowhead). An additional site of expression posterior to ps7 is observed in this preparation (white arrow). This is a normal site of *dpp* expression (St. Johnston and Gelbart, 1987), observed only in darkly stained preparations. (J) Stage 16 of an *s11* homozygote. Faint expression is observed at the evaginating gastric caecae (arrows).

1990). This same result is obtained with breakpoints mapping to approximately 76 (*s7*), suggesting that no unique regulatory information resides within the 82.5-76 interval.

Homozygous and *trans*-heterozygous *shv* combinations containing *s22* (at approximately 73, Fig. 1) show normal *dpp* expression in the VM at ps7 (Fig. 2E,F). The pharyngeal, esophageal and ps4 VM expression of *dpp* are still absent, indicating that spatial *cis*-regulatory information controlling these aspects of *dpp* expression are located distal to the *s22* breakpoint. In agreement with these expression data, *s22* mutant embryos have normal midgut constrictions, but lack gastric caecae (Fig. 2F). This result is supported by examination of one of only two internal deficiencies that exist in the *shv* region, *s6* (Fig. 1). In this mutation, ps7 VM staining is absent, and the embryos are missing the secondary constriction (Fig. 2G,H). Staining in the ps4 region of the VM appears to be reduced when compared to wild type (Fig. 2G), and is undetectable later in development (Fig. 2H), but these embryos nonetheless produce gastric caecae. The results obtained with *s6* also suggest that the ps7 VM *dpp* expression originates between coordinates 76 and 72. Finally, in embryos homozygous for *s11* whose proximal breakpoint is approximately 74 (Fig. 1), expression in ps7 is normal while expression in ps4 is reduced but detectable (Fig. 2I). The midgut constrictions of *s11* homozygotes are normal (Fig. 2J). However, the gastric caecae, although present, may be somewhat reduced in length. The *dpp* staining in the pharynx and esophagus is also missing in this genotype, placing their regulatory elements distal to the *s11* breakpoint.

In summary, these analyses allow us to crudely order regulatory regions within the *shv* region. The pattern of expression in ps7 suggests that it is controlled from the most proximal position, with expression in ps4 closely apposed but distal to it. The expression of *dpp* in the pharynx and esophagus must also be directed by the *shv* region with control elements located more distally than those directing ps4 expression.

Sequences in the *shv* region are both necessary and sufficient to direct *dpp*'s VM expression

To further define the sequence requirements for *dpp* gene expression in the VM, we compared two rescue constructs, P20 and P15 (Fig. 1). These two constructs differ only in their distal endpoints and contain the same set of *dpp* promoters. Both constructs extend to the end of the coding region (*Hin* region) but contain none of *dpp*'s extensive 3′ *cis* regulatory unit (*disk* region). P20 extends to 71.2 on the molecular map, a position further distal than the *s11* breakpoint. Given the result described above with *s11*, we predicted that P20 would contain the gastric caecae and ps7 response elements, and indeed, this construct rescues *shv*-associated larval lethality (data not shown.) In contrast, P15 extends distally only to 77.6 on the molecular map. The P15 construct expresses functional *dpp* as evidenced by its ability to rescue the dominant D/V patterning defects of *dpp* null mutations (data not shown). We compared the ability of these two constructs to drive *dpp* expression in the VM in homozygous *dpp* null backgrounds. We were unable to examine *dpp* transcript directly, as the *dpp* loss-of-function

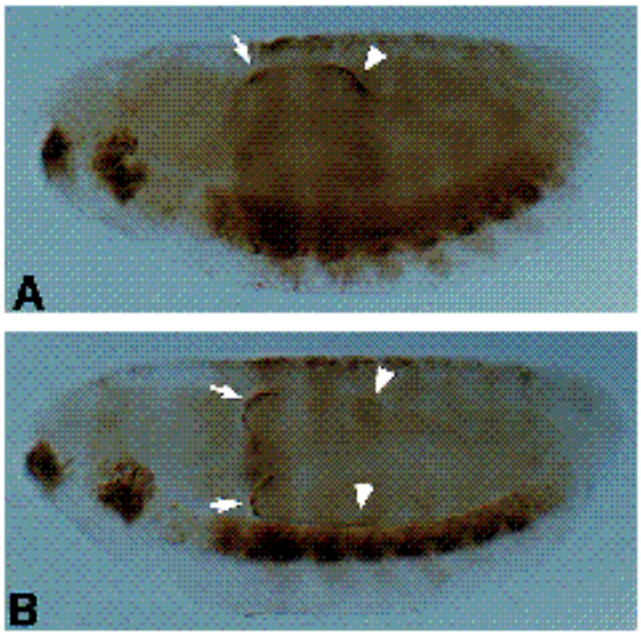

Fig. 3. Immunohistochemical detection of *Scr* and *Ubx* protein expression in embryos expressing *dpp* rescue constructs. (A) Lateral view, stage 15 embryo from P20 rescue construct line. *Scr* expression (arrow) is restricted to a small domain and strong *Ubx* expression (arrowhead) is observed at the site of the future secondary midgut constriction. (The ventral domains of *Scr* and *Ubx* are out of the plane of focus in this photo.) This phenotype was characteristic of all embryos in the P20 lines. (B) Lateral view, stage 15, of an embryo from a P15 rescue construct line. *Scr* expression domains (arrows) are expanded and extend to the anterior of the midgut. *Ubx* protein expression in ps7 (arrowheads) is reduced as compared to A.

alleles we used were not RNA nulls. We instead examined expression of *Scr*, *Ubx* and *lab*. Lack of *dpp* expression in ps4 causes an expansion of the *Scr* domain (Panganiban et al., 1990) and in ps7 causes an elimination of *lab* expression (Immerglück et al., 1990; Panganiban et al., 1990). In agreement with the results of Panganiban et al., (1990) we also find that in ps7, lack of *dpp* expression causes a reduction of *Ubx* expression. These markers could therefore be used as indirect assays for *dpp* gene activity.

In P20 backgrounds, we are unable to detect *Scr* domain expansion or loss of expression of *Ubx* (Fig. 3A) or *lab* (data not shown). All embryos examined have normal gut morphology. In contrast, in P15 backgrounds, approximately 1/4 of the embryos examined displayed *Scr* domain expansion (Fig. 3B). In these embryos, which were inferred to express *dpp* solely from the P15 rescue construct, *Ubx* expression in ps7 was reduced (Fig. 3B). Gut defects were observed in older embryos. When *lab* expression was monitored using digoxigenin in situ hybridization, approximately 1/4 of the appropriate stage embryos failed to express *lab* in the gut endoderm (data not shown).

We conclude that the sequences contained in P15 are insufficient for correct *dpp* expression in ps4 and ps7 of the VM. That is consistent with the results obtained with *shv* mutants and targets the region between 77.6 and 71.2 as critical in *dpp*'s VM expression. In addition, the ability of P20 to provide both spatially correct expression and rescue

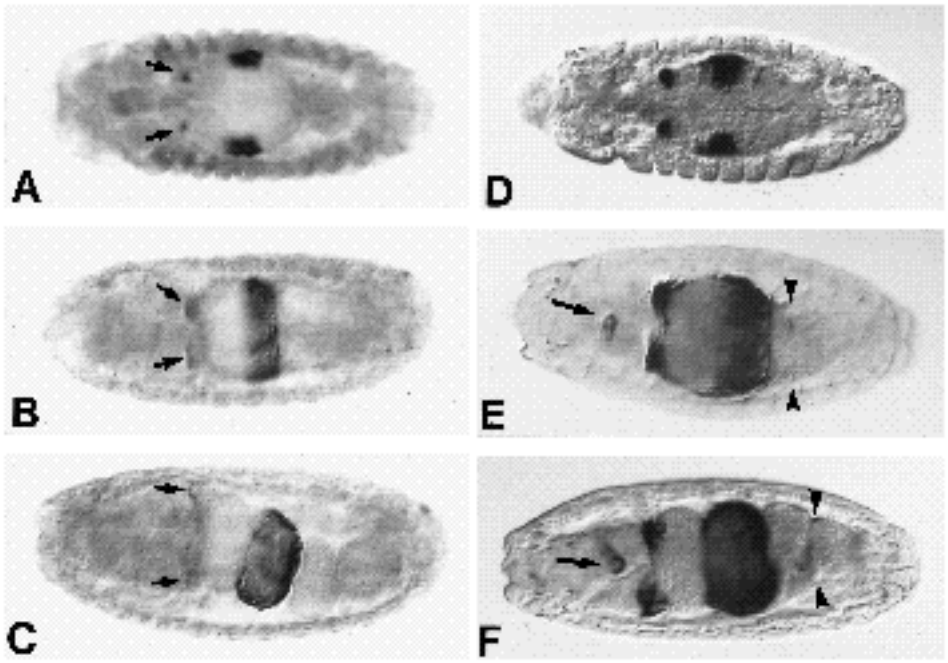

Fig 4. Immunohistochemical localization of β-galactosidase directed by the RD1 (A-C) or RD2 (D-F) reporter gene constructs. Horizontal views are shown. (A) Stage 13 embryo. The RD1 construct expresses primarily in ps7, although expression is detectable at a low level in the VM at ps4 (arrows). (B) Stage 15 embryo. The two blocks of ps7 expression have now fused to form a band around the midgut. Arrows indicate the low level of expression observed at the gastric caecal evaginations. (C) Stage 16. Expression now surrounds the second compartment of the midgut. Arrows indicate gastric caecal expression. (D) Stage 13, of an embryo expressing the RD2 transgene. The proportion of ps4 to ps7 expression is that observed for wild-type *dpp* expression. (E,F) Stage 15 and 16 embryos, respectively. Expression in ps4 and ps7 follows the normal dpp pattern (compare to Fig. 2A,B). In addition, expression is also observed in the developing esophagus (arrow) and, posterior to ps7, at the site of the tertiary midgut constriction (arrowheads).

of larval lethality indicates that no further sequences from the *dpp* gene are required for VM function and gut morphogenesis.

Location of *cis* elements directing *dpp* VM expression in the *shv* region

Our conclusions are supported and supplemented by two β-galactosidase reporter constructs, RD1 and RD2 (Fig. 1). RD1 uses the resident *dpp* promoter(s) to drive β-galactosidase expression, while RD2 places *shv* region sequences upstream of a minimal hsp70 promoter. The *lacZ* expression patterns produced by these constructs are shown in Fig. 4. RD1, whose coordinates are 75.1-81.4, is expressed primarily in ps7 (Fig. 4A-C). Some expression is detectable in ps4, indicating that a ps4 *cis*-regulatory element resides within it. However, this expression is at a much lower level than normal when compared to the ratio of expression in ps4 to ps7 observed in wild-type (Fig. 2A,B). This suggests that other elements required for wild-type ps4 pattern are absent in RD1. RD2 provides normal levels of expression in ps4 relative to ps7, suggesting that additional elements driving gastric caecae expression are located within RD2 but not within RD1 (Fig. 4D-F). In both constructs, the spatial restriction of expression is indistinguishable from *dpp* transcript accumulation. Consistent with our genetic analysis, in RD2, β-galactosidase expression is detectable in the embryonic esophagus (arrow, Fig. 4E,F). We also observe faint expression in the area where the tertiary midgut constriction will form (arrowheads, Fig. 4E,F). These are both sites of normal *dpp* expression (St. Johnston and Gelbart, 1987), although no defects have been ascribed to loss of *dpp* activity in these areas. Note that, overall, RD2 expresses more strongly than RD1.

dpp VM reporter gene constructs respond to BX-C gene products

The response of *dpp* expression in the VM to mutations in genes of the bithorax complex (BX-C) has been defined by the use of mutants and gene-specific probes (Immerglück et al., 1990; Reuter et al., 1990; Panganiban et al., 1990). These results indicate that *Ubx* is a positive activator of *dpp*'s VM expression, and that in embryos homozygous for *abd-A* mutations the domain of *dpp* expression expands posteriorly in a manner identical to that observed for *Ubx* itself (Bienz and Tremml, 1988). We wished to know if the *cis*-regulatory elements contained in RD1 and RD2 responded to homeotic genes in the same way. Fig. 5 shows the response of RD1 and RD2 to a loss-of-function allele of *Ubx*. (The allele shown is $Ubx^{9.22}$. The result with $Ubx^{6.28}$ is identical.) In the absence of *Ubx* gene function, the ps7-specific *lacZ* expression of RD1 and RD2 is abolished or reduced, respectively (Fig. 5B,F). (Low levels of expression from RD1 could be present but below detectable levels, due to its weaker wild-type expression.) Lack of *abd-A* activity yields ectopic expression of β-galactosidase in the VM surrounding the midgut (Fig. 5C,G). In Ubx^{109}, a deficiency removing both the *Ubx* and *abd-A* genes (Karch et al., 1985), strong expression is observed in the most posterior portion of the midgut from both RD1 and RD2 (Fig. 5D,H). A similar result has been observed for *Ubx* reporter genes in this genetic background (Bienz and Tremml, 1988). They demonstrate that this ectopic expression is caused by the *Abd-B* gene in the absence of functional *abd-A* gene product. Mutations that remove the entire *BX-C* do not display this ectopic expression. Our result indicates that *dpp*, like *Ubx*, responds to this activity of *Abd-B*.

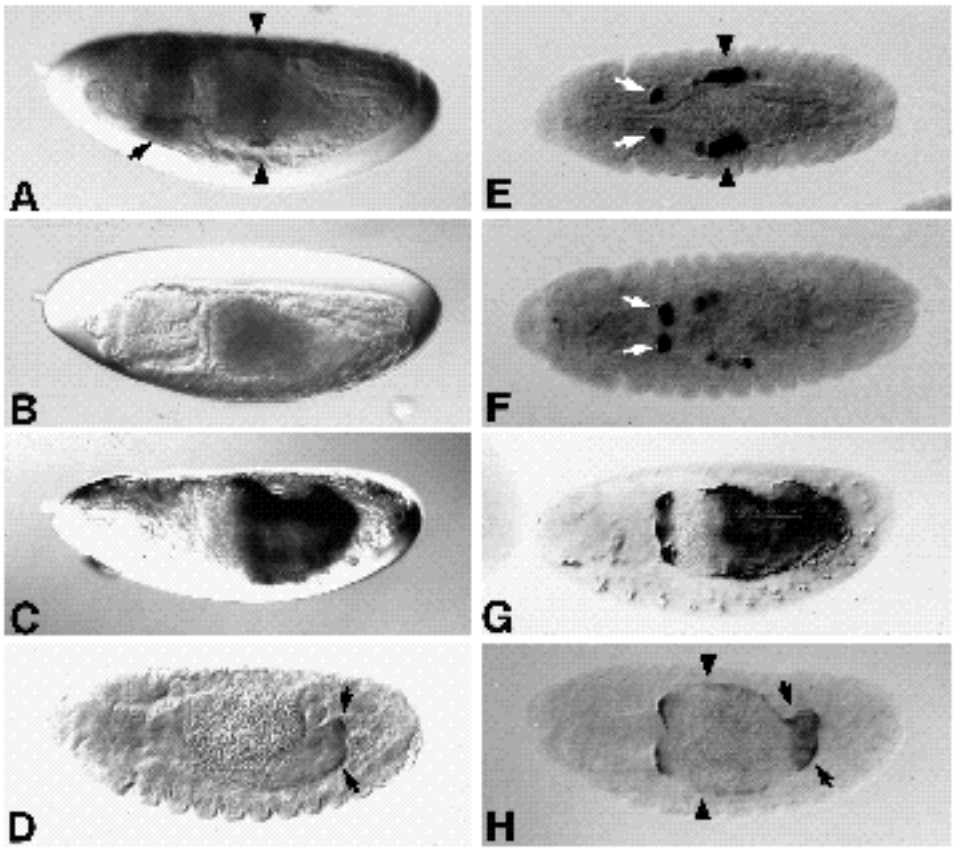

Fig.5. Localization of β-galactosidase expression in homeotic mutant backgrounds. Histochemical (A-C) or immunohistochemical (D) detection of β-galactosidase activity directed by RD1. (E-H) Immunohistochemical detection of β-galactosidase activity directed by RD2. A-D, G-H are lateral views, E-F are horizontal views. (A) Wild-type embryo, stage 14. Arrow indicates the stripe of expression directed by a *ftz*-β-galactosidase reporter gene marker for the BX-C^{+} balancer chromosome, (*TM3*). Expression in ps7 is normal (arrowheads). Expression in ps4 from RD1 is rarely detectable using X-gal. (B) Stage 14 *Ubx*$^{9.22}$ homozygote, from the same reaction as in A. (C) Stage 14 *abd-A*^{mx2} homozygote. (D) Stage 14 *Ubx*^{109} homozygote. Ectopic staining in the posterior-most VM is indicated by arrows. (E) Stage 13 wild-type embryo expressing the RD2 transgene. Arrowheads indicate expression in ps7. White arrows indicate expression in ps4. This expression is unaffected in these genotypes and serves as a control for staining intensity between E and F. (F) Stage 13, *Ubx*$^{9.22}$ homozygote from the same reaction as E. Expression in ps7 is reduced and patchy. (G) Stage 14 *abd-A*^{mx2} homozygote, showing ectopic expression of *lacZ*. (H) Stage 14 *Ubx*^{109} homozygote. Arrows indicate strong ectopic expression in most posterior VM. Arrowheads indicate anterior border of weak ectopic expression in posterior VM.

Surprisingly, in *Ubx*^{109}, the RD2 construct expresses ectopically in the remainder of the posterior midgut in a pattern like that observed for homozygous *abd-A* mutant embryos, albeit at a lower level (arrowheads, Fig. 5H). As this mutation deletes both the *Ubx* and *abd-A* genes, this ectopic expression cannot be mediated by de-repression of *Ubx*, and must therefore suggest an independent activity of *abd-A* on *dpp*. ps4 expression is unaffected in all these mutant backgrounds.

Autoregulation of *dpp* in the VM

TGF-β1 has been reported to positively regulate its own expression in both normal and transformed cell lines (Van Obberghen-Schilling et al., 1988). We examined the response of RD1 and RD2 in *shv* mutant backgrounds to ask if *dpp* also displays positive autoregulation. We used three alleles, each with a different VM expression profile. *s4* removes all *dpp* expression in the visceral mesoderm, *s6* removes only expression in ps7, and *s22* removes only expression in ps4 (Fig. 6). The response of RD1 in *s4* homozygous mutant embryos is shown in Fig. 6A,B, visualized by anti-β galactosidase immunohistochemistry. Reporter gene expression in ps7 is reduced relative to controls. We also examined *s6* and observed the same result (data not shown). In RD2, expression in both ps4 and ps7 was monitored. In *s4*, expression of RD2 is absent in ps4 and reduced in ps7 (Fig. 6C,D). RD2 reporter constructs in *s6* give normal expression in ps4, but reduced expression relative to the control in ps7 (Fig. 6E,F). This expression pattern follows the distribution of *dpp* in this mutant. In *s22*, normal levels of expression are seen in ps7 but none is detectable in ps4, again paralleling the expression of *dpp* in embryos of this genotype. Therefore, normal RD1 and RD2 reporter gene expression levels require endogenous *dpp* expression, demonstrating that a positive autoregulatory loop maintains *dpp*'s expression in both ps4 and ps7 of the VM.

Ubx reporter gene expression in the VM is coincident with and requires *dpp*

dpp's positive autoregulation must be mediated by one or more transcription factors. In ps7, *Ubx* is an obvious candidate gene, given its coincident expression and its ability to affect *dpp* expression (Fig. 5; also see Immerglück et al., 1990; Reuter et al., 1990). In addition, *Ubx* has been reported to be required for the maintenance of its own transcription in an 'autocatalytic' loop (Bienz and Tremml, 1988; Müller et al., 1989). However, as discussed in the Introduction, the effect of *dpp* on *Ubx* protein levels was unclear. Our results, as shown in Fig. 3, support those of Panganiban et al. (1990) and are in contrast to those reported by Immerglück et al. (1990). Thus we support the

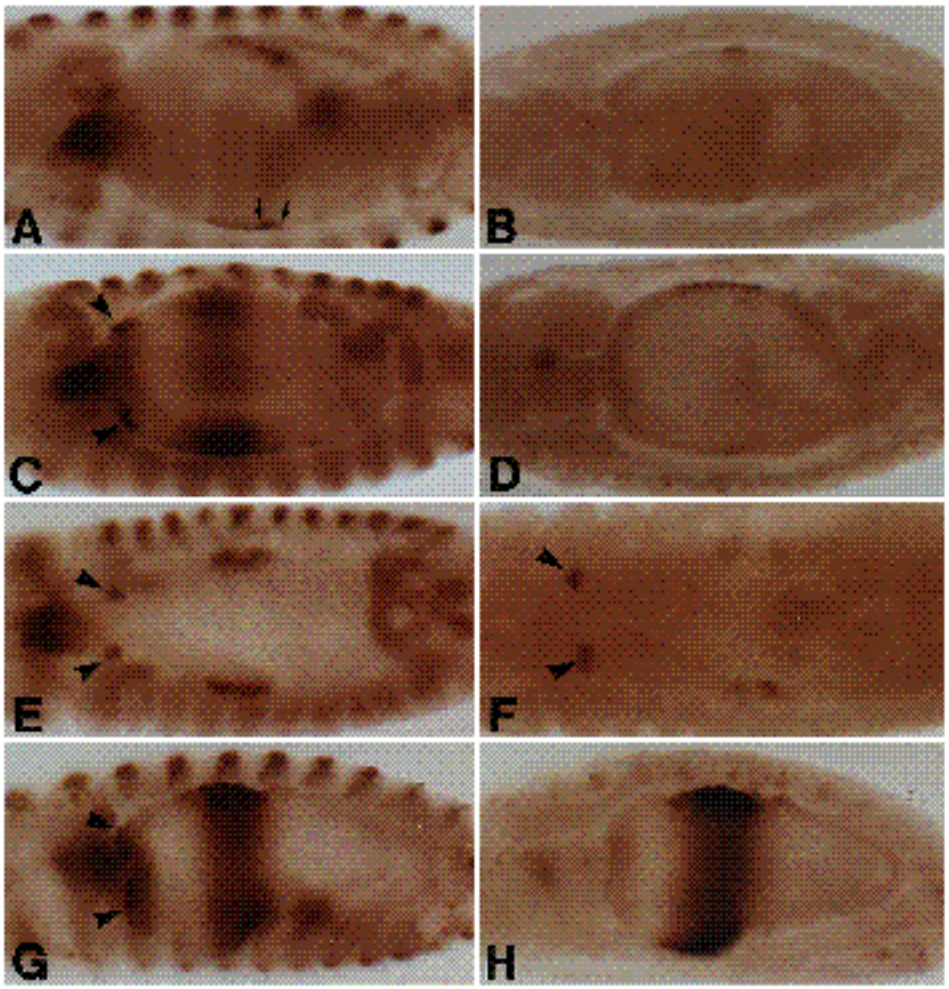

Fig. 6. Immunohistochemical detection of β-galactosidase expression directed by RD1 and RD2 in *shv* mutant backgrounds. A-D,G,H, lateral views. E,F, Horizontal views. (A-B) Expression from RD1. (C-H) Expression from RD2. (A) Stage 14 control embryo expressing the RD1 construct and also a *wg-lacZ* marker for the *CyO, dpp*$^+$ balancer. In addition to ectodermal stripes, *wg* is also expressed in the VM at ps8 (area between arrows), directly behind *dpp*. (B) Stage 14 homozygous *s4* mutant embryo from the same staining reaction as A. Expression from RD1 in ps7 is greatly reduced in this genotype. (C-D) Stage 13 *dpp*$^+$ and s4 homozygote embryos respectively, expressing the RD2 transgene. Arrowheads indicate expression in the gastric caecae from RD2. The contribution of the VM expression driven by the *wg-lacZ* reporter in these constructs is minimal, due to the intensity of RD2 expression. (E-F) Stage 13, *dpp*$^+$ and *s6* homozygote embryos, respectively. Expression in the gastric caecae from RD2 is indicated by arrowheads. (G-H) Stage 15 control and *s22* homozygote embryos, respectively. Gastric caecal expression in control (G) is marked by arrowheads.

contention that *dpp* affects the level of *Ubx* protein. However, these experiments do not determine the mechanism of this effect. We investigated whether *dpp* could influence *Ubx* transcription by examining the expression of a *Ubx-lacZ* reporter gene. In wild-type backgrounds *Ubx* β-galactosidase expression is seen in the VM surrounding the midgut at ps7, but to our surprise it is also observed in the evaginating gastric caecae at ps4 (Fig. 7A,B). The VM at the site of the gastric caecae is not a part of *Ubx's* in vivo expression (Akam and Martinez-Arias, 1985; White and Wilcox, 1985; Tremml and Bienz, 1989; and our unpublished observations). However, examination of the literature (Müller et al., 1989; Irvine et al., 1991), as well as our own observations of two additional reporter gene constructs (data not shown), suggest that *Ubx*-reporter gene constructs containing only the region 3-5 kb upstream of the start of *Ubx* transcription fused to *lacZ* are indeed expressed in both the normal ps7 location and in the ectopic location in ps4, although gastric caecae staining in these constructs has not been noted as such. Reporter gene constructs containing larger sequences of the *Ubx* gene (35 kb) do not exhibit this ps4 expression (Irvine et al., 1991), suggesting that the removal of upstream DNA derepresses *Ubx* expression in ps4. This expression precisely matches the expression of *dpp* in ps4 (compare Fig. 4 and Fig. 7A,B).

This *Ubx*-directed β-galactosidase expression in both ps4 and ps7 is dependent upon *dpp*. In *s4* embryos, which do not express *dpp* in the VM, no *Ubx* reporter gene activity is seen (Fig. 7C). In *s6*, where *dpp* is present in ps4 but absent in ps7, the reporter gene is expressed at wild-type levels in ps4, but expression is severely reduced in ps7 (Fig. 7D). The reciprocal expression pattern is produced by *s22*; expression is lost in ps4, but is normal in ps7. We do not feel that this is solely due to the absence of gastric caecal tissue, as Fig. 7A shows significant β-galactosidase expression prior to any substantial caecal evagination. In conclusion, the expression of these truncated *Ubx*-reporter genes is coincident with *dpp* in the VM, and like the *dpp* reporter genes RD1 and RD2, is dependent on *dpp* gene activity to achieve normal expression levels.

DISCUSSION

Properties of *cis*-regulatory elements directing VM in the *shv* region

We have localized *cis*-regulatory elements driving *dpp* expression in the VM surrounding the embryonic midgut by mutant breakpoints, rescue constructs, and β-galactosidase reporter genes. The properties of the *cis*-regulatory elements directing *dpp*'s expression in ps4 differ from those directing expression in ps7. Expression of *dpp* in ps4 is controlled

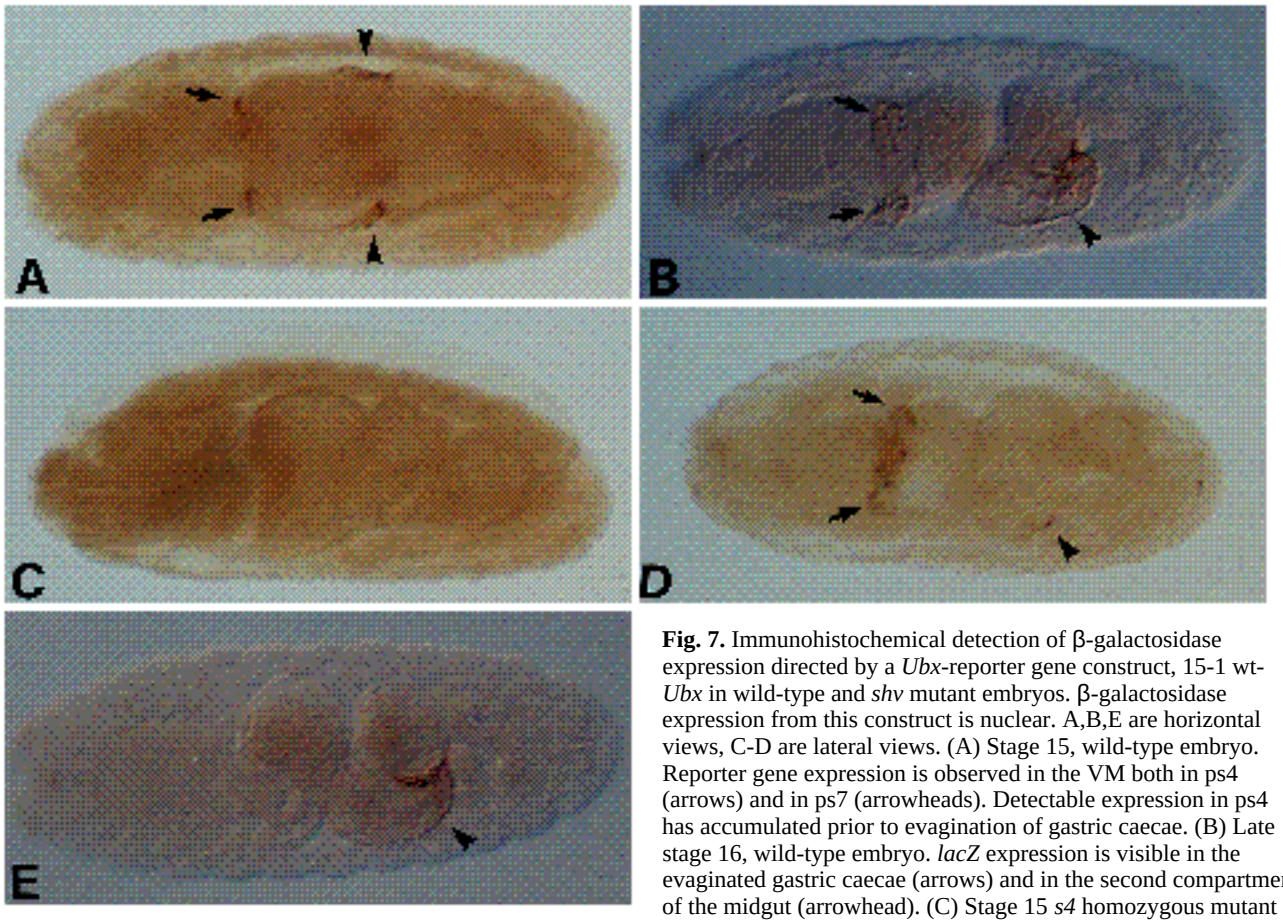

Fig. 7. Immunohistochemical detection of β-galactosidase expression directed by a *Ubx*-reporter gene construct, 15-1 wt-*Ubx* in wild-type and *shv* mutant embryos. β-galactosidase expression from this construct is nuclear. A,B,E are horizontal views, C-D are lateral views. (A) Stage 15, wild-type embryo. Reporter gene expression is observed in the VM both in ps4 (arrows) and in ps7 (arrowheads). Detectable expression in ps4 has accumulated prior to evagination of gastric caecae. (B) Late stage 16, wild-type embryo. *lacZ* expression is visible in the evaginated gastric caecae (arrows) and in the second compartment of the midgut (arrowhead). (C) Stage 15 *s4* homozygous mutant embryo. No *Ubx*-reporter gene expression is observed. (D) Stage 16 homozygous *s6* mutant embryo. Reporter gene expression in the gastric caecae is indicated by arrows, faint expression in the area where the second midgut compartment should have formed is indicated by an arrowhead. (E) Stage 16 homozygous *s22* homozygous mutant embryo.

by three or more separable elements within the *shv* region. First, the RD1 reporter gene construct is capable of directing a low level of ps4 β-galactosidase expression, and thus must contain ps4 regulatory information. This region in isolation appears to be insufficient to produce gastric caecae because in the *s22* mutation, which retains this region, we see no detectable expression of *dpp* transcript in ps4, a failure to produce gastric caecae and an expansion of the domain of *Scr* (data not shown). The slightly more distal *s11* mutation restores *dpp* RNA levels in ps4 to detectable but reduced levels, produces gastric caecae and represses *Scr* in ps4. It must therefore identify a second element required for correct *dpp* expression in this region, residing between the proximal end of *s11* and the proximal end of *s22*. However, the *s6* deficiency removes this element yet produces an RNA expression pattern similar to *s11*, produces gastric caecae and blocks *Scr* expression in ps4. We therefore conclude that at least one more element must reside distal to the *s11* breakpoint that is contributing to gastric caecal expression in *s6*. The RD2 reporter construct that produces wild-type levels of expression in ps4 extends over the entire domain defined as critical by the *s22*, *s11* and *s6* breakpoints. The ability of *s11* and *s6* to produce spatially correct but reduced levels of expression from mutually exclusive areas of the *shv* region suggests that these multiple elements function in the same way. We hypothesize that wild-type expression in ps4 is achieved through additive effects of multiple elements residing in the *shv* region.

In contrast to the situation in ps4, expression in ps7 behaves as if there is a small regulatory region that is both necessary and sufficient to direct *dpp* expression in ps7. Mutant analysis suggests that DNA from molecular coordinate 72.5 (the distal-most possible position of the *s22* breakpoint) to 76.6 (the proximal-most possible position of the *s7* breakpoint) is necessary to affect both expression of *dpp* transcript in ps7 and the formation of the secondary midgut constriction. No further defects arise by removal of more proximal DNA (*s12-s4*). The *s6* deletion removes this critical area and lacks ps7 expression, further supporting the notion of a discrete region controlling ps7, as does the inability of the P15 rescue construct to direct *dpp* expression in ps7. The RD1 and RD2 reporter gene constructs both direct correct spatial patterning in ps7 and their overlap includes a portion of this critical region. The most parsimonious explanation of these data is that critical ps7 elements are confined to the 800 bp between these two constructs: 75.1 and 75.9 on the molecular map. In summary, all these data are consistent with a discrete location immediately proximal to the *s22* breakpoint that is necessary to direct *dpp*'s expression in ps7.

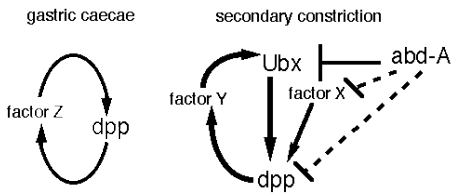

Fig. 8. Schematic diagram of a model for interactions of *dpp* with genes in the VM. Lines with arrowheads indicate positive interactions, lines with bars indicate negative interactions. Our data do not distinguish between *abd-A* acting through factor X on *dpp*, or acting on the *dpp* gene directly, so both possibilities are included (dashed lines).

dpp requires multiple factors to achieve correct gene expression in the VM

The transcription of β-galactosidase in *dpp* reporter constructs is modulated by homeotic genes of the BX-C (Fig. 5) in a manner identical to *dpp* transcript (Immerglück et al., 1990) or protein (Reuter et al., 1990). Thus both RD1 and RD2 contain sufficient homeotic gene-responsive elements to produce the expected patterns of *dpp* expression in ps7 of the VM. We have scanned the sequence of *dpp* (R. W. Padgett, M. de Cuevas, S. Findley and W. Gelbart, unpublished data) covering most of our reporter genes for sites that might be predicted to bind UBX, using a consensus binding site and related sites as defined in Ekker et al. (1991). Within the *dpp* sequence extending from 71.8 on the molecular map to the end of the coding exons (Fig. 1), 31 sites exist that would be predicted to bind UBX. Over a third of these sites are clustered in the interval between 74.8 and 76.8, in the region identified as critical to expression of *dpp* in ps7 of the VM. This abundance of sites in a gene that behaves as a target of *Ubx* is suggestive of a direct relationship; however, further work is required to determine if these sites are directly responsible for *Ubx's* effect on *dpp* expression.

Nevertheless, in homozygous mutant *Ubx* embryos, we see significant expression from the RD2 reporter construct in ps7. Residual expression of *dpp* protein in a $Ubx^{6.28}$ homozygote was reported by Reuter et al. (1990) who attributed this effect to partial activity by this allele as they failed to detect DPP in embryos homozygous for a deficiency of *Ubx* ($Df(3R)bxd^{100}$). However, $Ubx^{6.28}$ is a frame shift caused by a 32 bp deletion at codon 27 (Weinzierl et al., 1987) and unlikely to encode a functional protein. We see residual reporter gene expression in embryos homozygous for both the pseudopoint mutations $Ubx^{6.28}$ and $Ubx^{9.22}$, as well as for Ubx^{109}, a large deficiency removing both the *Ubx* and *abd-A* transcription units. Two conclusions must be drawn from these data. The first is that *Ubx* is not the sole factor required to initiate *dpp*'s expression in the VM. Some other factor or factors must work in concert with *Ubx* to turn on *dpp* in ps7, although the presence of *Ubx* greatly enhances the expression of *dpp*. The requirement for factors in addition to *Ubx* is also predicted by the fact that *dpp* is not activated by *Ubx* in any tissue outside the VM, even when a heat shock-*Ubx* construct is used (Reuter et al., 1990). Thus, positive transcription factors whose expression is limited to the VM could act with *Ubx* to initiate gene expression in this tissue and are capable of directing a low level of *dpp* expression in the absence of *Ubx*.

The second conclusion is based on the observation that ectopic expression of RD2 throughout the posterior VM occurs even in a deficiency for both *Ubx* and *abd-A* (Ubx^{109}). This expression is spatially identical to that observed in embryos mutant for the *abd-A* gene alone, although the level of expression is greatly reduced. This result suggests that *abd-A* acts on *dpp* by two independent mechanisms: through negative regulation of *Ubx* itself and through independent negative regulation of a second factor required for *dpp*'s ps7 VM expression. Thus even in the absence of both *Ubx* and *abd-A*, the derepression of the second factor triggers ectopic expression of the reporter gene. The ability of *abd-A* to act directly on *dpp* is also suggested by the observation that ectopic expression of *Ubx* by a heat shock construct is unable to activate *dpp* in the *abd-A* domain (Reuter et al., 1990). These results suggest that a cofactor necessary for initiating *dpp*'s ps7 gene expression is under negative repression by *abd-A* and that the posterior limit of *dpp* ps7 VM expression depends on several activities of *abd-A*.

There are at least two homeobox-containing genes that are expressed in the VM and are candidates for positive co-regulators, *H2.0* (Barad et al., 1988) and *msh-2* (Bodmer et al., 1990). The low level of activity produced by the co-regulator(s) alone would not generate sufficient *dpp* product to mediate biological activity, as all the *Ubx* loss-of-function alleles we tested appear to be fully penetrant for the loss of the secondary midgut constriction (data not shown).

dpp is required for both the maintenance of its own transcription and that of *Ubx* in the VM

Using mutations that independently alter specific aspects of *dpp*'s expression in the VM we have established that both sites of *dpp* transcript accumulation require *dpp* protein to achieve correct expression levels. In addition, our data indicates that *Ubx* autoregulation in ps7 appears to act through *dpp*. Work by Bienz and coworkers have demonstrated that sequences from −3.1 to +980 are required to provide correct *Ubx* expression in ps7 of the VM (Müller et al., 1989). We find that *Ubx* reporter genes containing such sequences express in both ps4 and ps7 of the visceral mesoderm in a pattern remarkably similar to that observed with the *dpp* RD2 reporter gene, although it should be noted that the initiation of gene expression from 15-1wt*Ubx* appears to occur later than RD2 as judged from the morphology of the anterior midgut. This promiscuous expression in the developing gastric caecae appears to be common among *Ubx* reporter gene constructs containing approximately this portion of the *Ubx* gene, both from our results and upon reexamination of the literature (Müller et al., 1989; Irvine et al., 1991). We therefore believe it is not an artifact of a specific construct, or chromosomal location, but reflects aberrant expression due to the absence of other *cis*-regulatory DNA that causes repression in anterior portions of the embryo. Other workers have described similar ectopic anterior expression in β-galactosidase reporter gene constructs from other regions of the *Ubx* gene (Simon et al., 1990; Qian et al., 1991), and they also attribute it to the removal

of *cis*-regulatory information that represses this expression in vivo. We surmise that short *Ubx* constructs, lacking the relevant repression domains, would not show the normal repression of *Ubx* in ps4. In the absence of active repression, the reporter gene might then be capable of responding to some activating network resident in ps4 VM cells. The coincidence of *Ubx* and *dpp* expression in the VM suggests that this network includes and absolutely requires the *dpp* gene. The result obtained supports this idea: VM expression from *Ubx* reporter genes is eliminated in *shv* mutant backgrounds lacking *dpp* gene expression in the VM. Thus the presence of DPP is a requirement for transcription from a *Ubx*-reporter gene both in ps7, in cells that express endogenous *Ubx* protein, and in ps4, in cells that do not.

Autoregulation in the VM

Autoregulation is invoked in models of pattern formation to account for the stable maintenance of gene expression initiated by transient signals (Meinhardt, 1982). Many *Drosophila* patterning genes exhibit this characteristic, such as *even-skipped* (*eve;* Harding et al. 1989; Goto et al.,1989) *fushi tarazu* (*ftz*; Hiromi and Gehring, 1987), *Deformed* (*Dfd;* Kuziora and McGinnis, 1988; Bergson and McGinnis, 1990) and *Ubx* (Bienz and Tremml, 1988; Müller et al., 1989). All these genes are DNA binding proteins that putatively act as transcription factors. Furthermore, all their genes contain sequences that are capable of binding their own gene products in vitro (Jiang et al., 1991; Pick et al., 1990; Regulski et al., 1991; Beachy et al., 1988; von Kessler and Beachy, unpublished observations cited in Beachy, 1990) so it has been tempting to speculate that such forms of autoregulation are direct, and that the products of autoregulatory genes act by binding to their own promoters to positively regulate transcription.

This appears to be the case for *eve*, *Dfd*, and *ftz*, where the sites within the autoregulatory elements that bind the respective gene products are required for correct autoregulation (Jiang et al., 1991; Regulski et al., 1991; Schier and Gehring, 1992) although it is conceivable that other homeotic genes with similar site specificity could be the effectors in vivo. In these cases a strong argument can be made for direct positive autoregulation.

The regulation of *Ubx* in the VM may be different. We have unambiguously demonstrated that *dpp* is required to maintain *Ubx* reporter gene transcription in the VM. In ps4 a pathway must exist for *dpp* to act on *Ubx* transcription independent of the presence of *Ubx* protein, which is not expressed in this location. In ps7 there are at least two possibilities: the simpler is that the same pathway obtains in both ps4 and ps7; that is the autoregulation of *Ubx* in the VM is indirect and acts through *dpp* and a presently unidentified transcription factor. We have schematized this model in Fig. 8. The other possibility is that *dpp* acts on *Ubx* transcription by activating a signal transduction network that post-translationally modifies the *Ubx* protein itself, allowing it to maintain its own expression. While we cannot distinguish between these two possibilities, we favor the former and raise the possibility that *Ubx* autoregulation in the VM may be indirect.

In summary, we have demonstrated that the expression of *dpp* in the VM of the *Drosophila* embryo is autoregulatory, and that it depends on the interplay of a variety of factors as diagrammed in Fig. 8. While *Ubx* controls the expression of *dpp* in ps7, an additional factor (X), whose spatial boundaries are independently controlled, is a necessary cofactor. The expression of *Ubx* in ps7 requires *dpp*, but does not absolutely require *Ubx* itself, invoking yet another regulatory factor (Y). Furthermore, *dpp*'s autoregulation both in ps4 and in ps7 indicate that *dpp*'s ability to stimulate its own transcription acts through both a *Ubx*-independent (ps4) and a *Ubx*-dependent (ps7) pathway. This may invoke yet another factor resident in ps4 (Z). Experiments with *Ubx*-heat shock constructs (Reuter et al., 1990), and our own experiments using *Ubx*-reporter gene constructs indicate that each gene has the capability to induce transcription of the other, and opens the question as to which gene is the primary activator in vivo. We conclude that there is no simple linear gene network regulating midgut morphogenesis, but a complex feedback mechanism maintaining gene expression in this tissue.

We would like to thank Thom Kaufman, Jim Mahaffey, Bob Holmgren, and Danny Brower for providing probes and antibodies. We are grateful to Phil Beachy for providing the *Ubx* reporter gene, and for drawing our attention to its anterior midgut staining. We thank Jim Masucci and Mike Hoffmann for the $Ubx^{6.28}$ stock and for communicating results prior to publication. We are grateful to Mark Mortin for assistance with antibody experiments, Lorraine and Diane Lukas for technical assistance, and Dawn Rowe for secretarial assistance. We thank Mark Mortin and Michele Sanicola for critical comments on the manuscript. Discussions with Phil Beachy and Welcome Bender were invaluable during the course of this study. This work was supported by Public Health Service grants to W. M. G. During the course of this work, D. A. H. was a National Institutes of Health Postdoctoral Fellow, and R. W. P. a Fellow of the Charles A. King Trust.

REFERENCES

Akam, M. E. and Martinez-Arias, A. (1985). The distribution of *Ultrabithorax* transcripts in *Drosophila* embryos. *EMBOJ.* **4**, 1689-1700.

Barad, M., Jack, T., Chadwick, R. and McGinnis, W. (1988). A novel, tissue-specific, *Drosophila* homeobox gene. *EMBO J.* **7**, 2151-2161.

Beachy, P. A. (1990). A molecular view of the *Ultrabithorax* homeotic gene of *Drosophila*. *Trends Genet.* **6**, 46-51.

Beachy, P. A., Krasnow, M. A., Gavis, E. R. and Hogness, D. S. (1988). An *Ultrabithorax* protein binds sequences near its own and the *Antennapedia* P1 promoters. *Cell* **55**, 1069-1081.

Bergson, C. and McGinnis, W. (1990). An autoregulatory enhancer element of the *Drosophila* homeotic gene *Deformed*. *EMBO J.* **9**, 4287-4297.

Bienz, M., Saari, G., Tremml, G., Müller, J., Züst, B. and Lawrence, P. A. (1988). Differential regulation of *Ultrabithorax* in two germ layers of Drosophila. *Cell* **53**, 567-576.

Bienz, M. and Tremml, G. (1988). Domain of *Ultrabithorax* expression in *Drosophila* visceral mesoderm from autoregulation and exclusion. *Nature* **333**, 576-578.

Blackman, R., Sanicola, M., Raftery, L. A., Gillevet, T. and Gelbart, W. M. (1991). An extensive 3′ cis-regulatory region directs the imaginal disk expression of decaptaplegic, a member of the TGF-β family in *Drosophila. Development* **111**, 657-665.

Bodmer, R., Jan, L. Y. and Jan, Y. N. (1990). A new homeobox-containing gene, *msh-2*, is transiently expressed early during mesoderm formation of *Drosophila*. *Development* **110**, 661-669.

Campos-Ortega, J. A., and Hartenstein, V. (1985). *The embryonic development of* Drosophila melanogaster. Berlin: Springer-Verlag.

Ekker, S. C., Young, K. E., von Kessler, D. P. and Beachy, P. A. (1991). Optimal DNA sequence recognition by the *Ultrabithorax* homeodomain of *Drosophila*. *EMBOJ.* **10**, 1179-1186.

Ghosh, S., Gifford, A. M., Riviere, L. R., Tempst, P., Nolan, G. P. and Baltimore, D. (1990). Cloning of the p50 DNA binding subunit of NF-κB: Homology to *rel* and *dorsal*. *Cell* **62**, 1019-1029.

Goto, T., MacDonald, P. and Maniatis, T. (1989). Early and late periodic patterns of *even skipped* expression are controlled by distinct regulatory elements that respond to different spatial cues. *Cell* **57**, 413-422.

Harding, K., Hoey, T., Warrior, R. and Levine, M. (1989). Autoregulatory and gap gene response elements of the *even-skipped* promoter of *Drosophila*. *EMBO J.* **8**, 1205-1212.

Hiromi, Y. and Gehring, W. J. (1987). Regulation and function of the *Drosophila* segmentation gene *fushi tarazu*. *Cell* **50**, 963-974.

Immerglück, K., Lawrence, P. A. and Bienz, M. (1990). Induction across germ layers in *Drosophila* mediated by a genetic cascade. *Cell* **62**, 261-268.

Irish, V. F. and Gelbart, W. M. (1987). The *decapentaplegic* gene is required for dorsal-ventral patterning of the *Drosophila* embryo. *Genes Dev.* **1**, 868-879.

Irvine, K. D., Helfand, S. L. and Hogness, D. S. (1991). The large upstream control region of the *Drosophila* homeotic gene *Ultrabithorax*. *Development* **111**, 407-424.

Jiang, J., Hoey, T. and Levine, M. (1991). Autoregulation of a segmentation gene in *Drosophila*: combinatorial interaction of the *even-skipped* homeo box protein with a distal enhancer element. *Genes Dev.* **5**, 265-277.

Karch, F., Weiffenbach, B., Peifer, M., Bender, W., Duncan, I., Celniker, S., Crosby, M. and Lewis, E. B. (1985). The abdominal region of the bithorax complex. *Cell* **43**, 81-96.

Karess, R. E. and Rubin, G. M. (1984). Analysis of P transposable element functions in *Drosophila*. *Cell* **38**, 135-146.

Kerridge, S. and Morata, G. (1982). Developmental effects of some newly induced *Ultrabithorax* alleles of *Drosophila*. *J. Embryol. exp. Morph.* **68,** 211-234.

Kieran, M., Blank, V., Logeat, F., Vandekerckhove, J., Lottspeich, F., Le Bail, O., Urban, M. B., Kourilsky, P., Baeuerle, P. A. and Israël, A. (1990). The DNA binding subunit of NF-κB is identical to Factor KBF1 and homologous to the *rel* oncogene product. *Cell* **62**, 1007-1018.

Kuziora, M. A. and McGinnis, W. (1988). Autoregulation of a *Drosophila* homeotic selector gene. *Cell* **55**, 477-485.

Lewis, E. B. (1978). A gene complex controlling segmentation in *Drosophila*. *Nature* **276**, 565-570.

Meinhardt, H. (1982). *Models of Biological Pattern Formation.* London: Academic Press.

Müller, J., Thüringer, F., Biggin, M., Züst, B. and Bienz, M. (1989). Coordinate action of a proximal homeoprotein binding site and a distal sequence confers the *Ultrabithorax* expression pattern in the visceral mesoderm. *EMBO J.* **8**, 4143-4151.

Padgett, R. W., St. Johnston, R. D. and Gelbart, W. M. (1987). A transcript from a *Drosophila* pattern gene predicts a protein homologous to the transforming growth factor-β family. *Nature* **325**, 81-84.

Panganiban, G. E. F., Reuter, R., Scott, M. P. and Hoffmann, F. M. (1990). A *Drosophila* growth factor homolog, *decapentaplegic*, regulates homeotic gene expression within and across germ layers during midgut morphogenesis. *Development* **110**, 1041-1050.

Pick, L., Schier, A., Affolter, M., Schmidt-Glenewinkel, T. and Gehring, W. J. (1990). Analysis of the *ftz* upstream element: germ layer-specific enhancers are independently autoregulated. *Genes Dev.* **4**, 1224-1239.

Qian, S., Capovilla, M. and Pirrotta, V. (1991). The *bx* region enhancer, a distant *cis*-control element of the *Drosophila Ubx* gene and its regulation by *hunchback* and other segmentation genes. *EMBO J.* **10**, 1415-1425.

Raftery, L. A., Sanicola, M., Blackman, R. K. and Gelbart, W. M. (1991). The relationship of *decapentaplegic* and *engrailed* expression in *Drosophila* imaginal disks: do these genes mark the anterior-posterior compartment boundary? *Development* **113**, 27-33.

Ray, R. P, Arora, K., Nüsslein-Volhard, C. and Gelbart, W. M. (1991). The control of cell fate along the dorsal-ventral axis of the *Drosophila* embryo. *Development* **113**, 35-54.

Regulski, M., Dessain, S., McGinnis, N. and McGinnis, W. (1991). High-affinity binding sites for the *Deformed* protein are required for the function of an autoregulatory enhancer of the *Deformed* gene. *Genes Dev.* **5**, 278-286.

Reuter, R., Panganiban, G. E. F., Hoffmann, F. M. and Scott, M. P. (1990). Homeotic genes regulate the spatial expression of putative growth factors in the visceral mesoderm of *Drosophila* embryos. *Development* **110**, 1031-1040.

Reuter, R. and Scott, M. P. (1990). Expression and function of the homeotic genes *Antennapedia* and *Sex combs reduced* in the embryonic midgut of *Drosophila. Development* **109**, 289-303.

Robertson, H. M., Preston, C. R., Phillis, R. W., Johnson-Schlitz, D. M., Benz, W. K. and Engels, W. R. (1988). A stable genomic source of *P* element transposase in *Drosophila melanogaster. Genetics* **118**, 461-470.

Rubin, G. M. and Spradling, A. C. (1983). Vectors for P element-mediated gene transfer in *Drosophila*. *Nucl. Acids Res.* **11**, 6341-6351.

Sánchez-Herrero, E., Vernós, I., Marco, R. and Morata, G. (1985). Genetic organization of *Drosophila* bithorax complex. *Nature* **313**, 108-113.

Schier, A. F. and Gehring, W. J. (1992). Direct homeodomain-DNA interaction in the autoregulation of the *fushi tarazu* gene. *Nature* **356**, 804-807.

Segal, D. and Gelbart, W. M. (1985). Shortvein, a new component of the decapentaplegic gene complex in *Drosophila melanogaster*. *Genetics* **109**, 119-143.

Simon, J., Peifer, M., Bender, W. and O'Connor, M. (1990). Regulatory elements of the bithorax complex that control expression along the anterior-posterior axis. *EMBO J.* **9**, 3945-3956.

Spencer, F. A., Hoffmann, F. M. and Gelbart, W. M. (1982). *Decapentaplegic*: A gene complex affecting morphogenesis in *Drosophila melanogaster. Cell* **28**, 451-461.

Spradling, A. C. (1986). P element-mediated transformation. In *Drosophila, a Practical Approach* (ed. D. B. Roberts), pp. 175-197. Oxford: IRL Press.

Steward, R. (1987). *Dorsal*, an embryonic polarity gene in *Drosophila*, is homologous to the vertebrate proto-oncogene, c-*rel*. *Science* **238**, 692-694.

St. Johnston, R. D. and Gelbart, W. M. (1987). *Decapentaplegic* transcripts are localized along the dorsal-ventral axis of the *Drosophila* embryo. *EMBO J.* **6**, 2785-2791.

St. Johnston, R. D., Hoffmann, F. M., Blackman, R. K., Segal, D., Grimaila, R., Padgett, R. W., Irick, H. A. and Gelbart, W. M. (1990). Molecular organization of the *decapentaplegic* gene in *Drosophila melanogaster*. *Genes Dev.* **4**, 1114-1127.

St. Johnston, R. D. (1988). The structure and expression of the *decapentaplegic* gene of *Drosophila*. Ph. D Dissertation, Harvard University, Cambridge, MA., USA.

Tautz, D. and Pfeifle, C. (1989). A non-radioactive in situ hybridization method for the localization of specific RNAs in *Drosophila* embryos reveals translational control of the segmentation gene *hunchback*. *Chromosoma* **98**, 81-85.

Thummel, C. S., Boulet, A. M. and Lipshitz, H. D. (1988). Vectors for *Drosophila* P-element-mediated transformation and tissue culture transfection. *Gene* **74**, 445-456.

Tremml, G. and Bienz, M. (1989). Homeotic gene expression in the visceral mesoderm of *Drosophila* embryos. *EMBO J.* **8**, 2677-2685.

Van Obberghen-Schilling, E. V., Roche, N. S., Flanders, K. C., Sporn, M. B. and Roberts, A. B. (1988). Transforming growth factor β1 positively regulates its own expression in normal and transformed cells. *J. Biol. Chem.* **263**, 7741-7746.

Weinzierl, R., Axton, J. M., Ghysen, A. and Akam, M. (1987). *Ultrabithorax* mutations in constant and variable regions of the protein coding sequence. *Genes Dev.* **1**, 386-397.

White, R. A. H. and Wilcox, M. (1984). Protein products of the bithorax complex in Drosophila. *Cell* **39**, 163-171.

White, R. A. H. and Wilcox, M. (1985). Distribution of *Ultrabithorax* proteins in *Drosophila*. *EMBO J.* **4**, 2035-2043.

(Accepted 6 January 1993)