Development 117, 807-822 (1993)
Printed in Great Britain © The Company of Biologists Limited 1993

An activity gradient of *decapentaplegic* is necessary for the specification of dorsal pattern elements in the *Drosophila* embryo

Kristi A. Wharton*, Robert P. Ray† and William M. Gelbart

Department of Cellular and Developmental Biology, Harvard University, 16 Divinity Avenue, Cambridge, Massachusetts 02138, USA

*Present address: Brown University, Division of Biology and Medicine, Providence, Rhode Island 02912, USA
†Present address:Princeton University, Department of Molecular Biology, Princeton, New Jersey 08544, USA

SUMMARY

***decapentaplegic* (*dpp*) is a zygotically expressed gene encoding a TGF-β-related ligand that is necessary for dorsal-ventral patterning in the *Drosophila* embryo. We show here that *dpp* is an integral part of a gradient that specifies many different cell fates via intercellular signalling. There is a graded requirement for *dpp* activity in the early embryo: high levels of *dpp* activity specify the amnioserosa, while progressively lower levels specify dorsal and lateral ectoderm. This potential for *dpp* to specify cell fate is highly dosage sensitive. In the wild-type embryo, increasing the gene dosage of *dpp* can shift cell fates along the dorsal-ventral axis. Furthermore, in mutant embryos, in which only a subset of the dorsal-ventral pattern elements are represented, increasing the gene dosage of *dpp* can specifically transform those pattern elements into more dorsal ones. We present evidence that the zygotic *dpp* gradient and the maternal *dorsal* gradient specify distinct, non-overlapping domains of the dorsal-ventral pattern.**

Key words: *Drosophila*, dorsal-ventral patterning, TGF- -related protein, *decapentaplegic*

INTRODUCTION

Within the past two centuries, two related models have been proposed as mechanistic solutions to the problem of organization and specification of cells in embryonic fields (Slack, 1991). According to the *localized determinant* model, embryonic pattern is established through the action of discrete morphogenetic substances that are localized to different regions of the egg cortex. During cleavage, these determinants are partitioned into different blastomeres, which consequently assume different states of differentiation. According to the *gradient* model, pattern is established by one or a few multifunctional regulatory substances, called morphogens, that are distributed in monotonic gradients. These morphogens specify cell fate in a concentration-dependent fashion such that cells acquire different fates based on the concentration of morphogen that they contain (Crick, 1970).

Embryological, genetic and molecular studies on *Drosophila* have provided the first concrete evidence for the gradient model. Two maternal morphogen gradients have been identified that establish aspects of the anterior-posterior and dorsal-ventral axes (reviewed by Nüsslein-Volhard, 1991). Specifically, along the anterior-posterior axis, the *bicoid* (*bcd*) gene product is distributed in a monotonic gradient with a high point at the anterior end of the embryo (Driever and Nüsslein-Volhard, 1988a,b). Along the dorsal-ventral axis, the *dorsal* (*dl*) gene product is differentially compartmentalized into a steep gradient with a high point ventrally (Steward et al., 1988; Roth et al., 1989; Rushlow et al., 1989; Steward, 1989).

The *dl* gradient specifies positional information along the dorsal-ventral axis by the differential activation and repression of downstream zygotic genes (Roth et al., 1989; Ray et al., 1991). *dl* directs the differentiation of ventral tissues by the activation of the zygotic genes *twist* (*twi*) and *snail* (*sna*), which are required for specification of the ventrally derived mesoderm (Simpson, 1983; Thisse et al., 1987). Conversely, *dl* directs the differentiation of dorsal tissues by repressing the expression of *dpp* and *zerknüllt* (*zen*) ventrally, thereby restricting the activities of these genes to the dorsal regions of the embryo where they are required for the specification of the dorsal ectoderm and amnioserosa (Irish and Gelbart, 1987; Wakimoto et al., 1984).

Significantly, the *dl*-directed activation and repression of these zygotic genes is restricted to the ventral regions of the embryo. Thus, while the *dl* morphogen may directly specify a number of different cell states in the ventral part of the embryo, it is apparently not involved in the specification of cell states within the dorsal part of the embryo. This is plainly evidenced by the fact that dorsal pattern elements, specifically dorsal epidermis and amnioserosa, are observed in embryos that completely lack *dl* function (Ray et al., 1991). Therefore, since the *dl* gradient appears to be the only maternal system defining dorsal-ventral polarity (Nüsslein-Volhard, 1991), it follows that the specification

of these dorsal pattern elements must be carried out by downstream zygotic genes.

Based on mutant phenotype, seven zygotic loci have been identified that are likely to be involved in the specification of dorsal pattern elements: *dpp*, *screw* (*scw*), *short gastrulation* (*sog*), *shrew* (*srw*), *tolloid* (*tld*), *twisted gastrulation* (*tsg*), and *zen* (reviewed by Rushlow and Arora, 1990). Loss-of-function alleles of these genes produce a variety of related mutant phenotypes, consisting, to a greater or lesser degree, of an expansion of lateral or ventral structures at the expense of more dorsal ones. For example, embryos bearing loss-of-function alleles of *sog*, *srw* and *tsg* show an expansion of the dorsal-most part of the dorsal epidermis at the expense of the amnioserosa. Similarly, embryos bearing loss-of-function alleles of *scw* and *tld* show an expansion of lateral aspects of the dorsal epidermis at the expense of the rest of the dorsal epidermis and amnioserosa. Null alleles of *dpp* produce the most severe mutant phenotype, in which ventral pattern elements are expanded at the expense of all dorsal pattern elements (Irish and Gelbart, 1987; Ray et al., 1991; Ferguson and Anderson, 1992; Arora and Nüsslein-Volhard, 1992).

Of these seven zygotic genes, *dpp* is unique, not only because of the severity of its mutant phenotype, which is reminiscent of the ventralized phenotypes produced by the maternal genes, but also because of the nature of its interactions with the other zygotic genes. For instance, the initial expression pattern of *dpp* in the dorsal-most 40% of the blastoderm embryo is autonomous with respect to the other genes. That is, *scw*, *srw*, *sog*, *tld*, *tsg*, and *zen* mutations do not affect the initiation or maintenance of *dpp* gene expression. By contrast, the expression of at least two of these genes, *zen* and *tld*, depends on *dpp* activity (Ray et al., 1991; R. Ray, unpublished data). These findings suggest that the genes of this group participate in a single pattern forming system, in which *dpp* is a central element.

In this report, we provide support for this proposal through a genetic and molecular analysis of *dpp* function in early embryonic development. Our analysis indicates that *dpp* is an integral part of a zygotic activity gradient that differentially specifies cell fate within the dorsal ectoderm. We show that the dorsal-ventral requirement for *dpp* is highly dosage sensitive, and that there is a graded requirement for the *dpp* product in the early embryo. Cells fated to the amnioserosa are most sensitive to reduction in *dpp* activity; cells fated to the dorsal epidermis are less sensitive; and cells fated to the ventral epidermis are the least sensitive. Furthermore, we show that the fate map of the embryo along the dorsal-ventral axis responds to decreases and increases in the level of *dpp* activity. Finally, we present evidence that the *dl* and *dpp* gradients control different aspects of the dorsal-ventral pattern, and that they elaborate dorsal and ventral pattern elements from a common, central ground state.

MATERIALS AND METHODS

Drosophila strains

Drosophila were cultured on standard cornmeal yeast extract dextrose medium at 25°C. In all cases, the strain referred to in the text as 'wild type' is Oregon-R. In the experiments reported here, ten ethyl methyl sulfonate (EMS) induced, and two -ray induced *dpp* alleles were used. The ten EMS induced alleles are: dpp^{hr4}, and dpp^{hr27}, which were generated in an F_2 lethal screen based on failure to complement the *dpp* disk alleles dpp^{d2} and dpp^{d14}, respectively (Spencer et al., 1982); dpp^{e87}, which was isolated in an F_2 lethal screen based on failure to complement the embryonic lethal *dpp* allele dpp^{hr4} (Irish and Gelbart, 1987); dpp^{e90}, formerly dpp^{IC}, which was isolated in an F_2 lethal screen as a mutation that affected the pattern of the larval cuticle (Nüsslein-Volhard et al., 1984); and dpp^{hr92}, dpp^{hr93}, dpp^{H91}, dpp^{H94}, dpp^{H95}, and dpp^{H96}, which were isolated as *dpp* alleles that failed to complement a transvection-sensitive chromosomal rearrangement of the disk mutation $dpp^{d\text{-}ho}$ (R. Blackman and W. Gelbart, unpublished data). The two -ray induced alleles, dpp^{hr56} and dpp^{H46}, were isolated in an F_1 screen based on failure to complement a rearrangement of $dpp^{d\text{-}ho}$ (Irish and Gelbart, 1987). A synthetic duplication of *dpp*, *CyO23*, was constructed by jumping a P-element rescue construct, P20 (R. Padgett, unpublished data), onto the balancer chromosome *CyO*. This new balancer chromosome completely rescues the haplolethality associated with null alleles of the *dpp* locus. The *dpp* stocks used in the experiments described in this report are as follows: dpp^{e87} (V. Twombly); *S* dpp^{e90} *cn/CyO* (C. Nüsslein-Volhard), *net* dpp^{hr4}*/CyO*, dpp^{hr4} *cn bw/CyO*; *net* dpp^{hr27}*/CyO*; dpp^{hr27} *cn bw/CyO*; dpp^{hr56} *Sp cn bw/CyO*; dpp^{hr56} *cn bw/CyO*; dpp^{hr92} *L/CyO*; dpp^{hr92} *cn bw/CyO*; dpp^{hr93} *L/CyO*; dpp^{hr93} *cn bw/CyO*; dpp^{H94} *L/CyO23*; dpp^{H94} *cn bw/CyO23*; dpp^{H95} *L/CyO23*; dpp^{H95} *cn bw/CyO23*; dpp^{H96} *L/CyO23*; dpp^{H96} *cn bw/CyO23*; dpp^{H91} *L/CyO23*; dpp^{H91} *cn bw/CyO23*; dpp^{H37} *cn bw/CyO23*; dpp^{H46} *Sp cn bw/CyO23*; *Dp(2;2)DTD48* (4 copies of *dpp*), *Dp(2;2)B16 dp cl cn bw* (4 copies of *dpp*, V. Twombly); *Dp(2;2)B16 dp cl* dl^{1} *cn sca/CyO*; dpp^{H46} *Sp* dl^{1} *cn sca/CyO23*.

Viability studies

To test for the dominant lethality of *dpp* mutations, males bearing the mutant alleles were crossed to virgin Oregon-R females. The genotype of the males was either *Dp(2;1)G146*, dpp^{+}*/Y; dpp*/CyO*, or *dpp*/CyO*, or *CyO23*. Flies were reared in small cages constructed by inverting a 150 ml tripour beaker (VWR) onto a 60 mm Petri dish (Falcon) filled with grape juice agar. Embryos were collected every 12 hours and individually laid in a grid pattern on grape juice plates. The grids were scored after 24 hours and the number of hatched and phenotypically mutant embryos were recorded. More than 300 embryos (n) were scored for every genotype in multiple, independent experiments. The numbers from all experiments were pooled, and the percentage lethality was determined. In order to make the embryonic data comparable with the adult data (described below) we devised the statistic 'percentage lethality of expected', that was calculated by dividing the number of phenotypically mutant embryos by half of the total number of embryos ($n/2$).

To test for the dominant lethality of the alleles as determined by adult survivorship, crosses identical to those performed for the embryonic viability studies were reared in bottles. Following eclosion, the adults were scored and the number of *Cy* and Cy^{+} flies were recorded. To determine the statistic 'percentage lethality', the number of Cy^{+} flies was subtracted from the number of *Cy* flies, and this difference, representing the predicted number of individuals that did not survive to adulthood, was divided by the number of *Cy* progeny. This approach necessarily introduces a bias if the number of survivors is very small, thus as the severity of the genotype approaches that of $dpp^{null}/+$ (haploinsufficient), the statistic often reaches or exceeds the theoretical maximum of 100%. In such cases, the percentage survivorship is indicated in the text to illustrate this fact.

Cuticle preparations

Cuticle preparations were done as follows: mutant embryos were picked from a grape plate after having aged for 36 hours at 25°C. They were dechorionated in 50% Chlorox (sodium hypochlorite) for 3 minutes in wire mesh baskets and then transferred to eppendorf tubes containing 1 ml of 0.1% Triton X-100. Dechorionated embryos were washed twice with Triton X-100 and then twice with 1:9, methanol: 50 mM EGTA (ME). After ME washes, 1 ml of fresh ME was added, followed by 0.5 ml n-heptane. The tubes were inverted until the devitellinized embryos sank to the bottom, after which the methanol and heptane were removed and replaced with 200 μl 1:4, glycerol: acetic acid. The tubes were then incubated at 60°C for 1 hour. The cuticles were mounted under a coverslip in a small drop of Hoyer's medium (Wieschaus and Nüsslein-Volhard, 1986). The slides were returned to 60°C for 24 hours, and a 100 g weight was placed on the coverslip. Cuticles were analyzed and photographed under phase contrast optics using an Olympus stereomicroscope and Kodak Tech Pan Film.

Antibody labelling

Embryos were removed from the grape juice agar plates, and then dechorionated in 50% Chlorox in wire mesh baskets and washed extensively with water. The dechorionated embryos were transferred to a glass scintillation vial containing 2 ml of 3.7% formaldehyde (Mallinckrodt) and 2 ml of n-heptane, and fixed for 15 minutes with constant agitation. After fixation, the lower phase (formaldehyde) was removed with a pasteur pipette and the embryos were washed twice with fresh heptane. Then, 3 ml of ME (see above) were added, and the vials were shaken until the devitellinized embryos sank to the bottom. The embryos were collected and washed in ME, 1:1 ME: 1× PBS (130 mM NaCl, 7 mM Na_2HPO_4 $7H_2O$, 3 mM NaH_2PO_4 H_2O), and 1× PBS for 10 minutes each. The tissue was blocked for 1 hour in PBT (1× PBS; 1% bovine serum albumin fraction V, Boehringer Mannheim; 0.1% Triton X-100, Boehringer Mannheim), and exposed to the primary antibody (anti-Kr, kindly provided by C. Rushlow) for 1 hour at room temperature or overnight at 4°C. The embryos were washed in PBT for 1.5 hours with four changes of buffer, and then exposed to the secondary antibody (peroxidase-conjugated goat anti-rabbit, Vector) using the same conditions as described above. After the secondary labelling, the embryos were washed for 1.5 hours with four changes of buffer. DAB staining solution (500 μg/ml DAB in PBT, 0.2% H_2O_2) was added, and the reaction was allowed to proceed for 10 minutes. Embryos were washed 3 times with PBT to stop the reaction, and then dehydrated with ethanol. They were mounted in methyl salicylate and photographed using a Olympus stereomicroscope and Kodak Tech Pan film.

RESULTS

Embryonic lethal *dpp* alleles fall into a simple allelic series

The dorsal-ventral patterning defect observed in *dpp* mutant embryos represents the first requirement for this gene during the development of the fly (St. Johnston et al., 1990). All embryos homozygous for a null *dpp* allele are 100% lethal, while embryos heterozygous for this same allele are approx. 95% lethal. This haploinsufficiency, or inability to survive as a heterozygote, demonstrates the sensitivity of the animal to the level of the *dpp* product. More than 30 embryonic lethal *dpp* mutations have been isolated that affect the dorsal-ventral pattern of the embryo (Spencer et al., 1982; Irish and Gelbart, 1987). The majority of these alleles behave as nulls, as they show a high percentage of dominant lethality when heterozygous (*dpp**/+). However, some of these alleles display a lower percentage of dominant lethality and represent a class of hypomorphic, or partial loss-of-function, alleles (Irish and Gelbart, 1987). We have used these hypomorphic alleles to determine the nature of the requirement for *dpp* in the early embryo.

To determine the relative severity of these hypomorphic *dpp* alleles, we have assayed for the level of dominant lethality specific to each allele and compared this to the level characteristic of null *dpp* alleles. In these experiments, the viability of each allele as a heterozygote in *trans* to wild type (*dpp**/+) was determined both in terms of the number of progeny that died as embryos, and the number of progeny that survived to adulthood (Fig. 1A,B). We have used the dpp^{H46} allele as a standard null, since it is known to be deficient for a significant portion of the *dpp* coding region (St. Johnston et al., 1990; Table 1). As a heterozygote, dpp^{H46} shows 98% dominant lethality in embryos, and 99.8% lethality in adults, consistent with the level expected for a null allele. Two of the other alleles we have tested, dpp^{H91} and dpp^{H96}, exhibit similar levels of dominant lethality in both embryos and adults, and thus are not distinguishable from nulls. The weakest allele we have identified is dpp^{e87}, which exhibits no dominant lethality (Fig. 1A,B) and is, in fact, 60% viable as a homozygote (Fig. 1C, Table 1). Two other alleles, dpp^{e90} and dpp^{hr56}, while 100% lethal as homozygotes, are nevertheless about 30% viable in *trans* to dpp^{e87} (Fig. 1C), and thus are also relatively weak alleles. Each of the remaining alleles tested exhibit a distinct level of dominant lethality which lies in between the level observed for dpp^{e87} and that observed for dpp^{H46}. Thus, these *dpp* alleles describe a simple allelic series. Based on these data, the order we have observed for this allelic series from weakest to most severe is: $dpp^{e87} < dpp^{e90} < dpp^{hr56} < dpp^{hr4} < dpp^{hr92} < dpp^{hr27} < dpp^{hr93} < dpp^{H94} < dpp^{H95} < [dpp^{H96} = dpp^{H91} = dpp^{H46}]$.

Based on these viability studies, we have arbitrarily designated those alleles that exhibit >50% dominant lethality dpp^{H}, and those that exhibit <50% lethality as dpp^{hr} alle-

Table 1. *dpp* alleles that affect dorsal-ventral patterning

Allele	Mutagen	Lesion	Reference
dpp^{H}			
dpp^{H46}	-ray	deletion*	Irish and Gelbart, 1987
dpp^{H91}	EMS	point	R. Blackman, unpublished data
dpp^{H94}	EMS	–	R. Blackman, unpublished data
dpp^{H95}	EMS	–	R. Blackman, unpublished data
dpp^{H96}	EMS	–	R. Blackman, unpublished data
dpp^{hr}			
dpp^{hr4}	-ray	point	Spencer, et al., 1982
dpp^{hr27}	-ray	point	Spencer, et al., 1982
dpp^{hr56}	-ray	deletion	Irish and Gelbart, 1987
dpp^{hr92}	EMS	point	R. Blackman, unpublished data
dpp^{hr93}	EMS	–	R. Blackman, unpublished data
dpp^{e}			
dpp^{e87}	EMS	deletion	Irish and Gelbart, 1987
dpp^{e90}	EMS	point	Nüsslein-Volhard, et al., 1984†

*The molecular analysis of these alleles is presented elsewhere: Wharton, et al., unpublished data.

†dpp^{hr90} was formerly named dpp^{IC} by Nüsslein-Volhard, et al.

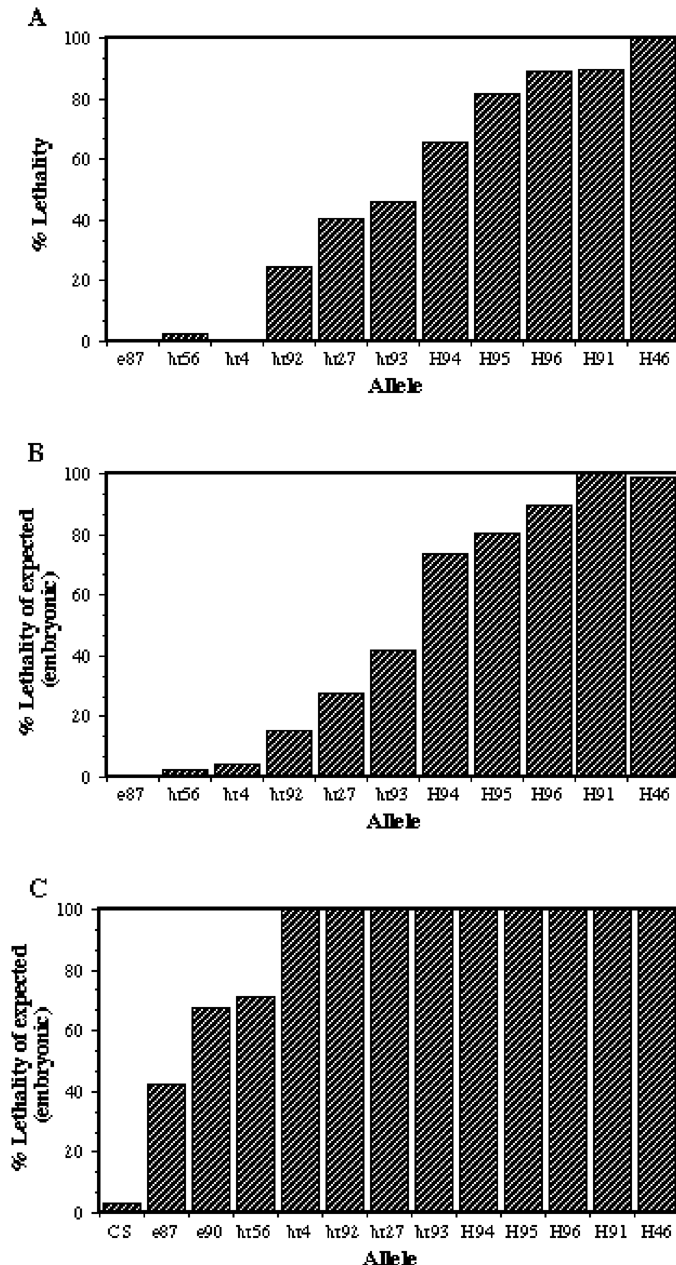

Fig. 1. Hypomorphic and amorphic *dpp* alleles exhibit different levels of dominant lethality.The percentage lethality of individuals heterozygous for various *dpp* alleles is shown, as measured in both adult (A) and embryonic (B) stages (see Materials and methods). In addition, the viability of embryos bearing each of the different *dpp* alleles in *trans* to dpp^{e87} was determined (C) to elucidate the relative severity of the alleles that showed no dominant lethality in A and B. As can be seen in A and B, individuals heterozygous for the weak allele dpp^{e87} are essentially 100% viable as compared with wild-type controls, while those heterozygous for the amorphic allele dpp^{H46} are nearly 100% lethal. The remaining hypomorphic alleles display different degrees of haplolethality which fall between these two extremes. As illustrated in C, dpp^{e87}, while viable in stock as a homozygote (see Materials and methods), is nevertheless about 40% lethal. The other weak alleles, i.e. dpp^{e90}, and dpp^{hr56}, also show a significant percentage of survivors in *trans* to dpp^{e87}. All of the remaining alleles were 100% lethal in *trans* to dpp^{e87}. While the percentage lethality as shown in the graphs varied from trial to trial of a particular cross, the degree of haplolethality relative to the other alleles was always the same. Thus, while the exact percentage lethality differed from experiment to experiment, the order of the allelic series did not change. The data plotted here is based on the total number of individuals (*n*) counted in multiple trials of a particular cross. The total progeny scored are as follows: (A) *n*=235 (dpp^{hr56}), 233 (dpp^{hr4}), 805 (dpp^{hr92}), 91 (dpp^{hr27}), 597 (dpp^{hr93}), 224 (dpp^{H94}), 464 (dpp^{H95}), 360 (dpp^{H96}), 569 (dpp^{H91}), and 574 (dpp^{H46}). (B) *n*=780 (dpp^{hr56}), 911 (dpp^{hr4}), 1251 (dpp^{hr92}), 399 (dpp^{hr27}), 489 (dpp^{hr93}), 674 (dpp^{H94}), 448 (dpp^{H95}), 331 (dpp^{H96}), 624 (dpp^{H91}), and 472 (dpp^{H46}). (C) *n*=1532 (CS), 574 (dpp^{e87}), 436 (dpp^{e90}), 475 (dpp^{hr56}), 568 (dpp^{hr4}), 410 (dpp^{hr92}), 379 (dpp^{hr27}), 346 (dpp^{hr93}), 501 (dpp^{H94}), 543 (dpp^{H95}), 563 (dpp^{H96}), 471 (dpp^{H91}), and 406 (dpp^{H46}).

les (Table 1). For simplicity, in this report we consider the designation dpp^{H46} to be equivalent to dpp^{null}. The two weak alleles, dpp^{e87} and dpp^{e90}, have been given a special designation based on their complementation behavior with other *dpp* alleles, as described elsewhere (Spencer et al., 1982).

The haploinsufficient nature of the *dpp* locus indicates that in a large percentage of dpp^{H46}/+ embryos, the single remaining copy of the *dpp* gene does not produce enough functional *dpp* product to meet the early dorsal-ventral requirement. Similarly, the lower levels of dominant lethality we observe for the hypomorphic alleles suggest that some of the embryos heterozygous for these alleles do not produce enough functional *dpp* product but that a significant portion of embryos must produce just enough product to survive. However, in the case of the hypomorphic alleles, the loss of *dpp* function does not appear to be due to a difference in the absolute level of protein (as was true for the null) or in the spatial regulation of *dpp* transcription. Our molecular studies indicate that at least six of these hypomorphic alleles result in single amino acid changes in the *dpp* protein sequence (Wharton et al., unpublished data), and thus they are not likely to affect the levels of *dpp* transcription. Furthermore, in whole-mount in situ hybridizations of mutant embryos, we have not observed any changes in the initial expression pattern, even in the null. Therefore, it is very likely that such alleles result in mutant *dpp* proteins of differing activities and not simply in different concentrations of the *dpp* product. Thus, we must conclude that the different levels of dominant lethality reflect different levels of *dpp* protein activity.

Phenotypic effects of hypomorphic *dpp* alleles that affect dorsal-ventral patterning

To address the role of *dpp* in the patterning of the dorsal ectoderm, we have examined the cuticular phenotypes of embryos mutant for the alleles discussed above as homozygotes and as *trans*heterozyogotes with other *dpp* alleles. Cuticle preparations representative of these data are shown in Figs 2, 3 and 4, and an analysis of the data is presented in Table 2.

The weakest *dpp* mutant phenotypes that we observe are produced by embryos bearing homo- and heteroallelic combinations of those alleles that show the lowest percentages of dominant lethality, specifically, dpp^{e87}, dpp^{e90}, and

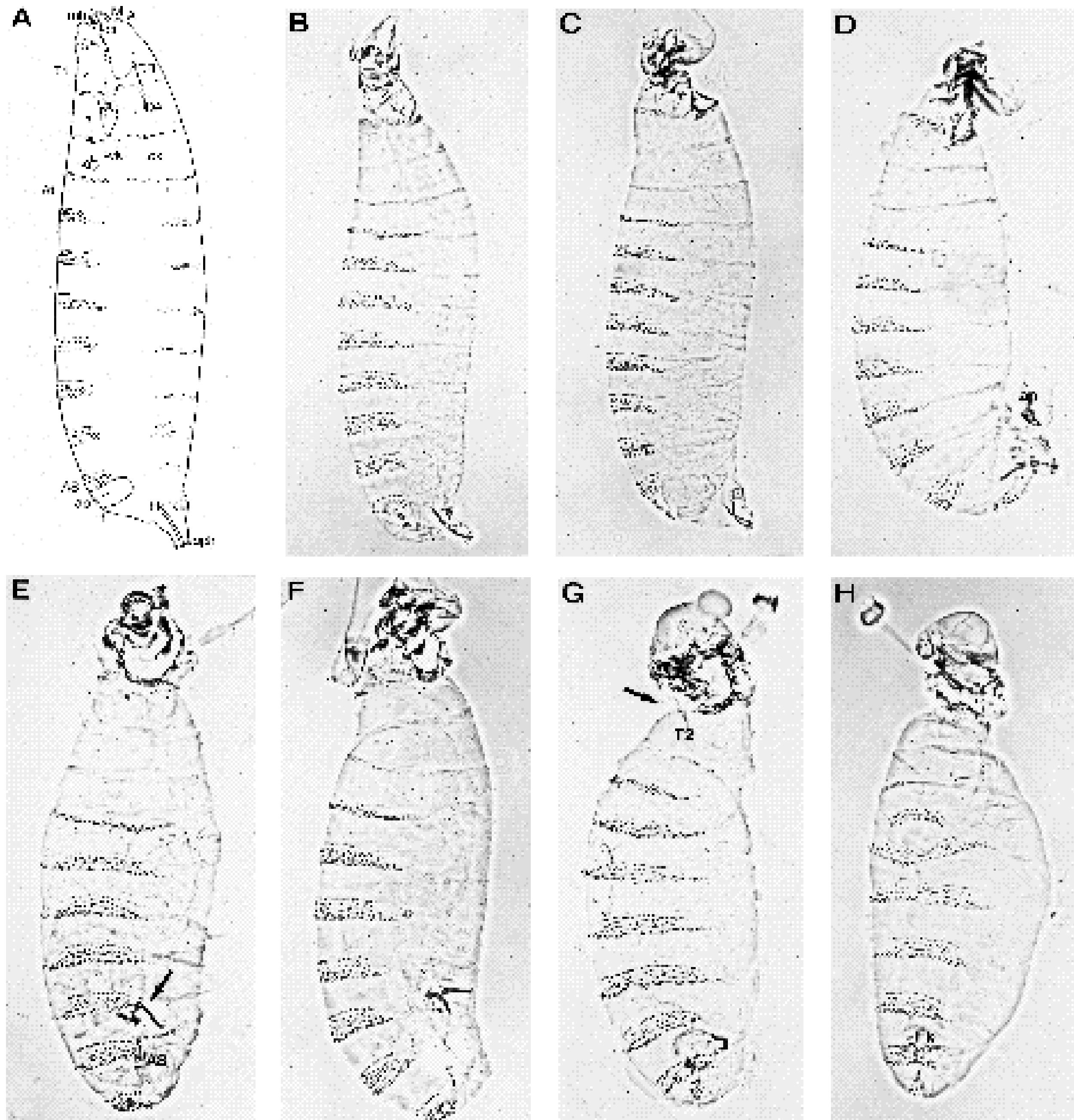

Fig. 2. Cuticle phenotypes of weak hypomorphic *dpp* alleles. (A) Schematic diagram of a wild-type larva (adapted from Lohs-Schardin et al., 1979). Dorsal is to the right and anterior is up. (B) Cuticle of a dpp^{H46}/+ embryo illustrating one of the mildest *dpp* mutant phenotypes consisting of minor deletions of dorsal-anterior structures of the head. (C) Cuticle of a homozygous dpp^{e87} embryo. This panel shows the most severe phenotype observed among the 40% of dpp^{e87} homozygotes that are embryonic lethal (see Fig. 1). The phenotype is similar to that shown in B. (D) Cuticle of a dpp^{e87}/dpp^{e90} embryo. Note the germ band is curled onto the dorsal side of the embryo suggesting a defect in germ band extension/retraction (see Fig. 5). In this preparation, the tuft and anal pads (ap) are displaced to a position above the Filzkörper. (E) Cuticle of a homozygous dpp^{hr56} embryo. The Filzkörper and eighth abdominal segment are internalized (arrow). (F) Cuticle of a dpp^{H46}/+ embryo illustrating the most severe phenotype associated with this genotype (Irish and Gelbart 1987). As in E, the Filzkörper and eighth abdominal segment are internalized. (G) Cuticle of a dpp^{H46}/dpp^{e87} embryo. The loss of ventral arms of the cephalopharyngeal skeleton results in an abrupt change in the morphology of the head, and a deep constriction appears in the first thoracic segment (arrow) that is characteristic of this and all of the more severe phenotypic classes (see text). (H) Cuticle of a homozygous dpp^{hr4} embryo. This phenotype is similar to that shown in G. The Filzkörper (Fk), which are progressively lost in the two succeeding genotypes (see Fig. 4A,B) are present here. Abbreviations are listed in the legend to Fig. 6.

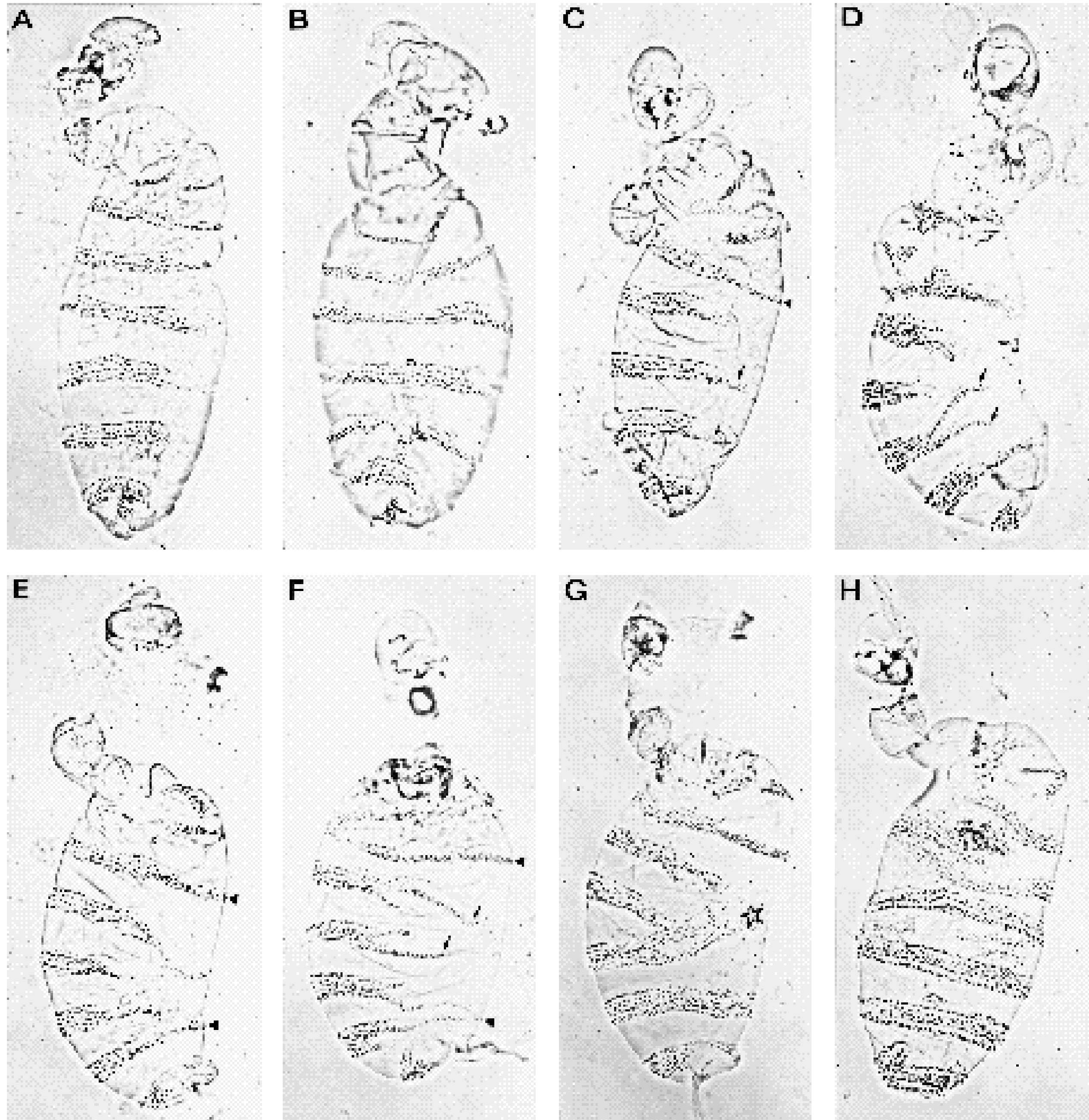

Fig. 3. Cuticle phenotypes associated with the strong hypomorphic and null *dpp* alleles. (A) Cuticle of a homozygous dpp^{hr92} embryo. The majority of embryos of this genotype have reduced or disorganized Filzkörper (Fig. 4H), though more severe examples that completely lack the Filzkörper are observed. This particular embryo is of the latter type: the eighth abdominal segment is internalized, and the Filzkörper are absent. (B) Cuticle of a homozygous dpp^{hr27} embryo. Slight expansion of the denticle belts is seen in this ventral view. (C) Cuticle of a homozygous dpp^{hr93} embryo. The Filzkörper are absent. Expansion of the denticle belts, particularly the tips, is apparent in this cuticle as well as in those shown in D-F. Here, A1 and A2 are disorganized, perhaps due to the constriction in the thoracic segments, and the tips of A3 have met on the dorsal side of the embryo (carat). Small arrows point to similar expansion of the tips of the more posterior denticle belts. (D) Cuticle of a homozygous dpp^{H94} embryo. This cuticle is similar to that shown in C, but greater expansion of the ventral denticle belts, particularly in the mid-abdominal region (small arrows), is observed. (E) Cuticle of a dpp^{hr92}/dpp^{H95} embryo. Significantly, this genotype produces a stronger phenotype than the dpp^{hr92} homozygote (A), thus the effects of different mutations appear to be additive; A1 and A2 are disorganized, and A3 and A7 encircle the entire embryo circumference. (F) Cuticle of a dpp^{H94}/dpp^{H95} embryo. In this cuticle, the characteristic expansion of the tips of the denticle belts is observed. A3, A6, and A7 meet on the dorsal side (carats, notably, A6 meets A7), and extensions of A4 and A5 are also observed (arrows). (G) Cuticle of a dpp^{H95}/dpp^{H91} embryo. Only a small patch of dorsal material remains (star). As in F, the thoracic segments are completely obliterated, though some head strucutres are still evident (see Fig. 4). (H) Cuticle of a homozygous dpp^{H46} embryo, which is null for *dpp*. No dorsal ectodermal structures are present and the ventral denticle belts encircle the entire embryo circumference. Abbreviations are listed in the legend to Fig. 6.

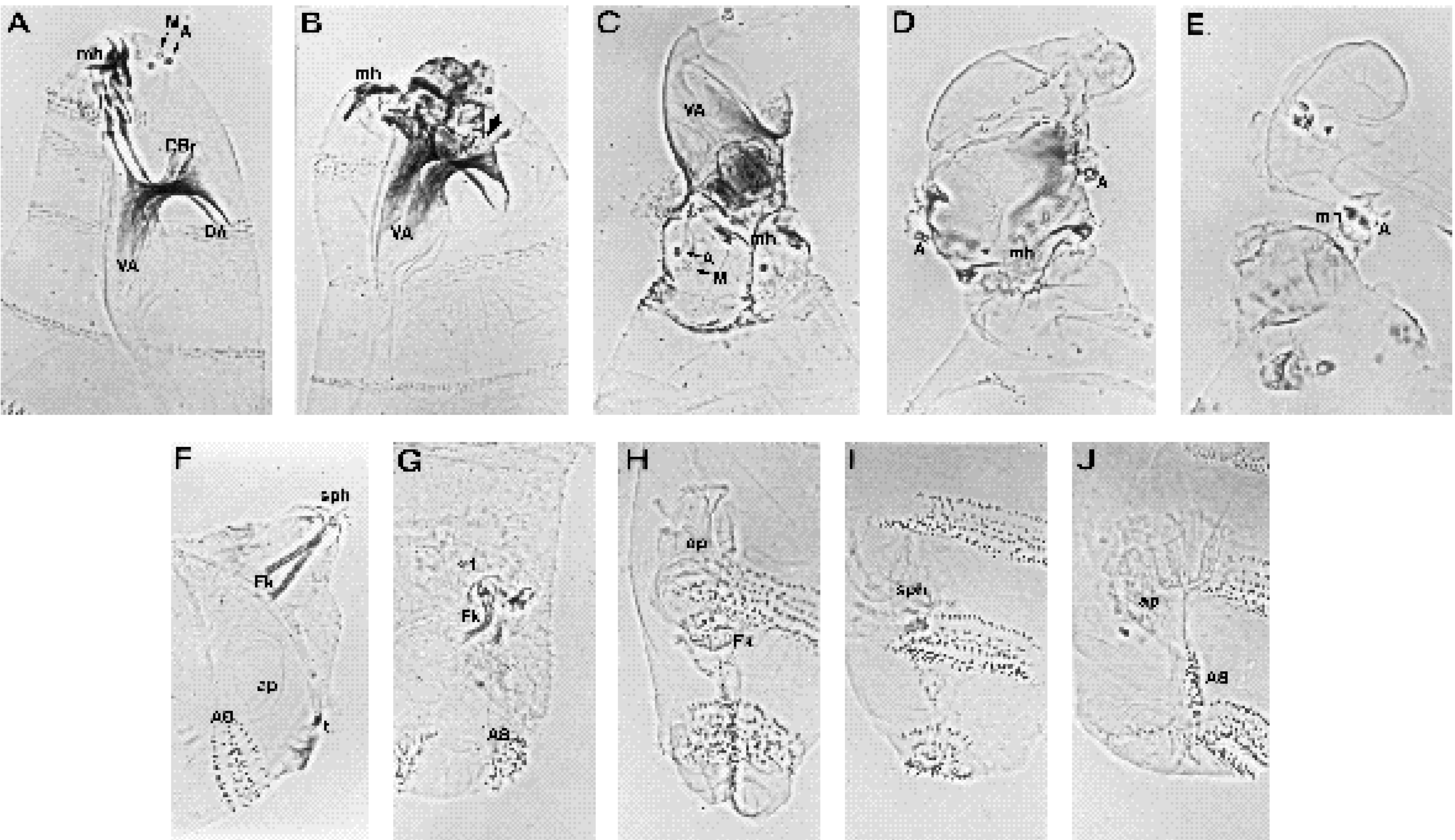

Fig. 4. Head and tail defects associated with hypomorphic *dpp* alleles. (A) Cuticle of a wild-type embryo illustrating the head region. Dorsal is to the right, and anterior is up. (B) Head of a homozygous dpp^{e87} embryo illustrating one of the weakest phenotypes observed for *dpp* mutant embryos. The dorsal bridge is deleted (arrow) and the two halves of the cephalopharyngeal skeleton have collapsed. The maxillary and antennal sense organs are still present, but cannot be clearly distinguished in this illustration (see Fig. 2C for a more severe dpp^{e87} phenotype in which the sense organs can be seen). (C) Head of a homozygous dpp^{hr56} embryo. In this example ventral arms (VA) and the walls of the pharnyx have been forced out of the body cavity and can be seen at the top of the figure. The dorsal arms and dorsal bridge are absent, and the two ventral halves of the head skeleton are not connected. Two pairs of mouth hooks (to the left and right of mh in the figure), antennal sense organs, and maxillary sense organs are evident. The cirri are also present, but cannot be seen in this figure. (D) Head of a homozygous dpp^{hr4} embryo. The only clearly evident head structures are the antennal sense organs and the mouth hooks. The scleretinized (i.e. dark grey) regions in the middle of the head most likely represent a disorganized remnant of the ventral arms. (E) Head of a homozygous dpp^{hr27} embryo. In this and more severe mutant phenotypes, the mouth hooks and antennal sense organs are either fused into a single structure (see Table 2) or positioned next to one another. In the most severe phenotypes, the antennal sense organ is lost. (F) Cuticle of a wild-type embryo illustrating the tail region. Posterior is to the right, and dorsal is up. (G) Tail region of a homozygous dpp^{hr56} embryo. The telson in internalized, but none of the scorable structures are deleted. In this, and the following panels, anterior is up (cf. Figs 2,3). (H) Tail region of a homozygous dpp^{hr92} embryo. All of the structures posterior of A7 are internalized. The Filzkörper are disorganized and appear as a cratered dome surrounded by spiracle hairs. (I) Tail region of a homozygous dpp^{hr27} embryo. In this embryo the Filzkörper are entirely absent, and only the spiracle hairs and anal pads are evident. In this embryo the eighth segment is internalized. (J) Tail region of a dpp^{hr92}/dpp^{H46} *trans*heterozygote. Only the anal pads are seen. Abbreviations are listed in Fig. 6.

dpp^{hr56} (see above). In addition, these same phenotypes are produced by embryos that die due to the dominant lethality associated with the stronger alleles. Thus, the mutant phenotypes resulting from *dpp* genotypes that exhibit dominant lethality (i.e. heterozygotes of the form *dpp**/+) are similar to those that are produced by embryos homozygous for the weak alleles.

The cuticular phenotypes characteristic of these allelic combinations consist of progressive deletion of the anterior-dorsal cuticular structures of the head, and invagination of the telson and posterior abdominal segments. In the head region, the labral sense organ, dorsal bridge and dorsal arms, which map to the dorsal anterior tip of the blastoderm embryo (Jürgens et al., 1986), are the first structures to be affected, and the only structures that are absent in embryos homozygous for *dpp*^{e87} and *dpp*^{e90} (Fig. 4B). No alterations in tail morphology are observed in these cuticles (Fig. 2B,C). In *dpp*^{e87}/*dpp*^{e90} *trans*heterozygotes and *dpp*^{hr56} homozygotes (Fig. 2D,E), deletions in the head region include structures that map to more ventral positions on the blastoderm fate map. Specifically, in addition to those structures absent from *dpp*^{e87} and *dpp*^{e90} homozygotes, these embryos also show defects in the vertical plates and the anterior portions of the ventral arms (Fig. 4C). Furthermore, these embryos show progressive changes in the morphology of the tail region. In *dpp*^{e87}/*dpp*^{e90} embryos, the telson and posterior abdominal segments are often curled onto the dorsal side of the embryo as if the germ band had failed to retract completely (Fig. 2D). In *dpp*^{hr56} embryos the defect is more severe, and the entire telson and eighth abdominal segment are found inside the embryo beneath the fifth and sixth abdominal segments (Fig. 4G).

Many of these weaker genotypes produce a continuum of mutant phenotypes. This is particularly true for those genotypes that produce both wild-type and mutant progeny. For example, 60% of the embryos that are homozygous for *dpp*^{e87} survive to adulthood, and 40% die as embryos (Fig. 1C). Of the 40% that die as embryos, some produce a cuticle like the one shown in Fig. 2C, that lacks most of the dorsal elements of the cephalopharyngeal skeleton, while others produce a cuticle that lacks only the labral sense organ and a small part of the dorsal bridge, and others still that lack only the labral sense organ (cf. Fig. 2C, 4B). Similarly, 80% of embryos of the genotype *dpp*^{hr92}/+ survive to adulthood, and 20% die as embryos (Fig. 1A,B). Of the 20% that die, some produce a cuticle like that shown in Fig. 2D (the most severe mutant phenotype observed for *dpp*^{hr92}/+), while others produce cuticles like those shown in Fig. 2B,C (data not shown). Finally, only 5% of the embryos of the genotype *dpp*^{H46}/+ (haploinsufficient) survive to adulthood, and 95% die as embryos (Fig. 1A,B). Of the 95% that die as embryos, some produce a cuticle similar to that produced by *dpp*^{hr56} homozygotes (Fig. 2F, cf. Fig. 2E), while others produce cuticles like those shown in Fig. 2C,D (for example, Fig. 2B). Thus, each of these genotypes produces a continuum of phenotypes that are representative of all or a portion of the range of phenotypes characteristic of homo- and heteroallelic combinations of the weak alleles.

A careful analysis of the phenotypes produced by embryos mutant for weak alleles, or those that are haploinsufficient, revealed that the progressive change in tail region morphology that we observed in the allelic series was not accompanied by the reduction or loss of any posterior cuticular structures. In light of this, we suspected that this aspect of the phenotype might be a consequence of defects elsewhere in the embryo. In particular, the phenotype of *dpp*^{e87}/*dpp*^{e90} embryos (Fig. 2D), which is indicative of a defect in germ band extension or retraction, suggested the possibility that the tail defect could be due to a progressive deletion of the dorsally derived amnioserosa. To test this hypothesis, we immunostained mutant embryos using an antibody directed against the *Krüppel* protein (Kr), which accumulates in amnioserosa cells shortly after gastrulation (Gaul et al., 1987). We find that the internalization of the posterior structures can be directly correlated with the progressive loss of amnioserosa cells. This result is best illustrated in haploinsufficient embryos of the genotype *dpp*^{H46}/+. Consistent with the variability characteristic of this genotype, we find embryos that have anything from 0 to 150 amnioserosa cells (Fig. 5). Furthermore, based on the morphology of these embryos, it appears that this reduction in the number of amnioserosa cells specifically results in the invagination of the posterior segments and telson. We have done similar immunostainings on embryos mutant for the weak hypomorphic alleles and we find that the more severe the allele or allelic combination, the fewer amnioserosa cells are found. Thus, in embryos mutant for *dpp*^{e87} and *dpp*^{e90}, the number of amnioserosa cells is essentially the same as that observed in wild-type controls (about 200). In the heteroallelic combinations between *dpp*^{e87}, *dpp*^{e90}, and *dpp*^{hr56}, the number of amnioserosa cells ranges from 20 to 100 cells. In *dpp*^{hr56} homozygotes few if any amnioserosa cells are observed (data not shown).

Taken together, the above results indicate that the weaker hypomorphic *dpp* alleles manifest progressive deletions of dorsally derived structures: weak alleles, like *dpp*^{e87}, only show deletions of anterior dorsal regions of the head; more severe allelic combinations like *dpp*^{e87}/*dpp*^{hr90}, and *dpp*^{hr56}, result in further deletions of head structures and progressive deletion of the dorsally derived amnioserosa. Thus, the weakest *dpp* alleles affect only the dorsal-most pattern elements on the blastoderm fate map.

The moderate and severe hypomorphic *dpp* alleles show further deletions in the head, as well as a progressive expansion of the ventral denticle belts which was not observed in the cuticles of the weak alleles. In addition, deletions of the structures in the tail region are observed (Table 2, Figs 2,3). There are several distinct transition points within this part of the allelic series that are evident due to the loss of prominent cuticular structures. The first is evidenced by a radical change in the morphology of the head and thorax. This is illustrated by the change in morphology between the cuticles of *dpp*^{H46}/+ and those of *dpp*^{H46}/*dpp*^{e87} and *dpp*^{hr4} (Fig. 2G,H). In this transition, the head loses most of the remaining cephalopharyngeal skeleton, in particular the ventral arms and pharnyx walls, and becomes globular. In fact, the only scorable head structures present in these embryos are the mouth hooks and antennal sense organs (Fig 4D). A characteristic defect associated with these changes is a prominent constriction in the first thoracic seg-

Table 2. Phenotypic analysis of *dpp* hypomorphic alleles and heteroallelic combinations

	92/+	46/+(w)	87	87/90	56	46/+(s)	46/87	4	92	27	92/46	93	94	91/95	46
LrSO†	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
DBr	+/–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
DArm	+	+/–	–	–	–	–	–	–	–	–	–	–	–	–	–
VertP	+	+	+/–	+/–	–	–	–	–	–	–	–	–	–	–	–
VArm	+	+	+	+	+/–	+/–	–	–	–	–	–	–	–	–	–
HO	+	+	+	+	+	+/–	–	–	–	–	–	–	–	–	–
T	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–
MxSO	+	+	+	+	+	+	+/–	–	–	–	–	–	–	–	–
ci	+	+	+	+	+	+				–	–	–	–	–	–
AnSO	+	+	+	+	+	+	+	+	+	+f	f	f	f	f	+/–
mh	+	+	+	+	+	+	r	r	r	rf	rf	rf	rfv	rfv	rfv
H	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
t-dlh	+	+	+	+	+	+									
dk	+	+	+	+	+	+	+	+	–c	–c	–c	–	–	–	–
t-lh	+	+	+	+	+	+									
vk	+	+	+	+	+	+	+	+	–c	–c	–c	–	–	–	–
t-vlh	+	+	+	+	+	+									
KO	+	+	+	+	+	+	+	+	+c	+c	+c	–	–	–	–
A1-3	+	+	+	+	+	+	+	+	+/–co	+/–co	co	co	co	co	co
A4-6	+	+	+	+	+	+	+	+	+	+	+	+	+	co	co
A7-8	+	+	+	+	i	i	i	co	co	co	co	co	co	co	co
sph	+	+	+	+	r–i	r–i	r–i	–	–	–	–	–	–	–	–
t	+	+	+	+	+i	+/–i	+/–i	–	–	–	–	–	–	–	–
Fk	+	+	+	+	+i	+i	+i	+i	+/–i	–	–	–	–	–	–
ap	+	+	+	+	+i	+i	+i	+i	+i	+i	+i	+i	+i	–	–

Allele designations: 4, *dpp*^{hr4}; 27, *dpp*^{hr27}; 56, *dpp*^{hr56}; 46, *dpp*^{H46} (null); 87, *dpp*^{e87}; 91, *dpp*^{H91}; 92, *dpp*^{hr92}; 93, *dpp*^{hr93}; 94, *dpp*^{H94}; 95, *dpp*^{H95}.

†Abbreviations for cuticle structures are listed in the legend to Fig. 6. A1-3, etc. refer to the denticle belts of abdominal segments 1-3, etc. Other abbreviations: (+) the structure is observed in all embryos (+/−) or (v) the structure is observed in some individuals; (−) the structure was never observed. ( ) the structure could not be scored due to morphological aberrations in the region; (f) a pair of symmetric structures (specifically mh and AnSO) are fused into a single structure; (r) the structure is reduced in size or proportion; (c) morphology in the region is constricted and the structure is either, (+) present, or (−) cannot be distinguished from similar structures in the region. (co) the denticle belt is confluent and encircles the embryo; (i) the structure is internalized, but not deleted (w) weak phenotype (s) severe phenotype. In some cases, the abbreviations are used combinatorially.

ment which essentially obscures all structures in this region of the cuticle (see Fig. 2G,H; Table 2).

The next transition occurs in the range of phenotypes produced by embryos homozygous for *dpp*^{hr4}, *dpp*^{hr27}, *dpp*^{hr92}, and heteroallelic combinations thereof. In this transition progressive loss of the posteriorly located Filzkörper is observed. In homozygous *dpp*^{hr4} embryos, at least one normally differentiated Filzkörper is observed in every case (Fig. 2H); in homozygous *dpp*^{hr92} embryos, the Filzkörper are either normally differentiated as in *dpp*^{hr4} (rare), irregular or partially deleted (Fig 4H), or entirely absent (Fig. 3A); in homozygous *dpp*^{hr27} embryos, small remnants of the Filzkörper are occasionally observed (Fig. 4I), but in general they are entirely absent (Fig. 3B). In addition to this defect, further degeneration of the mouth hooks and antennal sense organs is observed. The change here is particularly interesting as the pairs of mouth hooks and antennal sense organs are not lost, but rather fuse to form a single irregular mouth hook and antennal sense organ (Fig. 4E). These fused structures are present as such in all of the more severe genotypes discussed below.

Subsequent to the loss of the Filzkörper and the fusion of the mouth hooks and antennal sense organs, the most obvious transition that is observed in the cuticles is the progressive expansion of the ventral denticle belts. In embryos homozygous for *dpp*^{hr93} and *dpp*^{H94}, and in *trans*heterozygous combinations with *dpp*^{H46}, the tips of the denticle belts in the most anterior and most posterior segments are observed to meet at the dorsal side of the embryo (Fig. 3C,D,E,F) indicating a complete loss of dorsal structures in these regions. The tips of the denticle belts in the mid-abdominal segments are expanded, but in general, they do not completely encircle the embryo and a small patch of dorsal tissue remains on the dorsal side (Fig. 3D,E,F). In the more severe genotypes like *dpp*^{H95}/*dpp*^{hr93}, *dpp*^{H95}/*dpp*^{H94} and *dpp*^{H95}/*dpp*^{H91} further expansion of the ventral denticle belts is evidenced by an increase in the width of the denticles that reach the dorsal side of the embryo (Fig. 3G). Finally, in *dpp*^{H46} homozygotes (complete null) no dorsal structures are observed, and the ventral denticle belts encircle the entire embryo (Fig. 3H, Irish and Gelbart, 1987). The most ventral pattern elements in the tail region of the embryo, the anal pads (Jürgens, 1987), are observed in all mutant embryos except the null (Fig. 3H; Table 2).

A graded requirement for *dpp* in the early embryo

From our analysis of the phenotypic manifestations of these 12 hypomorphic *dpp* alleles, we draw the following conclusions. First, the different levels of *dpp* activity that we defined based on the relative percentage of dominant lethality give rise to distinct mutant phenotypes. Second, the severity of the mutant phenotype characteristic of a particular allele is inversely proportional to the level of residual

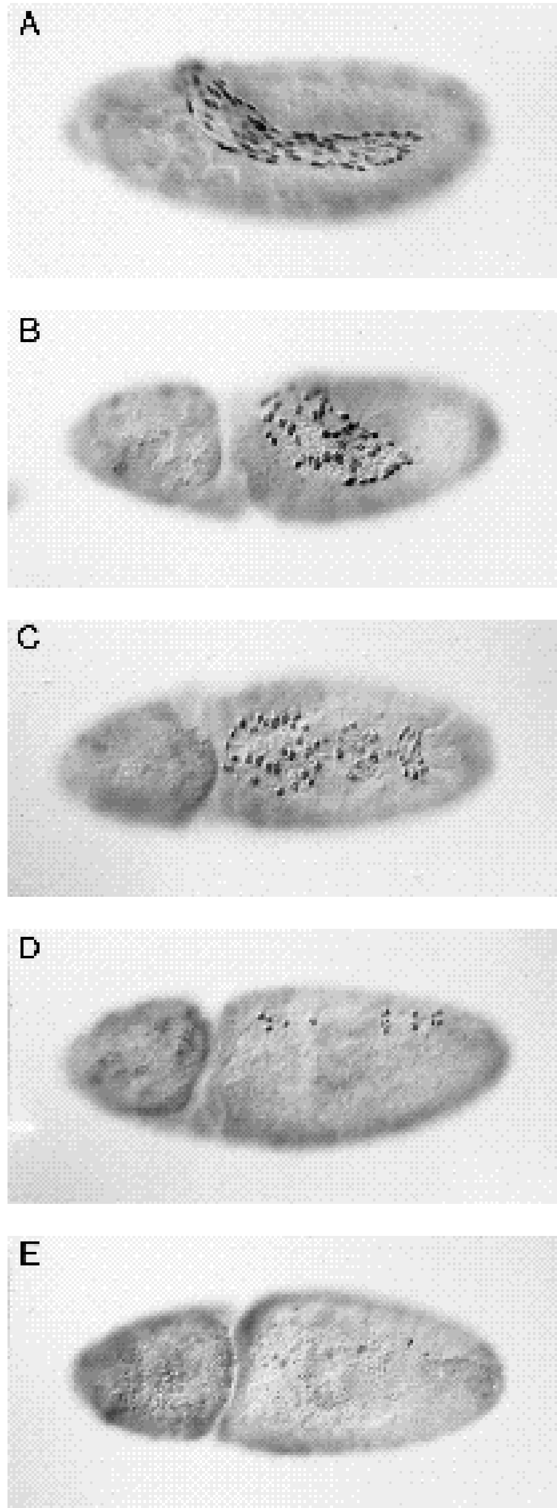

Fig. 5. Weak hypomorphic *dpp* alleles result in progressive deletion of the amnioserosa. For all embryos, anterior is to the left. (A) Lateral view of a stage 10 (Campos-Ortega and Hartenstein, 1985) wild-type embryo immunostained using an anti-Kr antibody to detect the amnioserosa cells. (B-E) A series of mutant embryos illustrating the range of phenotypes that result from the genotype *dpp*^{H46} *Sp cn bw*/+ (haploinsufficient). As is evident from the figure, these embryos show progressive reduction in the number of cells fated to the dorsally derived amnioserosa. The embryos shown have: (B) 90, (C) 55, (D) 16, and (E) 0 amnioserosa cells as revealed by Kr expression. This loss of amnioserosa cells is correlated with a failure to extend the germ band to the dorsal side of the embryo (cf. B and E). This germ band extension defect results in the invagination of the telson and posterior abdominal segments that is observed in the cuticles (see text). Similar Kr expression patterns are observed in embryos mutant for homo- or heteroallelic combinations of the other weak *dpp* alleles.

dpp activity it is thought to produce. Thus, *dpp* alleles that exhibit little or no dominant lethality (i.e. that have the highest level of residual activity) give rise to the mildest mutant phenotypes consisting of progressive deletions of the dorsally derived amnioserosa. Furthermore, those *dpp* alleles that exhibit intermediate percentages of dominant lethality, i.e. *dpp*^{hr4}, *dpp*^{hr27}, *dpp*^{hr92}, and *dpp*^{hr93}, give rise to more severe mutant phenotypes which consist of a complete loss of the amnioserosa and the progressive loss of the dorsal ectoderm. Finally, those alleles that exhibit high percentages of dominant lethality similar to the null alleles, give rise to mutant phenotypes which consist of near complete or complete loss of all dorsal structures (Fig. 6). Similar progressive dorsal-to-ventral deletions of cuticle structures are observed in the head and tail regions of the embryo as discussed above.

In light of this relationship between the activity levels, as defined by the dominant lethality assay, and the cuticular phenotypes, it is evident that there is a graded requirement for *dpp* activity in the early embryo. Peak levels of *dpp* activity are required for initial specification of the dorsally derived amnioserosa; intermediate levels are required for specification of the dorsal epidermis, and low levels are required for the specification of the ventral epidermis. Furthermore, the progressive nature of the phenotypes we observe, i.e. the progressive loss of the amnioserosa, and the progressive loss of the head structures and Filzkörper, as well as the fact that the range of phenotypes characterisitc of the different mutant genotypes overlap to such a great extent, imply that the graded requirement for *dpp* activity is continuous or nearly so. Thus, from these data, it is apparent that, like the ventral pattern elements, dorsal pattern elements in the *Drosophila* embryo are specified by a gradient. However, unlike the ventral pattern elements, which are specified by the maternal *dl* gradient, the dorsal pattern elements appear to be specified by a zygotic gradient system involving the *dpp* gene product.

Significantly, the range of mutant phenotypes that we have observed for *dpp* mutations alone encompasses the entire repertoire of phenotypes characteristic of all of the other zygotic genes that affect the dorsal-ventral pattern. The mildest phenotypes we observed are similar to those produced by embryos bearing loss-of-function alleles of *sog*, *srw*, *tsg*, and *zen*, while the more moderate *dpp* mutant phenotypes are representative of those produced by *tld* and *scw* alleles. In particular, *sog*, *srw*, and *tsg* embryos produce mutant phenotypes which are similar to those produced by *dpp*^{e87} and *dpp*^{e90} homozygotes and *trans*-heterozygotes (Fig. 2C,D). Null alleles of *zen* (e.g. *zen*^{w36}) produce mutant phenotypes virtually identical to that produced by *dpp*^{hr56} homozygotes (Fig. 2E). Finally, *tld* and

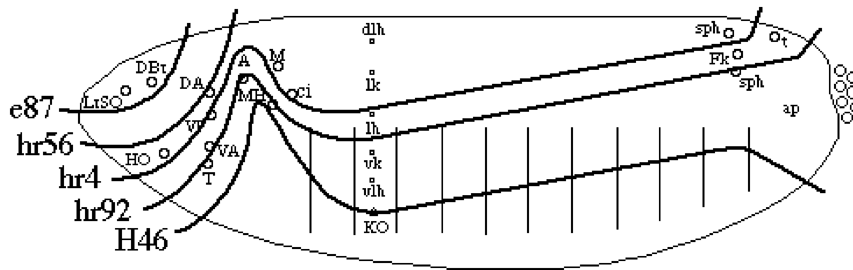

Fig. 6. Schematic illustration of pattern deletions associated with hypomorphic *dpp* alleles. Schematic fate map of a cellular blastoderm embryo showing the location of larval cuticular structures (Lohs-Schardin et al., 1979; Jürgens et al., 1986; Jürgens, 1987). Pattern deletions characteristic of homozygous dpp^{e87}, dpp^{hr56}, dpp^{hr4}, dpp^{hr92}, and dpp^{H46} embryos are shown. All structures that map above the line are absent in the particular mutant. Abbreviations for Figs 2, 4, 5 and Table 2: LrSO, labral sense organ; DBr, dorsal bridge; DArm or DA, dorsal arm; HO, hypopharyngeal organ; VertP or VP, vertical plate; A or AnSO, antennal sense organ; M or MxSO, maxillary sense organ; VArm or VA, ventral arms; T, T-bars; ci, cirri; mh, mouth hooks; H, H-piece; dlh, dorsolateral hair; dk, dorsal kölbchen; lh, lateral hair; vk, ventral kölbchen; vlh, ventrolateral hair; KO, Keilin's organ; sph, spiracle hair; Fk, Filzkörper; t, tuft; ap, anal pads.

scw embryos exhibit mutant phenotypes similar to those produced by embryos homozygous for dpp^{hr4}, dpp^{hr27}, and dpp^{hr92}(Fig. 3A,B). This similarity of the hypomorphic *dpp* mutant phenotypes and those of the other zygotic genes, as well as the demonstration that *tld*, *srw*, *scw* and *sog* show genetic interactions with the *dpp* gene (Ferguson and Anderson, 1992; V. Twombly, personal communication), strongly suggest that the gradient system that we have defined based on the loss-of-function mutant phenotypes of *dpp* must involve these other zygotic genes as well.

In light of the fact that these other gene products are likely to be involved in the gradient system defining dorsal pattern elements in the embryo, the nature of loss-of-function mutations, while clearly indicating the presence of a gradient, does not distinguish the central (i.e. graded) element in the system. That is, in a multicomponent gradient system like the one considered here, any element that affects the efficacy of the system as a whole will produce a graded allelic series like that we have described for *dpp* (and, in fact, such a series has been described for *tld*; Ferguson and Anderson, 1992). Thus, a graded series of mutant phenotypes only suggests that the particular gene product contributes to the gradient system as a whole; it does not imply that it is the graded element. In order to define the key element in a gradient system, it is necessary to define which element in the system is limiting, since this must, by definition, be the graded element. Given that any product already in excess is unlikely to have an effect on the system when present in even greater excess, the simplest demonstration that a particular element is limiting is to determine whether or not gain-of-function phenotypes are consistent with a general increase in the activity of the gradient. In the next section we address this issue.

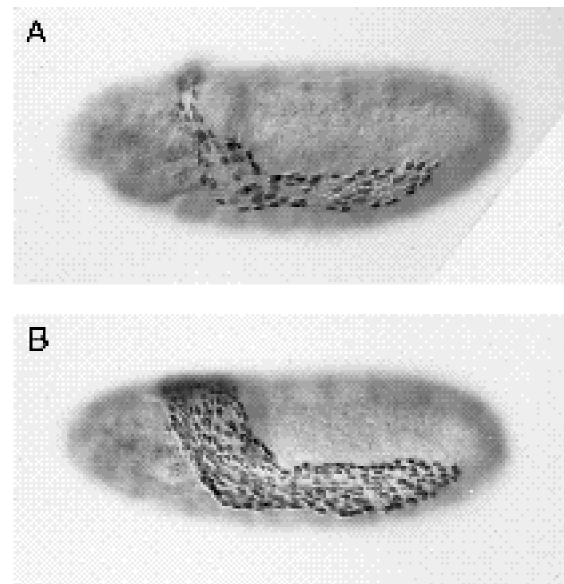

Fig. 7. Increasing the gene dosage of *dpp* results in expansion of the amnioserosa. Anterior is to the left. Dorsolateral views of embryos bearing 2 (A) and 4 (B) copies of *dpp*, immunostained using an anti-Kr antibody. The genotypes are (A) Canton-S and (B) *Dp(2;2)DTD48/Dp(2;2)DTD48*. The embryo shown in A has 140 amnioserosa cells (total), that in B, 400. On average, embryos bearing 4 copies of *dpp* gene have 2.5 times more amnioserosa cells than those with 2 copies. Despite the observed expansion, embryos with 4 copies are almost 100% viable (see text). Thus, the change in the allocation of cell fates along the axis does not affect the ability for the embryo to intercalate all the fates.

Increasing *dpp* gene dosage results in progressive expansion of dorsal pattern elements

As we have previously stated, *dpp* is a dosage sensitive gene. A deviation from the normal diploid dose of dpp^{+} is sufficient to induce a shift in the fate map of the early embryo along the dorsal-ventral axis, such that cell fates that map to the dorsal regions of the embryo are lost and are replaced by more ventral fates. In order to characterize the effects of increasing *dpp* dosage in the wild-type embryo, we have employed two insertional duplications of *dpp*, *Dp(2;2)DTD48* and *Dp(2;2)B16* (see Materials and methods). Since reducing *dpp* gene dosage affected the dorsally derived amnioserosa, we expected that increasing the gene dosage would also affect this tissue. So, we have used the Kr antibody to detect the domain of amnioserosa cells.

We have found that increasing the *dpp* gene dosage can cause shifts in the fate map along the dorsal-ventral axis. In wild-type embryos bearing two copies of the *dpp* gene (i.e. disomic) we have observed an average of 130 (n=36) amnioserosa cells (Fig. 7A). When the gene dosage is increased from two to four doses (i.e. tetrasomic), we find that the average number of amnioserosa cells increases from 130 to 325 (n=36), and in exceptional embryos the number can reach 400 or more (Fig. 7B). Thus, by doubling the

dpp gene dosage, we observe 2.5× the normal number of amnioserosa cells (on average). Notably, despite this dramatic change in the dorsal-ventral fate map, embryos tetrasomic for *dpp* are adult viable and exhibit no mutant phenotype. This fact implies a certain degree of autonomy of the more ventral fates (which are presumably specified by the *dl* gradient) as they are not simply overrun by the expansion of the dorsal pattern elements. We suspected that this ventral patterning system might be limiting the expansion of the dorsal fates, so we repeated the above experiment in embryos derived from *dl* mutant mothers. These embryos lack the ventral structures of the wild-type embryo. dl^{-} embryos that are monosomic for *dpp* (i.e. $dpp^{H46}/+$) completely lack amnioserosa cells (Fig. 8A), consistent with the phenotype we have observed in dl^{+} embryos that are monosomic for *dpp* (Fig. 5E). In dl^{-} embryos that are disomic for *dpp* (+/+) we observed 20-200 amnioserosa cells, as has been previously described (Fig. 8B; Ray et al., 1991). If the *dpp* gene dosage is increased further, progressively more amnioserosa cells are observed. In dl^{-} embryos trisomic for *dpp*, we observed 500-1000 amnioserosa cells (Fig. 8C), and in those tetrasomic for *dpp*, 2000 or more (Fig. 8D). Thus, increasing the dosage of *dpp* in the context of an apolar dl^{-} embryo completely transforms the segmented region of the embryo into amnioserosa.

From these results, it is clear that increasing the dosage of the *dpp* gene, and thus presumably the level of *dpp* activity in the embryo, alters the distribution of fates along the dorsal-ventral axis. Increasing the *dpp* gene dosage in the context of the wild-type embryo produces a corresponding expansion of the dorsally derived amnioserosa, and thus must result in a ventral shift in the fate map of the embryo. Increasing the *dpp* gene dosage in the context of an apolar dl^{-} embryo results in the transformation of a uniform field of dorsal epidermal cells into amnioserosa cells. Both of these experiments imply that cell fate in the embryo is very sensitive to the level of *dpp* activity, and that the *dpp* gene product has the potential to specify at least two different cell fates along the dorsal-ventral axis. In similar experiments, in which the dosage of *dpp* is reduced in the context of an apolar dl^{-} embryo by the introduction of loss-of-function *dpp* alleles, we find that a number of other cell types characteristic of dorsal and lateral ectoderm are produced (Fig. 9, data not shown). Thus, just as the hypomorphic series in the context of the wild-type embryo shows progressive, nested deletions of dorsal and lateral structures (Fig. 6), a hypomorphic series can be generated in the context of an apolar embryo that shows an ordered series of different cell states from dorsal to ventral as the level of *dpp* activity is reduced. We conclude that it is the activity of the *dpp* gene product that is graded along the dorsal-ventral axis, and that the gradient that defines pattern within the dorsal regions of the embryo is, in fact, a *dpp* gradient.

The *dpp* and *dl* gradients specify different aspects of the dorsal-ventral pattern

Along the anterior-posterior axis, distinct regions of the body plan are determined largely independently by three maternal patterning systems. The anterior system is required for specification of the head and thorax, the posterior system

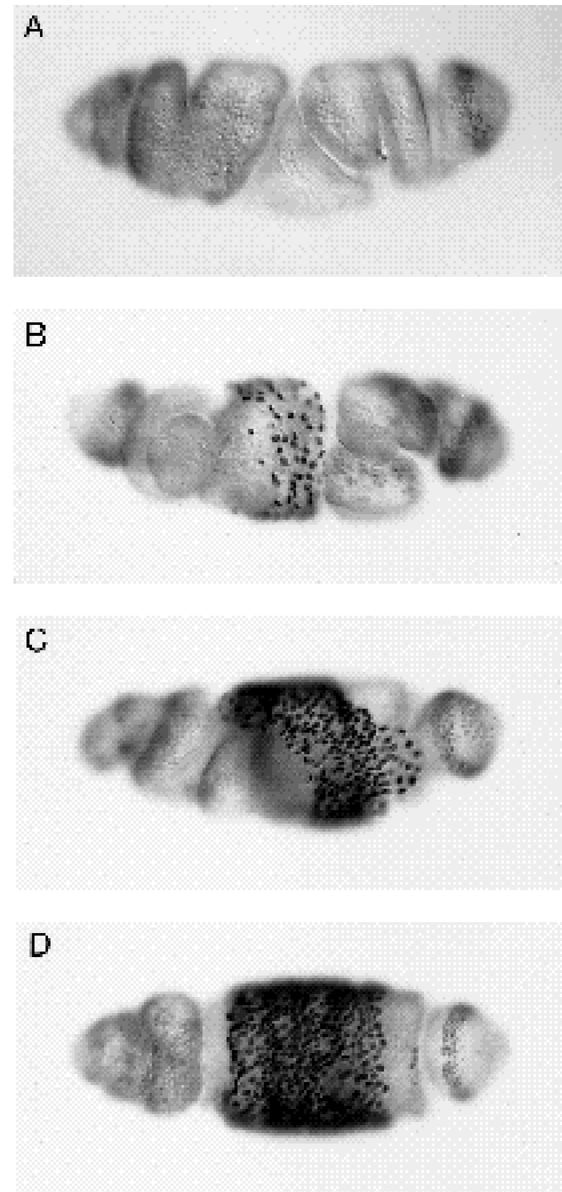

Fig. 8. Increasing the gene dosage of *dpp* progressively transforms dorsal epidermis to amnioserosa. Anterior is to the left. All embryos are derived from a cross between dl^{1} *cn sca*/dl^{1} *Dp(2;2)DTD48* females and dpp^{H46} *Sp cn/CyO23* males. The embryos were immunostained using an antibody directed against the Kr protein. The embryos shown carry a total of 1 (haploinsufficient) (A), 2 (B), 3 (C), and 4 (D) wild-type copies of the *dpp* gene. Their genotypes are: (A) dpp^{H46} *Sp cn bw*/dl^{1} *cn sca*, (B) dl^{1} *Dp(2;2)DTD48*/dpp^{H46} *Sp cn*, (C) dl^{1} *cn sca/CyO23*, (D) dl^{1} *Dp(2;2)DTD48/CyO23*. The number of amnioserosa cells present in each embryo are (A) 0, (B). 100, (C) approx. 400, and (D) >2000 (the number of amnioserosa cells in C and D could not be determined accurately because of folds in the amnioserosa region). Note the changes in embryonic morphology that accompany the increase in the number of amnioserosa cells. Embryos like those shown in C and D fail to lay down cuticle. While the embryos shown in this figure were derived from a single cross, the genotypes of such embryos have been verified by other crosses that specifically generate only one or two of the different genotypes (data not shown).

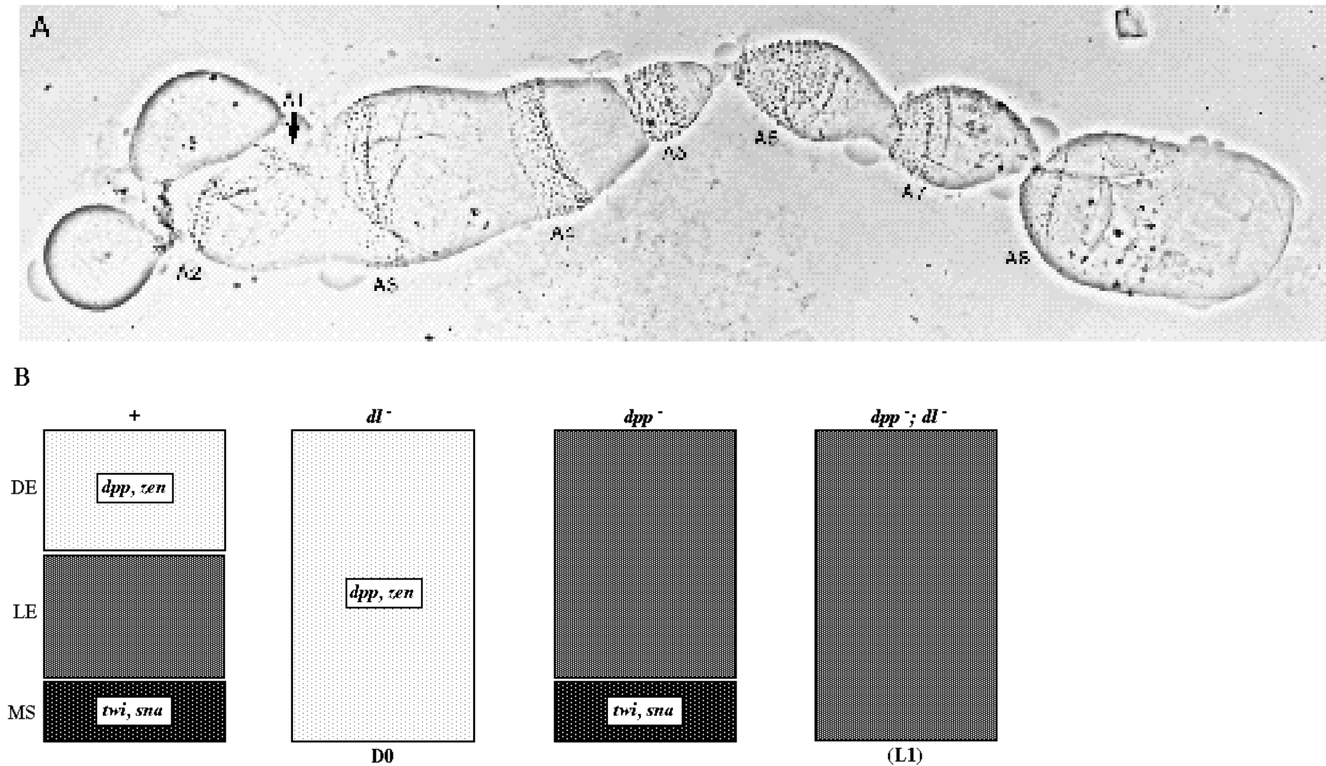

Fig. 9. *dpp* and *dl* specify different aspects of the dorsal-ventral pattern. (A) Cuticle of a homozygous dpp^{H46} embryo derived from a homozygous *dl* mother (a *dpp*, *dl* double mutant). The embryo is lateralized, showing neither dorsal nor ventral cuticular structures as they are mapped to the blastoderm embryo (see also Irish and Gelbart, 1987). (B) Schematic illustration of an interpretation of the mutant phenotypes of dl^- embryos, dpp^- embryos, and dl^-, dpp^- embryos. In the far left panel, the different dorsal-ventral fates of a wild-type embryo are shown. Zygotic genes that are expressed within a particular domain are indicated. In the second panel, the phenotype of a dl^- embryo is diagrammed. In this case, the MS and LE are not present, and the DE is expanded over the entire dorsal-ventral axis. Consistent with this change in cell fate, *dpp* and *zen* are expressed throughout (Ray et al., 1991). This maternal phenotype has been given the designation D0 (Anderson et al., 1985; Roth et al., 1991). In the third panel, the *dpp* mutant phenotype is diagrammed. Note that this is not the 'opposite' phenotype of that produced by dl^- embryos (specifically, *dpp* mutant embryos are not composed of a single pattern element, and thus have residual dorsal-ventral polarity). The MS is fated as in wild type, and appropriate expression of zygotic genes *twi* and *sna* is observed. The LE is expanded dorsally and takes the place of the DE which is not fated. The fourth panel illustrates the dl^- dpp^- double mutant shown in A. Both dorsal and ventral pattern elements (DE and MS) are lost, and the lateral fates (LE) are expanded over the entire dorsal-ventral axis. This phenotype is apolar and reminiscent of the L1 phenotype that has been described for certain maternal mutants (Anderson et al., 1985; Roth et al., 1991). Abbreviations: DE, dorsal ectoderm; LE, lateral ectoderm; MS, mesoderm.

for the abdomen and the terminal system for the unsegmented acron and telson. The independence of these systems is illustrated by the general additivity of the mutant phenotypes. If the activity of any two of the systems is disrupted, the resulting embryo shows a partial pattern that reflects the function of the remaining system. In the absence of all three systems no apparent pattern is formed (Nüsslein-Volhard, 1991). On the molecular level, the lack of all pattern in the cuticle of the triple mutant is a reflection of the uniform expression of the zygotic gap gene *Krüppel* along the entire anterior-posterior axis (Lehmann and Frohnhöfer, 1989). In light of this regulation, it has been suggested that the expression of Kr defines a central ground state from which the three maternal systems elaborate the anterior-posterior pattern.

In light of the results we have presented in this report, it would appear that the dorsal-ventral axis is specified by at least two gradients, one maternal and one zygotic, which are quite *dependent*, as discussed below. Nevertheless, like the anterior, posterior and terminal systems, the *dl* and *dpp* systems appear to control different aspects of the dorsal-ventral pattern. The *dl* gene product defines the ventral pattern elements by the activation of zygotic genes that are expressed ventrally and the repression of genes that are expressed dorsally, and the *dpp* system defines dorsal pattern elements via an intercellular signalling mechanism that has yet to be defined (see Discussion). However, do these two systems define the entire dorsal-ventral pattern? By analogy with the anterior-posterior system, this question should be answered by the phenotype of embryos that lack the function of both *dpp* and *dl*. Such embryos can be generated, and have been generated (Irish and Gelbart, 1987), and an example of such an embryo is shown in Fig. 9A. Embryos lacking the function of both *dpp* and *dl* are lateralized. Thus, as regards the cuticular phenotype, the *dpp* and *dl* systems do not account for all of the dorsal-ventral pattern elements. However, if we consider the molecular basis for the *dpp*, *dl* and *dpp*,*dl* double-mutant phenotypes,

this result is, in fact, in keeping with that of the triple mutant in the anterior-posterior system. First, the *dl* mutant phenotype, as noted above, results from the failure to activate the expression of genes, like *twi* and *sna*, that direct the development of ventral regions, and a failure to repress the expression of genes, like *dpp* and *zen*, that direct the development of dorsal regions of the embryo (in this way, the *dpp* system is dependent on the *dl* system). Based on the results presented in this report, it is this uniform expression of *dpp* in dl^- embryos that leads to the 'dorsalization' of the embryo. Second, in a *dpp* mutant embryo the dorsal fates are lost and they are supplanted by more lateral fates (see Fig. 3H), but the ventral fates are not affected since these are specified by the *dl* system. Finally, in the *dpp*, *dl* double mutant, both dorsal and ventral fates are absent, and both are supplanted by the lateral fates (Fig. 9B).

Based on this interpretation of the mutant phenotypes, the following conclusions can be made. The dorsal expansion of lateral pattern elements in *dpp* mutant embryos suggests that in the wild-type embryo, the activity of the *dpp* system serves to confine these elements to more lateral regions. In other words, the *dpp* system elaborates the pattern in the dorsal ectoderm with respect to the lateral fates. Further evidence for the negative effect that *dpp* function has on the lateral fates is revealed by the *dl* mutant phenotype. As at least some lateral fates can be specified in dl^- embryos (as evidenced from the phenotype of the *dpp*, *dl* double mutant), the failure to produce lateral structures in a *dl* mutant embryo must be the result of a negative effect from *dpp,* which is expressed throughout the embryo. Finally, the ventral expansion of the lateral pattern elements in the *dpp*,*dl* double mutant embryo implies that the ventral gene expression induced by the *dl* gradient also acts to confine lateral pattern elements to the lateral regions of the embryo. In other words, the *dl* system elaborates the pattern in the ventral ectoderm with respect to the lateral fates. Thus, like the *Kr* expression domain, which is defined entirely by negative regulatory cues, the lateral domain of the dorsal-ventral pattern is also defined entirely by negative regulatory cues. Thus, this pattern element is a ground state from which the *dl* and *dpp* gradients define ventral and dorsal pattern elements, respectively.

DISCUSSION

We have presented evidence supporting the hypothesis that the *dpp* gene product is an integral part of a dorsal-to-ventral activity gradient in the early embryo that specifies fates within the ectodermal anlagen. We have demonstrated that there is a graded requirement for the *dpp* gene product in the early embryo. A high level of *dpp* activity is necessary to specify the amnioserosa, and progressively lower levels of *dpp* activity are required to specify dorsal and lateral ectoderm. Furthermore, we have provided evidence that the *dpp* gene product is the central element of this system.

Significantly, the *dpp* system differs from the previously described maternal gradient systems in two ways. First, the *dpp* gene is strictly zygotic and has no germ-line dependent maternal activity (Irish and Gelbart, 1987). Thus, the *dpp* system is organized around a zygotic gene rather than a maternal one. Second, the *dpp* gene product is homologous to members of the TGF- superfamily of secreted growth factors (Padgett et al., 1987). Thus, *dpp* does not instruct cell fate based on direct transcriptional activation or repression of downstream genes. Rather the mechanism by which *dpp* specifies cell fate must involve intercellular signalling. Given that there is no paradigm for a gradient system organized around a growth factor molecule, it is of interest to consider how such a gradient would be established and how it would function.

For instance, it is possible that the *dpp* protein is distributed in a concentration gradient, analogous to the *bicoid* system. In such a scenario, the fate of a particular cell could be specified based on the number of receptors that are activated by the *dpp* ligand. As such, a cellular milieu containing high concentrations of *dpp* product would promote one cell fate, while a milieu with a lower concentration would promote a different cell fate. While such a model is, of course, hypothetical, concentration-dependent specification of cell fate by a TGF- -related molecule is not without precedent. Studies on *Xenopus* XTC-MIF (activin A) have indicated that this TGF- member has the potential to induce different cell types in a dose-dependent fashion (Green and Smith, 1990). However, if *dpp* protein was in fact distributed in such a concentration gradient, then this graded distribution must be established by transcriptional or translational regulation. Two possible types of transcriptional regulation can be eliminated. First, *dpp* transcripts do not accumulate in a concentration gradient. Based on in situ localization of *dpp* transcripts in wild-type embryos, the expression of *dpp* is uniform throughout the dorsal ectoderm (St. Johnston and Gelbart, 1987). Second, it is unlikely that the initial expression of *dpp* could serve as a localized source from which the protein might diffuse, since *dpp* is expressed in nearly all the cells that are affected in *dpp* mutant embryos. The possibility that the *dpp* gradient is regulated at the level of differential translation of message cannot be eliminated at the present time, and is a reasonable possibility.

As an alternative to a *bcd*-like concentration gradient, it is possible that the *dpp* protein is part of an activity gradient, such that the activity of the *dpp* protein is modulated along the dorsal-ventral axis. As such the *dpp* signal is positively or negatively modulated by interactions with extracytoplasmic factors, and that the modified *dpp* ligand or ligand-factor complex can elicit different cellular responses upon receptor binding. Evidence in favor of this type of model is derived from genetic studies on the interactions between *dpp* and the other zygotic genes which affect the patterning the dorsal ectoderm. Three of these genes, *tld*, *srw*, and *scw*, have been shown to interact genetically with *dpp*, and the nature of these interactions strongly suggests that the wild-type function of these gene products is to potentiate the *dpp* signal (Ferguson and Anderson, 1992; V. Twombly, personal communication). Based on molecular analysis of *tld*, it is conceivable that this gene could potentiate the *dpp* signal via post-translational modulation. It has been shown that *tld* encodes a secreted protein with sequence similarity to the vertebrate bone morphogenetic protein BMP-1. The regions of homology include a metalloprotease domain and several

motifs implicated in protein-protein interaction (Shimell et al., 1991). Based on potential interactions of BMP-1 and TGF- -related ligands (Wozney et al., 1988), Shimell et al. (1991) have suggested that the *tld* gene product may be involved in the post-translation modification of the *dpp* protein. Thus, it is compelling to hypothesize that this post-translational modification may be partially responsible for establishing the *dpp* gradient.

In light of the similarity between the phenotypes of *dpp* alleles and the other zygotic dorsal-ventral patterning genes, it is possible to construct a model in which these various factors interact in a combinatorial manner so as to generate such an activity gradient of *dpp*. For instance, if these genes were expressed in overlapping, nested domains centered on the dorsal midline, the combinatorial interactions between these potentiating gene products and the *dpp* ligand could serve to create an activity gradient of *dpp*. However, as *dl* is the only source of polarity in the dorsal-ventral system this type of model requires that these different overlapping expression domains be determined by the *dorsal* gradient, and at present there are no data, either genetic or molecular, to support this differential transcriptional regulation of *tolloid*, *shrew*, and *screw*. Furthermore, in situ localization of *tld* transcripts has revealed that this gene is expressed in the dorsal-most 40% of the blastoderm embryo, similar to *dpp* (Shimell et al., 1991). Therefore, with the present data it is not possible to pursue this type of model further.

Nevertheless, the models we have suggested above clearly illustrate a fundamental constraint on the *dpp* system. Inevitably the *dpp* gradient must be tied to the *dl* gradient since it is the only source of polarity in the dorsal-ventral system. Thus, regardless of whether a model is proposed in which *dpp* transcripts are translationally regulated, or one invoking the post-translational modulation of *dpp* activity by several potentiating gene products, the polarity of the *dpp* system must be derived from *dl*. In the first case, it is necessary to propose a translational regulator, perhaps an RNA binding protein, that is specified by *dl* and mediates the graded translation of the *dpp* message. In the second case, as we have discussed above, it is necessary to propose that the different expression domains of the post-translational modifiers are established by the *dl* gradient.

Returning to the post-translational modification model, if *dl* does not affect the differential expression of proteins that interact positively with *dpp*, the *dl* gradient might establish a new zygotic ventral-to-dorsal gradient that interacts antagonistically with the *dpp* system. Thus, *dl* may indirectly generate the *dpp* gradient. This is analogous to proposing a graded regulatory molecule which mediates the differential translation of the *dpp* message, but in this case, the regulatory molecule is a secreted protein that modulates *dpp* activity post-translationally.

This bipolar model would explain several puzzling results that have been obtained in studies involving the zygotic dorsal-ventral patterning genes. For instance, consider the two mesoderm specific zygotic genes *twist* and *snail*. A double mutant for *twi* and *sna* completely deletes the mesoderm and adjacent mesectoderm, yet this is not accompanied by a corresponding expansion of the neurogenic ectoderm. Rather, the neurogenic region is simply shifted ventrally (Rao et al., 1991). This could be explained in light of a bipolar gradient system in that rather than expanding the ventral fates, the entire axis is redistributed over a greater number of cells. Similarly, we have shown here that an intercalation of fates is observed in embryos bearing duplications of *dpp*. In this case, the expanded amnioserosa reduces the number of cells that can contribute to the epidermal fates, yet all of the fates are established.

The idea of a bipolar system is also made compelling by a genetic analysis of *sog*. The *sog* gene has been placed among the zygotic ventralizing genes, based on the effect it has on the specification of the amnioserosa and on the expression pattern of *zen* (Ray et al., 1991). However, the focus of *sog* activity has been fate mapped ventrally (Zusman and Wieschaus, 1985) suggesting that it is active in ventral and not dorsal regions of the embryo. Based on these two characters alone, it is tempting to propose that *sog* may be a component of a ventral gradient like the one proposed above. This proposal is supported by the fact that *sog* interacts antagonistically with *dpp* in a dosage sensitive fashion. It has been shown that increasing the dosage of the *dpp* gene in a *sog* mutant embryo effectively dorsalizes the embryo (Ferguson and Anderson, 1992). Furthermore, we have found that $sog/+;dpp^{H46}/+$ animals survive more frequently than sog^{+}/sog^{+}; $dpp^{H46}/+$ animals indicating that *sog* is a dominant suppressor of *dpp* haplolethality. Thus, *sog* is a gene that is presumably expressed ventrally and that acts antagonistically to the *dpp* gradient. The possibility that *sog* is a component in the proposed ventral gradient that establishes the *dpp* system must await further data.

It is important to note that the proposal that *sog* is part of a ventral-to-dorsal gradient is consistent with either a model invoking translational regulation of *dpp* or one invoking post-translational modification of the *dpp* protein. In the first case, it might be suggested that the *sog* gene encodes an RNA binding protein that sequesters *dpp* transcripts in more ventral regions. In the second case, a wide variety of protein types might be suggested. For instance, the ventral gradient could be composed of a specific protease or ligand binding protein, either of which could differentially inactivate the *dpp* protein along the dorsal-ventral axis. Alternatively, analogous to the activin/inhibin relationship, where the function of hetero- and homodimers differs dramatically (Vale et al., 1986; Ling et al., 1986), the ventral gradient could be composed of another TGF- -like molecule. In such a system, the position specific ratio of homo- to heterodimers could be involved in the specification of discrete cell fates.

We would like to thank H. Duncan for technical contributions. We are indebted to C. Rushlow for providing the *Krüppel* antibody and for helpful discussions. We would like to thank the members of the Schüpbach laboratory for help in preparation of the final figures, and Trudi Schüpbach for critical reading of the manuscript. During the course of this work, K. A. W. was supported by a National Institutes of Health postdoctoral fellowship, and R. P. R. by Office of Naval Research and National Institutes of Health predoctoral training fellowships. This work was funded by a National Institutes of Health Grant to W. M. G.

REFERENCES

Anderson, K. V., Jürgens, G. and Nüsslein-Volhard, C. (1985). The establishment of dorsal-ventral polarity in the *Drosophila* embryo: Genetic studies on the role of the *Toll* gene product. *Cell* **42**, 779-789.

Arora, K. and Nüsslein-Volhard, C. (1992). Altered mitotic domains reveal fate map changes in *Drosophila* embryos mutant for zygotic dorsoventral patterning genes. *Development* **114**, 1003-1024.

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

Crick, F. (1970). Diffusion in embryogenesis. *Nature* **225**, 420-422.

Driever, W. and Nüsslein-Volhard, C. (1988a). A gradient of *bicoid* protein in *Drosophila* embryos. *Cell* **54**, 83-93.

Driever, W. and Nüsslein-Volhard, C. (1988b). The *bicoid* protein determines position in the *Drosophila* embryo in a concentration-dependent manner. *Cell* **54**, 95-104.

Ferguson, E. L. and Anderson, K. V. (1992). Localized enhancement and repression of the activity of the TGF- family member, *decapentaplegic*, is necessary for dorsal-ventral pattern formation in the *Drosophila* embryo. *Development* **114**, 583-597.

Gaul, U. Seifert, E., Schuh, R. and Jäckle, H. (1987). Analysis of *Krüppel* protein distribution during early *Drosophila* development reveals posttranscriptional regulation. *Cell* **50**, 639-647.

Green, J. B. A. and Smith, J. C. (1990). Graded changes in dose of a *Xenopus* activin A homologue elicit stepwise transitions in embryonic cell fate. *Nature* **347**, 391-394.

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

Jürgens, G. (1987). Segmental organization of the tail region in the embryo of *Drosophila melanogaster. Roux's Arch. Dev. Biol.* **196**, 141-157.

Jürgens, G., Lehmann, R., Schardin, M. and Nüsslein-Volhard, C. (1986). Segmental organization of the head in the embryo of *Drosophila melanogaster*. *Roux's Arch. Dev. Biol.* **195**, 359-377.

Lehmann, R. and Frohnhöfer, H. G. (1989). Segemental polarity and identity in the abdomen of *Drosophila* is controlled by the relative position of gap gene expression. *Development* **Supplement** 21-29.

Ling, N., Ying, S.-Y., Ueno, N., Shinasaki, S., Esch, F., Hotta, M. and Guillemin, R. (1986). Pituitary FSH is released by a heterodimer of the -subunits from the two forms of inhibin. *Nature* **321**, 779-782.

Lohs-Schardin, M., Cremer, C. and Nüsslein-Volhard, C. (1979). A fate map for the larval epidermis of *Drosophila melanogaster*: localized cuticle defects following irradiation of the blastoderm with an ultraviolet laser microbeam. *Dev. Biol.* **73**, 239-255.

Nüsslein-Volhard, C. (1979). Maternal effect mutations that alter the spatial coordinates of the embryo of *Drosophila melanogaster.* In *Determinants of Spatial Organization*, (eds. S. Subtelny and I. R. Koenigsberg). pp. 185-211. New York: Academic Press.

Nüsslein-Volhard, C. (1991). Determination of the embryonic axes of *Drosophila*. *Development* **Supplement** 1-10.

Nüsslein-Volhard, C., Wieschaus, E. and Kluding, H. (1984). Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster*. I. Zygotic loci on the second chromosome. *Roux's Arch. Dev. Biol.* **193**, 267-282.

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- family. *Nature* **325**, 81-84.

Rao, Y., Vaessin, H., Jan, L. Y. and Jan, Y.-N. (1991). Neuroectoderm in *Drosophila* embryos is dependent on the mesoderm for positioning but not for formation. *Genes Dev.* **5**, 1577-1588.

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.

Roth, S., Stein, D. and Nüsslein-Volhard, C. (1989). A gradient of nuclear localization of the *dorsal* protein determines dorsoventral pattern in the *Drosophila* embryo. *Cell* **59**, 1189-1202.

Roth, S., Hiromi, Y., Godt, D. and Nüsslein-Volhard, C. (1991). *cactus*, a maternal gene required for proper formation of the dorsoventral morphogen gradient in *Drosophila* embryos. *Development* **112**, 371-388.

Rushlow, C. and Arora, K. (1990). Dorsal ventral polarity and pattern formation in the *Drosophila* embryo. *Seminars in Cell Bio.* **1**, 137-149.

Rushlow, C., Han, K., Manley, J. L. and Levine, M. (1989). The graded distribution of the *dorsal* morphogen is initiated by selective nuclear transport in *Drosophila*. *Cell* **59**, 1165-1177.

Shimell, M. J., Ferguson, E. L., Childs, S. R. and O'Connor, M. B. (1991). The *Drosophila* dorsal-ventral patterning gene *tolloid* is related to bone morphgenetic protein 1. *Cell* **67**, 469-481.

Simpson, P. (1983). Maternal-zygotic gene interactions during formation of the dorsoventral pattern in *Drosophila* embryos. *Genetics* **105**, 615-632.

Slack, J. M. W. (1991). From Egg to Embryo. Cambridge: Cambridge University Press.

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

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.

Steward, R. (1989). Relocalization of the *dorsal* protein from the cytoplasm to the nucleus correlates with its function. *Cell* **59**, 1179-1188.

Steward, R., Zusman, S. B., Juang, L. H. and Schedl, P. (1988). The *dorsal* protein is distributed in a gradient in early *Drosophila* embryos. *Cell* **55**, 487-495.

Thisse, B., Stoezel, C., El Messal, M. and Perrin-Schmitt, F. (1987). Genes of the *Drosophila* maternal dorsal group control the specific expression of the zygotic gene *twist* in presumptive mesodermal cells. *Genes Dev.* **1**, 709-715.

Vale, W., Rivier, J., Vaughan, J., McClintock, R., Corrigan, A., Woo, W., Karr, D. and Spiess, J. (1986). Purification and characterization of an FSH releasing protein from porcine ovarian follicular fluid. *Nature* **321**, 776-779.

Wakimoto, B. T., Turner, F. R. and Kaufman, T. C. (1984). Defects in embryogenesis in mutants associated with the Antennapedia gene complex of *Drosophila melanogaster. Dev. Biol.* **102**, 147-172.

Wieschaus, E. and Nüsslein-Volhard, C. (1986). Looking at embryos. In *Drosophila: A Practical Approach.* (ed. D. B. Roberts)., pp. 199-227. Oxford: IRL Press.

Wozney, J. M., Rosen, V. Celeste, A. J., Mitsock, L. M., Whitters, M. J., Kriz, R. W., Hewick, R. M. and Wang, E. A. (1988). Novel regulators of bone formation: molecular clones and activities. *Science* **242**, 1528-1534.

Zusman, S. B. and Wieschaus, E. (1985). Requirement for zygotic gene activity during gastrulation in *Drosophila melanogaster*. *Dev. Biol.* **111**, 359-371.

(Accepted 6 November 1992)