Identification in transgenic animals of the *Drosophila decapentaplegic* sequences required for embryonic dorsal pattern formation

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Mutant alleles of the *Drosophila decapentaplegic* (*dpp*) gene affect embryonic dorsal–ventral pattern formation, larval viability, and adult cuticle formation from the imaginal disks. The *dpp* DNA required for this array of functions spans almost 50 kb. We report that the embryonic lethal, ventralizing alleles of the *dpp* gene are rescued in transgenic animals by an 8-kb fragment of the wild-type *dpp* DNA. Full rescue, from embryonic lethality to adult viability, is obtained in two situations: in animals hemizygous for the haplolethal *dpp* gene, and in animals hemizygous for either of two recessive embryonic lethal alleles. In embryos null for *dpp*, the transformation of dorsal cuticle to ventral cuticle is blocked by one copy of the *dpp* transposon; two copies permit the hatching of the larvae. The portion of *dpp* sufficient for these embryonic functions encodes a protein with homology to the transforming growth factor-β (TGF-β) family of proteins (Padgett et al. 1987). The larval and imaginal disk functions of *dpp* are not rescued by the 8-kb portion of the gene and must require additional sequences from the 50 kb of DNA.

[Key Words: *Drosophila*, pattern formation; transgenic]

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The *Drosophila decapentaplegic* (*dpp*) gene is required for dorsal–ventral pattern formation in the early embryo [Gelbart et al. 1985; Irish 1986], for viability of the larva [Segal and Gelbart 1985], and for the growth of the imaginal disk cells that produce much of the cuticle of the adult fly [Spencer et al. 1982]. The gene spans almost 50 kb of DNA [F.M. Hoffmann, R.D. St. Johnston, and W.M. Gelbart, unpubl.] and is predicted to encode a protein product, on the basis of the DNA sequence, that is homologous to the mammalian regulatory factors transforming growth factor-β (TGF-β), inhibin-β, and Müllerian inhibiting substance [Padgett et al. 1987].

The *dpp* gene originally was identified by a series of mutant alleles that affect the development of the fly during embryogenesis and at metamorphosis [Spencer et al. 1982]. The disk alleles result in the failure to develop properly the adult cuticular structures derived from the imaginal disks. Striking defects in the adult cuticle derived from 15 of the imaginal disks led to the name of the gene. Each allele affecting the imaginal disks exhibits complete penetrance and uniform expressivity; however, a spectrum of phenotypes is seen among the group of disk alleles. Progressively more proximal cuticular structures along the proximal–distal axis of adult appendages are absent in animals with the more severe alleles [Spencer et al. 1982].

The embryonic alleles, a second group of *dpp* mutant alleles, fail to complement the disk alleles, as evidenced by the absence of some imaginal disk-derived cuticular structures in heterozygotes. These mutant alleles also disrupt embryogenesis. Genetically they are of two types: dominant lethals that can be rescued by a duplication of the *dpp* gene, called *dpp*⁺⁺ alleles for “haplo-insufficient-decapentaplegic” and recessive embryonic lethals, called *dpp*⁺⁻ and *dpp*⁻ alleles. In *dpp*-null embryos, in which both copies of the *dpp* gene are haplolethal alleles, the dorsal cuticle is transformed to a ventral cuticle. The dorsal-to-ventral transformation is characterized by the presence of ventral denticle hairs on lateral and dorsal surfaces, thereby forming rings of denticle hairs around the circumference of the embryo [Gelbart et al. 1985; Irish 1986]. Therefore, *dpp* function is required for proper embryonic development along the dorsal–ventral axis of the embryonic epidermis. A number of other *Drosophila* genes are also implicated in development along the dorsal–ventral axis of the embryo, including 10 maternal-effect genes in which loss-of-function mutations produce dorsalized embryos [Anderson and Nusslein-Volhard 1984]. A ventralized phenotype is observed in *Toll* dominant mutations [Anderson et al. 1985a,b]. The *dpp* gene is epistatic to three of the maternal-effect dorsalizing genes, *dorsal,*...
pelle, and Toll, so dpp may function on the same developmental pathway “downstream” of these genes [Irish 1986].

A third class of dpp mutations is made up of alleles that fail to complement a mutation originally called shortvein. These dpp alleles also fail to complement the recessive embryonic lethals at dpp and, in some cases, fail to complement dpp disk alleles. By these genetic criteria, shortvein and its alleles were defined as part of the dpp gene [Segal and Gelbart 1985]. Several of the dpp

The dpp gene was cloned by a chromosomal walk from an adjacent middle repetitive element (F.M. Hoffmann, R.D. St. Johnston, and W.M. Gelbart, unpubl.). Mapping of chromosomal rearrangements associated with dpp mutant phenotypes indicates that approximately 50 kb of genomic DNA is part of the dpp gene. Five overlapping transcripts, ranging in size from 3.5 to 5.0 kb, are differentially modulated during development [Gelbart et al. 1985]. The transcripts have two common 3’ exons, from the Hin domain, and several different 5’ exons, four of which map to the shortvein domain of the gene. None of the transcripts extends into the disk domain. The DNA sequence of cDNA clones reveals an open reading frame encoded entirely on the two 3’ exons from the Hin domain. The predicted protein sequence extends over 455 or 588 amino acids, depending on which of two in-frame initiation codons is used. The 100 amino acids at the carboxyl terminus have extensive homology with a family of mammalian growth factors or regulatory peptides that includes TGF-β, inhibin-β, and Mullerian-inhibiting substance [Padgett et al. 1987].

To understand the genetic complexity implicated by the three genetic domains and the molecular complexity implicated by the five transcripts, we are dissecting the gene into the individual segments required for specific functions. Our assay is the restoration of biological function in a dpp mutant animal. We begin our functional dissection of the gene with a piece of DNA spanning the Hin region because of the requirement for this region in all dpp functions.

**Results**

**Obtaining the dpp transgenic lines**

The hypothesis that the DNA between positions 83 and 91 on the DNA map might be sufficient for the role of dpp in embryonic pattern formation was based on the location of dpp mutant alleles on the DNA. dpp mutant lesions were mapped on the DNA by Southern blot analysis of mutant DNAs and by in situ hybridization of cloned dpp DNA to polytene chromosomes bearing dpp mutant chromosomal rearrangements [F.M. Hoffmann, R.D. St. Johnston, and W.M. Gelbart, unpubl.]. dpp

embryonic development lies between the shortvein and disk regions [Spencer et al. 1982; Segal and Gelbart 1985]. Recently, a number of dpp

The standard P-element-mediated DNA transformation strategy was used [Rubin and Spradling 1982]. The chromosomal locations of the integrated transposons were mapped genetically by segregation analysis with the dominant markers Curly [Cly] on the second chromosome and Ultrabithorax [Ubx] on the third chromosome. After establishment of balanced stocks of the transgenic lines, the sites of insertion were determined by in situ hybridization of 3H-labeled dpp DNA [fragment 83–91] to polytene chromosomes. The data for the transgenic lines carrying dpp8391 and, in one case, the opposite orientation dpp9183 are shown in Table 1.

**Transposon rescue of the Hin lethality**

To assay the transgenic lines for dpp function encoded on the transposon, we mated virgin females to males from stocks of several dpp

When males from these stocks are mated to females that
are wild type for dpp ("none", for no transposon; Table 2), the male progeny do not inherit the paternal X chromosome with the duplication of dpp. Therefore, males that inherit the paternal dppHm allele die as embryos because the one intact copy of the dpp gene they inherit is insufficient for embryonic development.

When the males with the X-linked duplication and the dppHm alleles were mated to females from the transgenic lines, the presence of the transposon-bearing chromosome permitted recovery of viable fertile males in spite of the fact that these males did not inherit the X-linked duplication of dpp (Table 2). The quantitative nature of the rescue by the transposon was determined by scoring the number of progeny in each of the progeny classes that did not involve transposon rescue of the mutant allele, calculating the total progeny in these classes, and dividing by the number of such classes to arrive at the average size of an individual progeny class. These are the denominators in Table 2. The numerator is the actual number of adult male flies scored in the experimental class, that is, the male progeny with the dppHm allele.

Each of the seven transgenic lines was able to rescue the haplo-lethality of five different dpp mutant alleles, including Df[2L]DfTD2, a large multigenic deletion that removes the entire dpp gene and portions of the chromosome on either side of dpp. dppHm37, dppHm46, dppHm47, and dppHm61 all contain a deletion or rearrangement breakpoint in their DNAs between coordinates 83 and 91 on the DNA (Gelbart et al. 1985). For the five rescued alleles, the actual number of rescued flies ranged from 66 to 100% of the expected number, based on sibling progeny classes. Although the different chromosomal locations of the transposons were expected to affect their level of expression and, thereby, their potency in rescuing mutant alleles, none of the transposon lines was significantly better or worse than the others in re-

### Table 1. dpp8391 transposon lines

| Plasmid injected | Transposon name | Mapped to chromosome | Location on polytene chromosome |
|------------------|----------------|----------------------|---------------------------------|
| pPADpp9183      | TnMH1         | 3                    | 64E,F                           |
| pPADpp8391      | TnJW1         | 3                    | 93A,B                           |
| pPADpp8391      | TnJW2         | 2                    | 23D                             |
| pPADpp8391      | TnJW3         | 2                    | 46C                             |
| pPADpp8391      | TnJfA1        | X                    | n.d.                            |
| pPADpp8391      | TnJfA2        | 3                    | 82A                             |
| pPADpp8391      | TnJfA3        | 3                    | 79E                             |

Progeny from seven different injected embryos survived the ethanol selection for the kdh gene. Single survivors from each of the seven embryos were used to establish a line of the transposon. The flies were crossed to an adh-null stock carrying the dominantly marked balancer chromosomes Cy0 and TM2. Ethanol-resistant progeny that were Cy and Ubx were backcrossed to an adh-null stock, and the segregation of the ethanol resistance was followed relative to the dominantly marked second and third chromosomes. After assignment of the transposon to a specific chromosome, a balanced stock of each of the transposon lines was established. The specific chromosomal location of the transposons was determined by preparation of salivary gland polytene chromosomes and hybridization with a 3H-labeled dpp probe [Pardue and Gall 1975]. Each of the lines used carried a single insertion of the transposon, as determined by in situ hybridization and by genomic Southern blot analysis of the DNA (data not shown).
scuing the haplo-lethality of one copy of the dpp gene.

For the transposons integrated on the X or third chromosomes, the experiments were designed so that the transposon segregated away from a dominant visible marker. Only one of the chromosomes in the transgenic mothers carried the transposon, and the homologous chromosome was dominantly marked with either Bar (B) eyes on the X, or Ubx or Tubby (Tb) on the third. In these cases, male progeny with the dppHm allele were viable only when they inherited the transposon-bearing maternal chromosome (Ubx+, Tb+, or B+). We did not recover males with the dpp mutation and the dominant marker (Ubx, Tb, or B) beyond the expected numbers of escapers from the haplo-lethality (Table 2).

The one exception was the failure of any of the transposon lines to rescue dppHm. However, this allele also failed to be rescued by Dp(2L)DTD48, a duplication of polytene bands 22E2-4 to 23A1 containing the entire dpp gene, one lethal complementation group proximal to dpp, and more than 70 kb of DNA, undefined genetically, distal to dpp. Because the duplication of the entire dpp gene did not yield dppHm adult progeny, it is not surprising that the small piece of the dpp gene on the transposon was unsuccessful. We expect that there may be some other lesion on the chromosome bearing dppHm that precludes viability with Dp(2L)DTD48 or the transposon dpp8391.

**Rescue of the ventralized null phenotype**

We concluded that one intact dpp gene and the transposon with only 8 kb of the dpp gene were able to provide all the dpp product required for correct development and survival to adulthood. However, mutations in dpp block at least three aspects of development: (1) embryonic dorsal–ventral pattern formation [Irish 1986], (2)...
larval growth and survival [Segal and Gelbart 1985], and (3) growth of imaginal disk tissues [Spencer et al. 1982]. We wished to determine which of the multiple functions of dpp were carried out by products expressed by the transposon.

To address this question, we produced animals heterozygous for two deletions of the dpp gene. These deletions should eliminate dpp products from both endogenous copies of the gene. The only intact copies of dpp protein-coding sequences in such animals would be supplied by the transposon. The deletions of dpp used in these experiments were Df(2L)DTD2, a large multigenic deletion that removes the entire dpp gene, and Df(2L)dppHm61, an internal deletion of 2.5 kb in the Hin domain [Spencer et al. 1982; Gelbart et al. 1985]. The Df(2L)dppHm61 allele does not remove DNA from the shortvein and imaginal domains, but it does behave genetically as a null allele for these functions in failing to complement mutant shortvein and disk dpp alleles. The deletion removes approximately 300 amino acids from the carboxyl terminus of the only reported protein-coding sequences at dpp [Padgett et al. 1987]. The ideal null allele would remove all dpp DNA without affecting flanking genes, but in the absence of such a deletion, we have used dppHm61 as the largest internal deletion of dpp sequences that behaves genetically as a null allele.

When the endogenous copies of dpp were eliminated by the two deletion alleles, the dead embryos had a completely ventralized cuticle [Gelbart et al. 1985]. The ventral denticle belts wrapped entirely around the circumference of the larval cuticle formed during embryogenesis [Fig. 2b]. Development of the extreme anterior and posterior ends of the embryo was affected severely: The posterior spiracles and the head structures, most prominently the cephalopharyngeal skeleton, were absent. Frequently, the most posterior denticle belts defined an unusual posterior invagination of the cuticle. There was usually a reduction in the number of segments as defined by the number of denticle belts.

The presence of one copy of the dpp transposon was sufficient to provide a normal ventral pattern of denticle belts [Fig. 2c]. However, the extreme anterior and posterior ends were still defective: The anterior end of the cuticle was often open as if head involution had not occurred, and, frequently, the posterior spiracles were not everted. The pattern of dorsal hairs was more variable than in a wild-type embryo; the anterior rows of thick dorsal hairs were usually present, but the broad areas normally covered with slender hairs were frequently bare. The cuticular phenotype was similar to that observed in animals with one Hin allele and one wild-type allele of dpp [Fig. 2d].

Two copies permit larval hatching

We quantitated the effect of the transposon on successful embryonic development and hatching of the larval form. Eggs from matings that would produce 25% dpp-null embryos with zero, one, or two copies of the transposon were collected, counted, and permitted to hatch. The chorion from the unhatched eggs and embryos was removed 48–72 hr after egg laying, and the extent of development was scored. Many of the unhatched eggs appeared to be undeveloped and exhibited the uniform opaqueness of an unfertilized egg in both experimental and control crosses (Table 3; undeveloped...
eggs). This was probably due to the highly inbred nature of the lines used in the experiment.

The remainder of the unhatched eggs contained dead embryos, with clear evidence of segmentation and cuticle formation even in the crosses that generated dpp-null animals with ventralized cuticles. The number of the unhatched, but developed, dead embryos was used as an indicator of the lethality due to the mutations in the dpp gene. By this criterion, null embryos with one copy of the transposon failed to hatch, independent of whether the transposon was derived from the maternal or paternal genome (Table 3). The embryos hatched to larvae when two copies of the transposon were present (Table 3). Thus, in the absence of the endogenous genes,

Table 3.  Transposon rescue of the dpp-null phenotype

| Parental genotypes [females × males] | Experimental class | Undeveloped eggs | Hatched embryos | Dead embryos | Percent dead |
|-------------------------------------|--------------------|------------------|-----------------|-------------|-------------|
| DfDTD2 × TnJW2                      | null              | 97               | 566             | 225         | 28          |
| DfDTD2 × TnMH1                      | null + 1Tn         | 94               | 472             | 136         | 22          |
| DfDTD2 × TnJA2                      | null + 1Tn         | 144              | 627             | 165         | 21          |
| DfDTD2 × TnJW1                      | null + 1Tn         | 207              | 694             | 207         | 23          |
| DfDTD2 × TnJW1                      | null + 1Tn         | 96               | 447             | 157         | 26          |
| DfDTD2 × TnJW2                      | null + 1Tn         | 74               | 639             | 134         | 17          |
| DfDTD2 × TnJW2                      | null + 1Tn         | 52               | 550             | 142         | 20          |
| DfDTD2 × TnJW1                      | null + 1Tn         | 81               | 423             | 26          | 6           |
| DfDTD2 × TnJW2                      | null + 2Tn         | 44               | 548             | 1           | <1          |
| DfDTD2 × TnJW1                      | null + 2Tn         | 67               | 494             | 23          | 4           |
| DfDTD2 × TnJW2                      | dpp* control       | 171              | 484             | 7           | 1           |
| DfDTD2 × TnJW2                      | dpp* control       | 65               | 498             | 3           | 1           |

Transposon rescue of the dpp-null phenotype. The dpp alleles used represent null mutations normally haplo-lethal but viable in the presence of one copy of the dpp transposon, as indicated here in the parental genotypes. The experimental class represents the 25% of the progeny that inherit both null alleles and from zero to two copies of the transposon. All of the other progeny classes generated in these crosses are adult viable. Control crosses (dpp ÷) with the dppH161 allele gave results comparable to those shown for DfDTD2. The large number of undeveloped eggs found in both experimental and control crosses are believed to be due to the inbred stocks used and independent of the specific transposon line or dpp alleles present. A binomial likelihood ratio test was used to examine the proportions of hatched versus dead embryos. Five out of six crosses that generated 'null + 1Tn' progeny produced significant decreases in the number of dead embryos (p < 0.005). The maternally derived TnMH1 produced no significant effect on the embryonic lethality in one copy (p = 0.15). Among the five null + 1Tn crosses that provided improved embryonic viability, there was no significant difference in the amount of viability provided by different transposon lines or by maternal vs. paternal inheritance of the transposon (p = 0.1–0.5).

Undeveloped eggs showed no development of cuticle or internal structures upon mechanical removal of the chorion 48–72 hr after egg lay; the appearance was the same as unfertilized eggs.

Dead embryos were removed mechanically from the chorion 48–72 hr after egg lay, ventral denticle belts were scored as an indication of cuticle formation.
the *dpp* sequences on the transposons provided sufficient product to permit embryonic development to the larvae. As in the case of the intact *dpp* gene, two copies of the transposon were necessary to provide enough of the embryonically required *dpp* products.

In experiments in which all progeny classes could be unambiguously identified at the adult stage by dominant visible markers, the homed animals in the progeny classes with two copies of the transposon did not develop to the pupal or adult forms. Although the *dpp* sequences on the transposon rescued the embryonic lethal phenotype, the rescued larvae did not develop further. We observed the same arrested larval development that was observed in animals trans-heterozygous for mutant *dpp*^{shv} alleles (Segal and Gelbart 1985).

We expected that a specific transposon might not provide sufficient product in two copies because the level of expression from a transposon may be reduced, compared with its parent gene, depending on the chromosomal position of the transposon (Hazelrigg et al. 1984). However, for the three transposon lines tested, all permitted hatching to larvae in two copies. The requirement for, and sufficiency of, two copies suggested that the quantitative level of expression of the *dpp* product from the transposon was similar to that from the intact gene.

Rescue of recessive embryonic lethals

The other *dpp* alleles that affect embryonic development are the recessive embryonic lethals (Spencer et al. 1982). These alleles must involve subtle changes in the DNA, as none of them is associated with a gross chromosomal rearrangement at *dpp*. These mutant alleles behave differently from the *dpp*^{Hin} alleles in that the recessive alleles are fully viable in the presence of one normal copy of *dpp*. However, the embryonic lethal alleles are similar to the *dpp*^{Hin} alleles in two respects: The embryonic lethal phenotype of an animal trans-heterozygous for two recessive lethal alleles is similar to the phenotype of an animal with one *dpp*^{Hin} allele and one wild-type *dpp* allele (Irish 1986); and, like the *dpp*^{Hin} alleles, most of the embryonic lethal alleles are also defective for shortvein and disk functions, as evidenced by a lack of genetic complementation. The recessive embryonic lethal alleles may be reduced, but not totally null, for the same *dpp* function eliminated by haplo-lethal alleles. If the recessive lethal alleles affect the same *dpp* product or products as do the haplo-lethal alleles, the *dpp* sequences on the transposon should rescue the embryonic lethality.

Two of the embryonic recessive lethal alleles, *dpp^{87}* and *dpp^{C38}*, were fully rescued to adulthood by one copy of the transposon in flies in which the other endogenous copy of *dpp* was deleted by Df[2L]DTC2 (Table 4). Unlike the other embryonic recessive lethal alleles, these two alleles complement lesions in both the shortvein and disk regions of *dpp*. The only *dpp* alleles they fail to complement are the other recessive embryonic lethal alleles. Because they were fully rescued by the transposon, we conclude that these mutations disrupt only the *dpp* product or products expressed from the DNA on the transposon.

The embryonic lethality in animals hemizygous for two other recessive lethal alleles, *dpp^{ain} and dpp^{n56}*, was overcome by the presence of two copies of the transposon (Table 4). Progeny heterozygous for the two recessive lethal *dpp* alleles were also fully rescued from the embryonic lethality by the presence of two copies of the transposon. However, the rescued animals did not survive to adults. In two cases, the number of dead pupae was approximately equal to the number of animals in the sibling progeny classes. These two cases involved the *dpp^{ain} allele, either as a hemizygote or as a heterozygote with *dpp^{n56}, in the presence of two copies of the transposon Tn/A2 (Table 4). The dead pupae exhibited a specific phenotype. The anterior portion of the pupal case was usually empty, and the posterior half was filled with amorphous tissue but not any adult cuticle. A similar phenotype was observed for severe (class V) imaginal disk *dpp* alleles.

Crossovers that generated progeny hemizygous for *dpp^{ain} with two copies of the transposon, or *dpp^{ain} with two copies of transposon Tn/A1, showed no significant pupal lethality (Table 4). From experiments in which all adult progeny classes could be identified unambiguously, we knew that these genotypes did not survive to adulthood. Therefore, these progeny classes must die as larvae. Although we have not examined the larval lethality quantitatively, we observed animals with arrested larval growth similar to that reported for the shortvein mutant alleles (Segal and Gelbart 1985).

The transposon does not affect adult viable imaginal and shortvein phenotypes

The larval and early pupal lethality observed in the presence of two copies of the transposon indicated that the transposon did not provide either shortvein or disk *dpp* functions. To test this hypothesis more fully, we generated flies that were hemizygous for a number of shortvein or imaginal disk mutant alleles by removing the other copy of *dpp* with Df[2L]DTC2 and providing *dpp* embryonic function with one copy of the transposon. In all cases, the shortvein and imaginal disk mutant phenotypes were observed, even for the phenotypically mildest shortvein (*dpp^{ahi}*) and disk (*dpp^{dho})* mutant alleles. A second copy of the transposon did not alleviate these mutant phenotypes.

Discussion

The technique of making transgenic *Drosophila* by P-element-mediated DNA transformation provides a way to assay the biological activity of specific DNA sequences in the whole animal. We have used this technique to show that a DNA fragment representing, at most, one-fifth of the *dpp* gene contains sufficient regulatory and structural sequences to rescue the ventralized embryonic phenotype and the embryonic lethality caused by a number of mutations in *dpp* (Fig. 3). The *dpp* function...
Table 4. Transposon rescue of the dpp recessive embryonic lethals

| Parental genotype (females × males) | Experimental class | Undeveloped eggs\(^a\) | Hatched embryos | Dead embryos\(^b\) | Dead pupae\(^c\) | Adults\(^d\) |
|-----------------------------------|--------------------|-------------------------|-----------------|------------------|-----------------|-------------|
| Gla / TnJW1 × dpp\(^{887}\) TnJW1 | dpp\(^{887}\) + 1Tn | nd                       | nd              | nd               | nd              | 60/59       |
| Gla / TnJW1 × dpp\(^{1338}\) TnJW1 | dpp\(^{1338}\) + 1Tn | nd                       | nd              | nd               | nd              | 53/53       |
| Gla / TnJW1 × dpp\(^{887}\) TnJW1 | dpp\(^{887}\) + 1Tn | nd                       | nd              | nd               | nd              | 60/59       |
| Gla / TnJW1 × dpp\(^{1338}\) TnJW1 | dpp\(^{1338}\) + 1Tn | nd                       | nd              | nd               | nd              | 53/53       |
| Gla / TnJW1 × dpp\(^{887}\) TnJW1 | dpp\(^{887}\) + 1Tn | nd                       | nd              | nd               | nd              | 60/59       |
| Gla / TnJW1 × dpp\(^{1338}\) TnJW1 | dpp\(^{1338}\) + 1Tn | nd                       | nd              | nd               | nd              | 53/53       |
| Gla / TnJW1 × dpp\(^{887}\) TnJW1 | dpp\(^{887}\) + 1Tn | nd                       | nd              | nd               | nd              | 60/59       |
| Gla / TnJW1 × dpp\(^{1338}\) TnJW1 | dpp\(^{1338}\) + 1Tn | nd                       | nd              | nd               | nd              | 53/53       |
| Gla / TnJW1 × dpp\(^{887}\) TnJW1 | dpp\(^{887}\) + 1Tn | nd                       | nd              | nd               | nd              | 60/59       |
| Gla / TnJW1 × dpp\(^{1338}\) TnJW1 | dpp\(^{1338}\) + 1Tn | nd                       | nd              | nd               | nd              | 53/53       |

Transposon rescue of the dpp recessive embryonic lethals. The dpp alleles tested are recessive lethals as trans-heterozygotes with each other. The dpp\(^{887}\) and dpp\(^{1338}\) alleles complement dpp imaginal disk and shortvein alleles, but dpp\(^{622}\) and dpp\(^{622}\) fail to complement most dpp imaginal and shortvein alleles. The alleles were tested as hemizygotes by use of Df(2L)DTD2 to remove the entire dpp gene. The proportions of hatched and dead embryos should be compared dpp\(^{+}\) control and dpp-null crosses in Table 3. Adult phenotypes were scored only for the first two crosses, because the other five crosses did not include sufficient visible marker mutations on the chromosomes to permit unambiguous assignment to progeny classes.

\(^a\) Undeveloped eggs showed no development of cuticle or internal structures upon mechanical removal of the chorion 48–72 hr after egg lay; the appearance was the same as unfertilized eggs.

\(^b\) Dead embryos were mechanically removed from the chorion 48–72 hr after egg lay; ventral denticle belts were scored as an indication of cuticle formation.

\(^c\) Number of dead pupae/expected number based on the number of sibling adult survivors.

\(^d\) Number of adults in experimental class/expected number based on total adult progeny.

Provided by the transposon rescues both the haplo-lethals and the recessive embryonic lethal alleles. The dominant lethality of the dpp\(^{887}\) alleles had confounded the genetic assignment of the haplo-lethal and recessive embryonic lethals to the same function, but the observation that both are rescued by the same defined piece of DNA is consistent with the idea that both affect the same dpp function. The difference in the genetic behavior of the two types of alleles is probably due to quantitative differences in the activity of the gene product produced by the mutant genes.

The line established with TnMH1 carries the dpp 83–91 EcoRI fragment in the opposite orientation relative to the adh gene on the transposon (Table 1). The orientation in the transgenic flies was confirmed by genomic Southern blotting. The orientation-independent rescue of the dpp\(^{887}\) alleles is consistent with transcription of the dpp sequences being driven from a promoter on the dpp fragment and not from the P element or adh promoters. We have not eliminated the possibility that cryptic promoter sequences present on the transposon are driving the expression of dpp transcription in either orientation. In addition, we have not determined whether the tRNA\(^{Tyr}\) gene on the transposon has any role in the rescue. Because most of the Hin alleles used in this work map within the sequences transcribed into mRNA products, and not near the tRNA sequences, we believe that the tRNA gene on the transposon is not responsible for the rescue of the dpp mutant phenotypes. Experiments in progress to define the dpp promoter and the RNA products made by the transposon will address these questions directly. It has been reported that four of the five dpp transcripts initiate in the shortvein domain (Padgett et al. 1987). We predict that the fifth transcriptional initiation site is within the 83–91 fragment.

The haplo-insufficiency of one intact copy of the dpp gene was rescued by one copy of the transposon, and the rescued animals survived to fertile, viable adults. We interpret this result to mean that there is a threshold quantity of dpp product required in the early embryo and that one copy of the dpp gene is incapable of supplying enough product. The dpp sequences on the transposon encode this dosage-sensitive product and supply additional quantities sufficient to attain the threshold level. Once the embryonic functions are accomplished, the survival to adulthood requires that the larval and imaginal disk functions of dpp are carried out. Because mutations in the imaginal disk and shortvein regions are strictly recessive in character, showing no haplo-insufficiency, it is likely that one intact copy of the dpp gene is sufficient to provide product for the imaginal disk and shortvein functions of dpp.
Mutant animals hemizygous for dpp alleles that affect only the embryonic function of dpp, dpp\textsuperscript{e87}, and dpp\textsuperscript{IC38} were also rescued to viable, fertile adults by the transposon [Fig. 3]. We should note that the chromosome bearing dpp\textsuperscript{IC38} used in these studies carries the dominant mutation Star (S) and the recessive mutation cinnebar (cn); when this chromosome is used in complementation tests, the dpp\textsuperscript{IC38} allele complements imaginal and shortvein alleles. It has been reported that dpp\textsuperscript{IC38} on a recombinant chromosome that does not carry S exhibits a temperature-sensitive lack of complementation with imaginal alleles (Irish 1986). We suspect that the transposon rescue to adults may be dependent on which dpp\textsuperscript{IC38} chromosome is used.

The effectiveness of the transposon dpp sequences was observed in the dpp-null embryo. First, one copy of the transposon in a dpp-null animal restored much of the dorsal pattern of the cuticle formed during embryogenesis. The circumferential pattern of the denticle bands was replaced by an essentially normal ventral pattern of the bands. However, the cuticle was not restored to a wild-type appearance. In particular, the dorsal hairs did not occupy as broad an area of the cuticle, and the extreme anterior and posterior cuticular structures were not normal. It will be of interest to characterize the role of dpp at the anterior and posterior poles of the embryo and to determine whether this function is coupled to the elaboration of dorsal–ventral pattern formation or whether it is a separate function of the dpp product.

The cuticular phenotypes and the failure of the animals to hatch from the egg case indicated that the dpp DNA on the transposon was as potent as, but no better than, one intact copy of the dpp gene. In fact, one copy of the transposon did provide a small, but statistically significant, decrease in the number of dead embryos [Table 3]. This is consistent with earlier observations that the haplo-lethality of dpp\textsuperscript{hm} alleles is leaky, yielding from 0 to 5% adult survivors, depending on the allele and its genetic background (Spencer et al. 1982). Thus, the transposon may give rise to approximately the same frequency of escapers as one intact copy of the dpp gene, although, for reasons discussed below, the transposon-induced escapers from the embryonic lethality do not survive to adults. The best indication of the sufficiency of the transposon sequences was the successful embryonic development and larval hatching imparted to the otherwise dpp-null embryo by two copies of the transposon.

The dpp\textsuperscript{hm} alleles and the dpp\textsuperscript{hm} recessive lethal alleles fail to complement lesions in the shortvein and imaginal disk functions of dpp. Therefore, although the earliest failure of the dpp\textsuperscript{hm} and dpp\textsuperscript{hm} alleles is during embryogenesis, the lesions also disrupt the later functions of dpp. The death of the mutant transgenic organisms at the larval stage indicated that the transposon did not provide the dpp functions needed for larval viability. Mutant alleles in the shortvein domain of dpp cause larval lethality (Segal and Gelbart 1985). The conclusion that the portion of dpp on the transposon is incapable of providing the dpp functions disrupted by short-vein mutant alleles is consistent with the fact that the transposon does not contain any of the dpp sequences in which shortvein mutant lesions have been mapped.

The survival to pupae was unexpected because the transposon does not contain sequences from the shortvein region. One explanation is that the dpp\textsuperscript{hm} recessive embryonic lethal allele is hypomorphic but not completely null for dpp shortvein function. When the hypomorphic allele is present, sufficient shortvein function is provided by the mutant gene to permit larval viability and survival to pupae. The animals die as early pupae with no evidence of adult cuticle formation, a phenotype very similar to that caused by the most severe lesions in the imaginal disk domain of dpp (Spencer et al. 1982). Neither the recessive embryonic lethal allele nor the dpp sequences on the transposon are able to provide the dpp imaginal functions necessary for growth of the imaginal disks.

However, the observation that only one of the two transposon lines allowed survival to pupae with the hemizygous dpp\textsuperscript{hm} allele raises the intriguing possibility that the product encoded on the transposon may be very
similar to that required for larval functions associated with the short vein domain. We have raised the point that different transposon lines can be expected to produce quantitatively different levels of protein because of position effects at the site of insertion. For those differences to be seen, the biological response has to be sensitive to the level of product. For the most part, we have not seen differences in the biological responses to the transposon, but the sensitivity may not have been high enough. The rescue to pupation by transposon line Tn/A2 is an indication that the level of expression from this transposon may be sufficiently high to provide product for the larval viability functions of dpp. Although the transposon does not contain any of the expected regulatory sequences from the short vein domain or the untranslated 5' exon from the short vein domain (Padgett et al. 1987), all of the known protein-coding sequences from the dpp gene are within the sequences on the transposon (Fig. 3). Thus, with enough copies being expressed at sufficiently high levels, some of the larval viability functions may be restored by the transposon.

In the early embryo, dpp may act in concert with a number of other Drosophila genes to establish or maintain the dorsal–ventral field of positional information, or dpp may respond to this field to specify the developmental fate of the cells along the dorsal–ventral axis. The identification of the portion of the dpp gene that exhibits the early embryonic function and the opportunity to assay the biological activity of this portion of the gene permit further molecular genetic manipulation of the dpp DNA sequences. The goal of these studies will be to define the molecular interactions between dpp and other components of dorsal–ventral pattern formation in the Drosophila embryo.

The dpp8391 transposon is not competent to provide the dpp product or products required for larval viability and growth of imaginal disk cells (Fig. 3). The dpp DNA fragment on the transposon is from the center of the dpp gene. There are at least 10 kb of DNA in the short vein domain to one side and more than 20 kb of DNA in the imaginal disk domain to the other side of the 83–91 fragment. The complementation patterns of mutant alleles could be explained if these flanking sequences contained cis-regulatory elements that acted upon the gene product encoded on the 83–91 fragment. For example, the reported cDNA structure indicates that alternate promoters and transcript initiation sites may reside in the short vein domain, even though the predicted protein-coding sequence is contained entirely within the 83–91 fragment (Padgett et al. 1987). The functional dissection of the dpp gene will be extended to the short vein and imaginal disk domains to identify sequences from these regions that are essential for dpp function in larval viability and imaginal disk growth.

**Experimental procedures**

**Genetic stocks**

Isolation of the dpp mutant alleles has been described previously (Gelbart 1982; Spencer et al. 1982; Segal and Gelbart 1985; Irish 1986). The TM6, B chromosome was described by Craymer (1984). All other genetic markers are described in Lindsley and Grell (1968).

**DNA manipulation**

Isolation and purification of plasmid DNA, restriction enzyme reactions, and DNA ligations were performed as described in Maniatis et al. (1982). Bacterial transformations into DH1 were performed as described by Hanahan (1983).

**DNA transformation**

Preblastoderm Drosophila embryos null for the adh gene were injected with DNAs from the plasmid pPAdpp8391 and from plasmid p25.7wc (Karess and Rubin 1984). The latter provides P-element transposase to catalyze integration of pPAdpp8391 but is unable to integrate itself. The animals that survive to adults are individually mated to flies null for adh, and the resulting progeny are screened for the transposon bearing the wild-type adh gene by tests for survival after a 24-hr exposure to 6% ethanol (Vigue and Sofer 1976). Individual survivors are used to establish transgenic lines by being mated to the adh-null stock.

**Cuticle preparation**

Unhatched embryos were dechorionated for 5 min in 50% bleach or by hand. The vitelline membrane was removed by a 10-min exposure to 10 ml of heptane : 9 ml of methanol : 1 ml of 0.5 M EDTA at pH 8.0. The embryos were treated with 4 : 1 glacial acetic acid : glycerol at 65°C for 12 hr and mounted in Hoyer's mounting medium (Van der Meer 1977).

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