The thick veins Gene of Drosophila Is Required for Dorsoventral Polarity of the Embryo

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ABSTRACT

We have discovered a new member of the class of genes controlling embryonic dorsoventral patterning. Mutants of the thick veins (tkv) gene have been described previously (as slater alleles) as embryonic lethal, lacking dorsal epidermis, but not as showing a recognizable dorsoventral phenotype. We show here that maternal alteration of function coupled with zygotic reduction of function of tkv is strongly ventralizing. In addition, in double heterozygous combinations in the mother, tkv mutations increase the ventralizing effect of dominant, weakly ventralizing alleles of the maternal effect, dorsoventral genes easter and cactus. An interaction is also seen with zygotic dorsoventral genes: tkv interacts maternally and zygotically in double heterozygotes with decapentaplegic and zygotically with screw in double homozygotes. We conclude that both maternally and zygotically supplied wild-type tkv product can play a role in dorsoventral patterning of the early embryo. On the basis of the phenotype of trans-heterozygous adult escapers, we propose that tkv might act by potentiating the activity of the zygotically acting decapentaplegic gene.

Establishment of dorsoventral polarity in the Drosophila melanogaster embryo is under the control of at least 12 maternal effect loci: the dorsal group genes and the cactus (cact) gene (Nüsslein-Volhard 1979; Anderson and Nüsslein-Volhard 1986; Schüpbach and Wieschaus 1989). Loss-of-function mutations in any of the dorsal group genes result in dorsalized embryos, i.e., an expansion of the dorsal regions of the embryo and reduction in ventral regions.

The product of the dorsal (dl) gene is a member of the rel/NFκB family of transcription factors (Steward et al. 1984; Steward 1987; Ghosh et al. 1990; Kieran et al. 1990) and is considered to be the dorsoventral morphogen. The 10 other maternal effect dorsal group genes act to positively regulate establishment of a nuclear concentration gradient of the dl protein (Roth et al. 1989a; Rushlow et al. 1989; Steward 1989). Cytoplasmic retention of the dl protein is mediated by the cact gene product, a member of the IκB family (Davis et al. 1991; Roth et al. 1991; Geisler et al. 1992; Kidd 1992; Nolan and Baltimore 1992). Loss-of-function mutations in the cact gene, as well as gain-of-function mutations in some of the other dorsal group genes, result in ventralization of the embryo, i.e., an expansion of ventral denticle belts and decrease in the amnioserosa.

The graded distribution of the dl protein is required for the spatially restricted expression of a small group of zygotic lethal pattern genes. The twist (twi) and snail (sna) genes are transcriptionally activated, and the zerknüllt (zen), decapentaplegic (dpp) and tolloid (tld) genes are transcriptionally repressed by dl (Doyle et al. 1986; Boulay et al. 1987; Hoffmann and Goodman 1987; Irish and Gelbart 1987; Rushlow et al. 1987; St. Johnston and Gelbart 1987; Thissen et al. 1987a,b, 1987, 1988, 1991; Ip et al. 1991; Kirson et al. 1994). This results in ventral expression (in the presumptive mesoderm) of twi and sna, and dorsal expression (in the presumptive amnioserosa and dorsal epidermis) of zen and dpp. Expression of these four zygotic dorsoventral patterning genes in spatially restricted domains is required for the formation of specific anlagen along the dorsoventral axis; mesoderm most ventrally, and amnioserosa most dorsally.

Genes that are transcriptionally activated in the embryo as a result of the dl protein gradient act to further refine the dorsoventral pattern and to establish anlagen along the dorsoventral axis. A gradient of dpp activity establishes fine-grained pattern in the dorsal 40% of the dorsoventral axis; the genes tld and short gastrulation (sog) function to regulate the dpp activity gradient (Ferguson and Anderson 1991, 1992a,b; Shimell et al. 1991; Wharton et al. 1993). Other zygotically acting genes that play a role in dorsoventral patterning of the embryo, in addition to the genes zen, twi and sna, mentioned above, are the genes shrew (sru) and screw (scw) (Tearel and Nüsslein-Volhard 1987; Arora and Nüsslein-Volhard 1992; Ferguson and Anderson 1992a). Loss-of-function mutations in the twi and sna genes...
genes lead to loss of mesendoderm-derived, i.e., most ventral structures, while loss of function mutations in dpp, tld, scw, zen, srw and sog lead to varying loss of dorsal-most pattern elements, i.e., amnioterosa and dorsal epidermis, and expansion of ventral elements (ARORA and NÜSSLIN-VOLHARD 1992; FERGUSON and ANDERSON 1992a).

We show here that the thick veins (tkv) gene is a previously undescribed member of the class of dorsoventral genes, and that, unlike other members of this class, it can play a role in embryonic dorsoventral patterning both maternally and zygotically. The original alleles of tkv were described as spontaneous, homozygous viable mutations resulting in thickening of the wing veins (LINDSLEY and ZIMM 1992). Screens for zygotic lethal mutants affecting pattern formation identified, in the region around tkv, alleles named slater (str) for which the mutant phenotype is a lack of dorsal epidermis (NÜSSLIN-VOLHARD et al. 1984); subsequently, these str alleles and multiple lethal alleles of tkv were found to belong to the same complementation group (SZIDONYA and REUTER 1988). The embryonic lethal phenotype of the tkv (str) gene is unique among described dorso-ventral genes: there is a dorsal hole in the cuticle, rather than an expansion of ventral (or dorsal) pattern elements of the embryo. Additionally, some alleles affect wing development. We describe here the phenotypic series and complex complementation pattern of all tkv alleles. Although loss of tkv function appears to be lethal in female germline clones, a maternal requirement for tkv is demonstrated by the fact that escaper females trans-heterozygous for two different tkv alleles produce moderately ventralized embryos. When this maternal reduction is coupled with zygotic loss of tkv function, strongly ventralized embryos result. The function of tkv as a dorsoventral gene is confirmed by its interaction in double mutant combination with the maternal effect genes easter (ea) and cact and with the zygotically acting genes dpp and scw. We discuss the similarity of the tkv phenotype to aspects of the dpp phenotype and consider the possibility that tkv acts by increasing dpp activity.

**MATERIALS AND METHODS**

**Strains:** The wild-type strain used was Oregon R. The tkv and dorsoventral mutations used are shown in Table 1. In the text and the table we use the previously published tkv allele designations (REUTER and SZIDONYA 1983; SZIDONYA and REUTER 1988), and tkv\(^1\) as the designation for the spontaneous tkv allele isolated by Nichols-Skoog, and originally referred to simply as tkv (LINDSLEY and ZIMM 1992). The str alleles str\(^{19}\) and str\(^{1B}\) (NÜSSLIN-VOLHARD et al. 1984), since they fail to complement multiple lethal tkv alleles (SZIDONYA and REUTER 1988), are now designated tkv\(^{19}\) and tkv\(^{1B}\).

The Df(2L)tkv\(^{43}\) allele is a deficiency that includes a strong Minute phenotype and a haplo-sterile site; thus Df(2L)tkv\(^{43}\)/SM1 females are sterile and the males semisterile (REUTER and SZIDONYA 1988). The strain carrying the Df(2L)tkv\(^{43}\) allele is maintained with the insertional transposition associated with the deficiency. From the inter se Df(2L)tkv\(^{43}\)/SM1; Tp(2;3)tkv\(^{43}\)/T(2;3)tkv\(^{43}\)/SM1 cross, two types of dead embryos are observed: among the Df/Dfembryos the tkv embryonic lethal phenotype is not easily recognizable since other lethals are included in the deficiency, in particular the schlaff gene, mutants of which show an abnormal arrangement of cuticle and a tilted head skeleton (NÜSSLIN-VOLHARD et al. 1984). The other dead embryos, namely Df/Df; Tp/TM3 and Df/Df; Tp/Tp, show the weak embryonic lethal tkv phenotype seen in Figure 1D.

The second chromosome balancers used were SM1, SM5, CyO or SM6b (LINDSLEY and ZIMM 1992). In the text these balancers are referred to collectively as Cy when more than one balancer was used. All crosses were performed at 25° unless otherwise specified.

**Cuticle and wing preparations:** Embryos were collected on yeasted agar plates (0.5% agar, 3.5% sucrose, 3.5% (vol/vol) acetic acid), dechorionated in 50% sodium hypochlorite and rinsed in H\(\text{2}O\). The embryos were then fixed for 25 min, with rotation, in a 1:1 two phase mixture consisting of (1 × PBS, 10% formaldehyde, 50 mM EGTA):heptane. After fixation, embryos were devitellinized by vortexing in 9:1 methanol:heptane and dehydrated in an ethanol series. They were then mounted in Hoyer’s medium (VAN DER MEER 1977) and incubated overnight at 60°. Wings were dissected, collected in 70% ethanol and mounted in Euparal.

**Germline clones:** Germline clones were generated using the F2/**ouv\(^{128}\) ovu/iov\(^{53}\); FMO; F(); ovu/iov\(^{11}\) 8.2/msl-1 msl-2 mle strain (MEVEL-NINIO et al. 1994) that carries two ovu\(^{11}\) alleles.

### Table 1
<table>
<thead>
<tr>
<th>Mutation</th>
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<td></td>
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<td>3</td>
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<td>REUTER and SZIDONYA (1983)</td>
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<td>scw(^{a})</td>
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<td>Described here</td>
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\(^{a}\) 1, Bowling Green Stock Center; 2, KATHRYN ANDERSON; 3, J. SZIDONYA and G. REUTER; 4, Tübingen Stock Center.
thick veins Is a Dorsoventral Gene

FIGURE 1.—Effect of alteration of tkv activity on embryonic phenotype. Cuticles were prepared as described in MATERIALS AND METHODS. The embryos, in this and subsequent figures, are oriented with anterior to the left and dorsal up unless otherwise specified. The arrows indicate the Filzkörper. The wild-type cuticular pattern is shown in (A). In (B) through (E) are shown embryos homozygous for various tkv alleles, and in (F) through (H) embryos carrying at least one tkv\textsuperscript{null} allele. Three classes of tkv mutant cuticle are seen. The null phenotype, characterized by an absence of dorsal epidermis, is seen in embryos homozygous for the deficiency tkv\textsuperscript{Sz2} (B) and for the allele tkv\textsuperscript{12} (C), as well as in embryos homozygous for the alleles tkv\textsuperscript{Sz3} (see Figure 5D), tkv\textsuperscript{12}, tkv\textsuperscript{10} and tkv\textsuperscript{11B} (not shown). A phenotype characterized by weak head defects, wrinkled dorsal epidermis and internalized and often disorganized Filzkörper is seen in embryos homozygous for the alleles tkv\textsuperscript{11D} (D) and tkv\textsuperscript{11S} (E). A weakly ventralized (V4) phenotype is seen in embryos carrying the semidominant tkv\textsuperscript{11} allele. An embryo from an inter se tkv\textsuperscript{11}/SM5 cross is shown in (F); strong head defects, internalized and disorganized Filzkörper, internalized seventh and/or eighth abdominal segments, and denticle belts slightly extended laterally are seen. A similar weakly ventralized phenotype is observed in heterozygotes, independently of whether the tkv\textsuperscript{11} allele is brought in from the mother or the father, as seen in the tkv\textsuperscript{11}/+ embryo shown in (G). A stronger ventralizing effect of the tkv\textsuperscript{Sz1} allele is seen at a higher temperature, as is seen in the moderately ventralized (V3) embryo from an inter se tkv\textsuperscript{Sz1}/SM5 cross at 29°: strong head defects, internalized and severely reduced Filzkörper, internalized terminal abdominal segments, and moderately laterally extended ventral denticle belts are observed (H).

RESULTS

Embryonic lethal phenotypes: The tkv zygotic embryonic phenotype, as originally described for the tkv\textsuperscript{10} (str\textsuperscript{10}) and tkv\textsuperscript{11B} (str\textsuperscript{11B}) EMS-induced alleles, consists of a dorsally open cuticle, a severely defective head and a constricted epidermis which gives the cuticle a rounded appearance (Nüsslein-Volhard et al. 1984; Tearle and Nüsslein-Volhard 1987; Figure 1, B and C). Additional tkv alleles were isolated as lethals (Reuter and Szidonya 1983; Szidonya and Reuter 1988); one of these, tkv\textsuperscript{Sz2}, is a small, cytologically visible deficiency (Reuter and Szidonya 1983) and can be considered to define the null phenotype. Cuticle preparations of embryos homozygous for the tkv\textsuperscript{Sz2} deficiency show a phenotype similar to that previously described for tkv\textsuperscript{10} and tkv\textsuperscript{11B} (Figure 1B). Two other tkv alleles isolated by Szidonya and Reuter (1988) show the same cuticular phenotype as the tkv\textsuperscript{Sz2} deficiency and can thus by this criterion be considered amorphs, namely tkv\textsuperscript{12} (Figure 1C) and tkv\textsuperscript{33} (see Figure 5D). The Filzkörper appear normal in cuticle preparations from all of these tkv alleles. Examination of sectioned tkv\textsuperscript{Sz2} embryos (not shown) reveals...
no obvious defects until stage 15, at which point it can be seen that dorsal closure and head involution are not occurring, the exposed amnioserosa is beginning to degenerate and internal organs are beginning to be extruded dorsally. Since germband retraction occurs normally, the contraction of the embryos seen in cuticle preparations (Figure 1, B and C) is probably due to a secondary effect of the failure of dorsal closure. The absence of dorsal epidermis-derived structures from the cuticle may be due to degeneration of the dorsal epidermis.

Three additional alleles isolated by Szidonya and Reuter (1988), tkv<sup>sl1</sup>, tkv<sup>sl3</sup> and tkv<sup>sl15</sup> (see Table 1), are also embryonic lethal as homozygotes. When placed over the loss-of-function allele tkv<sup>sl2</sup>, the alleles tkv<sup>sl1</sup> and tkv<sup>sl3</sup> give the typical “dorsal-open” phenotype (data not shown). The allele tkv<sup>sl15</sup>, although not lethal over the tkv<sup>sl2</sup> deficiency, is lethal over the other tkv null alleles, i.e., tkv<sup>y</sup>, tkv<sup>ib</sup>, tkv<sup>sl2</sup> and tkv<sup>sl3</sup>. In contrast to embryos homozygous for the tkv null alleles, however, tkv<sup>sl1</sup>, tkv<sup>sl3</sup> and tkv<sup>sl15</sup> homozygous embryos do not form the “dorsal open” cuticle. tkv<sup>sl2</sup> and tkv<sup>sl15</sup> embryos have a similar phenotype: the dorsal epidermis appears wrinkled and head involution is abnormal (although more head structures are formed than in tkv null embryos); the Filzkörper are often internalized and reduced in size (Figure 1, D and E).

The tkv<sup>sl1</sup> allele is unique among the tkv alleles in that it has a phenotype as a heterozygote: about 40% of the embryos from either tkv<sup>sl1</sup>/SM5 mothers or fathers crossed to wild type die; the head is severely defective, the Filzkörper are internalized and markedly reduced, the eighth abdominal segment is internalized, and the ventral denticle belts are extended laterally (Figure 1G; see Table 3). Similar phenotypes are observed among the progeny of flies trans-heterozygous combinations discussed below exclusively in females.

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We performed crosses to test for complementation among all tkv alleles. Our results, summarized in Table 2, confirm and extend the results of Szidonya and Reuter (1988). All lethal tkv alleles, as trans-heterozygotes with the tkv<sup>y</sup> allele, give viable progeny that show a wing phenotype. In addition, some trans-heterozygous combinations of lethal alleles give a small number of adult progeny that show a wing phenotype.

We have defined five classes of tkv wing phenotype and ordered them in terms of increasing strength. The weakest phenotype, class 1, corresponds to that seen in tkv<sup>y</sup> homozygotes (Figure 2B). In the class 2 phenotype, thickening of the wing veins is more extreme than in class 1 wings (Figure 2C). In going from class 3 to class 5, the wing becomes progressively shortened along the proximal-distal axis, and the tip of the wing becomes more rounded (Figure 2D–G). It is on the basis of these phenotypic alterations that we have placed the class 3 phenotype as an intermediate between 2 and 4, as other features of the class 3 phenotype make it difficult to place in a phenotypic series. In the class 3 phenotype, which is displayed by the tkv<sup>sl15</sup>/tkv<sup>y</sup> and tkv<sup>sl10</sup>/tkv<sup>y</sup> trans-heterozygotes, there is no vein thickening, but rather the wing veins L4 and L5 are interrupted and the transverse veins are partially missing (Figure 2D). The class 4 and 5 phenotypes constitute a clear continuum with the class 1 and 2 phenotypes in that they display an increasingly greater thickening of veins (Figure 2, E–G).
While viability of the trans-heterozygous class 1 to class 4 flies is not significantly different from that of their tkv/Cy sibs, the viability of the class 5 trans-heterozygous progeny is significantly reduced: the number of class 5 adults that appear can be as low as 5% (among females) relative to the number of tkv/Cy sibs. Consistent with our observation that the wing phenotype is stronger in females, viability of the trans-heterozygotes is lower for females than for males.

In the most extreme phenotypic category, class 5, seen only in combinations with the tkvsz3 allele, the wing veins are extremely thickened and the wings are also severely crumpled and blistered (Figure 2, F and G). In addition to these stronger wing defects, there are dramatic effects on the body seen only in class 5 flies: the scutellum is noticeably shorter along the anteroposterior axis; most significantly, a deep median furrow is present in the dorsal part of the thorax, and the tergites are incompletely fused along the dorsal midline of the abdomen (Figure 2H). An additional defect is seen in two allelic combinations of the class 5 category: tkv<sup>ns3</sup>/tkv<sup>10</sup> and tkv<sup>ns3</sup>/tkv<sup>10b</sup> female legs lack distal material, i.e., the tarsal claws and some tarsal segments are missing. Similar truncations of the distal portion of the leg are seen in strong dpp viable alleles (Spencer et al. 1982).

**Maternal phenotype:** To test whether there is a requirement for maternal expression of the tkv gene, we generated germline clones using two ovo<sup>D'</sup> genes transposed onto the second chromosome (MévéL-Niño et al. 1994). w/w; tkv<sup>+/</sup>/CyO virgin females were crossed to w<sup>+</sup> progeny were X- or γ-irradiated to induce mitotic recombination clones (see MATERIALS AND METHODS). The 290 irradiated w<sup>ovo<sup>D1</sup></sup>Y; P[w<sup>+</sup>ovo<sup>D1</sup>]/msl-1 msl-2 mle males. The progeny were X- or γ-irradiated to induce mitotic recombination clones (see MATERIALS AND METHODS). The 290 irradiated w<sup>ovo<sup>D1</sup></sup>Y; P[w<sup>+</sup>ovo<sup>D1</sup>]/msl-1 msl-2 mle males. The progeny were X- or γ-irradiated to induce mitotic recombination clones (see MATERIALS AND METHODS). The 290 irradiated w<sup>ovo<sup>D1</sup></sup>Y; P[w<sup>+</sup>ovo<sup>D1</sup>]/msl-1 msl-2 mle males. After irradiation of the first instar larvae, 165 w<sup>+</sup> females were recovered, of which 5% were fertile and produced w progeny. Our inability to obtain embryos from tkv/tkv germline clones indicates that the tkv/tkv genotype is lethal in the germline. While this inability could be due to the presence of a cell-lethal mutation other than tkv on the tkv<sup>10</sup> chromosome, the fact that atrophic ovaries are found in trans-heterozygous escaper tkv females (described below) is
Consistent with the idea that tkv function is required for oogenesis.

The availability of a small number of (escaper) females trans-heterozygous for several different tkv alleles (Table 2) provided another means of testing for maternal effect of the tkv gene. We examined the progeny of the class 5 (strongest wing phenotype) female escaper genotypes indicated in Table 2. Adult tkv$^{S3}$/tkv$^{FL}$ females are found at a very low frequency (5% of the expected number, compared to tkv/Cy sibs), are extremely weak, possess atrophic ovaries and do not lay eggs in the few days that they survive beyond eclosion. Adult tkv$^{S3}$/tkv$^{10}$ females are recovered at 20% of the expected number (compared to tkv/Cy sibs) and are the strongest viable trans-heterozygous combination that lays eggs. Embryos from these females crossed to wild-type males have a normal appearing egg shell with correctly positioned dorsal appendages and a normal appearing vitelline membrane. These embryos, however, die and show a moderately ventralized (V3) cuticular phenotype (Figure 3A, Table 3). The V3 phenotype is characterized by internalization of the posterior abdominal segments and Filzkörper; also, the Filzkörper are disorganized and reduced in size (often appearing as only two small dots). The ventral denticles are extended laterally. Also, the maxillary sense organs are missing and the head is not involuted; rather, there is a constriction between the head and trunk region and the head is extended and convoluted. This V3 phenotype is similar to that described for embryos carrying certain dpp alleles (Wharton et al. 1993).

Adult females of the genotype tkv$^{S3}$/tkv$^{a12}$ are seen at a higher frequency (50% of the expected number, compared to that of tkv/Cy sibs). A much weaker ventralizing maternal effect is observed among the progeny of these females crossed to wild-type males: only 9% of the embryos die and these are weakly ventralized (V4 in Figure 3B and Table 3). In the V4 phenotype, the head is not involuted, structures posterior to the seventh abdominal segment are internalized and the Filzkörper are disorganized. Of the other viable combinations indicated in Table 2, tkv$^{S1}$/tkv$^{a22}$ and tkv$^{S3}$/tkv$^{a15}$ females, when crossed to wild-type males, also produce embryos, a minor fraction of which (about 10%) are weakly ventralized.

We also examined the maternal effect of allelic combinations with the tkv$^{S1}$ allele. As was described above, the tkv$^{S1}$ allele has a semidominant zygotic effect that leads to weak ventralization of 40% of the embryos from tkv$^{S1}$/SM5 females crossed to wild-type males; thus about 80% of the embryos that carry the tkv$^{S1}$ allele are weakly ventralized (Figure 1G, Table 3). When tkv$^{S1}$/tkv$^{a12}$ females are crossed to wild-type males, most of the embryos die and are weakly ventralized (Figure 3D, Table 3).

When reduction of zygotic tkv activity is added to the maternal reduction described above, there is a significant increase in ventralization of the phenotype. This is seen most dramatically among the progeny of tkv$^{S3}$/tkv$^{10}$ mothers. When these females are crossed to wild-type males, almost all of the embryos produced are moderately ventralized, as described above. When the same females are crossed to tkv$^{S2}$/SM6b males, however, two types of embryos are obtained: half are moderately ventralized and the other half are strongly ventralized (V2) (Table 3). This strongly ventralized phenotype is comparable to that described for embryos homozygous for null alleles of dpp (Irish and Gelbart 1987; Årora and Nüsslein-Volhard 1992), and is characterized by the absence of Filzkörper and by ventral denticle bands that surround the entire embryo (Figure 3C). As strongly ventralized embryos were never observed in crosses of any tkv/Cy female to any tkv/Cy male, and since the strongly ventralized embryos constitute 50% of the progeny (the same fraction that is tkv/tkv), we conclude that the V2 phenotype is due to the combined effect of both maternal and zygotic loss of tkv function.

Strongly ventralized embryos are also observed in crosses of tkv$^{S1}$/tkv$^{a12}$ females to all tkv-lethal/Cy males except tkv$^{S15}$, i.e., in crosses to males carrying tkv$^{S3}$, tkv$^{S1}$, tkv$^{a12}$, tkv$^{a33}$, tkv$^{10}$ tkv$^{10}$ or tkv$^{S2}$ (Table 3; Figure 3C, and data not shown). Finally, when tkv$^{S1}$/tkv$^{S15}$ females are crossed to males of the same genotype, there is a slight increase in the number of dead embryos, and, at a low frequency, moderately ventralized (V3) embryos are seen (Table 3; Figure 3, E and F).

These increases in ventralization resulting from maternal and zygotic decrease in tkv activity can be detected relatively early in embryogenesis. Thus, observation of living embryos from tkv$^{S3}$/tkv$^{a12}$ inter se crosses
revealed a class of embryos in which germ band elongation was incomplete and in which there was a deep dorsal cephalic furrow. These embryos, when their cuticles were observed at a later stage, were strongly ventralized (data not shown).

The results, summarized in Table 3 and shown in Figure 3, indicate that a reduction of the \( tkv \) product maternally results in partially ventralized embryos. When this maternal reduction is coupled with zygotic reduction or loss of \( tkv \) activity, the degree of ventralization is increased.

**tkv interacts with dorsoventral patterning genes:** Further support for the notion that the \( tkv \) gene plays a role in establishing embryonic dorsoventral pattern would be the demonstration of an interaction between \( tkv \) mutations and mutant alleles in various well described dorsoventral patterning genes. Since there is a maternal component for \( tkv \) function, we tested interactions with ventralizing alleles of the maternally acting genes \( ea \) (Chasan and Anderson 1989) and \( cact \) (Rotth et al. 1991).

Maternal and zygotic reduction of \( tkv \) activity enhances the ventralizing effect of weakly ventralizing \( ea \) and \( cact \) alleles. The dominant gain-of-function \( ea \) allele, \( ea^{61.13} \), is incompletely penetrant and weakly ventralizing (Jin and Anderson 1990). As shown in Table 4, 62% of the embryos from \( ea^{61.13}/TM3 \) females crossed to wild-type males die, and are very weakly ventralized (Figure 4A). This phenotype, in which the Filzkörper appear normal, corresponds to the weakest ventralizing phenotype that we observed (V5 in Table 4). The only defect common to all of the dead embryos from this cross is a failure in head involution. A minority of the dead embryos (25%) have a tail-up phenotype, presumably due to incomplete retraction of the germ band. When \( tkv \) activity is also reduced, i.e., in \( tkv/+; ea^{61.13}/+ \) females, embryos are produced (in crosses to wild-type males) that almost all die and show an increased level of ventralization. All of the \( tkv \) null alleles, including the \( tkv^{62} \) deficiency, give similar results in this type of cross (Table 4): about 40% of the embryos remain very weakly ventralized (V5), roughly 60% are now weakly ventralized (V4), and a small fraction (about 1%) is moderately ventralized (V3).

When \( tkv \) activity is also removed zygotically, there is an increase in ventralization, i.e., when the same females
ventralized embryos: about 75% are moderately ventralized; ventral denticle belts surround the entire embryo; Filzkörper are disorganized, but rather the head and reduced; head structures are missing and the head is not invected (VOLHARD 1992). However, they produce more strongly ventralized (V4) and a significant proportion (15 to 22%, Table 4) are now moderately (V3; Figure 4, B and C) to strongly ventralized (V2, Figure 4D).

A similar effect is observed when tkv loss-of-function mutations are placed in heterozygous combination with the haploinsufficient dominant maternal effect allele cact99 (ROTH et al. 1991). As shown in Table 4, 6% of the embryos from cact99/Cyo females crossed to wild-type males die and are weakly ventralized (V4) (Figure 4E). When females are heterozygous for both a tkv null allele and the cact99 allele (tkv +/+ cact99 females crossed to wild-type males), however, they produce more strongly ventralized embryos: about 75% are moderately ventralized and 1% are strongly ventralized (Table 4). A fourth class of embryos (V1) is also observed among progeny from this cross. The V1 phenotype is very strongly ventralized, with patches of disorganized ventral denticles encircling the embryo, and holes in the cuticle (similar to the embryo shown in Figure 4H). This V1 phenotype appears similar to the phenotype described for embryos from TollE mothers (ANDERSON et al. 1985) or from germline clones of cact lethal alleles (ROTH et al. 1991). Note that the V1 phenotype as described by ROTH et al. (1991) has expanded mesoderm, while in TollE embryos there is no substantial mesoderm expansion. As was observed with the ec1611 allele, crosses of transheterozygous tkv +/+ cact99 females to tkv/Cyo males results in embryos in which the degree of ventralization is increased (Table 4). About 60% of embryos from such crosses are moderately ventralized (Figure 4F), 10% are strongly ventralized (Figure 4G) and 30% are very strongly ventralized (Figure 4H). No significant increase in ventralization was observed when crossing ec1611 +/+ or cact99 females to tkv/Cyo males; thus we were not able to detect a purely zygotic interaction of tkv with these ea and cact alleles.

The fact that reduction of tkv activity in mothers carrying weakly ventralizing alleles of ea and cact results in an increase in ventralization (seen in more than 50% of the progeny) supports the idea that there is a maternal requirement for tkv. The additional degree of ventralization brought about by removing tkv activity zygotically as well is consistent with the demonstrated zygotic requirement for tkv and further supports the notion that the tkv gene plays a role in establishing dorsoventral pattern.

**Interaction of tkv with the zygotic dorsoventral genes dpp and scw.** To further explore the function of tkv in establishing the dorsoventral axis, we tested the interaction of loss-of-function tkv alleles with loss-of-function alleles of the zygotic dorsoventral patterning genes dpp and scw.

The allele dppk82/+, which produces a moderately ventralized phenotype when homozygous (Figure 5B), has a haploinsufficient effect: about one-quarter of dppk82/ + zygotes die as embryos and show a very weakly to weakly ventralized phenotype (Figure 5A); a smaller
thick veins Is a Dorsoventral Gene

FIGURE 4.—Maternal and zygotic interaction of tkv null alleles with semidominant, weakly ventralizing ea and cact alleles. Cuticle preparation and orientation are as described in Figure 1. The phenotypic categories are described in Tables 3 and 4. The very weakly ventralized (V5) semidominant maternal effect of the ea<sup>61,13</sup> allele is seen in an embryo from the cross ea<sup>61,13</sup>/CyO females x +/+ males (A): the cuticle appears normal in all regards except that the head is not involuted. When, in addition, tkv activity is reduced twofold in the mother and eliminated zygotically, i.e., in roughly one-quarter of the embryos from the cross tkv<sup>02</sup>/+: ea<sup>61,13</sup>/+ females x tkv<sup>02</sup>/CyO males, an increased degree of ventralization is seen (B–D). This can range from a moderately ventralized phenotype (V3), in which the ventral segments are internalized and the head structures and Filzkörper are significantly reduced (B and C, both ventral views), to a strongly ventralized phenotype (V2), in which the ventral denticle belts surround the entire circumference of the embryo and Filzkörper are absent (D). The semidominant maternal effect of the cact<sup>99</sup> allele, leading to a weakly ventralized phenotype (V4), is seen in an embryo from the cross cact<sup>99</sup>/CyO females x +/+ males (E): there are head defects and a “tail-up” phenotype, probably due to defects in extension and/or retraction of the germ band. When tkv activity is concomitantly reduced in the mothers and eliminated in the progeny, i.e., in embryos from the cross tkv<sup>02</sup>+/+: cact<sup>99</sup> females x tkv<sup>02</sup>/CyO males, moderately ventralized (V3) to very strongly ventralized (V1) phenotypes are observed: the ventral denticle belts partially encircle the embryo in (F) (ventral view), and completely encircle the embryo in (G). Some embryos from crosses in which tkv is reduced maternally and eliminated zygotically in combination with cact<sup>99</sup> are very strongly ventralized (V1): the embryo from the cross tkv<sup>02</sup>+/+: cact<sup>99</sup> females x tkv<sup>02</sup>/CyO males is very small, with no head structures, only patches of ventral denticle belts are present, cuticular material is missing, and no Filzkörper are present (H).

fraction die before reaching adulthood (WHARTON et al. 1993). We have determined the percentage of tkv/dpp progeny relative to the total adult flies recovered. If there were no haploinsufficient effect of dpp<sup>h<sub>27</sub></sup> and no interaction between tkv and dpp, one-third of the adult flies should be tkv/dpp (the C<sub>y</sub>/C<sub>y</sub> genotype being lethal). If we take into account the decrease due to the haploinsufficient dpp effect, then approximately one-quarter of the adults should be tkv/dpp if there is no interaction between the genes. From crosses in which the tkv<sup>02</sup> or tkv<sup>jjb</sup> alleles were provided maternally and dpp<sup>h<sub>27</sub></sup> paternally, no adult dpp<sup>h<sub>27</sub></sup>+/+ tkv progeny were obtained (Table 5). In similar crosses with the tkv<sup>aa33</sup> allele, only a small fraction of the dpp<sup>h<sub>27</sub></sup>+/+ tkv progeny survived. With the weaker allele dpp<sup>h<sub>47</sub></sup>, an effect is seen when the tkv<sup>02</sup> allele (but not the tkv<sup>jjb</sup> and tkv<sup>aa33</sup> alleles) is provided maternally (Table 5). The adult lethality indicated in Table 5 can be accounted for by lethality during embryogenesis: this was determined by counting dead embryos (which have a weakly ventralized phenotype, see Figure 5C) from these crosses.

When the tkv allele is provided paternally, there is no apparent interaction with these dpp alleles (Table 5). This result might initially be interpreted to indicate that tkv plays only a maternal role in this interaction with dpp. The interaction of tkv with dpp is not exclusively
FIGURE 5.—Maternal and zygotic interaction of tkv with dpp and scw. Cuticle preparation and orientation as in Figure 1. The dpp<sup>h27</sup>/+ genotype gives a very weakly ventralized (V5) phenotype: head structures are abnormal, the embryo has a slight “tail-up” character, and the dorsal epidermis appears wrinkled and necrotic (A). The dpp<sup>h27</sup>/dpp<sup>h27</sup> genotype gives a moderately ventralized (V3) phenotype in which there are strong head defects, the A6, A7 and A8 segments are internalized, the Filzkörper are markedly reduced, and the ventral denticle belts are extended laterally (B); the ventral side of the embryo is tilted toward the viewer, increasing the apparent extent of ventralization. When maternal and zygotic reduction of tkv activity is combined with zygotic reduction of dpp activity (see text), i.e., in the double heterozygotes dpp<sup>h27</sup>+/tkv<sup>h27</sup>, a weakly ventralized (V4) phenotype is seen: the head is very defective, the A7 and A8 segments are internalized, the Filzkörper have an abnormal morphology and the ventral denticle belts are extended laterally (C). The tkv<sup>h33</sup>/tkv<sup>h33</sup> homozygote shows the typical tkv zygotic null phenotype lacking dorsal epidermis (D). The scw<sup>h</sup>/scw<sup>h</sup> homozygote gives a moderately ventralized (V3) phenotype typical for scw mutants: the head is very defective, terminal segments are internalized, Filzkörper material is strongly reduced and the ventral denticle belts are extended laterally (E). When these tkv and scw alleles are combined, in tkv<sup>h33</sup> scw<sup>h</sup>/tkv<sup>h33</sup> scw<sup>h</sup> embryos, a strongly ventralized (V2) phenotype is seen: the ventral denticle belts surround the embryo, and head structures and Filzkörper are absent (F).

maternal, however, since when tkv<sup>h33</sup>/SM66 females are crossed to dpp<sup>h27</sup>/CyO males, dpp<sup>+</sup>+/tkv<sup>+</sup> transheterozygous adults constitute only 3% (Table 5), while dpp<sup>h27</sup>/SM66 and tkv<sup>h33</sup>/CyO adults constitute 53% and 44%, respectively, of the total adult flies recovered. It was not possible to discriminate between the two categories in the other crosses because both balancers were CyO. We conclude that maternal and zygotic reduction of tkv activity increases the lethality caused by a zygotic decrease in dpp activity, while a zygotic reduction alone has no significant effect.

Zygotic loss of both tkv and scw activity has an additive effect on ventralization of the embryonic phenotype. This was discovered fortuitously, as we observed, among embryos produced by our tkv<sup>h33</sup> stock, some with a strongly ventralized phenotype (Figure 5F). The tkv<sup>h33</sup> allele alone shows a strong tkv null phenotype (Figure 5D). Further analysis revealed that an allele of scw (Teare and Nüsslein-Volhard 1987; Arora and Nüsslein-Volhard 1992), which we designate scw<sup>h</sup>, had arisen spontaneously in our stock. The scw<sup>h</sup> mutation behaves as a typical loss-of-function scw allele (Arora and Nüsslein-Volhard 1992); as a homozygote, it has a moderately ventralizing phenotype (Figure 5E). The doubly homozygous combination tkv<sup>h33</sup> scw<sup>h</sup> gives a distinctly different, strongly ventralized phenotype (Figure 5F). In crosses of tkv<sup>h33</sup> scw<sup>h</sup>/SM66 females to scw<sup>h</sup>/SM66 males, the resultant dead embryos have the typical moderately ventralized scw<sup>h</sup> phenotype. This indicates that the phenotype observed in doubly homozygous tkv scw embryos is not due solely to reduction of tkv activity maternally, but must also include loss of activity of both genes zygotically.

In summary, reduction of tkv activity both maternally and zygotically enhances the haploinsufficient ventralizing effect of certain dpp alleles, while elimination of
were crossed with the alleles brought in by the indicated parent and scored for each cross. The percentages correspond to the number of adult flies of the indicated genotype compared to the total number of adult flies recovered. Between 200 and 300 flies were counted from each cross.

### DISCUSSION

Previous characterization of the tkv (str) gene showed that it is required zygotically for differentiation of dorsal epidermis (Nüsslein-Volhard et al. 1984) and that various lethal tkv alleles in combination with the spontaneous allele tkv' affect the wing and the dorsal medial cuticle of the adult (Szidonya and Reuter 1988). Our work adds several crucial points to an understanding of the tkv gene function. First, reduction of function (via null, or loss-of-function alleles) and alteration of function (via a gain-of-function allele) lead to ventralization of the embryo. This indicates that tkv plays a role in correct dorsoventral patterning of the early embryo. Second, there is both a maternal and zygotic component of tkv function in this dorsoventral patterning. Third, tkv plays multiple roles in development, not only in embryonic dorsoventral patterning (described here) and dorsal epidermis formation, wing development and dorsal medial integument development (described previously), but also in oogenesis and in leg development.

### Maternal and zygotic requirement for tkv in early dorsoventral patterning

Various lines of evidence suggest the idea that tkv plays a required role in early dorsoventral patterning of the embryo. While embryos homozygous for null tkv alleles do not have a recognizable dorsoventral phenotype (see below), reduction of tkv activity both maternally and zygotically does affect dorsoventral pattern. Female escapers carrying a loss-of-function tkv allele and the hypomorphic tkv' allele give, when crossed to wild-type males, weakly to moderately ventralized embryos. When tkv activity is eliminated zygotically by crossing these females to tkv null allele bearing males, the degree of ventralization is increased (Table 3).

It could be argued that at least part of this ventralizing effect is due to the unusual nature of the tkv' allele. Ideally one would wish to examine embryos in which tkv activity was completely eliminated, both maternally and zytogically. This was not done here, however, as all combinations of tkv null alleles are lethal as adults (Table 2) and we were unable to generate tkv homozygous germ-line clones that gave rise to eggs. Nevertheless, we consider it unlikely that these maternal and zygotic effects are due to specific hypermorphic or neomorphic alleles of tkv, since all the available tkv alleles, including the total deficiency of the locus, have been shown to have a ventralizing effect, either alone (tkv'), in trans-heterozygous combinations, or in association with the tested genes of the dorsoventral group.

Interactions of tkv null alleles with ventralizing alleles of both maternal effect and zygotic lethal dorsoventral genes support a required role of tkv in dorsoventral patterning. Partial reduction of tkv function (tkv'/+/) in mothers enhances the ventralizing effect of dominant, weakly ventralizing alleles of ea and cact; additional reduction of tkv activity in the embryo (by crossing to tkv null heterozygous males) further increased ventralization (Table 4). Doubly heterozygous dpp +/+/ tkv embryos derived from tkv/+ mothers are weakly ventralized. Thus reduction of tkv activity both maternally and zygotically enhances the effect of reduced dpp activity. Other evidence in support of the zygotic requirement for tkv function in determining dorsoventral pattern is the additive interaction with scw. scw homozygous embryos are only moderately ventralized, while loss of both scw and tkv activity simultaneously gives strongly ventralized embryos.

### Complexity of the tkv locus; different alleles affect multiple processes in various ways

Our results suggest that the tkv gene is required in a number of developmental processes, in addition to the above described role in early embryonic dorsoventral patterning. A role for tkv in oogenesis is suggested by the failure to obtain eggs from ovo' females in which homozygous tkv clones were induced, and also by the fact that tkv'Stkv' escaper females have atrophic ovaries. The phenotype of tkv null embryos indicates that the gene is required for dorsal closure, a later event in embryogenesis (see below). The phenotype of trans-heterozygotes among various tkv alleles (Table 2) indicates that altered tkv activity affects development of adult structures: the wing, distal portions of the leg and the dorsal midline of the body.

Possibly related to this variety of functions of the tkv gene is the fact that certain tkv alleles, although classified as equivalent (null) on the basis of their homozygous embryonic lethal phenotype, behave somewhat differently from each other in other genetic contexts. Thus

<table>
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<th>tkv allele</th>
<th>Percent + tkv/dpp + adult flies</th>
<th>dpp&lt;sup&gt;372&lt;/sup&gt;/CyO from</th>
<th>dpp&lt;sup&gt;br6&lt;/sup&gt;/CyO from</th>
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<tr>
<td>tkv&lt;sup&gt;0&lt;/sup&gt;/CyO</td>
<td>3</td>
<td>36</td>
<td>27</td>
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<tr>
<td>tkv&lt;sup&gt;69&lt;/sup&gt;/CyO</td>
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<tr>
<td>tkv&lt;sup&gt;n3&lt;/sup&gt;/SM6b</td>
<td>2</td>
<td>31</td>
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| tkv/CyO or SM6b and dpp/CyO flies carrying the indicated alleles were crossed with the alleles brought in by the indicated parent and the number of + tkv/dpp +, tkv/CyO and dpp/CyO or SM6b progeny scored for each cross. The percentages correspond to the number of adult flies of the indicated genotype compared to the total number of adult flies recovered. Between 200 and 300 flies were counted from each cross.

### TABLE 5

**Interaction between tkv and dpp**

<table>
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<tr>
<th>tkv allele</th>
<th>From</th>
<th>Percent + tkv/dpp + adult flies</th>
<th>dpp&lt;sup&gt;372&lt;/sup&gt;/CyO</th>
<th>dpp&lt;sup&gt;br6&lt;/sup&gt;/CyO</th>
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<td>tkv&lt;sup&gt;n3&lt;/sup&gt;/SM6b</td>
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the alleles tkv\textsuperscript{10} and tkv\textsuperscript{ap2} give different wing phenotypes in transheterozygous combination with tkv\textsuperscript{1} (Table 2); also, in transheterozygous combination with tkv\textsuperscript{3}, the tkv\textsuperscript{10} allele has a stronger maternal effect than the tkv\textsuperscript{ap2} allele (Table 3). Among the alleles considered to be null on the basis of their embryonic cuticular phenotype, tkv\textsuperscript{10}, tkv\textsuperscript{11} and tkv\textsuperscript{12} (but not tkv\textsuperscript{ap2} and tkv\textsuperscript{3}) give escaper progeny when in transheterozygous combination with tkv\textsuperscript{3} allele. Thus tkv\textsuperscript{10}, tkv\textsuperscript{11} and tkv\textsuperscript{12} may not be true null alleles, or may not be affecting all of the same processes.

On the basis of their embryonic cuticular phenotype the $Sz1$ and $Sz2$ alleles were not classified as null alleles; furthermore, they display a nonlinear pattern of complementation with presumed point mutations (Table 2). As they are associated with chromosome rearrangements and are presumably due to breakpoints within the tkv gene, it is possible that these mutations disrupt regulatory regions of the gene and do not affect functional domains of the protein.

The fact that the tkv gene appears to be involved in multiple functions, and that different alleles and allelic combinations have different effects on phenotype and lethality (Table 2), all tend to argue that tkv is a genetically complex locus.

Role of tkv in dorsal closure: Although various tkv alleles (including nulls) interact with the alleles tkv\textsuperscript{ap3} and tkv\textsuperscript{ap2} as well as with both maternal effect and zygotic lethal ventralizing alleles of other genes to give a more ventralized phenotype, the null (loss-of-function) tkv phenotype is not on its own a recognized dorsoventral phenotype. Thus the tkv dorsal open phenotype is not seen for any of the other weakly ventralizing mutants. Conversely, the internalization of Filzkörper and posterior segments, as well as the defects in germband extension and retraction, seen in many weakly ventralized alleles of the dorsoventral class are not seen for the tkv null alleles. This distinctly different null phenotype raises the question of whether the effect of tkv loss-of-function mutants on dorsal closure is due to incorrect dorsoventral patterning early in embryogenesis, or whether it is due to the tkv gene playing a later role specifically in the dorsal closure process. The fact that, of the host of ventralizing mutations, which give a great range of phenotypes from weakly to strongly ventralized, none give a defect in dorsal closure, tends to argue that the role of the tkv gene in dorsal closure may be additional to its role in dorsoventral patterning.

Characterization of dorsoventral patterning at the blastoderm stage in tkv null embryos could give insight into this question. We found that expression of the zen protein is normal, i.e., present in the dorsalmost 40% of the embryo (Rushlow et al. 1987), in tkv null embryos (data not shown). This supports the idea suggested above, i.e., that the failure of dorsal closure in tkv embryos does not stem from a failure in early dorsoventral patterning, but rather from the failure to provide correct tkv activity during a later stage in embryonic development (perhaps during or just prior to the process of closure itself).

Model for tkv function: We propose that both maternal and zygotic transcription of tkv can contribute to establishment of correct dorsoventral patterning in the early embryo. This makes tkv unique among described dorsoventral patterning genes, which act either exclusively maternally or exclusively zygotically (in terms of their effect on dorsoventral patterning) (Gerttula et al. 1988; Letsou et al. 1991; Roth et al. 1991; Shelton and Wasserman 1988; Hecht and Anderson 1993).

To explain the fact that tkv null embryos, although they lack dorsal epidermis, do not have a characteristic dorsoventral phenotype, we must assume that one-half of the normal complement of maternally provided tkv activity is sufficient to support normal dorsoventral patterning, if no other components of the system are reduced. A similar situation has been described for the gene caudal, which plays a role in anteroposterior patterning: provision of the caudal product either maternally or zygotically is sufficient to give an almost normal phenotype (Macdonald and Struhl 1986). Once the dorsoventral patterning system is sensitized, however, by the reduction of activity of another dorsoventral patterning gene such as ea or cact (maternally) or dpp or scw (zygotically), then reduction of the zygotically provided tkv activity leads to increased ventralization.

We must next consider whether, in the simplest case, tkv interacts with the maternal or the zygotic dorsoventral system, for which the graded morphogens are the nuclear localized dorsal protein and dpp activity, respectively. One possibility is that the function of both maternal and zygotic tkv activity is to down-modulate the dpp activity gradient or its interpretation. This could explain the interaction with the ventralizing ea and cact alleles: these alleles lead to increased ventralization; loss of tkv function, by increasing dpp activity, would increase this ventralization further. This model for tkv function could also explain the interaction with loss-of-function dpp and scw alleles: decrease of tkv activity would lead to increased activity of dpp, and hence increased repression of both dpp and scw (we make the assumption that dpp represses scw as well as dpp), leading to increased ventralization.

Alternatively, the observed genetic interactions could also result if tkv functions, both maternally and zygotically, in potentiating the dpp signal transduction pathway. The interaction with the ea and cact alleles could then be the additive effect of increased dpp activity caused by those alleles and decreased activity of the dpp pathway caused by the tkv mutations. The increased ventralization seen when loss-of-function dpp and tkv alleles are combined would be expected if tkv potentiates (increases) the dpp signaling system in some way. Since scw...
ANDERSON 1992a), this postulated function for tkv could be deduced from analysis of cuticular phenotypes; this type of analysis is believed to act in the dpp pathway (FERGUSON and ANDERSON 1992a), this postulated function for tkv could be deduced from analysis of cuticular phenotypes; this type of analysis is believed to act in the dpp pathway.

The increased ventralization of the embryo, which indicates the genetic interactions discussed above, was deduced from analysis of cuticular phenotypes; this type of analysis is believed to act in the dpp pathway. Since mesoderm is expected to expand if the dpp gradient is altered (CHASAN and ANDERSON 1993), but not if the dpp gradient is altered (ARORA and NÜSSLIN-VOLHARD 1992), analysis of twi expression might be a way to make this distinction, i.e., expansion of the twi domain in tkv embryos might indicate that tkv interacts with the dpp pathway, while a normal twi expression domain might indicate that interaction is with the dpp pathway.

While our current information on embryonic phenotypes is equivocal with regard to a model, the phenotype of the tkv adult trans-heterozygous escapers leads us to favor a model in which tkv interacts primarily with the dpp signal transduction pathway. An interaction between tkv and dpp would explain many aspects of this phenotype [except for effects in the germline, where tkv is lethal while dpp has no known requirement (IRISH and GELBART 1987)]. The disk class of dpp alleles affects all imaginal discs and shows a phenotypic series in which disc size is diminished; these phenotypes can be explained by an increasingly greater loss of distal material (SPENCER et al. 1982). A number of the phenotypic features observed in the most severely affected (class 5) trans-heterozygous tkv adults, namely reduced wing size, loss of tarsal claws and segments, and dorsomedial cleft in the notum (Table 2), are among the variety of defects observed in the class II through class IV dpp disk mutants (SPENCER et al. 1982). Another feature seen in the wing of tkv trans-heterozygous adults, the loss of the terminal portion of the LA vein (class 3, Table 2), is considered to be the characteristic phenotype of one allele of the shortvein class of dpp alleles (SEGAL and GELBART 1985). These similar defects in the wing and leg imaginal disc derivatives could be the result of tkv potentiating the activity of dpp in certain, but not all imaginal disks.

The tkv wing defect is not merely a reduction in wing size, but also involves wing vein thickening. For this reason, tkv has been placed in a category with other genes (Noiich, Delta) for which loss-of-function alleles cause wing thickening; it has been proposed that all of these genes are involved in cell-cell communication that affects cell proliferation in the vein anlagen (DIAZ-BENJUMEA and GARCIA-BELLIDO 1990). Since the dpp protein is a member of the transforming growth factor-β family (PAdGETT et al. 1987) it is possible that tkv might affect cell proliferation via its proposed interaction with dpp.


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