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The *decapentaplegic* gene: a TGF-β homologue controlling pattern formation in *Drosophila*

WILLIAM M. GELBART

Department of Cellular and Developmental Biology, Harvard University, 16 Divinity Avenue, Cambridge, MA 02138-2097, USA

Summary

The type β transforming growth factor (TGF-β) family of secreted factors encompasses a wide range of structurally related proteins that control the state of determination or differentiation in a wide variety of cell types. For all members of the family that have been studied at the protein level, the active moieties arise as dimers of the *C*-terminal ~110 amino acid fragment derived from much longer precursor polypeptides. The hallmark of the family is a series of 7 completely conserved cysteine residues in the *C*-terminus; other conserved amino acid sequences generally cluster in the vicinity of 6 of these 7 cysteines. This report focuses on our current understanding of the genetic structure and developmental role of the *decapentaplegic* (*dpp*) gene in *Drosophila*, the only member of the TGF-β family thus far identified in invertebrates. The *dpp* polypeptide bears a sufficiently close relationship to two bone morphogenesis proteins (BMP-2A and BMP-2B) identified in mammals (Wozney *et al.* 1988, *Science* 242, 1528–1534) to warrant the suggestion that *dpp* and the BMP-2s are the descendants of a common ancestral gene. The protein-coding information for *dpp* is contained within a 6 kb DNA segment. An elaborate *cis*-regulatory apparatus, encompassing a >55 kb DNA segment, has evolved to control expression of the *dpp* gene, which is required for determination of dorsal ectoderm in the early embryo, for normal distal outgrowth of the adult appendages, and for sundry other developmental events, which are currently less well-defined. Studies of chimeric individuals and observations of transcript accumulation *in situ* have demonstrated that the *dpp* gene is expressed along the A/P boundary of the imaginal disks. A possible role of *dpp* in elaborating positional information in imaginal disk development is discussed.

Key words: intercellular communication, pattern formation, positional information, TGF-β, *Drosophila*, *decapentaplegic* gene, imaginal disk development.

Introduction

The TGF-β family comprises a diverse set of 12 polypeptides having profound (generally inhibitory) effects on the growth and/or differentiation of many cell types. Typically, there are no obvious common physiological themes which would have predicted the relationship of these polypeptides to one another, but rather their homology has been revealed through searches of available protein data bases. In the four years following the reported sequencing of the prototypical member of the family, now called TGF-β1 (Derynck *et al.* 1985), the family has grown to include one polypeptide identified in the amphibian *Xenopus laevis* [a vegetal pole specific maternal transcript, Vg1 (Weeks and Melton, 1987)], 9 other polypeptides identified in humans and other mammals [a Vg1 cross-homologous gene, Vgr-1 (Lyons *et al.* 1989), TGF-β2 (Marquardt *et al.* 1987), TGF-β3 (Dijke *et al.* 1988), the inhibin α and β subunits (Mason *et al.* 1985; Mayo *et al.* 1986), Müllerian inhibiting substance, MIS (Cate *et al.* 1986), three bone morphogenesis proteins BMP-2A, BMP-2B and BMP-3 (Wozney *et al.* 1988)], and the decapentaplegic product in *Drosophila* [dpp (Padgett *et al.* 1987)].

The mammalian polypeptides are processed to generate secreted dimeric proteins which are thought to bind to cell surface receptors on target cells (Cheifetz *et al.* 1986). The first functional demonstration of a biologically active receptor for TGF-β1 and TGF-β2 has recently been reported (Boyd and Massagué, 1989). No definitive information is available on signal transduction systems which are activated in target cells in response to ligand-receptor binding. Several members of the TGF-β family have been implicated as key factors in determinative or differentiative decisions, and therefore, this family of intercellular signalling molecules is of special interest to developmental biologists. In this report, I will briefly review the current state of information regarding the TGF-β family, and will then focus on our current view of the one member of the family thus far identified in invertebrates: the decapentaplegic gene in *Drosophila*.

The biological effects of the vertebrate members of the TGF-β family

In general, the members of the TGF-β family have been isolated on the basis of specific *in vitro* assays: growth inhibition, differentiation inhibition, asymmetric distributions of transcripts. Upon further study, however, it has been found that at least some of these polypeptides have profound and singular effects on the growth or differentiation of many other cell types. Furthermore, by *in situ* localization of transcripts or by immunohistochemistry, it has been shown that at least some TGF-β family gene products are localized in many discrete sites during vertebrate development. However, in most cases, specific *in vivo* roles remain uncertain.

TGF-β1, TGF-β2 and TGF-β3

TGF-β1 was discovered as one of two proteins (the other being TGF-α, which is a member of the EGF family of growth factors) present in conditioned medium of transformed mammalian cell lines which would cause phenotypic transformation of the immortalized NRK (normal rat kidney) cell line, such that it would now exhibit anchorage-independent growth in soft agar (Roberts *et al.* 1985). Since its initial discovery based on this phenotypic transformation assay, TGF-β1 activity and responsiveness have been found in many normal cell types *in vivo* and in cell culture. It is especially abundant in bone and platelets (Roberts *et al.* 1981; Assoian *et al.* 1985). Its physiological functions *in vivo* have not been established. In addition, its effects *in vitro* have proven quite wide ranging. Under many conditions of treatment, it turns out to be growth inhibitory rather than growth promoting (Roberts *et al.* 1985). In other circumstances, it leads to the differentiation of particular cell lines in culture (Ignotz and Massagué, 1985). In part, the effects of TGF-β1 seem to depend upon the profile of other factors which are added along with it (Assoian *et al.* 1984). Many of the actions of TGF-β1 can be explained as the indirect consequences of changes in the architecture of the extracellular matrix (Ignotz and Massagué, 1986; Allen-Hoffmann *et al.* 1988; Montesano and Orci, 1988).

Recent observations have uncovered two proteins (TGF-β2 and TGF-β3) which are close relatives of TGF-β1. Both TGF-β1 and TGF-β2 homodimers have been identified. They are somewhat different in their binding to putative TGF-β receptors (Cheifetz *et al.* 1987), suggesting that they might have different biological activities. TGF-β3 was recovered on the basis of its cross-homology to TGF-β1 and TGF-β2 (Dijke *et al.* 1988). No information is available on its structure or biological effects.

Inhibin/activin

Inhibin, a secretion found in ovarian fluid, suppresses the release of follicle-stimulating hormone (FSH) by the pituitary gland (Ramasharma *et al.* 1984), thereby interfering with ovulation. It is an α/β heterodimer in which both subunits bear structural homologies to the TGF-β's (Mason *et al.* 1985). In pigs, two genes encoding related β-chains have been found (Mason *et al.* 1985). β-chain homodimers, or heterodimers of these β-chains, have been found to have the opposite effect of promoting FSH release; these proteins have been termed 'activins' (Ling *et al.* 1986; Vale *et al.* 1986).

Müllerian inhibiting substance

Müllerian inhibiting substance (MIS) is a homodimer secreted by the fetal testis that contributes to conversion of the indifferent urogenital system into the male reproductive system, by causing regression of the Müllerian ducts (Picon, 1969). It is thus a key factor in the determination of the somatic sexual phenotype of the mammalian fetus. Molecular analysis has revealed it to be a divergent member of the TGF-β family (Cate *et al.* 1986).

Bone morphogenesis proteins

These proteins were identified as products that have the ability to induce bone growth, using the ability to induce infiltration and differentiation of osteoblasts in demineralized bone as an assay system (Wang *et al.* 1988). Two of the proteins, BMP-2A and BMP-2B, are highly conserved (and hence their names), while BMP-3 is more divergent (Wozney *et al.* 1988). No information is available on the *in vivo* roles of these three proteins.

The vegetal pole specific transcript, Vg1

The sequencing of Vg1 cDNAs has revealed that the protein encoded by Vg1 transcripts is a member of the TGF-β family (Weeks and Melton, 1987). Vg1 RNA was first identified as the only transcript that was highly concentrated at the vegetal pole of unfertilized *Xenopus* oöcytes (Melton, 1987; Pondel and King, 1988). As the embryo begins development, the maternal Vg1 RNA becomes incorporated into vegetal cells, which will eventually form the endoderm. At this stage of gastrulation, endodermal cells induce ectoderm to form mesoderm. A TGF-β-like molecule has been implicated in this induction process (Slack *et al.* 1987; Kimelman and Kirschner, 1987; Rosa *et al.* 1987), using TGF-β1 and TGF-β2 polypeptides from mammalian sources. Given the distribution of Vg1 RNA, it has been suggested that Vg1 protein is a strong candidate to be the natural mesoderm-inducing factor (Weeks and Melton, 1987). Characterizations of Vg1 and of mesoderm-inducing factor are the subjects of other reports in this volume.

Recently, using Vg1 probes, Lyons *et al.* (1989) were able to isolate a cross-homologous mammalian gene from a mouse cDNA library. This gene, called Vgr-1 (Vg1-related), is expressed in numerous tissues throughout development. No information is available on the structure of the Vgr-1 protein, or on its biological role.

A structural comparison of the polypeptides of the TGF-β family

In every case in which the protein has been characterized, TGF-β family members are active as secreted dimers. The subunits of these dimers are about 110–130 amino acids in length and derive from longer precursors, ranging in size from approximately 300–600 amino acids. These precursors share several structural features. They all have *N*-terminal secretion signal sequences and several internal potential *N*-glycosylation sites; both of these features are consistent with the secreted nature of the polypeptides. No transmembrane domains are apparent in the protein sequences. The precursors all contain several basic residue pairs (arginine–arginine, arginine–lysine, lysine–lysine), at least some of which are the sites of cleavage in the processing of the precursor (*e.g.* Derynck *et al.* 1985). The amino acid conservations that characterize the TGF-β family are confined to the *C*-terminal 110 amino acid residues. As noted above, it is this *C*-terminal region that dimerizes to form the mature secreted product. In general, the extensive regions *N*-terminal to the mature product are poorly conserved between different family members. Nonetheless, if the same member is examined in different vertebrate species, strong homologies in more *N*-terminal regions are identified as well (*e.g.* Derynck *et al.* 1986). In the case of TGF-β1, a cleavage product of the *N*-terminal domain remains noncovalently associated with the *C*-terminal dimer in a latent form of the secreted molecule (Lyons *et al.* 1988).

With the recent expansion of the membership of the TGF-β family, it has been possible to discern subgroups on the basis of conserved or divergent structural features of the *C*-terminal portion of the polypeptides. The conservations among the members of the family generally reside in three domains surrounding six of the seven conserved cysteines present in each polypeptide. TGF-β1, TGF-β2, TGF-β3 and inhibin-β have two additional conserved cysteines, placing these molecules in a different subfamily from the others. MIS and inhibin-α show only a low level of conservation to the other polypeptides and to each other (~20–25%). BMP-2A, BMP-2B, BMP-3, Vg1, Vgr-1 and dpp exhibit at least 45% conservation (at the level of amino acid identities), and appear to be in their own subfamily. Below, we will discuss evidence that BMP-2A, BMP-2B and dpp are very closely related, and may actually represent the dipteran and mammalian derivatives of the same ancestral gene. The relationships of the different TGF-β family members to one another are demonstrated by a comparison of primary amino acid sequences of their *C*-termini (Fig. 1).

In summary, in the course of the four years since the first of these sequences – TGF-β1– was published, this vertebrate polypeptide family has come to include a diverse and physiologically unrelated set of factors with a range of very intriguing developmental effects. The homology of the decapentaplegic (dpp) polypeptide,

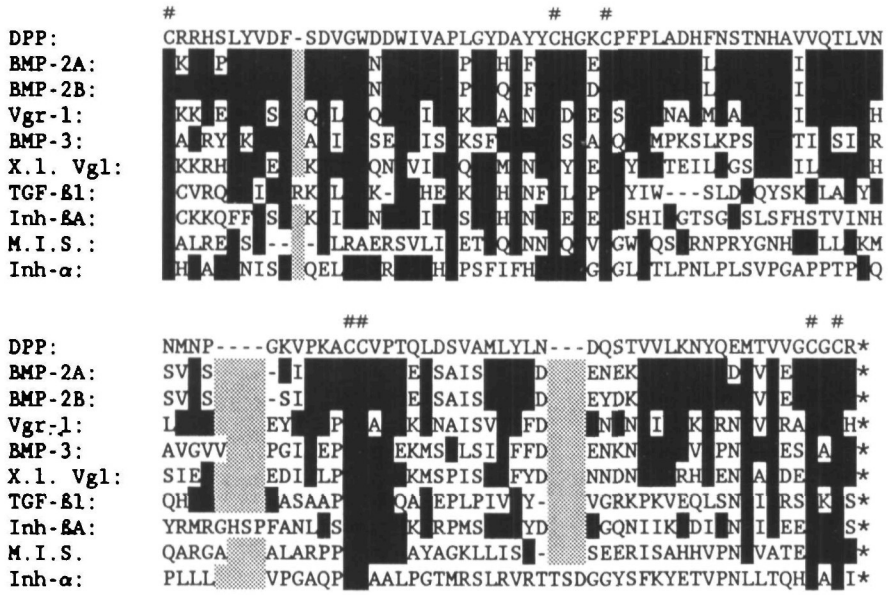

Fig. 1. A comparison of the *C*-terminal sequences (~100 amino acids) of the decapentaplegic polypeptide and several of the vertebrate members of the TGF-β family. The dpp polypeptide from *Drosophila melanogaster* is presented in the top line of the figure. Other members of the family, described in the text are listed below, in approximate rank order of amino acid similarity. The Vg1 sequence is from *Xenopus laevis*. All other sequences are from mammals. Origins of sequences are given in the text. Amino acid positions that are blacked out (■) are identical to the dpp amino acid. Positions that are shaded (□) represent gaps shared with a gap in the dpp sequence. Thus, the more a sequence is blacked out or shaded, the more similar it is to the dpp polypeptide. A # symbol identifies the 7 completely conserved cysteines in the *C*-termini of all TGF-β family members. The asterisk at the end of each sequence indicates the presence of a stop codon at aligned positions for each polypeptide.

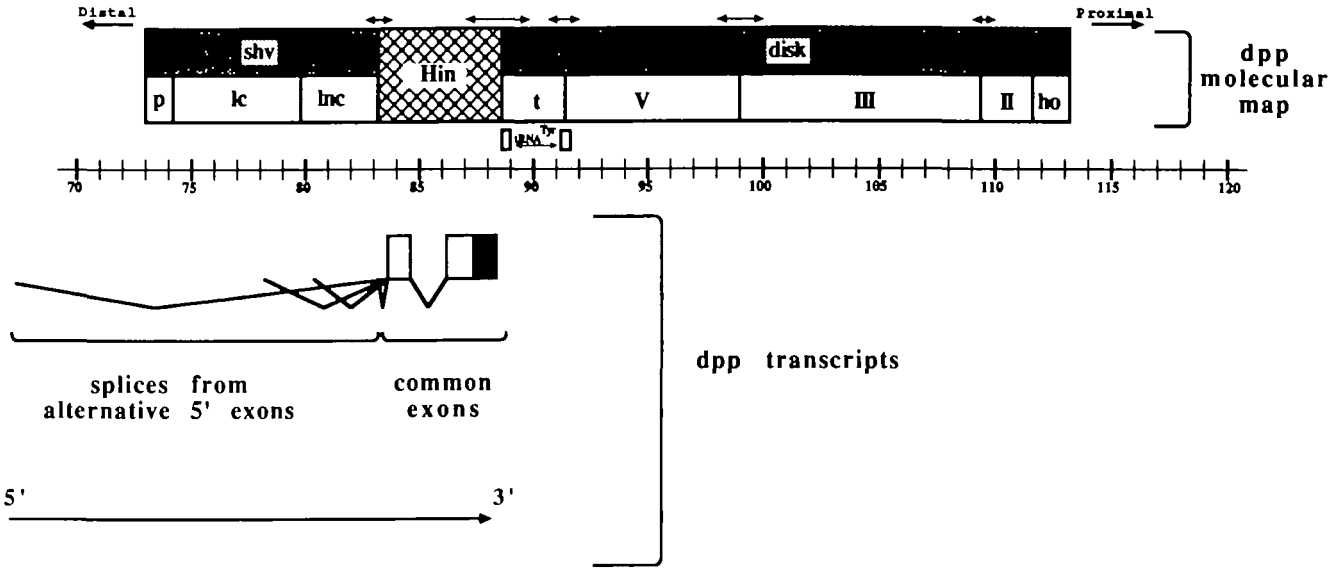

Fig. 2. The molecular map of the *dpp* gene. This map summarizes information presented by St. Johnston *et al.* (1989). The positions of the major segments of the *dpp* gene (**shv-Hin-disk**) and of the subdivisions of the **shv** and **disk** regions are depicted, and are based on the molecular mapping of mutant breakpoints. Double- headed arrows above the molecular map indicate uncertainties in the positions of these boundaries. Distal and proximal indicate the orientation of the *dpp* gene relative to the chromosome 2 (left arm) telomere and centromere, respectively. Below the molecular map are noted the positions of two $tRNA^{Tyr}$ genes within *dpp* (Suter *et al.* 1990). A standard molecular scale, from 70–120 kb, is depicted. The positions of the two exons common to all *dpp* transcript species, falling within the **Hin** region, are shown. The open boxes within these exons represents the extent of the open reading frame of the *dpp* transcripts, and the black box represents 3′ untranslated material (Padgett *et al.* 1987; Padgett *et al.* in preparation). At least 5 different 5′ exons have been identified in different *dpp* cDNAs.

which will be described in the next section, demonstrates that at least one ancestor of this gene family predates the divergence of arthropods from vertebrates, and provides yet another set of important developmental functions controlled by a representative of the TGF-β family.

The *decapentaplegic* polypeptide

We have now characterized several cDNAs corresponding to 5 different classes of overlapping *dpp* transcripts (Padgett *et al.* 1987; St. Johnston *et al.* 1989; Padgett *et al.* in preparation). Each transcript appears to encode the same polypeptide. We have not addressed the questions of possible heterogeneity among the transcripts in terms of stability or translational efficiency. The structures of the cDNAs that correspond to these transcripts are depicted in Fig. 2. These five transcript classes have three exons apiece, and they differ only in their first (*i.e.* 5′) untranslated exon. These untranslated exons appear to be initiated by different promoters and range in size from about 150 bases up to 2 kb. Each transcript class utilizes the same splice acceptor site in the second exon. There is only a single pattern of splicing between the second and third exons.

The first AUG in the open reading frame (ORF) of each transcript is located near the beginning of the common second exon. The ORF extends through the middle of the third exon. If translation initiated with the first AUG, the polypeptide that was produced would be 588 amino acids in length (Padgett *et al.* 1987). We think it likely that the first AUG of the ORF does indeed represent the actual translation initiation codon because (a) it conforms to the consensus sequence for *Drosophila* initiation codons and (b) unlike other AUGs in the ORF, it is followed by a consensus *N*-terminal signal secretion sequence (Watson, 1984), as expected for a secreted TGF-β-like polypeptide.

Several potential glycosylation sites can be found within the *dpp* ORF, consistent with the view that the *dpp* polypeptide is itself a secreted protein. Several dibasic residues are found within the ORF, suggesting that the primary polypeptide product might be proteolytically cleaved to generate a smaller mature product. The positions of these sites are diagrammed in Fig. 3.

The relationship of the *dpp* polypeptide to other members of the TGF-β family

The *C*-terminal ~110 amino acids of the *dpp* polypep-

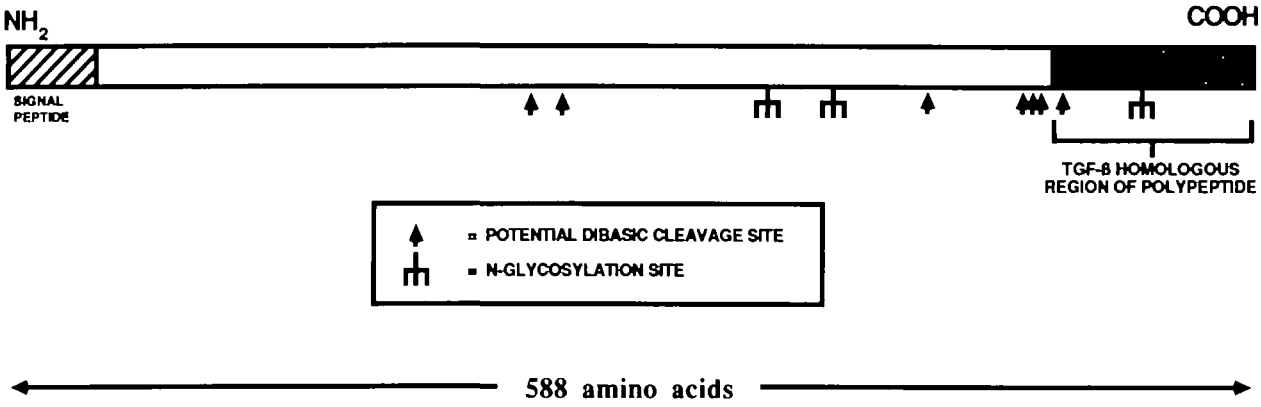

Fig. 3. A schematized view of the dpp polypeptide. The full-length product of the dpp ORF is a 588 amino acid protein containing an *N*-terminal signal peptide and a *C*-terminal region exhibiting high conservation with other members of the TGF-β family. Positions of dibasic residues, some of which may serve as cleavage sites for serine proteases, and *N*-glycosylation sites are shown as indicated. While the dpp polypeptide is the longest member of the family, the basic structure of the molecule parallels the structures of the other family members.

tide show extensive similarity to the comparable regions of the vertebrate TGF-β family members (Fig. 1). Three arginine–arginine dipeptides reside just *N*-terminal to this region of high conservation; these potential proteolytic cleavage sites are thus positioned analogously to the known cleavage sites in most of the other family members. The dpp polypeptide contains the same seven cysteines in its putative *C*-terminal fragment as are shared by the vertebrate family members. The locations of the similarities between the *dpp* polypeptide and the vertebrate members of the TGF-β family conform to the regions of similarity among the vertebrate family members themselves. These conservations reside in three domains surrounding six of the seven *C*-terminal cysteines. We presume this pattern of conservation reflects a common structural motif in this family of molecules.

With the recent report of the primary sequences of human BMP-2A and BMP-2B (Wozney *et al.* 1988), we now have two very strong candidates for the vertebrate analogues of *dpp*. The *C*-termini of BMP-2A and BMP-2B are each 75% identical in amino acid sequence to the corresponding region of the *dpp* polypeptide. Moreover, within the *N*-terminal precursor region, there is 25–30% amino acid identity. The only other polypeptides in the family which show any significant conservation in the *N*-termini are between BMP-2A and BMP-2B themselves, and among TGF-β1, TGF-β2 and TGF-β3. Based on these considerations, we think it very likely that the *dpp* gene of *Drosophila* and the BMP-2A and BMP-2B genes of humans are the modern highly conserved derivatives of a gene which was present in an ancestor common to the arthopod and vertebrate lineages.

The *dpp* gene

The organization of the dpp *locus*

Numerous observations on the structure and function of the *dpp* gene have coalesced into a clear picture of its organization. In brief, the *dpp* gene (map location 2–4.0; 22F1,2) is a rather large genetic unit (>55 kb), which produces a single TGF-β-homologous polypeptide (St. Johnston *et al.* 1990; Padgett *et al.* in preparation). This polypeptide is encoded by at least 5 overlapping transcripts (St. Johnston *et al.* 1990; Padgett *et al.* in preparation). These transcripts are initiated by different promoters and contain different 5′ untranslated exons. Each transcript has its own temporal (and presumably spatial) pattern of expression (Gelbart *et al.* 1985; St. Johnston and Gelbart, 1987; St. Johnston *et al.* 1989; Posakony *et al.* 1990). The bulk of the genetic information contained in the >55 kb of *dpp* DNA consists of *cis*-regulatory elements controlling the timing, location and quantity of *dpp* transcription (Gelbart *et al.* 1985; St. Johnston *et al.* 1990; Blackman *et al.* in preparation). These *cis*-regulatory elements must drive a very complex pattern of expression, as the *dpp* gene is required for numerous developmental events, including determination of dorsal ectoderm along the dorsoventral axis of the early embryo (Irish and Gelbart, 1987; St. Johnston and Gelbart, 1987), proper morphogenesis of the adult appendages derived from the imaginal disks (Spencer *et al.* 1982; Posakony *et al.* 1990; Spencer *et al.* in preparation), and other, less well-defined events necessary for proper development of the larva and adult (Segal and Gelbart, 1985).

Some mutations in the *dpp* gene interfere with the production of an active dpp polypeptide product. They are identified as lesions that affect all developmental events controlled by *dpp* (Irish and Gelbart, 1987). Null (amorphic) mutations are called dpp^{Hin} alleles, because they are Haplo-insufficient that is, $dpp^{Hin}/+$ animals are lethal, apparently because there is too little *dpp* product in the early embryo to produce proper determination along the dorsoventral axis. Most of these are likely to act by altering the structure of the dpp polypeptide, while the remainder probably derange the structure of all of the transcripts. Some dpp^{Hin} alleles have lesions that can be identified by restriction mapping; these lesions cluster in a region of about 6 kb

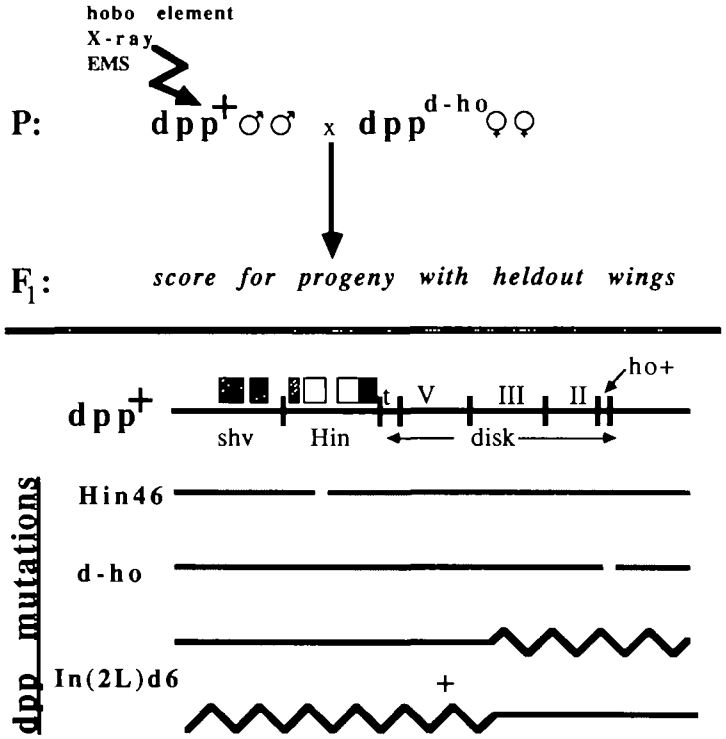

Fig. 4. The origins and types of mutations recovered in heldout mutagenesis screens. TOP: The basic scheme for generating *dpp* mutations as alleles of the original heldout ($dpp^{d\text{-}ho}$) lesion is shown. Several permutations of this screen, in which different mutagens (X-rays, *hobo* transposon mobilization, ethyl methanesulfonate [EMS]) were employed, extra copies of the **Hin** region were included to recover dpp^{null} alleles, and/or rearrangements were included on the $dpp^{d\text{-}ho}$ tester chromosome to obviate *dpp* transvection effects (Gelbart, 1982). BOTTOM: Specific examples of the three major types of *dpp* lesions recovered as heldout alleles are depicted below a molecular map of the *dpp* region (St. Johnston *et al.* 1990). On the molecular map, the subdivisions of the **disk** region are included. The boxes above the map indicate the position of relevant *dpp* exons. The two common exons are indicated in open and black boxes, as in Fig. 2. The striped boxes indicate the positions of some of the alternative 5′ exons containing untranslated information. The $dpp^{d\text{-}ho}$ mutation is a ~2.8 kb deletion from molecular position 112–114.8 on the molecular map of *dpp*. It presumably deletes a *cis*-regulatory element necessary to elaborate the portion of the dorsal base of the wing which includes a group of mechanoreceptors called the sensilla campaniformia-25 (Sc25) (Spencer *et al.* 1982). Other lesions which delete this region of the gene also confer the heldout phenotype and lack the Sc25. The dpp^{Hin46} lesion is a ~0.5 kb deletion within the first of the two common exons, and hence within the *dpp* ORF. It behaves as a null allele of *dpp* (although it is transvection-sensitive) and hence was recovered as a heldout allele (Irish and Gelbart, 1987). $In(2L)dpp^{d6}$ is an example of the largest class of heldout alleles recovered by X-irradiation. It is a cytologically visible inversion of the $dpp^{disk\text{-}III}$ class, and has one breakpoint at ~102 on the molecular map. $In(2L)dpp^{d6}$ splits the *dpp* gene into two pieces. The fragment containing the **shv-Hin** region retains its residual activities of the **shv**, **Hin** and **disk-V** regions. The fragment containing the **disk-III**, **disk-II** and **disk-ho** *cis*-regulatory regions is no longer adjacent to the *dpp* transcription unit, and is thus are inactive. Hence, this inversion was recovered as a heldout allele. Most dpp^{disk} mutations recovered as heldout alleles are such rearrangement breakpoints falling between the **disk-ho** *cis*-regulatory element and the *dpp* transcription unit.

which we term the **Hin** region (St. Johnston *et al.* 1990). This region also contains the two exons encoding the open reading frame for the *dpp* polypeptide. Leaky (hypomorphic) mutations are not haplo-insufficient; because they map to the **Hin** region but are recessive, they are called $dpp^{hin\text{-}r}$ alleles. All $dpp^{hin\text{-}r}$ alleles have normal restriction maps in the **Hin** region. We think it likely that some of the dpp^{Hin} and many of the $dpp^{hin\text{-}r}$ lesions are point mutations altering the amino acid sequence of the dpp polypeptide.

Other *dpp* mutations affect only a subset of the diverse array of *dpp* functions. We think that these mutations (virtually all of which are inversions, translocations or deletions: Spencer *et al.* 1982; Segal and Gelbart, 1985; St. Johnston *et al.* 1990) represent lesions disrupting batteries of *cis*-regulatory elements necessary for the proper spatial and temporal deployment of *dpp* transcripts. This is best exemplified by a consideration of the **disk** region, which is a >25 kb untranscribed region 3′ to the polyadenylation sites of all *dpp* transcripts.

Mutations in the **disk** region are recessive lesions that disrupt proper *dpp* expression in the imaginal disks, leading to the production of distally incomplete appendages. Several classes of such mutations have been described, with the most severe removing all but the most proximal regions of each appendage. All mutations of the **disk** region are recessive to wild-type.

The mildest alleles of the **disk** region are defective only in wing posture. These 'heldout' ($dpp^{d\text{-}ho}$) mutations cause the wings to be held out laterally and perpendicular to the body axis; this phenotype is associated with the loss of specific pattern elements (a group of 25 sensory structures called the sensilla campaniformia-25 or Sc25) on the dorsal base of the wing (Spencer *et al.* 1982). Most mutations of the **disk** region have been recovered as $dpp^{d\text{-}ho}$ alleles (Fig. 4). When examined in homozygotes, most have a more severe array of mutant phenotypes (Spencer *et al.* 1982; Irish and Gelbart, 1987; Blackman *et al.* 1987). These mutations are generally associated with gross chromosomal rearrangements. Mutant homozygotes lack structures derived from distal portions of the adult appendages. Mild alleles ($dpp^{disk\text{-}II}$) only affect the wing, halter and male genital derivatives. Intermediate alleles ($dpp^{disk\text{-}III}$) affect all major appendages (wing, halter, leg, eye, antenna, proboscis, male and female genitalia), but sufficient appendages remain to permit $dpp^{disk\text{-}III}$ homozygotes to survive to adulthood. Severe alleles ($dpp^{disk\text{-}V}$) cause such severe defects in all of the appendages that they engender death early in pupation. The defects in the adult structures are due to corresponding abnormalities in the imaginal disks (the precursors of the adult appendages). Distal structures of

adult appendages are known to derive from central regions of the imaginal disks (Bryant, 1978). These central regions are defective in these *dpp* homozygotes. The amount of imaginal disk material lacking in these mutant individuals parallels the severity of the adult defects (Spencer *et al.* 1982).

We have several lines of indirect evidence that the **disk** region is *cis*-regulatory in nature. (a) When duplications covering the **Hin** region, but not the **disk** region are used to rescue pseudopoint dpp^{Hin} mutations past the Haplo-lethal embryonic period, dpp^{Hin} mutations exhibit a phenotype indistinguishable from the most severe **disk** region phenotype, exhibited by $dpp^{disk\text{-}V}$ homozygotes. (b) The pattern of expression of the *dpp* transcripts emerging from the **shv-Hin** region is indistinguishable from the location of imaginal disk cells requiring normal **disk** region expression for proper appendage formation (Spencer *et al.* in preparation; Posakony *et al.* 1990). (c) We have no clear evidence of transcriptional activity within the **disk** region (St. Johnston *et al.* 1989). (d) The closer to the transcribed **shv-Hin** region that mutant breakpoints in the **disk** region fall, the more severe are their mutant phenotypes (Fig. 2). All of these mutations break within the **disk** region, and can be viewed as removing all **disk** region *cis*-regulatory DNA proximal to the breakpoint from the neighborhood of the *dpp* transcription unit (Fig. 4).

Based on these observations, we conclude that the **disk** region acts by controlling the expression patterns of **shv-Hin** transcripts in the imaginal disks. Similar considerations lead us to conclude that the **Hin** region must contain *cis*-regulatory elements for the embryonic ectodermal expression of the *dpp* transcripts (St. Johnston and Gelbart, 1987; Irish and Gelbart, 1987; Hoffmann and Goodman, 1987), and the **shv** region must contain *cis*-regulatory elements driving still other spatial and temporal patterns of *dpp* expression (Segal and Gelbart, 1985; St. Johnston *et al.* 1990).

On the contribution of *dpp* to imaginal disk development

The nature of the dpp protein product, combined with the developmental effects of *dpp* mutations, has led us to speculate that *dpp* contributes to *Drosophila* development at the level of intercellular signalling of positional information (Padgett *et al.* 1987). By analysis of chimeric individuals containing marked dpp^{-} tissue, we have made several observations (chiefly on wing disks and their derivatives) which bear on possible models of *dpp*'s developmental role (Spencer *et al.* in preparation; Posakony *et al.* 1990). (1) In mosaics that are mixtures of genotypically dpp^{+} and $dpp^{d\text{-}III}$ tissue, each imaginal disk solely determines the *dpp* phenotype of its consequent adult appendage. (2) In those mosaics in which a single imaginal disk contains a mixture of genotypically wild-type and mutant cells, the derivative adult appendages may be fully normal, even though 50% or more of the pattern elements that would be lacking in wholly mutant appendages are of the mutant genotype. (3) Alternatively, such disks of mixed *dpp* genotype can sometimes give rise to appendages that are partially or fully mutant in phenotype. (4) The *dpp* phenotype of a genotypically mixed imaginal disk depends solely upon the genotype of the cells in the region of the disk just anterior to, and abutting, the anterior–posterior (A/P) compartment boundary. We term this region the 'focus' of *dpp* disk expression. (5) *dpp* transcripts accumulate in a pattern concordant with the position of the *dpp* focus, *i.e.* along the A/P compartment boundary.

Based on these results, we conclude that *dpp* cannot act as an exocrine function, secreted from one organ to act on distant target tissues. However, within an imaginal disk primordium, we cannot determine if the primary action of *dpp* is strictly local, or if it acts on target cells throughout the disk.

One surprising aspect of our observations on *dpp* expression within the imaginal disks is its dissimilarity to the phenotypes *dpp* elicits in adult appendages. While the basic phenotype engendered by dpp^{disk} mutations are the loss of tissue in a center to peripheral (radial) direction within the disk, transcript accumulation and localization of the *dpp* focus occur as a stripe cutting across the entire disk at the level of the A/P compartment boundary. In other words, there is no apparent asymmetry of *dpp* expression along the radial axis of the imaginal disk. How then does *dpp* cause pattern deletions along the radial axis, leading to distally incomplete appendages?

One possible explanation is suggested by Meinhardt (1982). Based on theoretical considerations, he supposed that intersections of compartment boundaries (A/P and dorsal/ventral) would identify unique sets of cells, which would then serve as point sources of radial positional information. Given our observations on *dpp* expression in disks, I suggest that the strip of expression of *dpp* (which is constrained by the position of the A/P compartment boundary) serves as a reference line for a series of intersections which lead to the elaboration of radial positional information (detailed in Fig. 5). A gradient of *dpp* product within the disk may be one component of this positional signalling system, or alternatively, the localized expression of *dpp* along the A/P boundary may contribute to the activation of a different positional signalling molecule. In either case, it would be the integrated information from the intersection of *dpp* expression and the expression of other localized molecules within the disk that would lead to the elaboration of radial positional information. The *wingless* gene, which encodes an apparent secreted factor and which is expressed in a different but also highly localized pattern in all disks, is an intriguing candidate for another component of the positional signalling system (Cabrera *et al.* 1987; Rijsewijk *et al.* 1987; Baker, 1988).

In essence, then, I am proposing that *dpp* contributes to the establishment of central positions within developing imaginal disks. The establishment of these central positions would then, directly or indirectly, lead to the elaboration of radial positional information. Perhaps the establishment of central positions, together with the

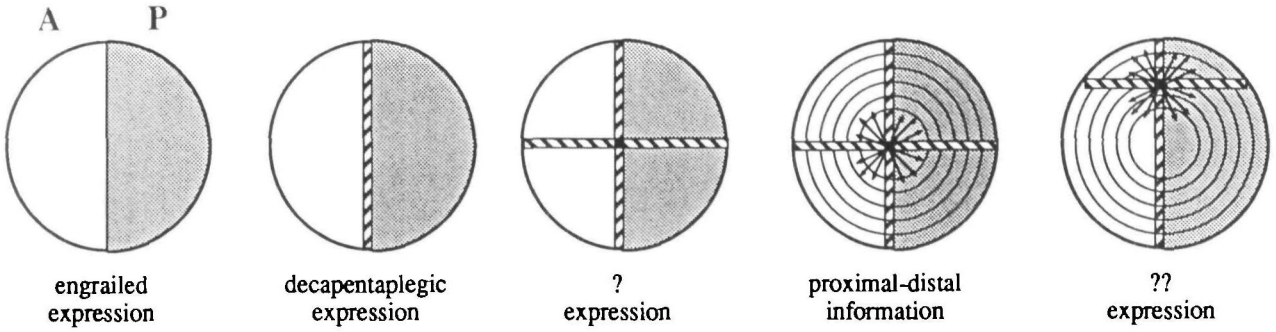

Fig. 5. A possible sequence of events in the elaboration of radial positional information within developing imaginal disks. In this scheme, first the disk primordium is divided into anterior (A) and posterior (P) compartments by the differential expression of the *engrailed* gene (Lawrence and Morata, 1976; Kornberg *et al.* 1985). *dpp* expression then occurs just anterior to the A/P boundary, forming a reference line running from the dorsal to the ventral margin of the disk proper. Another gene product is then locally activated in another orientation within the disk, such that it intersects the *dpp* reference line. The cells at the intersection point have the unique property of expressing both gene products. These cells might then elaborate a radially graded positional signal involving some third molecule, or radial information may be transmitted as direct gradients of the products of *dpp* and this other, undefined gene. In the last frame, another, developmentally later product intersecting the *dpp* reference line is shown, to indicate that multiple rounds of intersections and graded signals are likely to be necessary to elaborate the structures typical of an asymmetric primordium such as the wing disk. The basic idea for this model derives from the suggestions of Meinhardt (1982) and from the observations on *dpp* expression by Posakony *et al.* (1990).

pre-existence of peripheral positional values, could lead to the elaboration of intermediate positions through a process of intercalation. While there are some difficulties in fitting the range of *dpp* adult appendage phenotypes into such a model, it does have the attractive feature of relating to observations coming from so-called 'competence' experiments in imaginal disk development. As first noted by Schubiger (1974) in transplantation experiments in which immature leg disks were forced to metamorphose precociously, there is a regional hierarchy in the abilities of disk cells to differentiate adult cuticular structures. In the youngest disks at all competent to elaborate adult structures, structures from the very periphery and the very center of the disk differentiate. In somewhat older disks, larger swatches of peripheral and of central tissue differentiates. As the age of the disk increases, the size of the swatches increases until finally, in mature disks, the entire radial pattern is differentiated. A similar scenario holds for the wing disk (Bownes and Roberts, 1979). It is as if the cells forming center and periphery are first specified during development, and as if further specification occurs by intercalation. Curiously, the first structures from the leg and the wing that are removed by the mildest *dpp* alleles affecting those tissues are *precisely* the first structures competent to be differentiated from the central regions of those disks (the tarsal claws of the leg in $dpp^{d\text{-}III}$ homozygotes, and the Sc25 of the dorsal base of the wing in $dpp^{d\text{-}ho}$ homozygotes). Thus, *dpp* mutations and competence experiments appear to identify the same set of structures as central within the developing disk. Whether this relationship is of importance in understanding the molecular basis of positional specification within the developing disks must await future experimentation.

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