The *screw* gene encodes a ubiquitously expressed member of the TGF- β family required for specification of dorsal cell fates in the *Drosophila* embryo

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The *decapentaplegic* (*dpp*) gene product, a TGF- β related ligand, acts as an extracellular morphogen to establish at least two cellular response thresholds within the dorsal half of the *Drosophila* embryo. Null mutations in the *screw* (*scw*) gene are phenotypically similar to moderate *dpp* mutants and cause dorsal cells to adopt ventral fates. We show that *scw* encodes a novel TGF- β protein and is an integral part of the signal that specifies dorsal pattern. Although *scw* is expressed uniformly during blastoderm stages, its effect on development appears graded and is restricted to the dorsal side of the embryo. Our results indicate that DPP activity alone is insufficient to specify different dorsal cell fates. We propose that SCW and DPP act together to establish distinct response boundaries within the dorsal half of the embryo, perhaps by forming heterodimers that have higher activity than homodimers of either molecule alone.

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A set of zygotically active genes, *short gastrulation* (*sog*), *twisted gastrulation* (*tsg*), *zerknüllt* (*zen*), *shrew* (*srw*), *tolloid* (*tld*), *screw* (*scw*), and *decapentaplegic* (*dpp*) are required for pattern specification in the dorsal half of the *Drosophila* embryo (Jürgens et al. 1984; Nüsslein-Volhard et al. 1984; Wakimoto et al. 1984; Wieschaus et al. 1984; Zusman and Wieschaus 1985; Irish and Gelbart 1987). Null alleles of these genes produce related mutant phenotypes that vary in severity but are characterized by the loss of dorsal pattern elements and an expansion of structures that normally derive from lateral or ventral regions of the fate map (Arora and Nüsslein-Volhard 1992). Genetic analysis suggests that these zygotic genes are involved in establishing, or responding to, a morphogen gradient that has its highest point in the dorsal-most region (Ferguson and Anderson 1992a; Wharton et al. 1993).

Several lines of evidence suggest that *dpp* functions as the primary signal in the dorsal half of the embryo. Null mutations in *dpp* result in the most severe phenotype, that is, the loss of all dorsal structures. In addition, mutations in *dpp* are lethal even in heterozygotes, indicating that the level of *dpp* product is critical for development (Irish and Gelbart 1987). Recent experiments in which *dpp* gene dosage was manipulated have revealed that changes in *dpp* concentration cause cells to adopt different dorsal cell fates (Ferguson and Anderson 1992a;

Wharton et al. 1993). These studies provide compelling evidence that DPP behaves as a concentration-dependent morphogen.

DPP belongs to the transforming growth factor- β (TGF- β) family of secreted molecules and is related most closely to the bone morphogenetic proteins BMP-2 and BMP-4 (Padgett et al. 1987; Wozney et al. 1988). Because *dpp* message is uniformly distributed in the dorsal 40% of the embryo, it is believed that a gradient of *dpp* protein or activity is established post-transcriptionally (Ferguson and Anderson 1992a; Wharton et al. 1993).

Two other genes, *scw* and *tld*, play a major role in the specification of dorsal cells and appear to be involved in regulating DPP activity (Shimell et al. 1991; Arora and Nüsslein-Volhard 1992; Ferguson and Anderson 1992b). Mutations in these genes cause premature loss of *zen* expression, a downstream gene that specifies the dorsal most cell type, the amnioserosa (Rushlow and Levine 1990; Ray et al. 1991). Extra copies of the *dpp* gene can suppress the phenotype of partial loss-of-function mutations in *scw* and *tld* but not that of mutations in *zen*. Thus, it is likely that *scw* and *tld* act upstream of, or in conjunction with, *dpp* to enhance its activity (Ferguson and Anderson 1992b). Support for this view comes from the fact that *tld* encodes a metalloprotease that shares extensive sequence similarity with mammalian BMP-1 (Shimell et al. 1991). BMP-1 was originally identified in

demineralized bone extracts as part of a complex that includes BMP-2, the mammalian DPP homolog (Wozney et al. 1988), and it has been suggested that TLD may modulate DPP activity proteolytically (Shimell et al. 1991).

We have cloned and characterized the *scw* gene and show that it encodes a novel BMP-like member of the TGF- β superfamily. Thus, it is likely that SCW functions as an intercellular signaling molecule. Surprisingly, although the *scw* transcript is expressed ubiquitously only in the early embryo, SCW activity is required only in dorsal cells. We speculate that the activity of SCW is spatially restricted by its interaction with either TLD or DPP. We propose that the combinatorial action of both SCW and DPP, perhaps as heterodimers, is required to specify the entire range of cell fates in the dorsal half of the embryo. This is an alternative to the single morphogen gradient model in which DPP activity alone specifies different dorsal cell fates. Our demonstration that multiple TGF- β -like signaling molecules are involved in early dorsal patterning in the embryo suggests many parallels with mesoderm patterning in *Xenopus* and osteogenesis in vertebrates (Sive 1993; Kingsley 1994a).

Results

scw affects the ability of dorsal cells to differentiate amnioserosa and dorsal cuticle in a graded manner

The *scw* gene was identified in a screen for second chromosome zygotic lethal mutations that affect embryonic patterning (Nüsslein-Volhard et al. 1984). Blastoderm cells in the presumptive thoracic and abdominal regions of the wild-type embryo differentiate into one of four cell types according to their position along the dorsoventral (DV) axis: Ventral-most cells invaginate to give rise to the mesoderm; ventrolateral cells form the nervous system and the ventral ectoderm; dorsolateral cells differentiate into dorsal ectoderm; and the cells at the dorsal-most position become the extra embryonic amnioserosa (Fig. 1; Lohs-Schardin et al. 1979). Mutations in *scw* cause an expansion of the ventral ectoderm at the expense of amnioserosa and part of the dorsal ectoderm, indicating that the gene is required for normal dorsal development.

Different *scw* alleles can be ordered into a phenotypic series that shows a progressive loss of dorsal structures (Arora and Nüsslein-Volhard 1992). Cuticle preparations of the weakest mutant alleles indicate a loss of pharyngeal skeleton and other dorsally derived structures such as the antennomaxillary sense organs and the cirri. The filzkörper, a paired dorsolaterally derived structure in the tail of the larvae, are reduced or absent (Jürgens et al. 1986; Jürgens 1987; Fig. 1A,B). In *scw* null embryos the amnioserosa, as well as a significant part of the dorsal ectoderm, are lost. The change in dorsal cell fate in mutant embryos is reflected in the increased width of the ventral denticle bands (Fig. 1B,C). By comparison, in null

Figure 1. *scw* mutations result in partial ventralization of the blastoderm fate map. Anterior is up and ventral side is facing the viewer. (A) Cuticle of a wild-type larva. The internal pharyngeal skeleton that derives from anterior dorsal regions is marked with an arrow. The dorsolaterally derived filzkörper are marked with an arrowhead. (B) Cuticle of a *scw*^{G76} mutant illustrating the weak ventralized phenotype. Loss of the cephalopharyngeal structures results in the unusual morphology of the head. The terminal structures are displaced into the interior of the embryo because of defects in germ-band movement. Filzkörper are fused. Slight expansion of the ventral denticle belts is apparent, especially in the anterior abdominal segments. (C) Cuticle of a *scw*^{S12} mutant. This allele represents the null phenotype caused by mutations at this locus. Anterior dorsal structures are missing, as is the amnioserosa. The filzkörper are significantly reduced. The loss of dorsal pattern is accompanied by expansion of ventral structures. Ventral denticle belts are extended laterally. (D) Cuticle of a *dpp*^{H48} homozygous mutant. These embryos do not differentiate any dorsal cuticle, instead dorsal cells differentiate bands of ventral denticles like ventrolateral cells in a wild-type larvae. (E) Schematic drawings of the blastoderm fate maps of the wild-type and mutant embryos shown in A–D. (am) Amnioserosa; (de) dorsal ectoderm; (ve) ventral ectoderm; (me) mesoderm.

dpp embryos, the entire dorsal ectoderm is lost and all dorsal cells differentiate as ventral ectoderm (Fig. 1D; Irish and Gelbart 1987).

Mutations in *scw* affect embryonic pattern in a graded manner. This is demonstrated most dramatically by the progressive loss of the amnioserosa. A wild-type embryo differentiates ~200 amnioserosa cells (Fig. 2A). However, embryos derived from *scw* alleles of increasing phenotypic strength have from 120 cells to only a few amnioserosa cells (Fig. 2B,C). The most severe *scw* alleles do not differentiate any amnioserosa (Fig. 2D). The graded pattern defects in *scw* mutants are very similar to those that have been described for partial loss-of-function alleles of *dpp* (Wharton et al. 1993), suggesting that these two genes may act in a common process.

Figure 2. Partial loss-of-function *scw* alleles result in progressive deletion of the amnioserosa. Embryos have been immunostained using an anti-*Kr* antibody to detect amnioserosa cells. All embryos are oriented anterior to the left and dorsal up. (A) Lateral view of a wild-type stage 13 embryo (staging according to Campos-Ortega and Hartenstein 1985). Mutant embryos representing partial loss-of-function alleles *scw*^{IG76} (B), *scw*^{OS} (C), and *scw*^{S12} (D). In the wild-type embryos, typically 200 cells stain with the anti-*Kr* antibody. The mutant embryos show a progressive reduction in the number of cells fated to form the dorsal-most pattern element, the amnioserosa. There is some variability in the amnioserosa phenotype. The embryos shown here reflect the phenotype of the majority of embryos in an egg lay from the respective allele.

The first morphological evidence of altered development in *scw* mutants is seen early in embryogenesis at the beginning of gastrulation (Arora and Nüsslein-Volhard 1992). This places the time of requirement for *scw* function at or prior to gastrulation. Fate mapping studies in mutant embryos have shown that the loss of dorsal structures does not result from cell death but is caused by a change in cell fate such that dorsally located cells behave like cells from a ventral position in a wild-type embryo (Arora and Nüsslein-Volhard 1992). Taken together, the phenotypic analysis demonstrates that *scw* gene function is not simply required for the differentiation of a specific pattern element but, rather, must be

involved in organizing the overall pattern within the dorsal region of the embryo.

Genetic and molecular mapping of the *scw* locus

We first localized *scw* to the polytene band 38A1,2 on the second chromosome by complementation mapping using deficiency chromosomes (data not shown). Two overlapping deficiencies, *Df(2L)OD16* and *Df(2L)scw/[E1r2]*, fail to complement mutations in *scw*, thus positioning the *scw* locus in the interval defined by the distal breakpoint of *Df(2L)OD16* and the proximal breakpoint of *Df(2L)scw/[E1r2]* (Fig. 3A). Molecular entry into this region was provided by a DNA clone corresponding to the gene *brain-specific-homeobox*, which maps within *Df(2L)OD16* (Jones and McGinnis 1993). A total of ~120 kb of genomic DNA distal to the starting clone was isolated from a genomic library of wild-type *Drosophila* DNA. The deficiency *Df(2L)pr65* was used to orient the direction of the walk (Jones and McGinnis 1993; data not shown). Mapping and hybridization experiments were used to identify the distal breakpoint of *Df(2L)OD16* and the proximal breakpoint of *Df(2L)scw/[E1r2]*. These breakpoints define an ~50-kb interval that must contain at least a part of the *scw* gene (Fig. 3A).

Molecular identification of the *scw* gene

Three different transcription units were identified in the ~50-kb interval between the two breakpoints using reverse Northern blots (Fig. 3A). Genomic fragments from the walk were used to isolate cDNA clones corresponding to these transcribed regions. Full-length cDNA representatives of all but the longest (7.4 kb) of these transcripts were obtained from 0- to 4-hr cDNA libraries, and their positions on the genomic map were determined by restriction enzyme analysis and hybridization experiments (Fig. 3A). The 7.4-kb message corresponds to the previously identified *dlar* gene (Streuli et al. 1989; Saito and Streuli 1991). The *dlar* transcription unit is ~90 kb long and contains 16 introns (N. Krueger and H. Saito, pers. comm.). This gene spans both deficiency breakpoints that define the minimal *scw* region. A second class of cDNAs maps within the fourth intron of the *dlar* gene and is transcribed in the opposite direction with respect to *dlar*. The third cDNA species from the region abuts the proximal breakpoint of *Df(2L)scw/[E1r2]*.

To determine which of the three candidate transcripts encoded *scw*, the genomic regions corresponding to the 1.4-kb and the 3.0-kb cDNAs were cloned into the P element vector pCaSpeR and inserted into the *Drosophila* genome by P element-mediated transformation (Fig. 3A; Rubin and Spradling 1982; Pirrotta 1988). The 7.4-kb *dlar* cDNA was cloned into pCaSpeR-*hsp83* and the pCaSpeR-*hsp70* vectors, which place the cDNA under control of the *hsp83* and the heat shock-inducible *hsp70* promoter, respectively (Pirrotta 1988; Govind et al. 1993). Several independent transformant lines were obtained for each construct and tested for their ability to rescue *scw* mutants. Only constructs that included the

Figure 3. Molecular map of the *scw* region. (A) Molecular organization of the region containing the *scw* gene. Distal is to the left and proximal to the right. An *EcoRI* (R) restriction map of part of the chromosomal walk is shown. The DNA contained in the phage that were used to generate the map are shown. The shaded lines indicate the extent of the deficiencies *Df(2L)OD16* and *Df(2L)scw[E1r2]* (Raftery et al. 1994; see Materials and methods). The heavy black arrows above the restriction map mark the positions of the three cDNAs that map to the region of overlap between the breakpoints. (0) The 5' position of the *scw* cDNA. Position of the *dlar* introns are approximate, and a number of small introns are not shown (based on N. Krueger and H. Saito, pers. comm.). The black bars indicate the genomic fragments used for the P element rescue experiments [(B) *Bam*HI; (S) *Sal*II]. (B) Developmental profile of *scw* transcripts. A Northern blot of RNA from the different stages indicated was probed with the *scw*-2A cDNA and RP49 ribosomal protein gene (control for loading). Two *scw* transcripts, 1.4-kb and 1.7-kb in size, are detected. Both transcripts are also detected by *scw*-N cDNA clone. On prolonged exposure very low levels of the 1.4-kb *scw* transcript are visible at all stages (data not shown).

1.4-kb cDNA were capable of rescuing homozygous *scw* mutant embryos to viability (Fig. 3A). We concluded that the entire *scw* gene is contained within the 7.5-kb genomic region defined by the overlap between the two constructs and that the 1.4-kb cDNA is sufficient for *scw* function.

To determine the temporal pattern of *scw* expression, the 1.4-kb cDNA was used as a probe on developmental Northern blots. Two transcripts of 1.4 and 1.7 kb were identified (Fig. 3B). The 1.4-kb transcript is highly abundant in embryos at 2–4 hr of development, consistent with its early requirement in the embryo prior to the onset of gastrulation (Arora and Nüsslein-Volhard 1992). Prolonged exposure reveals a very low level of the 1.4-kb transcript at all embryonic stages (data not shown). The 1.7-kb transcript is significantly less abundant and is seen only in the 2- to 4-hr lane. Transcripts seen in the 0- to 2-hr lane are attributed to early zygotic transcription rather than to maternal contribution, as the lane containing RNA derived from female adults shows no detectable signal.

DNA sequence and conceptual translation of the *scw* gene

The complete nucleotide sequence of one *scw* cDNA isolate (*scw*-2A) accounts for the first 1413 bp shown in Figure 4A. The length of the cDNA is in close agreement with the transcript size of 1.4 kb observed on the Northern blots. Another isolate (*scw*-N) is identical to *scw*-2A in the region of overlap (1.3 kb) but is 306 bp longer at the 3' end (Fig. 4A), suggesting that it may correspond to the 1.7-kb message on the Northern blot. The cDNA sequence contains a single large open reading frame (ORF) of 1200 nucleotides, flanked by 114 bp of 5'-untranslated sequences and 405 bp of 3'-untranslated sequences.

The first in-frame methionine codon matches the *Drosophila* translation start consensus of ANN(C/A)A(A/C)(A/C) (Cavener 1987), and the predicted translation product is a 400-amino-acid protein with an approximate molecular mass of 44,000 kD. Hydropathy analysis revealed one hydrophobic region at the amino terminus of the protein that conforms to the rules that

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AAAATGCAGTTGCCACTGATGCAATTAACCTACCTGTTTCCATATATAAGACTATTCCATGACCAAGGAAA
ACAGTATTTCTTTCAGTCTCTCTATGAGAACACACAAAATGTTGAAGCTGTTTATTAACCAAGCTCTTTTAT
M L N V F L L T S L F Y>
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GCTGCTAGTGCCACCATATGTGACCAACCAATATACATCGAGATGCCATATACAGAGCGCTCTTTGAGC
A A S A T T Y V T T N N H I E M P I Y Q K R P L S>
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GAGCAATGGAGATGATTGATATCTGGATTGGGTGACCTCCGAGACGACAGGCGGACCAATCTCCCAAT
E Q M E M I D I L D L G D R P R R Q A E P N L H N>
62
TCAGCATCGAAATTCCTCTCGAAGTTTACACGAGATTAGCGAGGACCAAGAGCCCAAGAGGTCCTGCATCAG
S A S K F L L E V Y N E I S E D Q E P K E V L H Q>
87
CGCCACAAACGCTCACTGGACGATGACATTTGATTTCACACGAGGATCGACAGGAGATTGCCATTCGCAACGC
R H K R S L D D D I L I S N E D R Q E I A S C N S>
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ATCCTTACTCTTTTCGAGCGCTTGAACCCGAGCACTGGACACGAGCTTGATATGACATTAACCTTCAACACC
I L T F S S R L K P E Q L D N E L D M H I T F N T>
137
AACGATGTGCCAGTGGATTGAGTTTGGTTTGGTTCAGGCAATGCTCAGGATATACAGCAACCAAGTCCTGGTGACAGA
N D V P V D L S L V Q A M L R I Y K Q P S L V D R>
162
AGGGCGAATTTACCGTGCTGCTGACGAAATGCGCAATGCGCAGGACTTTTCTTATCGCATCTCTGGGCTCT
R A (N) F T V S Y R K L D N R Q D F S Y R I L G S>
187
GTGAATACACGAGCAGCAACGCTGGCTGGCTGGAATTCATTTGACAGATACGCTGCGGTATTGGTTACACAA
V (N) T T S S Q R G W L E F (N) L T D T L R Y W L H N>
212
AAGGACTCGCAACGCTCAATGAGCTAAGGATATCAATGGGCGATTCCAGCTGAGACCTTTCGACGTGAGATTG
K G L Q R R N E L R I S I G D S Q L S T F A A G L>
237
GTGACACACAGCGCAGCGCAGCTTTAGAGCCCTTTATTTGTCGGCTATTTCAACGCGCCCGAGCTGCTGCTG
V T P Q A S R T S L E P P I V G Y F N G P E L L Y>
262
AAAATACAGAAATCTGTTTCAAGAGATCTCGAGAACGAGGCGCGTGGTGGTTTCGCCACACCTCTCTCG
K I Q K L R F K R D L E K R R A G G G S P P P P P>
287
CCGCGCGCGTGGTCTGATACAGACCCGCGCTCTTCGAGCGCTTAACTTCACTGAGATTTCAAGGAGCTG
P P E V D L Y R P P Q S C E R L (N) F T V D F K E L>
312
CACATGCACTGGGTGATAGCGCCCAAAAGTTGAGGCTATTTTCGCGCGCGCTGCAATTCGCGCTG
H M H N W V I A P K K F E A T F C G G G C H F P L>
337
GGCACCAGATGATGCGCACCACCGCCATGCTCCAAACCTGATGACCTGAAGCAACCGCATCTGCCCAAG
G T K M (N) A T N H A I V Q T L M E L K Q P H L P R>
362
CCCTGCTGCTGCAAGTTTGGTTCGATCACTATTCGCGGTATCTCAATGAGGACATCATGACCTGACC
P C C V P T V L G A I T I L R Y L N E D I I D L T>
387
AAATACCAAGGAGCTGCGCAAGGAGTGCAGTGCATTAGATTAAATGCTTATTCGTTTGTGATGACATA
K Y Q K A V A K E C G C E * *
400
GTCAGGGTGATGTTATAGGTTTAAATAGTAAATACAAAGTAACTTCTAAACCTGACATCTGTGTTT
AAACCTAGGCGGTGTCGATATCAACTGTTTAAAGAGGCTTTCAAAATATCCATCGATGCTTAAAGTAACTTAC
TAAACCTCTTCAATAACTCAAGACCACTAGTGTGACACTTACGAGAACCTAAGCTATTTGAATATTGTCG
TGCAAGACTTTTATGCTTATTTAGCACGAACCTTACTGTTTACGCTGCGTTTATGACCTGCTTTGCTAATGGG
CACAGGGGTTTAAACGTAACAGACTCTGAAGATCTTAGATTATTAATAAATTGAAAGTCAATGATG

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sky and Burks 1988], revealed that the predicted *scw* protein displays extensive homology to members of the TGF- β superfamily of secreted proteins. These include the prototypical TGF- β molecules, members of the DPP/Vg1/BMP family, and activins and inhibins [for review, see Kingsley 1994a]. Proteins of this class are typically synthesized as inactive dimers that undergo proteolytic cleavage to generate a mature carboxy-terminal segment that forms the ligand molecule [for review, see Massagué 1990]. Two other *Drosophila* genes that encode TGF- β -like proteins are *dpp* and *60A* [Padgett et al. 1987; Wharton et al. 1991; Doctor et al. 1992]. The primary structure of SCW contains all of the features characteristic of this family of proteins. The putative precursor contains an amino-terminal signal sequence and several amino-linked glycosylation sites, consistent with the idea that the *scw* protein is secreted (Fig. 4A). As in other TGF- β -like proteins, the conserved carboxy-terminal region is immediately preceded by a series of basic residues that could form a site for proteolytic cleavage of the precursor protein [Celeste et al. 1990; Barr 1991]. In the case of SCW, two potential multibasic cleavage sites at positions 268 (RFKR) and 275 (KRR) would result in a ligand molecule with ~22–28 amino acids upstream from the first conserved Cys. One exceptional feature of the *scw* protein is a string of eight Pro residues at the amino terminus of the predicted mature protein (Fig. 4A).

As is typical for members of this family, the similarity of the derived SCW sequence to other TGF- β -like proteins is highest at the carboxyl terminus. An alignment of the 101 carboxy-terminal residues of the predicted SCW protein with the *Drosophila* *60A* and *dpp* gene products is shown in Figure 4B. A distinguishing feature of these proteins is the presence of seven invariant Cys residues in the carboxy-terminal region of the molecule. The structure of TGF- β 2 indicates that disulfide bonds formed between these residues are important for the structure and dimerization of the protein [Daopin et al. 1992; Schlunegger and Grütter 1992]. In addition to the invariant Cys residues, *scw* contains other conserved amino acids in the carboxyl terminus, such as the Pro residue at position 22 and the Gly at position 32, that have been identified as critical for the correct folding and secondary structure of TGF- β 2 [Daopin et al. 1992; Schlunegger and Grütter 1992; numbering of residues as in SCW].

Members of the TGF- β superfamily can be grouped into subfamilies with highly related sequences. The deduced evolutionary relationship indicates that SCW belongs to the DVR [Decapentaplegic-Vg related] family that includes DPP, Vg1, and the BMPs [Fig. 4C; Padgett et al. 1987; Weeks and Melton 1987; Wozney et al. 1988; Celeste et al. 1990; Lyons et al. 1991]. Overall, between 18% and 53% of the amino acid residues in the conserved active domain of SCW are identical with those in other TGF- β -like proteins. SCW shares 53% identity with 60A and 40% identity with DPP, in the carboxy-terminal region. Interestingly, the mammalian homologs of DPP, BMP-2, and BMP-4, are 75% identical to DPP in the mature signaling portion of the molecule

[Padgett et al. 1987; Wozney et al. 1988]. In an analogous manner BMP-5, BMP-6, and BMP-7, which share ~75% residues within the group, are 73% identical to 60A [Celeste et al. 1990; Wharton et al. 1991; Doctor et al. 1992]. By comparison, the greatest sequence conservation between the predicted *scw* protein and a vertebrate gene (49% with BMP-6) is considerably less. The divergence between SCW and other TGF- β -like proteins suggests that *scw* is not an ortholog of a known vertebrate member of the TGF- β superfamily.

The prodomain of TGF- β proteins is usually poorly conserved across different subfamilies. However, the sequence conservation between SCW, 60A, and BMP-6 extends into the proregion of the protein as well. SCW shares 36–39% identity in the amino-terminal region with BMP-6 and 60A, respectively [data not shown].

scw transcripts are uniformly distributed in blastoderm-stage embryos

Hybridization of digoxigenin-labeled *scw* probe to wild-type embryos [Tautz and Pfeifle 1989] revealed that the *scw* gene is ubiquitously expressed during early stages of embryogenesis but shows very tight temporal regulation [Fig. 5]. Early cleavage-stage embryos contain no detectable message [Fig. 5A]. Moderate levels of *scw* mRNA are first detected in a stage 4 embryo at ~1.5 hr of develop-

Figure 5. Spatial distribution of *scw* RNA during early embryogenesis. Embryos were hybridized with digoxigenin-labeled riboprobe derived from the *scw*-2A cDNA clone in the antisense (A–E) and the sense direction (F). Anterior is to the left and dorsal is up. (A) Stage 2 embryo, to show the absence of maternal transcript. (B) Syncytial blastoderm embryo at stage 4 (nuclear cycle 11) shows ubiquitous expression of *scw* mRNA. (C) Syncytial blastoderm embryo at stage 4 (nuclear cycle 12) with very high levels of expression. There is some modulation of *scw* expression in the middle region of the embryo along the anterior–posterior axis. (D) Cellular blastoderm embryo at stage 5, with very low levels of transcript. (E) Stage 7 embryo at gastrulation; *scw* transcripts are below levels of detection. (F) Embryo at the same stage as the embryo in C hybridized with a sense riboprobe as control, reveals essentially no background staining.

ment, toward the end of nuclear cycle 10 [data not shown; staging according to Campos-Ortega and Hartenstein (1985)]. During syncytial blastoderm, at nuclear cycle 11–12, the level of message rapidly increases (Fig. 5B,C). The pole cells, the progenitors of the germ line, do not accumulate *scw* transcript. Levels of *scw* transcript then decline very rapidly to below detection in embryos at cellular blastoderm stage 5 (at ~2.5 hr, Fig. 5D). During gastrulation and the rest of embryonic development, *scw* mRNA is below the detection threshold (Fig. 5E). Control embryos hybridized with sense riboprobe derived from the *scw* cDNA show no signal (Figure 5F). Thus, the *scw* gene is expressed during early zygotic development consistent with its mutant phenotype. However, the uniform expression of *scw* transcripts is curious, as the mutant phenotype suggests that *scw* activity is required only in dorsal cells. In this respect, *scw* RNA distribution differs strikingly from that of other genes involved in dorsal cell specification, that is, *dpp*, *tld*, and *zen*, which are transcribed only in the dorsal half of the embryo at cellular blastoderm (Rushlow et al. 1987; St. Johnston and Gelbart 1987; Ray et al. 1991; Shimell et al. 1991).

scw expression on the dorsal side is sufficient for normal development

To test whether expression of *scw* in dorsal cells alone is sufficient for pattern specification, we used the *tld* promoter to restrict *scw* transcription to the dorsal side at the blastoderm stage of embryonic development. A 0.8-kb fragment of the *tld* promoter is capable of directing expression in dorsal cells, in a pattern reminiscent of the endogenous *tld* gene (Shimell et al. 1991; Kirov et al. 1994). A P-element transformation construct containing the *tld* promoter fused upstream of the 1.4-kb *scw* cDNA was injected into embryos and transgenic animals were recovered (Fig. 6). The expression pattern of the *tld* promoter–*scw* fusion was assayed in embryos homozygous for the deficiency *Df(2L)OD16*, which do not contain any endogenous *scw* mRNA. Hybridization with a digoxigenin-labeled *scw* probe confirmed that the *tld* promoter was driving *scw* expression only in dorsal cells (Fig. 6).

Three independent P-*tld*[*scw*] transformant lines were tested for their ability to rescue severe *scw* alleles. In each case, homozygous *scw* mutants were rescued to viability and fertility, demonstrating that dorsal expression of *scw* is sufficient to allow the elaboration of a normal dorsal pattern. In particular, the absence of *scw* expression on the ventral side has no developmental consequences. This result has important implications because it suggests that SCW function is restricted to dorsal regions, perhaps by its interaction with a localized component of the signaling pathway.

Injected *dpp* RNA rescues the *scw* mutant phenotype

Genetic studies have demonstrated that *scw* and *dpp* mutants display similar abnormalities in embryonic pat-

Figure 6. *tld* regulatory sequences restrict *scw* expression to dorsal cells. The *tld* promoter–*scw* construct is an ~800-bp fragment that contains the *tld* regulatory sequences from –728 to +68, fused to the leader of the *scw* cDNA. Dorsolateral view of an embryo carrying a copy of the fusion construct and homozygous for a deficiency, *Df(2L)OD16*, which deletes the endogenous *scw* gene. The embryo was hybridized with digoxigenin-labeled riboprobe to detect *scw* transcript. *scw* transcripts are expressed in a dorsal on/ventral off pattern typical of *tld* expression (Shimell et al. 1991). Some expression is seen at the poles of the embryo.

tern and that both genes have a role in specifying cell fate in the dorsal part of the embryo (this work; Irish and Gelbart 1987; Wharton et al. 1993). Given the structural similarity of SCW and DPP to secreted proteins involved in inductive processes, it is plausible that both gene products act as signals to induce dorsal cells to differentiate. However, the *dpp* mutant phenotype is more severe than the *scw* phenotype, suggesting a more stringent requirement for *dpp*. In addition, embryos mutant for both *scw* and *dpp* do not have a stronger phenotype than *dpp* null embryos (Arora and Nüsslein-Volhard 1992). Thus, it is possible that SCW function may be required to enhance DPP activity. Because *dpp* expression is unaltered in *scw* mutants (Ray et al. 1991), the effect of *scw* on DPP activity cannot be attributable to transcriptional regulation. To establish the relationship between *dpp* and *scw*, we determined whether injection of *dpp* message into *scw* null embryos was sufficient to restore dorsal pattern.

Our results indicate that excess *dpp* can overcome the requirement for *scw* function (Fig. 7). In vitro-transcribed *dpp* mRNA was injected into *scw* null embryos prior to cellularization. The mutant chromosome bears a P-*Krüppel* (*Kr*)–*lacZ* insert that marks amnioserosa cells (see Materials and methods). Uninjected mutants do not differentiate an amnioserosa and hence do not express the *lacZ* marker (Fig. 7B). Following injection with 75 µg/ml of *dpp* message, 60% of the *scw* null mutants differentiate a large number of amnioserosa cells (Fig. 7C). An additional 22% display a fairly normal morphology of the embryo suggesting partial rescue, even though the number of amnioserosa cells in these embryos was small (data not shown). Thus, in embryos that lack SCW

Figure 7. Response of *scw* mutant embryos to injected *dpp* RNA. Injection of in vitro-transcribed *dpp* RNA into precellular blastoderm *scw* embryos results in suppression of the *scw* mutant phenotype. The presence or absence of the dorsal-most pattern element, the amnioserosa, was assayed by expression of the P(*Kr-lacZ*) construct in which the amnioserosa element of the *Kr* upstream regulatory element drives expression of *lacZ*. The P *lacZ* marker was recombined into the chromosome carrying the *scw*^{S12} allele used in these experiments (see Material and methods). Expression was assayed by immunostaining with an antibody directed against *lacZ*. Embryos are oriented dorsal side facing the viewer, anterior to the left. (A) Wild-type embryo at stage 13 showing *lacZ* staining almost exclusively in the amnioserosa cells. (B) *scw*^{S12} embryo showing no amnioserosa cells. The few cells staining in the mutant are neuroblast precursors that are displaced in the ventralized embryo. (C) 60% of the *scw*^{S12} embryos ($n=145$) injected with 75 μ g/ml of *dpp* RNA showed an expanded amnioserosa, as in the embryo shown here. A small group of injected mutant embryos (22%) elicited a weaker response; only a small patch of stained cells resembling the amnioserosa were observed (data not shown). However, the morphology of the embryos in this group was quite normal, suggesting partial rescue. Mutant embryos were recognized by the absence of expression of a *wg-lacZ* marker present in the balancer chromosome (see Materials and methods).

activity, increased levels of *dpp* can suppress the *scw* mutant phenotype and restore the dorsal-most pattern element, the amnioserosa. This demonstrates two critical aspects of *scw* function: (1) *scw* does not act down-

stream of *dpp*; and (2) the *dpp* signal transduction pathway is functional in *scw* mutant embryos. We conclude that *scw* may enhance DPP activity in the embryo and that the function of both genes is required to organize pattern in the dorsal part of the embryo.

Discussion

One of the fundamental questions in developmental biology is how are cells in an embryonic field specified? It is becoming clear that there are a number of possible answers. One solution is that cell fates can be established through the graded distribution of transcription factors. For example, in *Drosophila* the gradients of DNA-binding proteins, *bicoid* and *dorsal*, specify the anterior-posterior and dorsal-ventral axes of the embryo, respectively (Nüsslein-Volhard 1991; St. Johnston and Nüsslein-Volhard 1992). An alternative mechanism is that cell fates are assigned through the activity gradient of a diffusible ligand. Recent work has suggested that the dorsal region of the *Drosophila* embryo is specified by an activity gradient of DPP, a protein that belongs to the family of transforming growth factors (Ferguson and Anderson 1992a; Wharton et al. 1993). We have cloned and characterized the *scw* gene, which encodes a new member of the TGF- β family, that is also required for specification of dorsal cells. In *scw* mutants, the activity of DPP alone is insufficient to specify extreme dorsal cell fates. Our results suggest that the diversity of dorsal cell fates depends on the combinatorial action of two signaling factors, SCW and DPP, rather than the activity of a single ligand molecule.

scw activity is required only in dorsal cells

Phenotypic analyses indicate a requirement for *scw* activity in early embryonic development. In agreement with this, *scw* transcripts are detectable in a stage 4 embryo, ~1 hr prior to the time developmental defects become apparent in *scw* mutants (Arora and Nüsslein-Volhard 1992). In wild-type embryos the level of *scw* mRNA is sharply reduced by stage 5, ~50 min after the transcripts are first visible. Despite the tight temporal regulation of *scw* transcription, ectopic expression of the gene does not appear to affect patterning. In transgenic animals in which the *tld* promoter drives *scw* expression, *scw* transcripts are detected in the dorsal ectoderm at stages 9–11, as well as in imaginal discs and the optic lobe of the brain during larval development (Shimell et al. 1991; Nguyen et al. 1994). The misexpression at late stages does not cause any overt developmental defects in adult flies. Analysis of germ-line clones suggests that *scw* function is not required in the germ line (Arora and Nüsslein-Volhard 1992).

It is apparent from the *scw* mutant phenotype and fate mapping studies, that the loss of *scw* function affects dorsal cells while ventral cells develop normally. In addition, the progressive loss of amnioserosa and dorsal ectoderm in alleles of increasing severity suggests that the embryo is sensitive to reduction in the levels of the

scw product (Figs. 1 and 2). These data indicate that despite the uniform distribution of *scw* transcripts, the dorsal-most cells are more sensitive to loss in *scw* function than dorsolateral cells.

The expression of *scw* RNA in ventral embryonic cells may not be essential. We have shown that expression of the *scw* cDNA in the dorsal half of the embryo, under a heterologous *tld* promoter, is sufficient to rescue *scw* mutants to viability. Although SCW encodes a secreted protein, by analogy with DPP and TGF- β 1, we do not expect it to diffuse extensively or be long lived. During embryonic gut development, *dpp* expression in the mesoderm activates *labial* expression in the adjacent endodermal layer. This effect on *labial* expression is confined to the two to three cells closest to the *dpp*-expressing cells (Immergluck et al. 1990; Reuter et al. 1990). In the case of TGF- β 1, the active ligand is known to be bound by extracellular matrix components and is rapidly cleared from circulation (Coffey et al. 1987; Massagué 1990).

The spatial restriction of SCW activity

The discrepancy between the uniform distribution of *scw* transcript and the restricted effect of mutations in the gene suggests that functional *scw* protein may be localized or that it interacts with a localized component of the signal transduction pathway. The activity of SCW could be restricted by translation of *scw* message only in dorsal cells or by post-translational modification of SCW in a limited region of the embryo. This could occur by positive regulation of SCW activity in the dorsal half of the embryo or by negative regulation in ventral cells, as suggested for *dpp* (Ferguson and Anderson 1992b).

On the basis of its structural similarity to other TGF- β proteins, SCW is likely to be activated by proteolytic cleavage at the basic residues preceding the active ligand domain (see Fig. 4A; Celeste et al. 1990). *tld* encodes the *Drosophila* homolog of the vertebrate BMP-1 metalloprotease (Shimell et al. 1991) and, thus, is a candidate protein that may be involved in activation of SCW. Mammalian BMP-1 copurifies with other TGF- β -like BMPs and is thought to participate in activating the latent form of the ligand (Wozney et al. 1988). The similarity of the *scw* and *tld* mutant phenotypes suggests that both genes may affect the same event in pattern formation. Because *tld* transcripts are confined to the dorsal 40% of the embryo at the blastoderm stage, the restriction of SCW activity may result from its interaction with *tld* (Fig. 8A). Along similar lines, recent studies suggest that a key step in generating the body axis in *Xenopus* may be the post-translational activation of Vg1 in a restricted region of the embryo (Thomsen and Melton 1993). Interestingly, a *tld*-like gene has been identified in *Xenopus* as well (Maéno et al. 1993).

Another explanation for the restriction of *scw* function to dorsal cells may be that *scw* and *dpp* act together to specify dorsal structures. *dpp* behaves as a graded morphogen in the dorsal half of the embryo and establishes at least two cellular response thresholds: one for speci-

fication of the dorsal ectoderm, and a second, higher threshold for the specification of amnioserosa (Ferguson and Anderson 1992a; Wharton et al. 1993). In *scw* mutants, the higher threshold for amnioserosa is not achieved and the lower threshold for establishment of dorsal epidermis is shifted to a more dorsal position with respect to wild-type embryos (see fate map in Figs. 1 and 8C). Thus, the phenotypic analysis suggests that in the absence of *scw* function, DPP alone is unable to establish both thresholds in their normal position.

Given the structural similarity between DPP and SCW, we suggest that *scw* functions as a signaling molecule by forming heterodimers with DPP (Fig. 8B). *scw* transcripts are expressed uniformly in early embryos, so SCW homodimers could be formed in all cells along the DV axis. Expression of *dpp*, however, is confined to the dorsal 40% of the embryo, and thus SCW/DPP heterodimers would only form in this restricted region. We propose that DPP homodimers are capable of signaling, but SCW/DPP heterodimers elicit a stronger response. In *dpp* mutants no dorsal pattern is specified, suggesting that SCW homodimers alone are ineffective in specifying pattern (Fig. 8B,C). In contrast, the ability of DPP homodimers to elicit a basal response may explain why the *scw* mutant phenotype is less severe than that of *dpp* (Fig. 8C). A prediction of this model is that excess quantities of DPP homodimers should be sufficient to override the requirement for the SCW/DPP heterodimer. This might explain why injections of *dpp* message are capable of rescuing the *scw* mutant phenotype. Thus, in wild-type embryos, the activity gradient that specifies dorsal pattern may be composed of both SCW/DPP heterodimers and DPP homodimers (Fig. 8C). The greater potency of SCW/DPP heterodimers compared with the DPP homodimers could result from a higher affinity of the heterodimer for a common set of receptors. The recent identification of BMP receptors in *Drosophila*, that may bind SCW and DPP with different affinities could allow us to test this model (Brummel et al. 1994; Nellen et al. 1994; Penton et al. 1994). The interaction of SCW with DPP might also be the basis for the graded nature of the *scw* mutant phenotype. In a multicomponent gradient system, the loss of any element that affects the efficacy of the system will produce a graded effect, even if the lost component is not graded.

An example of how the composition of a TGF- β -like molecule can drastically alter its activity is provided by the inhibins and activins, which share common subunits. Activins that are made up of β subunits have the opposite biological activity to the inhibins, which consist of both α and β subunits, demonstrating the diversity of function that can be obtained from a combination of ligand subunits (Yu et al. 1987; Petraglia et al. 1989). TGF- β 1/2, TGF- β 2/3 and some of the BMPs (BMP-3, BMP-6, and BMP-7) have been shown to form heterodimers (Cheifetz et al. 1987; Massagué 1990, 1992; Ogawa et al. 1992, and references therein). Similarly, functional bovine osteogenic protein is composed of heterodimers of OP-1 and BMP-2 (Sampath et al. 1990).

The genetic interaction observed between certain *scw*

Figure 8. Model of *scw* gene function in the specification of dorsal pattern. *A* and *B* outline two mechanisms by which *scw* activity may be localized to dorsal cells. Diagrams represent cross sections through an embryo at cellular blastoderm stage with the dorsal (d) and ventral (v) sides indicated. The outer circle represents the vitelline membrane that surrounds the inner embryonic membrane. The arrows depict secretion of gene products into the fluid-filled extracellular perivitelline space. The small circles represent nuclei. (*A*) The *scw* gene is transcribed in all nuclei, whereas only dorsal nuclei (solid circles) express *tld*. The restricted expression of *tld* leads to the formation of active SCW ligand only in the dorsal half of the embryo. (*B*) Nuclei on the dorsal side express both *scw* and *dpp* (solid circles), whereas ventral nuclei (shaded circles) express only *scw*. We propose that DPP homodimers and DPP/SCW heterodimers, which are active in signaling, are formed only on the dorsal side. SCW homodimers, which are inactive, are formed on both dorsal and ventral sides. (*C*) Schematic representation of the activity gradient that defines pattern in the dorsal half of the embryo. The vertical axis represents the height of the gradient with its high point in the dorsal-most cells. The positions marked represent the activity thresholds necessary for the specification of dorsal ectoderm (I) and the amnioserosa (II). The horizontal axis depicts the tissue types differentiated in response to the signaling activity and their position along the DV axis. (AM) Amnioserosa; (DE) dorsal ectoderm; (VE) ventral ectoderm. In the wild-type embryo SCW/DPP heterodimers and DPP homodimers contribute to the activity gradient. The mechanism by which the activity gradient is established is not completely understood. In the *scw* mutants only DPP homodimers are formed and only the lower threshold is achieved. Thus, dorsal ectoderm is shifted dorsally, instead of at its normal dorsolateral position. In a *dpp* mutant neither DPP homodimers nor DPP/SCW heterodimers are formed, resulting in the total absence of signaling activity; therefore, all dorsal cells develop as ventral ectoderm.

and *dpp* alleles is consistent with a possible physical association of the proteins encoded by these genes. Specific alleles of *scw* have been identified that fail to complement a recessive, partial loss-of-function *dpp* allele (Raftery et al. 1994). Embryos carrying a single copy of both mutations (trans-heterozygotes) die with a partially ventralized phenotype. In contrast, embryos carrying a deficiency for the *scw* locus and the same allele of *dpp* are completely viable, indicating that the defective product encoded by the gain-of-function *scw* allele can block the activity of the remaining functional copy of *dpp*. The gain-of-function alleles of *scw* may be incapable of signal transduction but still sequester active *dpp* molecules and thus reduce the effective levels of *dpp* in the embryo.

The haplo-insufficiency of the *dpp* locus reflects the sensitivity of the embryo to reduction in levels of *dpp* activity (Irish and Gelbart 1987; Ferguson and Anderson 1992a; Wharton et al. 1993). If *scw* and *dpp* act together, it might be expected that the *scw* locus would also be haplo-insufficient. However, embryos with a single functional copy of *scw* are viable. An explanation may be that only *dpp* is limiting in the embryo while *scw* is present in excess.

Multiple TGF- β signaling molecules in development: implications for function

Several members of the TGF- β superfamily of secreted

proteins are involved in key developmental events in vertebrate and invertebrate systems (Lyons et al. 1991; Kingsley 1994a). An emerging theme is that many of these molecules act combinatorially to specify cell fate. For example, in *Xenopus* embryos the dosage-dependent induction of dorsal mesoderm by activin can be potentiated by the addition of other factors such as basic-fibroblast growth factor (b-FGF). It has been suggested that activin and FGF may act synergistically to specify the mesoderm (Kimelman and Kirschner 1987; Green and Smith 1990; Kimelman et al. 1992).

The BMPs are an example of a group of structurally related proteins that function in a common pathway of tissue differentiation. Although initially identified as a copurifying mixture of proteins, several of the BMPs are capable of inducing de novo bone formation individually (Wozney 1992; Kingsley 1994b, and references therein). Given this result, the functional significance of the existence of multiple BMPs is not clear. The overlapping spatial expression of several BMPs in murine embryos has led to the suggestion that these genes may combinatorially regulate aspects of vertebrate development (Lyons et al. 1990; Jones et al. 1991). In mice, the *short ear* (BMP-5) and *brachypodism* (GDF-5) mutations cause very specific defects in skeletal development, despite the broad expression of these genes in other regions of the embryo (Kingsley et al. 1992; Storm et al. 1994). It is possible that the regions affected are those in which a particular BMP is uniquely required and active. Alternatively, broad regions of the embryo may be specified by basal levels of a BMP signaling pathway, whereas limited regions may require a higher threshold of activity that is only provided by a combination of ligand molecules. Our data strongly support the latter alternative. Mutations in *dpp* have a severe effect on development, suggesting that it is the basal signal for dorsal patterning in the *Drosophila* embryo. In contrast, despite its uniform expression, the effects of mutations in *scw* are restricted to the region of overlap with *dpp* expression. These results may be of general relevance in view of the striking structural and functional conservation between the genes that specify dorsal pattern in *Drosophila* and the homologous genes required for osteogenesis in vertebrates (Padgett et al. 1993; Sampath et al. 1993).

We have demonstrated that the specification of dorsal pattern in the *Drosophila* embryo requires at least two TGF- β -like signaling molecule, SCW and DPP. Our results indicate that both proteins must be present to achieve the levels of activity required to specify the entire range of patterns that derive from dorsal embryonic cells. The interactions between SCW and DPP, two structurally related TGF- β -like proteins that have a common function and overlapping domains of expression, could provide a conceptual frame work for understanding related processes in vertebrates.

Materials and methods

Drosophila stocks

The X-ray induced deficiency *Df(2L)scw[E1r2]* was isolated and

genetically characterized by V. Twombly and L. Raftery (Raftery et al. 1994). The *dpp^{H48}/In(2LR)CyO*, P23 stock was provided by W. Gelbart (Harvard University, Cambridge, MA). For RNA injection experiments, embryos were obtained from a stock carrying the *scw^{S12}* allele as well as a P-*Kr-lacZ* insert recombined onto the same chromosome. The line was maintained over a *CyO* balancer carrying a P-*lacZ* insert in the *wingless* (*wg*) gene. Homozygous *scw* embryos were unambiguously identified by the absence of *wg-lacZ* expression. Flies carrying the P-*lacZ* inserts used to construct the injection stock were obtained from G. Struhl (Columbia University, NY) and S. Cohen (EMBL, Heidelberg, Germany).

Cuticle preparations were performed as described in Wieschaus and Nüsslein-Volhard (1986). A description of the other mutants and the balancer chromosomes used can be found in Lindsley and Zimm (1992) and Arora and Nüsslein-Volhard (1992). Fly stocks were obtained from the Bloomington Stock Center, Indiana.

Cloning of *scw*, isolation of cDNA, and sequencing

Genomic DNA clones corresponding to λ E86, a *brain specific homeobox*-containing clone that maps to the 38A region (Jones and McGinnis 1993), were isolated from a Canton-S λ EMBL 4 library provided by A. Preiss (Biozentrum, Basel, Switzerland). These clones were used as the starting point for a genomic walk spanning much of the 38A1,2 subdivisions. Standard protocols for library screening, Southern hybridization, and phage purification were employed (Sambrook et al. 1989).

For the reverse Northern blot, poly(A)⁺ RNA extracted from 2- to 6-hr embryos was used in reverse transcription reactions primed with random hexanucleotides and hybridized to Southern blots of genomic clones corresponding to the 50-kb region between the distal breakpoint of *Df(2L)OD16* and the proximal breakpoint of *Df(2L)scw[E1r2]*. Two cDNA libraries, a 0- to 4-hr embryonic library (Brown and Kafatos 1988) and a commercially available 2- to 4-hr embryonic library (Novagen, Inc.), were screened with DNA from the 50-kb minimal genomic region. The longest cDNA corresponding to each separate transcription unit was used as a probe in Southern blots to generate the data presented in Figure 3A.

Clone *scw*-2A is a full-length cDNA isolated from the 0- to 4-hr library, and the clone *scw*-N was isolated from the 2- to 4-hr library. The sequence of cDNA isolates, 2a and N, was determined on both strands by the dideoxy chain termination method (Sanger et al. 1977), using Sequenase version 2.0 (U.S. Biochemical).

Plasmids and constructs

For genomic rescue constructs, the 12.6-kb *SalI* fragment (pKA45), the 11.7-kb *BamHI* fragment (pKA55), and the ~12-kb *BamHI* fragment (pKA66; Fig. 3A) were subcloned into the pCaSpeR vector (Pirrotta 1988). Construct pKA45 includes the 1.4-kb cDNA as well as an additional 6 kb of 5' and 5 kb of 3'-flanking sequences. The construct pKA55 also contains the 1.4-kb cDNA but has only 2 kb of 5'-flanking sequences. Construct pKA66 contained the 3.0-kb cDNA. The *dlar* cDNA containing the entire coding region (7.4-kb) was subcloned into pCaSpeR-*hsp83* and pCaSpeR-*hsp70* vectors for transformation rescue constructs. The 900-bp *BamHI*-*RsaI* fragment of the *hsp83* promoter in pCaSpeR drives constitutive expression in the embryo (Zimmerman et al. 1983; Govind et al. 1993). The *hsp70* promoter is heat shock inducible (Pirrotta 1988). The full-length *dlar* cDNA was the gift of H. Saito (Dana-Farber Cancer Center, Boston, MA). For the *tld* promoter-*scw* fusion

construct, the entire coding region was isolated as a *HindIII*–*NotI* fragment from the *scw* cDNA clone 2A in pNB40 and inserted downstream of the 796-bp *Bam*HI–*Kpn*I fragment containing the *tld* regulatory sequences [Kirov et al. 1994] in pCaSpeR.

P-element transformation and rescue

A homozygous *Df(1)w, yw^{67c23}* stock was used as a recipient for germ-line transformation [Rubin and Spradling 1982]. For transformation rescue experiments, homozygous viable transformant lines that carried an insert in the third chromosome were used. Male flies of the genotype *scw^{S12}/CyO*; P-transgene/*TM3* were crossed to females *scw^{IG76}/CyO*; P-transgene/*TM3*, or *Df(2L)OD16/CyO*; P-transgene/*TM3*. The progeny from this cross were scored for the survival of *scw^{S12}/scw^{IG76}* or *scw^{S12}/Df(2L)OD16* offspring. In the cases where rescue was observed, survival was 60–90% of that expected. Expected survival was calculated as half the total number of *CyO* flies hatching from an individual cross. Control crosses lacking any transformant resulted in 0% survival of *scw^{S12}/scw^{IG76}* or *scw^{S12}/Df(2L)OD16* progeny.

Analysis of scw RNA expression

For the Northern blot, total RNA was isolated from staged embryos, larvae, and adults by the hot phenol method [Jowett 1986]. Poly(A)⁺ RNA was purified using the Poly A Tract mRNA Isolation System (Promega). Approximately 5 μ g of RNA was loaded per lane. Hybridization with random primed probes was carried out according to the conditions of Church and Gilbert [1984].

Embryos were fixed for in situ hybridization as described by Tautz and Pfeifle [1989]. Hybridization and detection were carried out using standard conditions with the modification that hybridization temperature was 55°C. Sense and antisense *scw* probes, derived from the 1.4-kb *scw*-2A cDNA in pBluescript II (Stratagene), were labeled with digoxigenin-UTP (Boehringer Mannheim).

RNA injections and antibody labeling

The complete *dpp* cDNA sequence, isolated as a 2.7-kb *Eco*RI fragment from plasmid E55 [Padgett et al. 1987], was inserted into pBluescript II. For *scw* RNA, the plasmid used for in situ localization was used (see above). Linearized plasmids were transcribed to generate capped RNA transcript in the sense orientation, according to Sambrook et al. [1989]. After digestion with DNase, the RNA was ethanol precipitated and the concentration determined spectrophotometrically. RNA transcripts were injected into precellular blastoderm embryos at the posterior pole. Embryos were aged until stage 13 prior to assaying expression. Expression of *Kr* and/or *lacZ* in injected embryos was assayed using polyclonal antibodies [Ferguson and Anderson 1992a]. Antibody directed against the *Kr* protein was provided by R. Warrior (University of Southern California, Los Angeles) and C. Rushlow (Roche Institute, Nutley, NJ), and a commercially available *lacZ* antibody was used (Promega).

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Note added in proof

The sequence data described in this paper have been deposited to the EMBL/GenBank data libraries.

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