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.

[Key Words: Dorsal–ventral pattern, BMP, signal transduction]

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A set of zygotically active genes, short gastrulation (sog), twisted gastrulation (tsg), zerknullt (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]. 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

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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 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 dorsal-ventral (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 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 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<sup>527</sup> 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<sup>s27</sup> 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<sup>l(1)Eh</sup> 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 larva. (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.
Gene function is not simply required for the differentiation; the phenotypic analysis demonstrates that behavior like cells from a ventral position in a wild-type by a change in cell fate such that dorsally located cells in mutant embryos have shown that the loss of dorsal function at or prior to gastrulation. Fate mapping studies here reflect the phenotype of the majority of embryos in an egg variability in the amnioserosa phenotype. The embryos shown dorsal-most pattern element, the amnioserosa. There is some progressive reduction in the number of cells fated to form the progressive 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 promoters, 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 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)Jp65 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 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.

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...
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1.4-kb cDNA were capable of rescuing homozygous screw mutant embryos to viability (Fig. 3A). We concluded that the entire screw 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 screw function.

To determine the temporal pattern of screw 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 screw gene

The complete nucleotide sequence of one screw 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
define signal sequences, suggesting that scw encodes a secreted protein (Fig. 4A; Kyte and Doolittle 1982). A probable signal peptide cleavage site can be positioned after Ala-16 (von Heijne 1985). There are five potential sites for amino-glycosylation. Two in-frame termination codons at positions 1315 and 1318, respectively, are located at identical positions in both cDNAs, suggesting that the two transcripts differ only in their 3'-untranslated region and use alternate polyadenylation sites. Both cDNAs contain poly(A) addition signals 18–25 bp upstream from the start of the poly(A) tail.

scw encodes a novel member of the TGF-β family most closely related to the bone morphogenetic proteins

A data base search using the BLAST Network Service and the translated GenBank data base (release 80, Bilof-
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-β2 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-β2 [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](https://via.placeholder.com/150)

**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 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.

Injected dpp RNA rescues the scw mutant phenotype

Genetic studies have demonstrated that scw and dpp mutants display similar abnormalities in embryonic pattern 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]. Un.injected 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
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

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-
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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 heterologous protein, by analogy with DPP and TGF-β1, 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 most cells closest to the dpp-expressing cells [Immergluck et al. 1990; Reuter et al. 1990]. In the case of TGF-β1, 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 [Thomson and Melton 1993]. Interestingly, a tld-like gene has been identified in Xenopus as well [Maeno 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 specification 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-β1/2, TGF-β2/3 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). 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 *brachypond* (GDF-5) mutations cause very specific defects in skeletal development, despite the broad expression of these genes in other regions of the embryo (Kimelman 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[E1z2]* was isolated and genetically characterized by V. Twombly and L. Raftery (Raftery et al. 1994). The *dppl{h40/Ih[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 *scwV12* 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).

Culture 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 AE86, a brain specific homebox-containing clone that maps to the 38A region (Jones and McGinnis 1993), were isolated from a Canton-S X 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 38A, 1,2 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[E1z2]*. 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 dyeodeoxy 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*–*RsoI* 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 *tdl* 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 BamIII–KpnI fragment containing the tld regulatory sequences [Kirov et al. 1994] in pCaSpeR.

P-element transformation and rescue

A homozygous Df(1)w, yw^67C3 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^{512/2}/CyO, P-transgene/TM3 were crossed to females scw^{512/2}/CyO, P-transgene/TM3, or Df(2L)OD16/CyO, P-transgene/TM3. The progeny from this cross were scored for the survival of scw^{512/2}/scw^{512/2} or scw^{512/2}/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 (Lwoff 1986). Poly(A)^+ RNA was purified using the Poly A Tract mRNA Isolation System [Promega]. Approximately 5 µg 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 EcoRI 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.

References


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


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

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