The control of cell fate along the dorsal–ventral axis of the Drosophila embryo

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Summary

We have analyzed the contributions made by maternal and zygotic genes to the establishment of the expression patterns of four zygotic patterning genes: decapentaplegic (dpp), zerknillt (zen), twist (twi), and snail (sna). All of these genes are initially expressed either dorsally or ventrally in the segmented region of the embryo, and at the poles. In the segmented region of the embryo, correct expression of these genes depends on cues from the maternal morphogen dorsal (dl). The dl gradient appears to be interpreted on three levels: dorsal cells express dpp and zen, but not twi and sna; lateral cells lack expression of all four genes; ventral cells express twi and sna, but not dpp and zen. dl appears to activate the expression of twi and sna and repress the expression of dpp and zen. Polar expression of dpp and zen requires the terminal system to override the repression by dl, while that of twi and sna requires the terminal system to augment activation by dl. The zygotic expression patterns established by the maternal genes appear to specify autonomous domains that carry out independent developmental programs, insofar as mutations in the genes that are expressed ventrally do not affect the initiation or ontogeny of the expression patterns of the genes that are expressed dorsally, and vice versa. However, interactions between the zygotic genes specific to a particular morphological domain appear to be important for further elaboration of the three levels specified by dl. Two of the genes, dpp and twi, are unaffected by mutations in any of the tested zygotic dorsal–ventral genes, suggesting that dpp and twi are the primary patterning genes for dorsal ectoderm and mesoderm, respectively.

Key words: gene expression, pattern formation, decapentaplegic, zerknillt, twist, snail.

Introduction

In Drosophila, embryonic pattern formation depends on contributions from both maternal and zygotic genes. Aspects of this pattern are elaborated along two embryonic axes, the anterior–posterior (A/P) and the dorsal–ventral (D/V). The establishment of the A/P pattern requires three sets of maternal genes, the anterior, posterior, and terminal systems. Each of these systems is responsible for a particular aspect of the body plan: the anterior for the head and thorax (Frohnhofer and Nüsslein-Volhard, 1986, 1987), the posterior for the abdomen (Lehmann and Nüsslein-Volhard, 1986, 1987), and the terminal for the unsegmented acroan and telson (Schüpbach and Wieschaus, 1986; Klingler et al. 1988). By contrast, the D/V axis requires only one set of genes, the dorsal–ventral system, which includes the eleven genes of the dorsal group and cactus (cact) (Anderson, 1987; Roth et al. 1989; Rushlow and Arora, 1990). The molecular asymmetries that define the two axes are set down by the maternal genes in the form of gradients. The A/P axis is organized in part by a gradient of bicoid protein (Driever and Nüsslein-Volhard, 1988), and the D/V axis by a gradient of differentially compartmentalized dorsal (dl) protein (Steward et al. 1988; Rushlow et al. 1989; Steward, 1989; Roth et al. 1989). The two axes are established independently of one another, as mutations that disrupt one axis do not significantly affect the other (Nüsslein-Volhard, 1979).

The overall pattern along the A/P axis is generated by the hierarchical activities of several classes of genes that sequentially subdivide the embryo into smaller units. The maternal coordinate genes act to establish broad overlapping domains of zygotic gap gene expression. These domains, in turn, interact to specify the double segment pattern of the pair-rule genes. The interactions between pair-rule genes lead to the single
expression of these two genes: dorsalizing mutations manipulations of the \( dl \) gradient results in shifts in the\( \text{et al.} \) \( \text{twi} \) expressed dorsally, neither of the genes are expressed protein defines a simple pattern of three regions, \( \text{zen} \) is \( \text{wild-type embryo, the distribution of} \text{twi} \text{zen} \text{and} \text{embryo responds to the state of the} \text{zen}\text{twi} \text{and} \text{expression to the AS}. \text{twi and} \text{sna will be regulated by} \text{dl}. \text{In particular, it has been shown that} \text{dl}. \text{several lines of evidence suggest that these genes may be regulated by} \text{dl}\text{strating that the maternal coordinate gene for the D/V patterning has not been entirely established (see} \text{et al.} \text{1987}; \text{Roth} \text{1989}). \text{This} \text{et al.} \text{et al.} (\text{Simpson, 1983}). \text{zen}\text{twi}\text{and} \text{sna,} \text{zen,} \text{twi}, \text{sna,} \text{zen, twi,} \text{have been studied in detail, and} \text{zen, twi,} \text{and} \text{twi}\text{zen}\text{expressed throughout the DE, AS, AP and PP. After refinement} \text{dpp}\text{expression is confined to the DE and} \text{zen}\text{expression to the AS.} \text{twi and} \text{sna are both initially expressed throughout the MS, AM, AP and PP. After refinement, expression is restricted to the MS and some aspects of the AP. The late patterns of} \text{sna}\text{will appear in the VE and DE (see Results and Fig. 2).}

Fig. 1. Dorsal–ventral fate map of the blastoderm embryo. A lateral view is shown at left, and a cross section on the right. Dorsal is up and anterior to the left. In the central region of the embryo, the dorsal most positions along the D/V axis give rise to the cells of the amnioserosa (AS), which will be covered over by the dorsal epidermis during dorsal closure and then incorporated into the dorsal vessel (Campos-Ortega and Hartenstein 1989). Lateral positions contribute to the dorsal epidermis and ventral ectoderm. The dorsal epidermis (DE) will give rise to dorsal structures like the trachea, and Filzkörper; the ventral ectoderm (VE), to ventral epidermis and the ventral nerve cord. In the context of this work, the dorsal ectoderm includes both the dorsal epidermis and the amnioserosa. The ventral most cells contribute to the mesoderm (MS) and its derivatives. These cells invaginate in the ventral furrow during gastrulation and do not contribute to structures of the larval cuticle. The poles of the embryo contribute to distinct aspects of the body plan. The posterior pole (PP) invaginates with the poles cells (PC) during germ band extension and gives rise to the proctodeum and posterior midgut; the region anterior to the cephalic furrow (CF) will give rise to head segments, cephalic mesoderm and neuroblasts, and to the anterior midgut (AM); the anterior pole proper (AP) will give rise to the labrum and head skeleton. Initially, \text{dpp} and \text{zen} are expressed throughout the DE, AS, AP and PP. After refinement \text{dpp} expression is confined to the DE and \text{zen} expression to the AS. \text{twi and} \text{sna are both initially expressed throughout the MS, AM, AP and PP. After refinement, expression is restricted to the MS and some aspects of the AP. The late patterns of} \text{sna}\text{will appear in the VE and DE (see Results and Fig. 2).}

segment pattern which is then further subdivided by the expression of segment polarity genes (for reviews, see \text{Akam, 1987}; \text{Ingham, 1988}).

By contrast, the hierarchy of genes involved in D/V patterning has not been entirely established (see \text{Ingham, 1988}). While there is ample evidence demonstrating that the maternal coordinate gene for the D/V axis is \( dl \) (\text{Steward et al. 1988}; \text{Rushlow et al. 1989}; \text{Steward, 1989}; \text{Roth et al. 1989}), it is not yet clear which zygotic genes are direct targets of \( dl \) function. Mutations in several zygotic loci affecting D/V pattern have been identified that may be such targets (\text{Nüsslein-Volhard et al. 1984}; \text{Wieschaus et al. 1984}; \text{Jürgens et al. 1984}), and in general, these loci affect one of three D/V pattern elements (Fig. 1): dorsal ectoderm (\text{Irish and Gelbart, 1987}; \text{Rushlow et al. 1987b}), ventral ectoderm (\text{Mayer and Nüsslein-Volhard, 1988}), and mesoderm (\text{Simpson, 1983}).

Four of these zygotic D/V patterning genes, \( \text{dpp, zen, twi, and} \text{sna, have been studied in detail, and several lines of evidence suggest that these genes may be regulated by} \text{dl.} \text{In particular, it has been shown that the distribution of} \text{zen} \text{and} \text{twi} \text{protein in the early embryo responds to the state of the} \text{dl} \text{gradient. In the wild-type embryo, the distribution of} \text{twi} \text{and} \text{zen} \text{protein defines a simple pattern of three regions.} \text{zen} \text{is expressed dorsally, neither of the genes are expressed laterally, and} \text{twi} \text{is expressed ventrally (\text{Rushlow et al. 1987b}; \text{Thisse et al. 1987}; \text{Roth et al. 1989}). Genetic manipulation of the} \text{dl} \text{gradient results in shifts in the expression of these two genes: dorsalizing mutations result in a loss of} \text{twi} \text{expression and a corresponding expansion of} \text{zen} \text{expression, lateralizing mutations result in a loss of both} \text{twi} \text{and} \text{zen} \text{expression, and ventralizing mutations result in a loss of} \text{zen} \text{expression and a corresponding expansion of} \text{twi} \text{expression (\text{Rushlow et al. 1987a; Roth et al. 1989}). Furthermore, recent molecular studies have established that the} \text{dl} \text{protein has the potential to bind to specific sites in regulatory sequences upstream of the} \text{zen} \text{promoter that mediate the ventral repression of} \text{zen} \text{(\text{Doyle et al. 1989}; \text{Ip et al. 1991})}. \text{As} \text{dpp and} \text{sna are expressed in domains similar to that of} \text{zen} \text{and} \text{twi respectively, it seems reasonable to presume that these genes are also targets of} \text{dl.}

We have analyzed the distribution of \( \text{dpp, zen, twi, and} \text{sna} \text{transcripts in whole-mount embryos mutant for a representative sample of maternal and zygotic genes affecting both D/V and A/P pattern so as to elucidate aspects of the regulatory hierarchy involved in D/V pattern formation. Consistent with the hypothesis that} \text{dpp and} \text{sna are regulated by} \text{dl}, \text{their expression patterns reflect shifts in the} \text{dl} \text{gradient. Furthermore, the shifts observed in the} \text{dpp} \text{and} \text{sna} \text{patterns are precisely those observed for} \text{twi} \text{and} \text{zen suggesting that these genes are responding to the same thresholds of} \text{dl} \text{activity. Thus, the} \text{dl} \text{gradient appears to subdivide the embryo into only three domains. Polar expression of} \text{dpp, zen, twi, and} \text{sna requires the function of the genes of the terminal system. These cues appear to act in conjunction with the cues from the dorsal–ventral system to specify zygotic gene expression at the termini.}
After the initial patterns of zygotic gene expression have been established, refinement and maintenance of these patterns depends on zygotic gene activity. These events are specific to each of the three domains specified by the dl gradient. Thus, zygotic ventralizing mutations may affect the refinement and maintenance of other zygotic ventralizing genes, but not of zygotic dorsalizing genes and vice versa. In the dorsal ectoderm, interactions between dpp and zen suggest that subdivision of this domain into dorsal epidermis and amnioserosa depends on the refinement of the zen pattern by dpp and the other ventralizing genes. In contrast, the expression of dpp is unaffected by mutations in these genes. Similarly, the sna pattern depends on twi for normal expression, but the reciprocal effect is not observed. Thus, we propose that dpp and twi are likely to be the primary patterning genes for dorsal ectoderm and mesoderm, respectively.

Materials and methods

Drosophila strains

The alleles, or combinations thereof, used to represent the various mutant genotypes were as follows: maternal ventralizing; Tl^{108}, TpQ^{6}, ea^{30}/ea^{10}, cact^{12}; maternal lateralizing; ea^{15}, TpQ^{6}/Tpa^{10}; maternal dorsalizing; snk^{203}/snk^{209}, tub^{118}/tub^{238}, TpQ^{6}/Df(3)Tl, dl^{1}; terminal: tor^{PM1}, trk^{PM1}, zen, dpp, tld, BIBL, tsg, srw, 10E, Q, tub*, Tl^{reQ}, Tl^{51}, tor^{™}, tub*. The alleles, or combinations thereof, used to represent the various mutant genotypes were as follows: maternal ventralizing; Tl^{108}, TpQ^{6}, ea^{30}/ea^{10}, cact^{12}; maternal lateralizing; ea^{15}, TpQ^{6}/Tpa^{10}; maternal dorsalizing; snk^{203}/snk^{209}, tub^{118}/tub^{238}, TpQ^{6}/Df(3)Tl, dl^{1}; terminal: tor^{PM1}, trk^{PM1}, zen, dpp, tld, BIBL, tsg, srw, 10E, Q, tub*, Tl^{reQ}, Tl^{51}, tor^{™}, tub*.

Mutant embryos in most zygotic genotypes were not distinguishable from wild-type siblings at cellular blastoderm. So the analysis of the expression patterns at these stages was done by examining a large number of stained embryos and looking for consistent aberrations that were observable in 25% of the embryos. In some cases, mutant embryos were identified with the aid of marked balancer chromosomes. All flies over 20°C were staged according to Campos-Ortega and Hartenstein (1985).

Flies were cultured on standard Drosophila cornmeal yeast extract sucrose medium in 25 mm x 95 mm shell vials or quarter pint urine specimen bottles. Crosses were reared at 25°C. Embryo collections were done at 25°C, in inverted 150 ml tripour beakers covered with a 60 mm plastic Petri dish filled with apple juice or grape agar. Cuticle preparations were performed according to standard techniques (Wieschaus and Nüsslein-Volhard, 1986).

Antibody labelling

Immunological staining of whole-mount embryos was carried out as described by MacDonald and Struhl (1986) using the Avidin/Biotin ABC System (Vector). Antibodies directed against the dl protein and the Kr protein were provided by C. Rushlow. Stained embryos were mounted in methyl salicylate against the dl protein were provided by C. Rushlow. Stained embryos were mounted in methyl salicylate.

Labelling with digoxigenin

Gel purified cDNA fragments were labelled with digoxigenin according to the protocol accompanying the kit (Boehringer Mannheim, Cat. 1093-657), using 30 ng of template DNA in an overnight reaction at room temperature. After incubation, the probe was run over a standard G-50 spin column equilibrated with water, and lyophilized to dryness. The pellet was resuspended in 10 µl distilled water, boiled for five minutes, and 2 µl of this probe solution was used for each hybridization.

Whole mount in situ hybridizations

Hybridizations were done essentially as described by Tautz and Pfeifle (Tautz and Pfeifle, 1989), with some minor modifications. Stained embryos were mounted in Permount (Fisher) or in 90% glycerol for photography.

Results

Expression patterns of dpp, zen, twi, and sna in wild-type embryos

In this report, we distinguish between the different phases of expression of the four zygotic genes. We use the term initial pattern to refer to the pattern that is evident prior to nuclear cycle 13 for dpp, zen, and twi, and nuclear cycle 12 for sna. After this initial phase, each of these patterns sharpens into a refined pattern which is a derivative of the initial pattern. For dpp and sna, we will use the term late patterns to refer to novel expression patterns that appear after the refined patterns. Although the wild-type expression patterns for zen, dpp and twi have been previously described (St. Johnston and Gelbart, 1987; Rushlow et al. 1987a; Thisse et al. 1987), we include a brief description of them for comparison with the mutant patterns.

Prior to nuclear cycle 14, dpp and zen are both expressed in the dorsalmost 40% of the blastoderm embryo with transcripts extending around both the anterior and posterior poles to label more ventral cells (Fig. 1; Fig. 2A,E; St. Johnston and Gelbart, 1987; Rushlow et al. 1987a). Expression at the anterior pole is consistently less intense than that at the posterior pole for both genes (Fig. 2E). Despite this similarity in initial pattern, the refined patterns of dpp and zen are quite different. zen expression refines during cellulariz-
Fig. 2. Expression of dpp, zen, twi, and sna in wild-type embryos. In all cases, dorsal is up and anterior to the left unless otherwise specified. (A–D), dpp; (E–F), zen; (G–H), twi; (I–L), sna. (A) Initial pattern for dpp. Lateral view of a late stage 4 (nuclear cycle 14) syncytial blastoderm embryo showing expression of dpp on the dorsal surface and at the poles. Expression at the anterior pole is notably less intense than that at the posterior pole (B) Refined pattern for dpp. Lateral view of a late stage 8 embryo. dpp transcripts have been excluded from the amnioserosa and are expressed only in the dorsal ectoderm. (C) Late pattern for dpp. Stage 10 embryo showing the double-stripe pattern in the thoracic and abdominal segments. The dorsal stripe consists of the single dorsalmost row of cells in the germ band. (D) Stage 13 embryo. Late pattern of dpp after germ band retraction. The dorsal and lateral stripe are now continuous through the head segments. Internal expression in the clypeolabrum, pharynx, esophagus, and two sites in the visceral mesoderm are visible. (E) Initial pattern for zen. Lateral view of a late stage 4 embryo. Expression at the anterior pole is less intense than at the posterior pole. (F) Refined pattern for zen. Dorsal view of a stage 5 embryo, cellular blastoderm. Expression is confined to cells of the presumptive amnioserosa and two regions in the head (the mohawk and head spots). (G) Initial pattern for twi. Ventral view of a late stage 4 embryo. This domain of expression is approximately 18 cells wide. (H) Lateral view of a mid stage 8 embryo. Expression of twi in the mesodermal anlage and the anterior midgut primordium. Expression at the anterior pole persists. (I) Initial pattern for sna. Lateral view of a ‘‘early’’ stage 4 embryo. (J) Refined pattern for sna. Lateral view of stage 5 embryo, cellular blastoderm. (K) Ventral view of a late stage 8 embryo showing the pattern of sna expression in the neuroblasts. (L) Expression of sna in a late stage 12 embryo showing the expression in the dorsal ectoderm and in the primordia of the wing and haltere discs.

About the time zen expression is fading, the dpp pattern refines. As the germ band extends, dpp transcripts are progressively excluded from the dorsally located amnioserosa, and by full germ band extension are located exclusively in the dorsal epidermis (Fig. 1; Fig. 2B). This pattern is maintained until midway through slow germ band extension, when it is succeeded by occupation of the dorsoapical 10% of the egg circumference in a narrow band 5–6 cells wide, the mohawk, and two patches of expression anterior to the cephalic fold, the head spots (Fig. 2F; Rushlow et al. 1987a, 1987b). This refined pattern fades during fast germ band extension, and no further expression of zen is observed.
by the late pattern which consists of two stripes of spots running parallel to the A/P axis, the first of which appears dorsally, followed by the second which appears laterally (Fig. 2C). This pattern is maintained throughout stages 10–14, though during late germ band extension and germ band retraction, these stripes become continuous lines of expression, elaborated with segmental modulations (Fig. 2D).

Transcripts of *twi* and *sna* are first detected during nuclear cycle 11–12 in a single continuous stripe, comprising the ventral most 20% of the embryo, that extends up to and around both poles (Fig. 1; Fig. 2G, and 2I, respectively; Thirse et al. 1987, 1988). Shortly after this initial pattern appears, *sna* transcripts are excluded from the polar regions, particularly from the posterior pole (Fig. 2J; this refinement is complete by nuclear cycle 13). The boundaries of the *sna* pattern are sharply delineated and a distinct sinus appears at the site of the presumptive cephalic furrow in late stage 4 embryos (see Fig. 5A). Cells expressing *sna* at this stage invaginate with the ventral furrow, but this expression in the presumptive mesoderm is lost after fast germ band extension (stage 8). However, as expression is lost in the mesoderm, *sna* transcripts are observed to accumulate in subsets of cells in the ventral ectoderm and in cells of the procephalic neurogenic ectoderm (Fig. 2K). Based on their segregation pattern, these cells appear to be neuroblasts (Campos-Ortega and Hartenstein, 1985). Later, during germ band retraction, this pattern is supplanted by a new round of expression in the dorsal ectoderm consisting of a single row of segmentally repeated spots from T2 to A8, the thoracic spots being collinear with the lateral *dpp* stripe, described above, and the abdominal spots being slightly more dorsal. The thoracic spots persist until well after dorsal closure (data not shown).

The initial pattern of *twi* expression becomes more restricted at the posterior pole from a position just above the pole cells in stage 4 embryos to a position just beneath them in stage 5 embryos (Fig. 2H; Thirse et al. 1988). Unlike the *sna* expression pattern, the boundaries of the *twi* domain are less sharp; cells at the edges express lower levels of transcript, relative to those along the ventral midline, suggesting that the *twi* pattern tapers off in a graded fashion. Most of the *twi* cells invaginate with the ventral furrow during stage 6, and *twi* expression persists in the mesodermal derivatives until late in embryogenesis (Thirse et al. 1988).
Expression patterns in embryos mutant for maternal effect genes of the dorsal-ventral system

In the following sections, information on mutant phenotypes precedes the description of expression patterns. Cuticular phenotypes engendered by representative maternal loci are shown in Fig. 3. We will use the term mutant embryos to refer to embryos derived from mothers mutant for a particular locus. All staging is according to Campos-Ortega and Hartenstein (1985). In general, specific alleles will not be referred to in the text, but all genotypes tested in this study are listed in the Materials and methods.

Ventralizing alleles

Ventralized embryos are produced by mothers bearing certain alleles of three maternally acting genes: Toll (Tl), easier (ea), and cact. This phenotype is characteristic of loss-of-function alleles of cact and select dominant gain-of-function alleles of Tl and ea. Unlike the cuticle of wild-type embryos, which consists of ventral denticle belts and dorsal hairs (Fig. 3A), ventralized mutant embryos (that produce cuticle) have rings or patches of ventral denticles along the entire D/V axis (Fig. 3C; Anderson et al. 1985a). Strongly ventralized embryos do not lay down cuticle (Fig. 3B).

Predicted fate shifts in the mesodermal anlage vary depending on the particular allele. The dominant allele Tl105 shows the most severe ventralization, in which all blastomeres behave as mesodermal cells (Fig. 3B; Leptin and Grunewald, 1990). A weaker fate shift is observed in cact mutant embryos, which show expansion of the mesoderm at the expense of the dorsal ectoderm (Fig. 3C; Roth et al. 1989). Phenotypic analysis of the dominant ventralizing allele Tl9® clearly shows a global shift primarily in the ectoderm (Anderson et al. 1985a).

Expression of dpp and zen in ventralized embryos is initiated at the normal time, but the pattern is altered. Transcripts for both genes accumulate at the termini, but not over the dorsal surface (Fig. 4A,B,E,F). zen expression at the anterior pole is transient and appears distinctly later than that at the posterior pole (Fig. 4E,F). dpp transcripts show a similar asymmetry in the initiation of expression at the two poles. Neither dpp nor zen are expressed in embryos that have entered stage 5, and the late patterns of dpp expression are not observed (data not shown).

The expression patterns of twi and sna in the ventralizing alleles correlate well with the effect a particular allele has on the expansion of the mesodermal anlagen. Mutant embryos derived from Tl105/+
mothers show uniform expression of \textit{twi} and \textit{sna} over the entire D/V axis (Fig. 4I,M). \textit{caec} mutants show an expansion of the \textit{twi} and \textit{sna} domain (Fig. 5D), which is consistent with the gastrulation phenotype (Roth \textit{et al.} 1989; Roth, 1990). We observe no expansion of the mesoderm in embryos derived from \textit{T}^{P0}/+ mothers (Fig. 4J,N; Fig. 5B). Regardless of the maternal genotype, the \textit{sna} pattern refines normally during late stage 4, and thereafter is not expressed at the poles (Fig. 4N). \textit{sna} expression appears to be initiated in late stage embryos, but no specific pattern is evident (data not shown).

\textbf{Lateralizing alleles}

Lateralized embryos are produced by mothers bearing certain alleles of \textit{Tl} or \textit{ea}. The cuticle of the mutant embryos is an elongated tube covered with rings of denticles that appear to be derivatives of the lateral regions of the wild-type denticles (Fig. 3D). Lateralized embryos fail to form a ventral furrow, and the cephalic folds are prominent both dorsally and ventrally. Thus, these alleles are expected to show an expansion of lateral fates at the expense of both more dorsal and more ventral fates (Anderson \textit{et al.} 1985a).

The initial expression patterns of \textit{dpp} and \textit{zen} in lateralized embryos are similar to those seen in ventralized embryos; \textit{dpp} and \textit{zen} are expressed only at the poles (Fig. 4C,G). The initial expression of \textit{dpp} and \textit{zen} at the poles is lost by the onset of stage 5, after which no further expression of \textit{zen} is observed. However, initiation of the late patterns of \textit{dpp} expression is observed (data not shown).

Lateralized embryos lack the initial pattern of \textit{twi} transcripts ventrally, but expression is observed at the poles (Fig. 4K). The expression of \textit{twi} at the posterior pole fades by the onset of gastrulation, while that at the anterior pole persists (data not shown). This persistence of \textit{twi} expression is consistent with the observation that, in wild type, the expression at the anterior pole is retained until germ band extension, while that at the posterior pole fades earlier (our observations, and Thisse \textit{et al.} 1988). \textit{sna} transcripts only appear transiently at the poles if at all (Fig. 4O). This is probably a reflection of the rapid refinement of the \textit{sna} pattern observed in wild type. Expression of \textit{sna} later in embryogenesis is observed (data not shown), consistent with the onset of the late patterns.

\textbf{Dorsalizing alleles}

Null alleles of each of the maternal effect genes in the dorsal group produce a characteristic embryonic lethal phenotype that is entirely dorsalized. The cuticle of mutant embryos reflects a fate shift in the D/V axis such that all cells along the axis behave like dorsal cells of the wild-type embryo. Mutant embryos differentiate as long, thin tubes of cuticle covered with fine dorsal hairs (Fig. 3E). Ventral denticle belts are entirely lacking. At the time of gastrulation, these embryos do not form a ventral furrow, and the cells normally recruited for this purpose fold to mirror the characteristic dorsal and transverse folds (Nüsslein-Volhard, 1979).

In dorsalized embryos, \textit{dpp} and \textit{zen} are expressed uniformly in all cells of the blastoderm stage embryo (Fig. 4D,H). The \textit{zen} pattern sharpens along the A/P axis, but not along the D/V axis: in stage 5 mutant embryos, \textit{zen} is expressed in two narrow stripes anteriorly, and a single broad stripe in the middle of the embryo, reminiscent of the head spots and mohawk seen in wild type (see Fig. 6C; cf. Fig. 6A). By contrast, the initial pattern of \textit{dpp} does not refine at all. Yet, in late stage embryos, new rounds of \textit{dpp} expression are observed, consistent with the normal onset of the late patterns (data not shown).

\textit{twi} is not expressed in dorsalized mutant embryos during the early stages of embryonic development (Fig. 4L). While \textit{sna} transcripts cannot be detected prior to the onset of gastrulation (Fig. 4P), later in development, mutant embryos show hybridization in the segmented region of the embryo, which can be correlated with the onset of thelate patterns (data not shown).

\textbf{Amnioserosa cells in \textit{dl} embryos}

The uniform expression of \textit{zen} in embryos derived from \textit{dl} mothers is not entirely consistent with the cuticular phenotype: while the expression of \textit{zen} in a \textit{dl} mutant embryo predicts that the blastomeres should be fated as amnioserosa, the cuticular phenotype of these mutant embryos implies that some of the cells expressing \textit{zen} in fact secrete dorsal cuticle. This can be accounted for, in part, by the fact that in the wild-type embryo, it is the mohawk that is coincident with the prospective amnioserosa, and not the initial expression pattern or the refined expression in the cephalic segments. Thus, since the \textit{zen} pattern refines along the A/P axis in \textit{dl} embryos (see above), it is only the centrally located region, corresponding to the mohawk, that should produce amnioserosa fates. These results suggest that a broad central domain of amnioserosa cells should appear in dorsalized embryos. To determine if this is the case, we have stained dorsalized embryos with an antibody directed against the \textit{Krüppel (Kr)} protein. Analysis of the wild-type distribution of \textit{Kr} protein has shown this antigen to be present in all amnioserosa cells from the onset of germ band extension until the completion of germ band retraction. Although other cells express \textit{Kr} protein at this time, the amnioserosa cells are morphologically distinct in that they have particularly large nuclei (Fig. 6B, Gaul \textit{et al.} 1987).

In late stage \textit{dl} embryos, we observe a subset of cells producing the \textit{Kr} antigen that are morphologically similar to wild-type amnioserosa cells. These cells form a broad ring in the middle of the embryo that is located in approximately the same position where the expanded mohawk appeared earlier in development (Fig. 6D; cf. Fig. 6B). While the position of these cells is relatively constant, the number of cells is not; some dorsalized embryos show as many as 200 amnioserosa cells (Fig. 6D), others as few as 40 (Fig. 6E). We suspect that this variation reflects the extinction of \textit{Kr} expression in the amnioserosa that is observed in wild type embryos during stage 13 (Gaul \textit{et al.} 1987). Nevertheless, our...
Fig. 4. Expression patterns in ventralized, lateralized and dorsalized embryos: lateral views. Each row represents a different probe: (A–D) dpp, (E–H) zen, (I–L) twi, and (M–P) sna. Each column represents a different genotype: (A, E, I, and M) Tl[106] (strongly ventralized), (B, F, J, and N) T[Q] (ventralized), (C, G, K, and O) T[10]/T[10] (lateralized), and (D, H, L, and P) dl (dorsalized) (compare with cuticle preparations shown in Fig. 3). All embryos are in stage 4 except for (L), (N), and (P), which are in stage 5 (cellular blastoderm). In ventralized and lateralized embryos, dpp and zen are expressed only at the poles (A–C, E–G, respectively). In dorsalized embryos, they are expressed uniformly throughout the embryo (D, H). twi and sna are expressed uniformly in strongly ventralized embryos (I, M). In (M), the sna pattern has refined. In embryos derived from T[Q] mothers (J, N), twi and sna are expressed in the wild-type pattern. In the lateralized embryos depicted in (K) and (O), twi expression is confined to the poles, while sna expression is absent. The absence of detectable sna transcripts in this genotype most likely reflects the rapid refinement of this pattern that is observed in wild type (see text, Fig. 2J). In dorsalized embryos, neither twi nor sna are expressed. For further details on the ontogeny of these patterns, see the text.
results do indicate that, consistent with the refined expression pattern of zen, some cells in dl embryos behave as amnioserosa cells and that these cells are expressed in the region of the embryo predicted by the refined expression pattern of zen.

**Expression patterns in embryos mutant for maternal effect genes of the terminal group**

Based on the preceding results, it might be expected that the positional value of cells at the poles of the embryo would be ambiguous or undefined with respect to their position along the D/V axis, insofar as genes that are exclusively expressed either dorsally or ventrally throughout the segmented region of the embryo (i.e. dpp, zen, twi, and sna), share a common domain of expression at the poles. This hypothesis is substantiated by the expression patterns of dpp and zen in the maternal ventralizing genotypes, and by the expression patterns of twi in the maternal lateralizing genotypes. In all of these cases, expression at the poles persists despite dramatic shifts of positional values along the D/V axis, suggesting that control of gene expression at the poles requires functions that are not specified by the D/V patterning genes. A similar singularity of the poles is observed in the regulatory
network controlling A/P pattern formation, and the genes of the terminal system have been shown to mediate this effect (Nüsslein-Volhard et al. 1987). Two representatives of this group, trunk (trk) and torso (tor) (Schüpbach and Wieschaus, 1986), are analyzed here.

The cuticular phenotype of embryos derived from mutant trk or tor mothers indicates the role these genes play in the specification of the unsegmented acron (anterior pole) and telson (posterior pole). The differentiated mutant embryos lack the labrum, the head skeleton is reduced in size, and all structures posterior to the seventh abdominal segment are deleted (Fig. 3F, Klingler et al. 1988). The first manifestation of

the mutant phenotype is observed at the time of gastrulation when the ventral furrow can be seen to invaginate over the entire length of the embryo rather than between 20 and 65% egg length, as seen in wild type (Schüpbach and Wieschaus, 1986).

Expression of dpp and zen in mutant embryos derived from tor or trk mothers is initiated at the proper time, but not in the proper pattern. Rather than extending over the dorsal surface and around both poles, expression is confined to the dorsal surface. Neither dpp nor zen transcripts are detected at the poles (Fig. 7A,C). Notably, this pattern of expression does not correspond to the fate shifts predicted from the cuticular phenotype, which predicts an expansion of the segmented region of the embryo (Klingler et al. 1988). The lack of terminal gene function does not affect the refinement of these patterns along the D/V axis or the initiation of late patterns. In stage 5 embryos, refinement of the zen pattern is observed along both axes, though the refined pattern is condensed along the A/P axis, and the anterior-most head spot is absent (Fig. 7D, cf. Fig. 2F). In late stage embryos, dpp is expressed in the characteristic double stripe pattern (Fig. 7B, cf. Fig. 2C).

As seen for dpp and zen, the expression of twi and sna is also initiated at the proper time, but not in the proper pattern. Both twi and sna transcripts are detected in early stage 4 embryos in the characteristic ventral domain. The width of the domain remains 18 cells wide, as observed in wild type, and the sinus in the sna pattern at the cephalic furrow is observed (Fig. 5D). However, the pattern along the A/P axis is aberrant. Transcripts are detectable in the ventral cells up to the middle of the poles, but no dorsal cells are labelled (Fig. 7E,G). This alteration is consistent with the A/P fate shifts predicted from the cuticular phenotype and the expression patterns of pair rule genes in these mutant embryos (Klingler, 1989; Casanova, 1990). Notably, the sna pattern does not refine (Fig. 7G). After stage 5, both twi and sna transcripts can be detected in the derivatives of the mesoderm (Fig. 7F, for twi), and later in development sna transcripts accumulate in the cells of the ventral and dorsal ectoderm, characteristic of the

Fig. 5. Expression patterns of sna in ventralized embryos and tor mutant embryos. (A) Ventral view of a wild-type stage 5 embryo illustrating the refined pattern. The domain expressing sna is approximately 18 cells wide. (B) Ventral view of a late stage 5 embryo derived from a TPQ/ + mother. The width of the domain is not affected. (C) Ventral view of a stage 5 embryo derived from a homozygous torPM mother. The width of the ventral domain of expression is also not affected, despite the fact that the domain appears slightly larger at the posterior pole. Cell counts indicate that this expansion is not statistically significant. The phenomena appears to be a consequence of the fact that the sna pattern does not refine in this mutant background (cf. Fig. 7G). (D) Ventral view of a stage 5 embryo derived from a homozygous cac + mother. In this embryo, the width of ventral domain is expanded to approximately 26 cells.
Dorsal-ventral patterning in Drosophila

Fig. 6. The fate of amnioserosa cells in wild-type and dorsalized embryos. (A,C) Whole mount RNA *in situ* probed with a *zen cDNA*. (B,D,E) Whole mount embryos stained with DAB; primary antibody directed against the *Kr* antigen. (A) Dorsal view of a stage 5 wild-type embryo showing the refined pattern of *zen*. The two head spots and the mohawk are clearly evident. (B) Dorso-lateral view of a stage 13 wild-type embryo showing localization of the *Kr* antigen. Amnioserosa cells are present centrally at the top of the embryo, and are distinguishable by virtue of their large nuclei. (C) Lateral view of a stage 5 *dl~* embryo. Refinement is observed along the A/P axis, but not along the D/V axis. The bracket indicates the region predicted to give rise to amnioserosa based on the wild-type pattern (see Fig. 1). (D,E) Localization of *Kr* antigen in late stage *dl~* embryos. The embryo in (D) has approximately 200 cells expressing *Kr*, located in the central region of the embryo, that are morphologically similar to the amnioserosa cells in (B). (E) Shows another *dl~* embryo with only about 50 cells stained for *Kr*. As it is unclear whether or not these embryos are of the same age, this difference could represent the extinction of *Kr* expression in late stage embryos that is observed in wild pe (see text).

In this last respect, *dpp, zen, twi*, and *sna* are similar: improper expression at the poles does not affect the ontogeny of the refined and late patterns in the segmented region of the embryo.

Taken together, the above results indicate that the polar expression of both dorsally expressed genes like *dpp* and *zen*, and ventrally expressed genes like *twi* and *sna*, show a requirement for the genes of the terminal group.

Expression patterns in embryos mutant for zygotic genes

In the following two sections, we report on the initial and refined expression patterns of *dpp, zen, twi*, and *sna* in embryos mutant for zygotic genes affecting D/V patterning. Representative cuticle preparations for these zygotic genotypes are shown in Fig. 8. We will use the term decapentaplegic group (*dpp* group) to refer to those genes that are required for the specification of dorsal structures, and the term twist group (*twi* group) to refer to those genes required for the specification of the ventral structures.

Decapentaplegic group genes

In addition to *dpp* and *zen*, the *dpp* group includes five other zygotic loci: screw (*scw*), tolloid (*tld*), shrew (*srw*), twisted gastrulation (*tsg*), and short gastrulation (*sog*). Mutations in these loci are associated with a general loss of amnioserosa, dorsal ectoderm, and dorsolaterally derived structures of the acron and telson (Anderson, 1987; Rushlow and Arora, 1990; K. Arora and C. Nüsslein-Volhard, in prep). Accompanying this loss of dorsal structures is an expansion of ventrolateral pattern elements (Fig. 8C–E). The seven genes can be subdivided into three classes based on a comparison of the phenotype of the most severe loss-of-function allele of each (R. Ray, K. Arora, and W. Gelbart, in prep):
Fig. 7. Expression patterns in tor embryos. (A,B) dpp; (C,D) zen; (E,F) twi; (G,H) sna. (A) Lateral view of a stage 4 mutant embryo probed with a dpp cDNA. Expression is clearly restricted from the poles. (B) Lateral view of a late stage mutant embryo showing the late pattern of dpp expression. The ventral midline (indicated by the arrowhead) lies between the two rows of spots corresponding to the lateral stripe of dpp (cf. Fig. 2C). On either side of the two lateral stripes, the fine dorsal stripe is evident. (C) Expression of zen in a late stage 4 embryo. Like dpp, zen expression does not extend to the poles. The weak staining in the dorsal side of the anterior pole is an artifact of the photography (cf. Fig. 10). (D) Dorsal view of a stage 5 embryo showing the refined zen pattern in a tor~ embryo. The anterior head spot is absent and the remaining pattern is condensed. (E) Lateral view of a late stage 4 embryo showing the expression of twi. Expression extends up to the poles, but not around. No refinement of the twi pattern is observed. (F) Lateral view of a stage 7 embryo probed with a twi cDNA. The ventral furrow has invaginated over the full length of the embryo. (G) Lateral view of an early stage 5 embryo showing the expression of sna. Similar to twi, sna transcripts accumulate ventrally up to, but not around the poles. The sna pattern does not refine. (H) A late stage mutant embryo illustrating the normal D/V pattern in the late expression of sna in the neuroblasts. The ventral midline is indicated by the arrowhead.
Dorsal–ventral patterning in Drosophila

Fig. 8. Cuticular phenotypes associated with zygotic mutations. (A) Wild type cuticle. Dorsal is to the right and anterior is up. (B) Cuticle of a sna mutant embryo. Slight truncations in the ventral denticle belts are visible, particularly in the posterior abdominal segments. The characteristic twist appears in the thoracic segments. The head is disorganized, but the Filzkörper are present. (C) Ventral view of the cuticle of a weakly ventralized embryo (zen). Some expansion of the ventral denticle belts is observed. The head has not involuted, and the structures posterior to A8 have not everted. (D) Cuticle of a moderately ventralized embryo (sew). Greater expansion of the ventral denticle belts is observed. Some dorsal ectoderm is still present. (E) Cuticle of a strongly ventralized embryo (dppH). The denticle belts are expanded over the entire D/V axis.

Table 1. Expression patterns of dpp, zen, twi and sna in embryos mutant for zygotic loci affecting dorsal–ventral patterning

<table>
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<tr>
<th>Probe</th>
<th>dpp</th>
<th>l ld</th>
<th>scw</th>
<th>srw</th>
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<th>sog</th>
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<tr>
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<td>+</td>
<td>not refined</td>
<td>+</td>
<td>not refined</td>
<td>+</td>
<td>not refined</td>
<td>+</td>
</tr>
<tr>
<td>twi</td>
<td>not maintained&lt;sup&gt;a&lt;/sup&gt;</td>
<td>not maintained</td>
<td>not maintained</td>
<td>not maintained</td>
<td>not refined</td>
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<sup>a</sup>The specific alleles that were used for each of these genotypes are listed in the Materials and methods.

<sup>b</sup>For each probe, the top line refers to the effect of the mutation on the initial pattern and the bottom line to the effect on the refinement of that pattern.

<sup>c</sup>+ refers to the wild type pattern.

<sup>d</sup>In the stronger ventralizing mutations the initial pattern of zen is not maintained, and thus not refined; in tsg and sog, the pattern is maintained, but not refined.

<sup>e</sup>In this case, the initial pattern is refined, but the refined pattern is not maintained (see Fig. 9 and Table 2).

weak loci (sog, tsg, and zen; Fig. 8C), moderate loci (l ld, scw, and srw; Fig. 8D), and a single strong locus (dpp; Fig. 8E). Loss-of-function mutations in these loci result in a characteristic disruption of germ band extension that leads to the invagination of the posterior segments into the interior of the embryo (our observations, see Fig. 8).

The initial expression patterns of all four zygotic genes are unaffected by mutations in any of the dpp group genes (Table 1). Thus, the expression of each gene appears to be independently initiated by dl. In the case of twi and sna, proper refinement, and initiation of late patterns is also observed (Table 1). For dpp and zen, however, maintenance and refinement of the initial patterns is aberrant (see also Rushlow and Levine, 1990). A comparison of the effects of weak, moderate and strong dpp group mutations on the dpp and zen expression patterns indicate that these two genes have reciprocal effects on their refinement. For instance, in a wild-type gastrulating embryo, expression of zen is confined to the dorsal-most region of the embryo, the presumptive amnioserosa, while dpp expression is excluded from this domain (Fig. 9A,D). In embryos mutant for weak loci of the dpp group, zen expression is
Fig. 9. Comparison of expression patterns of *zen* and *dpp* in embryos mutant for weak and moderate dpp group genes. (A) Dorso-lateral view of a wild type stage 8 embryo probed with the *zen* probe. The expression is confined to the dorsally located presumptive amnioserosa. (B) Dorso-lateral view of a *sog* mutant embryo probed with *zen* at a slightly earlier stage than that of the embryo in A. Expression has refined along the A/P axis, but not along the D/V axis. Phenotypically, this embryo does not properly fate the amnioserosa. (C) Expression of *zen* in an embryo null for *dpp*, the strong locus of the dpp group (see text). Initially, *zen* is expressed in the normal domain (data not shown), but by cellularization (and gastrulation, as shown here) *zen* transcripts are not observed. This same pattern of expression is observed for *zen* in embryos mutant for *tld, srw*, or *scw*. (D) Lateral view of a late stage 8 embryo showing the refined *dpp* expression pattern: no staining is observed in the dorsally located amnioserosa. (E) Lateral view of a late stage 8 *sog* embryo probed with *dpp*. Transcripts can be detected in the region normally occupied by the amnioserosa. (F) Lateral view of a stage 8 *tld* embryo showing the dorsal expression of *dpp* in this mutant background.

maintained until germ band extension, but the pattern fails to refine along the D/V axis, and expression is observed over 40% of the dorsal surface (Fig. 9B). *dpp* also fails to refine in these embryos, and expression is observed in the amnioserosa (Fig. 9E). In embryos mutant for moderate and strong loci of the dpp group, *zen* expression is lost throughout the dorsal ectoderm prior to cellularization, and thus is never seen to refine (Fig. 9C). Again, *dpp* expression is normal but does not refine and is expressed throughout the dorsal ectoderm (Fig. 9F).

Several points can be made from these results. First, all of the dpp group genes appear to be required for the normal ontogeny of the *zen* pattern and the fating of the amnioserosa. Second, the weak and moderate ventralizing loci have different effects on the expression pattern of *zen; dpp, tld, scw*, and *srw* are required for the maintenance of the *zen* expression pattern, while *tsg* and *sog* are required for its refinement. While these functions are distinct, the terminal phenotype is the same: loss of the amnioserosa. This failure to fate the amnioserosa undoubtedly accounts for the failure of the *dpp* expression pattern to refine, since the cells normally fated to the amnioserosa would be expected to behave like cells of the dorsal epidermis in the mutant embryos.

Thus, the subdivision of the dorsal ectoderm into amnioserosa and dorsal epidermis depends only on the dpp group genes and is subordinate to the initial specification of dorsal ectoderm by *dl*. Furthermore, there appears to be a regulatory loop involved that has the following organization. Moderate and strong dpp group genes are required for the proper ontogeny of *zen* expression, which determines the fate of the amnioserosa. Once this domain has been established, genes specific to the dorsal epidermis, like *dpp*, are excluded from it. Notably, *zen* has no role in its own refinement, as the ontogeny of *zen* expression is normal in *zen* mutant embryos. Similarly, null mutations of *dpp* do not affect the expression of *dpp*, excepting that since the amnioserosa is not fated, no refinement of the *dpp* pattern is observed (Table 1).

**Twist group genes**

Loss-of-function alleles of *twi* and *sna* produce embryos that lack all derivatives of the mesoderm. In such mutant embryos, the cells on the ventral side do not invaginate and no ventral furrow is formed (Simpson, 1983; Leptin and Grunewald, 1990). In spite of this defect, *twi* and *sna* mutant embryos produce fairly normal cuticles with only minor truncations of ventrally derived pattern elements (Fig. 8B).
The initial expression patterns of all four zygotic genes are not affected by mutations in twi or sna (Table 1). Thus, in conjunction with the results obtained for the dpp group genes, it seems clear that the initiation of expression of dpp, zen, twi, and sna is entirely controlled by the maternal morphogen dl. In particular, we note the initiation of twi expression is essentially normal in sna mutant embryos, and the initiation of sna expression is normal in twi mutant embryos (Fig. 10A,C,E,G). Thus, these two genes are not sequential elements in a hierarchical series, and neither depends entirely on the other. As was true for twi and sna expression in dpp group genes, refinement and initiation of late patterns is observed for both dpp and zen in twi and sna mutant embryos. In contrast, the ontogeny of twi and sna expression in twi group mutant embryos is aberrant, as discussed below.

twi expression is aberrant in both twi and sna mutant embryos. In twi mutant embryos, no effect is observed until the pattern begins to deteriorate during cellularization, at which time the level of expression drops and the pattern begins to develop gaps in a segmental fashion (Fig. 10B). By the beginning of germ band extension, twi expression is no longer detectable. Notably, twi mutations do not affect the width or refinement of the twi pattern, only its perdurance (Table 2).

In sna mutant embryos, twi expression appears in the normal pattern, but throughout stages 4 and 5, the mutant embryos can be distinguished from their wild-type siblings by the fact that the level of expression is reduced (Fig. 10C, Table 2). As was observed in twi mutant embryos, twi expression is extinguished in the presumptive mesoderm by the beginning of germ band extension. Notably, the expression of twi in these mutant embryos is extinguished only within the domain of the presumptive mesoderm; expression in the cephalic segments and telson is not affected and must be under separate control.

The expression of sna in twi mutant embryos reveals that sna function is at least partially subordinate to twi. In such embryos initiation of sna expression is normal, but the width of the pattern never reaches the characteristic 18 cells, and the edges, which are sharp and distinct in wild type, are diffuse and irregular (Fig. 10E, Table 2). Furthermore, stage 5 mutant embryos still show weak expression of sna at the poles suggesting that the refinement of the sna pattern is also aberrant. Thus, twi appears to be required for the proper ontogeny of the sna expression pattern, while the reciprocal, that sna is required for the ontogeny of twi, was not observed. The sna expression patterns described here have two features in common with the

<table>
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<tr>
<th>Probe</th>
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<tr>
<td>twi</td>
<td>twi</td>
<td>+</td>
</tr>
<tr>
<td>sna</td>
<td>sna</td>
<td>+</td>
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<td>sna</td>
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a The alleles used in these experiments were twiL006, an EMS-induced allele that is protein null (unpublished), and snaL005 which is a small deletion in the coding region of the sna gene (Boulay et al. 1987).

b This refers to the width of the initial expression domain, i.e., 18–20 cells in wild type (see Fig. 2).

c This refers to the sharpness of the edge of the sna expression domain.

d This refers to the point in development at which expression can no longer be detected. In wild type, sna expression is extinguished in the mesoderm at the end of fast germ band extension (see text). twi expression normally persists in the mesoderm until late in development (Thiss et al. 1987).

f + implies that this aspect of the pattern is indistinguishable from wild type.

Not applicable. twi expression domain does not have a sharp edge in wild type, but rather tapers off in a graded fashion.

b The timing of germ band extension in the mutant embryos does not correspond to that in wild type (unpublished).
Fig. 10. Expression patterns of twi and sna in twi and sna mutant embryos. In all embryos anterior is to the left, and ventral is up except in C which is viewed ventro-laterally. (A–D) Expression of twi at two stages of development in embryos mutant for twi (A,B) and sna (C,D). (A) Stage 4 embryo showing the initial pattern of twi expression in a twi mutant. The width and intensity of expression are as observed in wild-type. (B) Stage 5 embryo showing the waning twi pattern in a twi mutant. The deterioration of the initial pattern appears in a segmental fashion. Weak expression can be detected in these embryos until the onset of germ band extension. (C) Ventro-lateral view of a stage 4 sna embryo probed with twi. Such mutant embryos can be distinguished from their wild-type siblings by virtue of a uniform lower level of expression. Nevertheless, the overall pattern does not differ from wild type. (D) Late stage 7 sna embryo showing the deterioration of the sna pattern. The staining in the cephalic segments will persist after the expression in the mesoderm is lost. (E–H) Expression of sna at two stages of development in embryos mutant for twi (E,F) and sna (G,H). (E) Ventral view of a stage 4 twi mutant embryo probed with sna. The ventral domain is narrow and the edges are rough. The pattern never reaches its full extent. (F) Expression of sna in a late stage 7 twi mutant embryo. Expression in the mesoderm is almost entirely absent, but the expression in the cephalic segments and in the telson persists. (G) Expression of sna in a stage 4 sna mutant embryo. The width of the domain is as in wild type, but the edges fail to refine and are rough. (H) Extinction of the sna expression pattern in the sna embryo. The timing of this loss is essentially the same at that observed for (B), (D), and (F). See also, Table 2.
twi patterns: sna expression is extinguished by the beginning of germ band extension, and the expression in the cephalic segments and telson are under a separate control (Fig. 10F).

Consistent with the hypothesis that the width of the sna domain depends on twi, sna expression in sna mutant embryos is as broad as in wild type (Fig. 10G). However, the edge of the domain is rough, as was observed in the twi mutant embryos. This latter effect suggests that sna is required for the refinement of its own expression, at least as far as the sharpness of the edges is concerned. As was true for sna expression in twi mutant embryos, the expression of sna in sna embryos is not detectable after the beginning of germ band extension (Fig. 10H). Thus, regardless of probe and genotype, the expression patterns are extinguished at approximately the same time, suggesting that the failure of the patterns to be maintained in the mutant embryos may not be an effect of the mutations themselves, but rather a programmed point in development after which other fates are established in the mesodermal domain if twi and sna are not both expressed.

Discussion

Two sources of maternal information are required for the initial expression of dpp, zen, twi, and sna

We have shown that two sources of maternal information are required to generate the initial expression patterns of dpp, zen, twi, and sna. This information is provided by genes of the dorsal and terminal systems. In the segmented region of the embryo, the expression of all four zygotic genes is controlled exclusively by the dorsal group genes and cact. In the polar regions, the situation is more complex and requires the activity of the terminal system. In particular, dpp and zen require the activity of the terminal system alone, while twi and sna require the coordinate action of both the dorsal and the terminal systems, as will be discussed below. These two maternal controls are all that is required for the specification of the initial expression of all four zygotic genes.

dl activity subdivides the D/V axis into three broad domains

The dl gradient appears to be interpreted on three levels. Ventrally, dl activates twi and sna expression and represses dpp and zen expression, so only twi and sna are expressed. Laterally, the reduced activity of the dl protein is not sufficient to activate twi and sna, but can still repress dpp and zen, so none of the four genes are expressed. Dorsally, the activity of dl is so low that it can neither activate twi and sna nor repress dpp and zen, so only dpp and zen are expressed (Fig. 10). Thus, there are two discernible thresholds of dl activity along the D/V axis. twi and sna respond to the higher threshold. If dl activity is above this threshold, twi and sna are expressed; if it is below, they are not. dpp and zen respond to the lower threshold. If dl activity is above this threshold, dpp and zen are repressed; if it is below, they are not.

In the absence of double staining experiments or alternate sections, we cannot say that twi and sna, on the one hand, and dpp and zen on the other are responding to precisely the same level of dl activity. In fact, careful analysis of the twi and sna patterns suggests that the graded expression of twi may extend more dorsally to label cells that are not expressing sna (unpublished). Similarly, the ventralmost limit of dpp and zen expression may not coincide precisely. Nevertheless, within the resolution of the experiments presented here, the two thresholds are respected. The fact that all four of the genes analysed here appear to be responding to only two thresholds of dl activity suggests that the dl gradient may only be involved in defining these three broad domains of the embryo. Further subdivision of the embryo within each of these domains undoubtedly depends on the interactions between the zygotic gene products that are specifically required for each domain. Our results on the interactions between zygotic genes suggest that this is in fact the case. In particular the relationship between dpp and zen in the subdivision of the dorsal ectoderm into dorsal epidermis and amnioserosa suggests that only the zygotic genes of the dpp group are responsible for this partitioning event.

dpp is a primary gene required for the specification of dorsal ectoderm

Although the initial expression pattern of zen suggests a primary role for this gene in the organization of the D/V pattern, our data strongly suggest that this gene plays a subordinate role to dpp. The most notable of these is that the cuticular phenotype associated with zen mutations, which consists of a loss of amnioserosa and deletions in the optic lobes (Wakimoto et al. 1984), does not reflect a general requirement for zen in all cells of the dorsal ectoderm, as predicted by the initial expression pattern. Instead, the deleted structures correspond precisely to those regions of the fate map delimited by the refined expression pattern. Since the establishment of this refined pattern depends on the function of all other dpp group genes, it must be concluded that zen acts at a level further down the hierarchy. By contrast, the failure of other zygotic genes to affect the initial expression pattern of dpp, and the severity and singularity of the dpp phenotype, are strong indicators of the primary role this gene plays in the specification of the dorsal ectoderm.

twi and sna carry out complementary roles in mesoderm specification

The specification of the ventral domain requires both twi and sna. Our results indicate that these two genes are independently specified by the dl gradient, and the interactions between the two genes suggest that, despite their respective homologies to DNA binding proteins (Murre et al. 1989; Boulay et al. 1987), they are not sequential elements in a hierarchical series. Nevertheless, the expression of sna depends on the normal
expression of twi, and in this respect, sna is subordinate to twi. Thus, we propose that twi is the primary patterning gene for mesoderm. However, further studies on the roles these genes play in mesoderm development suggest that they carry out complementary roles. In particular, twi and sna have different effects on the expression of genes in mesodermal and surrounding cells. For instance, twi has been shown to be responsible for the activation of mesoderm specific genes like msh-2 (Bodmer et al. 1990) and PS-2 integrin (unpublished). In contrast, sna has been shown to be involved in repression of genes that are expressed in the mesectoderm and ventral ectoderm like single-minded (Nambu et al. 1990) and rhomboid (unpublished). Thus, it seems clear that twi and sna carry out complementary, not redundant, functions in the specification of the mesoderm.

**Lateral fates**

Although we have only analyzed a small number of zygotic genes which might be targets of dl activity, we speculate that the dl gradient may only be responsible for coarse subdivision of the embryo into the three domains described above. While it seems clear the determination of the dorsal and ventral fates are specified directly by dl, there is some evidence to suggest that lateral positional values appear to arise as a default state in the absence of the zygotic gene expression characteristic of the dorsal and ventral positional values. The support for this hypothesis comes from the cucticular phenotype of double mutant embryos lacking the maternal function of dl and the zygotic function of dpp. Such double mutant embryos do not express twi or sna due to the loss of dl function, and also manifest zygotic gene expression characteristic of the dorsal and ventral domains described above. While it seems clear the twi, sna and dpp carry out complementary, not redundant, functions in the specification of the mesoderm.

**Polar expression of the zygotic genes**

We have shown that the initial expression pattern of twi at the poles requires the activity of both the dorsal-ventral system and the terminal system: twi expression is entirely lacking in dorsalized embryos, and aberrant at the poles in tor embryos. The failure for twi expression to appear in dorsalized embryos clearly indicates that these two control mechanisms are not independent. As discussed below, it appears that the ability for the terminal system to act on twi depends on the activity of dl. We have therefore proposed that the terminal system acts through or with dl to affect expression of twi at the poles.

Expression of twi is lacking in all genotypes tested that produce dorsalized embryos. However, it is not the presence or absence of dl protein, per se, that is necessary for the activation of twi by the terminal system, since dorsalized embryos derived from Tl, snk, or tub mothers produce dl protein but nevertheless lack expression of twi at the poles. Thus, the effect of the terminal system on twi depends on the activity of the dl protein. This point accounts for the polar expression of twi in lateralized embryos, as dl is at least partially active (Steward et al. 1989; Roth et al. 1989; Roth, 1990) in these embryos, and thus twi can be expressed. This conclusion presumes that the expression of twi in lateralized embryos depends on terminal system function. In fact, this is the case, as we find that a lateralized embryo that is also lacking the function of one of the maternal terminal system genes does not express twi (unpublished). Furthermore, we can rule out the possibility that the terminal system is acting to alter the distribution of dl protein at the poles (i.e., causing the nuclear localization of dl at the poles) as no accumulation of dl protein is observed in the terminal nuclei of lateralized embryos (unpublished).

Further evidence to suggest that dl and the terminal system act in conjunction comes from analyses of twi expression in double mutants of loss-of-function alleles of the dorsal group genes and cact. Such double mutant mothers produce lateralized embryos (Roth et al. 1989; Roth 1990), and, like the lateralized embryos produced by mothers bearing lateralizing alleles of dorsal group genes, these embryos express twi at the poles. However, in the double mutant combinations with cact, the alleles of the dorsal group genes are nulls, and thus the dorsal pathway is not active. Thus, the lack of twi expression at the poles in a dorsalized embryo appears to be due to interference by cact. As it has been proposed that cact interacts with the dl gene product, we can account for our data by assuming that the interaction between the cact and dl gene products interferes with the action of the terminal system.

Two mechanisms could account for these data. First, two positive regulators may be required to activate twi: dl and another transcription factor, either maternal or zygotic, downstream of tor. We must presume that neither of these factors are sufficient to activate twi expression alone, in order to account for the fact the dorsalized embryos do not express twi at all. This simple model is appealing, but at present, there is no candidate for the second transcription factor. An alternative explanation is that dl is the only transcription factor required, and that dl activity is modified by the action of tor or downstream genes. While we know that this does not occur by redistribution of dl protein, it could occur by modulation of dl activity (i.e., directly acting on dl protein), thus making the lower concentration of dl protein normally found in polar nuclei sufficient to activate twi. At present, these two hypotheses cannot be distinguished.

With regard to the control of sna expression, the initial pattern is clearly regulated by the same network as described for twi. However, since the refinement of this pattern also depends on the terminal system, we must conclude that the genes of this system play a role
in the repression as well as activation of \textit{sna} transcription. Thus, there are two superimposed controls: the terminal system and \textit{dl} activate \textit{sna} expression initially, and then later in development other terminal genes act to refine this pattern. As the latter regulatory feature appears to override the effect of the former, we suspect that the transient polar expression of \textit{sna} is not a reflection of a specific requirement for \textit{sna} gene activity at the poles, but rather a consequence of these overlapping control mechanisms.

Finally, as we have noted earlier, the problem that arises from expressing \textit{twi} and \textit{sna} at the poles by affecting the activity of \textit{dl} is how \textit{dpp} and \textit{zen} can be expressed in the same domain when these two genes are repressed by \textit{dl}. We have shown that this effect is also mediated by the terminal system, and that this activity does not depend on \textit{dl}. The latter fact is clearly illustrated by the expression patterns of \textit{dpp} and \textit{zen} in embryos derived from \textit{Tl}^{1}\textit{Ob} mothers. Although such pattern clearly illustrates the three levels of interpretation gradient (see text). This pattern depends only on the \textit{dl} the polar regulation of the zygotic genes such that the \textit{D/V} and \textit{sna}. \textit{zen} function removes patterns away from the poles (Fig. 11), an effect which due to \textit{dl}. This assertion is supported by the expression \textit{zen} and \textit{dpp} owing to the terminal system overrides the repression of \textit{dl} protein to all blastoderm nuclei, the expression of \textit{dpp} and \textit{zen} persists at the poles. Thus, the activation of \textit{dpp} and \textit{zen} by the terminal system overrides the repression due to \textit{dl}. This assertion is supported by the expression patterns we observe in \textit{tor} embryos. These embryos show a retraction of the \textit{dpp} and \textit{zen} expression patterns away from the poles (Fig. 11), an effect which is not consistent with the fate changes predicted by the cuticular phenotype (Klingler et al. 1987; Casanova, 1990). However, though inconsistent with the \textit{tor} phenotype, this pattern of expression is consistent with the repression of \textit{dpp} and \textit{zen} by the virtually normal gradient of \textit{dl} protein in the mutant embryos (unpublished).

We conclude that the terminal system is required for two layered functions that are necessary to achieve expression of the four zygotic genes at the poles. First, the terminal system is required to express \textit{twi} and \textit{sna} at the poles by modulating the activity of \textit{dl}. Then, other functions of the terminal system activate \textit{dpp} and \textit{zen}, and repress \textit{sna} at the poles. Both of these latter effects override aspects of \textit{dl} regulation that are required for normal pattern formation in the segmented region of the embryo. The combination of these two functions can account for the modulations observed in the wild type patterns.

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