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The Polarity of the Dorsoventral Axis in the Drosophila Embryo Is Defined by an Extracellular Signal

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Summary

Twelve maternal effect loci are required for the production of Drosophila embryos with a correct dorsoventral axis. Analysis of mosaic females indicates that the expression of the genes *nudel, pipe*, and *windbeutel* is required in the somatic tissue, presumably in the follicle cells that surround the oocyte. Thus, information coming from outside the egg cell influences dorsoventral pattern formation during embryogenesis. In transplantation experiments, the perivitelline fluid from the compartment surrounding the embryo can restore dorsoventral pattern to embryos from females mutant for *nudel, pipe*, or *windbeutel*. The positioning of the transplanted pervitelline fluid also determines the polarity of the restored dorsoventral axis. We propose that the polarizing activity, normally present at the ventral side of the egg, is a ligand for the *Toll* receptor. Presumably, local activation of the *Toll* protein by the ligand initiates the formation of the nuclear concentration gradient of the *dorsal* protein, thereby determining dorsoventral pattern.

Introduction

The establishment of the primary axes of the Drosophila embryo is accomplished by four systems of maternal information (Nüsslein-Volhard and Roth, 1989). Along the anterioposterior axis three independent systems of maternal effect genes determine the anlagen of the anterior (head and thorax), posterior (abdomen), and terminal (acron and telson) elements of the larva, respectively (Nüsslein-Volhard et al., 1987). In contrast to anterioposterior development, the determination of the pattern elements along the dorsoventral axis is accomplished by a single system of positional information (Anderson and Nüsslein-Volhard, 1986; Anderson, 1987). These pattern elements are as follows, from ventral to dorsal: the mesoderm; the ventral neuroectoderm, which gives rise to the central nervous system and the ventral hypoderm, including the conspicuous ventral denticles; the dorsolateral ectoderm, from which the tracheae and dorsal hypoderm are derived; and the amnioserosa.

Females lacking the function of any 1 of the 11 dorsal group genes, *dorsal* (*dl*), *easter* (*ea*), *gastrulation defective* (*gd*), *nudel* (*ndl*), *pipe* (*pip*), *pelle* (*pll*), *snake* (*snk*), *spätzle* (*spz*), *Toll* (*Tl*), *tube* (*tub*), and *windbeutel* (*wind*), give rise to embryos that are completely dorsalized, lacking ventral, ventrolateral, and dorsolateral pattern elements (Anderson and Nüsslein-Volhard, 1986; Schüpbach and Wieschaus, 1989). The egg shape, however, is not affected by mutants of the dorsal group genes and displays a normal dorsoventral polarity as seen in the morphology of the chorion. The dorsalized phenotype can be detected during gastrulation when cell movements characteristic of those seen on the dorsal side of the wild-type embryo occur all around the circumference of the mutant embryo. In contrast, females lacking the function of a 12th gene, *cactus (cact)*, give rise to embryos exhibiting an expansion of ventral structures at the expense of dorsolateral and dorsal structures (Schüpbach and Wieschaus, 1989; Nüsslein-Volhard and Roth, 1989).

The interaction of the products of the dorsal group genes and *cact* causes the formation of a nuclear concentration gradient of the product of the *dl* gene (Roth et al., 1989; Rushlow et al., 1989; Steward, 1989). A comparison between the nuclear distribution of *dl* protein and the developmental fate map as reflected in the expression of zygotic dorsoventral genes in mutant and wild-type embryos indicated that *dl* protein acts as a morphogen by regulating zygotic gene expression in a concentration-dependent manner. The role of *dl* as a transcription factor is supported by its sequence homology (Steward, 1987) with several predicted (Gelinas and Temin, 1988) and demonstrated (Bours et al., 1990; Ghosh et al., 1990; Kieran et al., 1990) transcription factors and by the demonstration in vitro that *dl* protein binds directly to a specific target DNA sequence (Ip et al., 1991).

In embryos from lack-of-function mutants of any of the other 10 members of the dorsal group genes, the *dl* protein does not form a nuclear concentration gradient. Instead, it is found in the cytoplasm at all positions along the dorsoventral axis (Roth et al., 1989; Rushlow et al., 1989; Steward, 1989). In contrast, embryos produced by females mutant for *cact* exhibit elevated levels of *dl* protein in the nuclei at the lateral and dorsal regions of the embryo. These observations suggest that *cact* functions to inhibit the nuclear uptake of the dorsal protein, while the dorsal group gene products act in concert to facilitate the nuclear concentration. During normal development, the maximal nuclear concentration of *dl* protein in the embryo is found at the ventral side of the egg. The dorsal group genes, therefore, transduce an intrinsic dorsoventral polarity of the egg into embryonic dorsoventral polarity and consequently define positions along the circumference at which the *dl* protein is taken up.

Several experimental approaches have been taken to define the distinct roles of the individual genes during dorsoventral pattern formation and to order them tentatively in a functional hierarchy. Some restoration of dorsoventral pattern can be seen following injection of wild-type cytoplasm into mutant embryos of several of the dorsal group genes (Santamaria and Nüsslein-Volhard, 1983; Anderson and Nüsslein-Volhard, 1984a; Anderson et al., 1985b), and the inability to rescue embryos derived from females mutant for *ndl, pip, wind*, and *gd* (Anderson and Nüsslein-

Volhard, 1984a) has suggested that these gene products have a role during oogenesis that cannot be restored by altering the cytoplasmic content of the mature mutant egg. For most of the dorsal group genes in which cytoplasmic injection results in rescue (*dl, ea, tub, pll, snk*, and *spz*), the polarity of the restored pattern is normal and corresponds to the orientation of the egg axis rather than determined by the site of injection. This indicates that despite their dorsalized phenotype, mutant embryos retain an intrinsic polarity. The gene *Tl* behaves strikingly different in cytoplasmic rescue experiments since the polarity of rescued embryos in this case is determined by the site of injection of the rescuing activity (Anderson et al., 1985b). However, no significant spatial asymmetry of rescuing activity in donor embryos was observed. These experiments single out the *Tl* gene product as an important mediator of dorsoventral polarity.

In addition to the dorsalized lack-of-function alleles, there exist dominant gain-of-function alleles of *Tl* that display a ventralized phenotype, which retains polarity. Ventral pattern elements occupy an expanded portion of the dorsoventral circumference at the expense of lateral and dorsal pattern elements. Females that are doubly mutant for the gain-of-function allele Tl^{9Q} and for *dl, tub*, or *pll* give rise to dorsalized progeny (Anderson et al., 1985a; K. Anderson, unpublished data). In contrast, Tl^{9Q} mutant females that are additionally mutant for *ea, gd, ndl, pip, snk, spz*, or *wind* produce embryos that are partially ventralized and apolar (the "lateralized" phenotype). This indicates that these genes act upsteam of *Tl* in establishing the polarity of the embryo, while *tub* and *pll* presumably act downstream of *Tl* and transmit the polarizing signal received by *Tl* into a gradient of nuclear localization of *dl* (K. Anderson, unpublished data). The *Tl* gene has been cloned and found to encode a putative transmembrane protein that is evenly distributed in the membrane surrounding the embryo (Hashimoto et al., 1988; unpublished data).

An important clue to the understanding of *Tl* function was provided by studies of the gene *torso (tor)*, which plays a key role in the terminal system of axis determination. Like *Tl*, there also exist *tor* gain-of-function alleles with a phenotype opposite to the lack-of-function alleles (Klingler et al., 1988). *tor* encodes a transmembrane protein with homology to receptor tyrosine kinases (Sprenger et al., 1989) that is evenly distributed in the egg membrane (Casanova and Struhl, 1989; Stevens and Nüsslein-Volhard, in press). Determination of the embryonic termini is thought to result from activation of *tor* by a ligand produced by polar populations of somatic follicle cells, during the formation of the egg (Stevens et al., 1990). By analogy, *Tl* protein could act as a receptor for an extraembryonic signal produced under the control of a ventral population of follicle cells.

We have investigated whether the polarity along the embryonic dorsoventral axis is initiated by a signal originating outside of the embryo, with the *Tl* gene product playing the role of a receptor. Schüpbach (1987) observed that the expression of the maternal effect locus *torpedo (top)* is required in the follicle cells for the elaboration of correct dorsoventral polarity of both the eggshell and the future embryo. This suggested that the somatic follicle cells surrounding the developing oocyte might provide a signal that would later polarize the embryo and that this signal might involve the function of 1 or more of the dorsal group genes. Here, we show that of the 11 dorsal group genes, the expression of *ndl, pip*, and *wind* are required in the somatic tissue but not in the germline of the developing egg follicles. Recently, Schüpbach has also obtained evidence that members of the dorsal group act in the soma (as cited in Manseau and Schüpbach, 1989). By transplantation of fluid from the compartment bounded by the egg membrane and the vitelline coat (the perivitelline fluid) we have identified an activity that is able to restore the formation of a dorsoventral axis to mutant *ndl, pip*, or *wind* embryos. This activity can only be detected in eggs that lack the *Tl* gene product; furthermore, the location at which the perivitelline fluid is deposited into the perivitelline compartment of the recipient egg determines the polarity of the rescued embryo. This suggests that the perivitelline fluid contains a ligand that is produced in limited quantities, that binds to the extracellular domain of the *Tl* protein. We propose that during normal development this ligand is produced at the ventral side of the egg and mediates the ventrally restricted actiation of the *Tl* protein, thereby triggering a signal transduction cascade that culminates in the graded nuclear uptake of *dl* protein.

Results

ndl, pip, and *wind* Expression Is Required in the Somatic Tissue of the Adult Female

To determine within which tissues expression of the dorsal group genes is required, mosaic females were constructed in which the germline was mutant for a dorsal group gene, while the somatic tissue was wild type, or vice versa. Homozygous mutant pole cells were transplanted into recipient embryos that were wild type for all dorsal group genes but carried the dominant female sterile mutation *Fs(1)* ovo^{D1} (Busson et al., 1983). *Fs(1)*ovo^{D1} females are sterile due to the failure of the germ line to develop; after transplantation, only those females in which donor pole cells have populated the germline lay eggs. The production of dorsalized embryos by such females indicates a germline requirement for the dorsal group gene under investigation, while the production of normal progeny indicates that expression of the gene is not required in the germline. Transplantations of this type indicated that the genes *ea, snk, pll*, and *tub* must be expressed in the germline during oogenesis (Table 1). In contrast, expression of the genes *ndl, pip*, and *wind* is not required in the germline. The reciprocal transplantation of wild-type pole cells into females that were homozygous for *pip* or *ndl* and were also heterozygous for *Fs(1)*ovo^{D1} resulted in the production of dorsalized progeny (Figure 1), indicating that expression of these two genes is required in the somatic tissue. The donor embryos carried a *ftz–lacZ* transgene (Hiromi et al., 1985). β-Galactosidase staining in the dorsalized progeny of fertile females confirmed that these embryos arose from the transplanted (wild-type) pole cells (Figure 1). We could not carry out a similar reciprocal transplantation to confirm

Table 1. Production of Ovarian Mosaics by Pole Cell Transplantation

Parental Genotype of Pole Cell Donors	Parental Genotype of Pole Cell Recipients	Number of Recipients Injected	Number of Surviving Adult Females	Number of Fertile Adult Females	Phenotype of Progeny Embryos/Tissue Dependence
ea^{1}/*ea*^{2}	*ovo*^{D} males × oreR females	420	137	8	Dorsalized/germline
ndl^{046}/*ndl*^{093}	*ovo*^{D} males × oreR females	200	55	4	Wild type/germline excluded[a]
pll^{078}/*pll*^{rm8}	*ovo*^{D} males × oreR females	434	131	22	Dorsalized/germline
pip^{366}/*pip*^{864}	*ovo*^{D} males × oreR females	925	256	10	Wild type/germline excluded[a]
snk^{073}/*snk*^{229}	*ovo*^{D} males × oreR females	370	107	10	Dorsalized/germline
tub^{118}/*tub*^{238}	*ovo*^{D} males × oreR females	398	119	10	Dorsalized/germline
wind^{RP}/*wind*^{T6}	*ovo*^{D} males × oreR females	445	107	21	Wild type/germline excluded[a]
Tf(3;2)4304, ry^{+} *βgal*^{ftzP}	*ovo*^{D};*ndl*^{093}/TM3 males × *ndl*^{046}/TM3 females	568	62	7[b]	Dorsalized/soma
Tf(3;2)4304, ry^{+} *βgal*^{ftzP}	*ovo*^{D};*pip*^{366}/TM3 males × *pip*^{864}/TM3 females	360	42	9[b]	Dorsalized/soma

Pole cell transplantation and analysis of mosaic females were carried out as described in the Experimental Procedures. Parental genotypes of donor and recipient embryos are given only with respect to dorsal group genes and *Fs(1)ovo*^{D1}. Other workers have previously established the germline-dependent expression of the dorsal group genes *gd* (Konrad et al., 1988b), *dl*, *Tl* (Schüpbach and Wieschaus, 1986), and *spz* (Seifert et al., 1987).

[a] The production of wild-type progeny in these cases indicates that the expression of these genes is not required in the germline. While it suggests a requirement for expression in the soma, this result does not constitute a proof.

[b] In the investigations of the somatic dependence of the genes *ndl* and *pip* (bottom two transplantations in the table), the number of surviving adult females and fertile females refers only to the informative transheterozygous females (i.e., *Fs(1)ovo*^{D1};*ndl*^{046}/*ndl*^{093} and *Fs(1)ovo*^{D1};*pip*^{366}/*pip*^{864}).

the somatic requirement for *wind* expression due to the semilethal zygotic phenotype of the *wind* locus (Schüpbach and Wieschaus, 1989). We consider it likely, however, that the requirement for *wind* expression is also restricted to somatic tissues.

Recovery and Transplantation of Perivitelline Fluid

To protect the Drosophila egg following oviposition, the somatic tissues of the egg follicle produce the eggshell coverings, the vitelline coat, and the chorion. During early development, the membrane of the embryo remains inti-

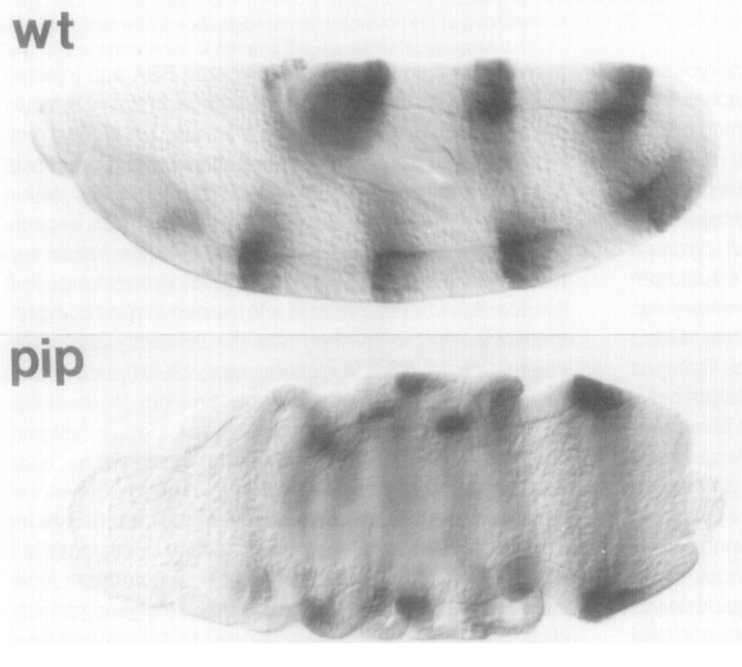

Figure 1. Gastrulation Patterns of the Embryos Developing after Transplantation of Wild-Type Pole Cells into *pip* Mutant Females

Transplantation of pole cells from embryos of the genotype *Tf(3;2)4304,ry*$^{+}$*βgal*^{ftzP} (Hiromi et al., 1985) into recipient embryos homozygous for mutant alleles of the genes *pip* or *ndl* was carried out as described in the Experimental Procedures. The top panel shows a gastrulating embryo of the genotype *Tf(3;2)4304,ry*$^{+}$ *βgal*^{ftzP}. It was fixed and stained using an antibody directed against E. coli β-galactosidase, which illustrates the *ftz* promoter–directed expression of the *lacZ* transgene. The gastrulation pattern is wild type. The lower panel shows a gastrulating embryo produced by a female that was somatically *pip/pip* but received pole cells of the genotype *Tf(3;2)4304,ry*$^{+}$*βgal*^{ftzP} (as confirmed by the striped pattern after staining with anti-*lacZ* antibody) by transplantation. The embryo is dorsalized. A similar result was obtained when *Tf(3;2)4304,ry*$^{+}$ *βgal*^{ftzP} pole cells were transplanted into females lacking *ndl* gene activity. Embryos are oriented with the anterior end at left and the dorsal surface upward.

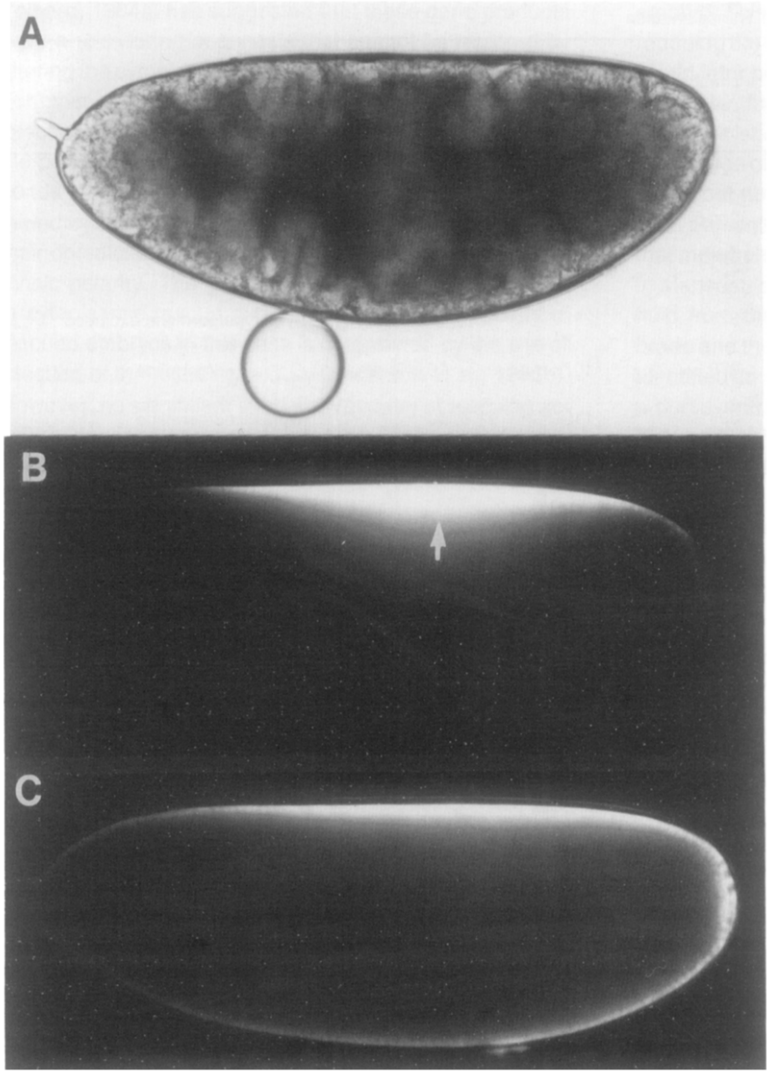

Figure 2. Isolation and Transplantation of Perivitelline Fluid

(A) shows a dorsalized embryo derived from a female lacking *Tl* activity (genotype *Df(3R)ro*^{XB3}/*Tl*^{sbRE}) that was pricked ventrally using an unbroken needle, allowing a drop of perivitelline fluid to leak out. (B) and (C) show an embryo injected dorsally (see arrow) in the perivitelline space with perivitelline fluid containing FITC-conjugated BSA (Sigma) 2 min (B) and 10 min (C) after injection. Similar results have been shown previously by Warn and Magrath, 1982. Eggs are oriented with the anterior end at the left and the dorsal surface upward.

mately associated with the vitelline coat. As embryogenesis progresses, the embryonic membrane detaches from the vitelline coat at various points, thereby defining a fluid-filled perivitelline compartment.

We attempted to detect activities present in this perivitelline fluid that are capable of restoring pattern elements and polarity along the dorsoventral axis to *ndl*, *pip*, and *wind* mutant embryos. We obtained perivitelline fluid from eggs by pricking them at the points at which the embryonic membrane had detached from the vitelline coat during gastrulation, thus allowing the fluid to escape. Embryos undergoing a dorsalized gastrulation are particularly well suited as donors due to the formation of folds all around the circumference that give rise to large gaps between the vitelline coat and the embryo. Figure 2A shows an embryo from a *Tl*$^{-}$ female whose vitelline coat was pricked during gastrulation, leaving the embryo intact and allowing a clear drop of perivitelline fluid to escape.

To determine the feasability of introducing liquid into the perivitelline space of a recipient embryo and to monitor the distribution of successfully transplanted fluid, we mixed perivitelline fluid with FITC-conjugated BSA and injected this mixture into the perivitelline compartment of a recipient embryo (see also Warn and Magrath, 1982). Two minutes after injection, the transplanted material is present in a bolus around the site of injection (Figure 2B). Within 10 min, however, the fluorescing material has become completely distributed within the perivitelline space surrounding the embryo (Figure 2C). This demonstrates that it is possible to inject material into the perivitelline compartment of the egg and furthermore, that relatively large molecules such as BSA (molecular size, 66 kd) can diffuse rapidly within the perivitelline space.

An Activity Present in the Perivitelline Fluid Determines Dorsoventral Polarity

Initially, embryos derived from *Tl* amorphic mutant females were used as donors for perivitelline fluid transplantation. While the dorsalized phenotype of these embryos facilitates easy removal of perivitelline fluid, this genotype was chosen mainly because we reasoned that if a ligand for *Tl* were present in perivitelline fluid, in *Tl*$^{+}$ embryos it might

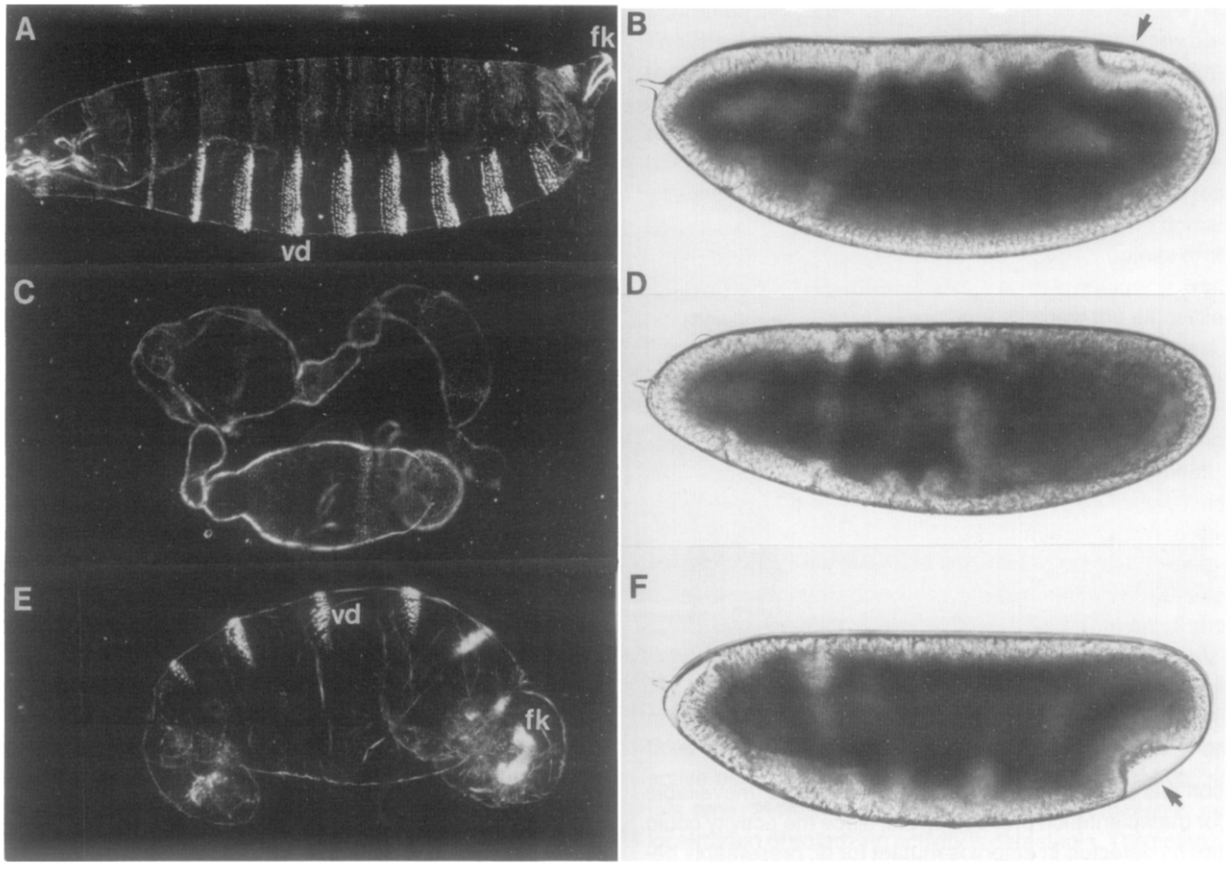

Figure 3. The Effect of Transplanted Perivitelline Fluid from Donors Lacking *Tl* Activity upon the Cuticular Pattern and Gastrulation Movements of *pip*-Deficient Recipients

Perivitelline fluid was obtained from embryos lacking *Tl* activity (maternal genotype $Df(3R)ro^{XB3}/Tl^{58RE}$) and injected dorsally into the perivitelline space of embryos lacking *pip* activity (parental genotype pip^{386}/pip^{664}) as described in the Experimental Procedures. The cuticular pattern and the gastrulation movements of representative injected embryos can be seen in (E) and (F), respectively. As controls, (A) and (B) show cuticle and gastrulation movements of wild-type embryos (strain Oregon R), while (C) and (D) show the differentiated cuticular pattern and gastrulation movements of uninjected dorsalized embryos produced by females of the genotype pip^{386}/pip^{664}. Note the presence of filzkörper (fk) and ventral denticles (vd) in the embryo that was injected with perivitelline fluid. Additionally, note that the injected embryos display polarized gastrulation movements (F), but that these movements show a reversed orientation within the eggshell from that seen for wild-type embryos due to the dorsal deposition of the rescuing perivitelline fluid. Note particularly the ventral extension of the germband (see arrow) with respect to the eggshell and compare that with the wild-type dorsal extension of the germband seen in (B). The embryos (A, C, and D) and the eggs (B, D, and F) are oriented with the anterior ends at left and the dorsal surfaces upward.

be bound and unavailable for transplantation. On the other hand, in embryos completely lacking *Tl* protein it might remain a diffusible and transplantable component of the perivitelline fluid long after the time at which it normally functions during wild-type development. When perivitelline fluid from Tl^- embryos was transplanted into the perivitelline space of embryos from *pip* mutant females, a large proportion of the recipient embryos exhibited a polar gastrulation. At the end of embryogenesis, these embryos display ventrolateral and ventral cuticular structures and thus, an almost complete restoration of pattern along the dorsoventral axis (Figure 3E, Table 2). The establishment of ventral fate after injection of perivitelline fluid can also be observed in the early embryo using cell-specific markers as demonstrated by the expression of *twist*, a zygotic gene normally expressed in the mesoderm in wild-type embryos (Thisse et al., 1988) (Figure 4). Strikingly, when the perivitelline fluid is transplanted into the perivitelline space on the dorsal side of the recipient egg, gastrulation occurs in reverse orientation with respect to the polarity of the eggshell, and the ventralmost elements of the dorsoventral axis form dorsally at the site of injection (Figure 3; Figure 4). Similar results were obtained after injection of perivitelline fluid from Tl^- embryos into embryos from females mutant for *ndl* or *wind*. As in the case of *pip*, the position at which the transplanted perivitelline fluid was placed determined the ventralmost point of the restored pattern (Table 2). These results suggest that somatically required dorsal group genes provide the signal that defines the polarity of the dorsoventral axis during normal development.

A Polarizing Activity in the Perivitelline Fluid May Correspond to the *Tl* Ligand

As described above, polarizing activity is present in the perivitelline fluid of embryos from Tl^- female. The assump-

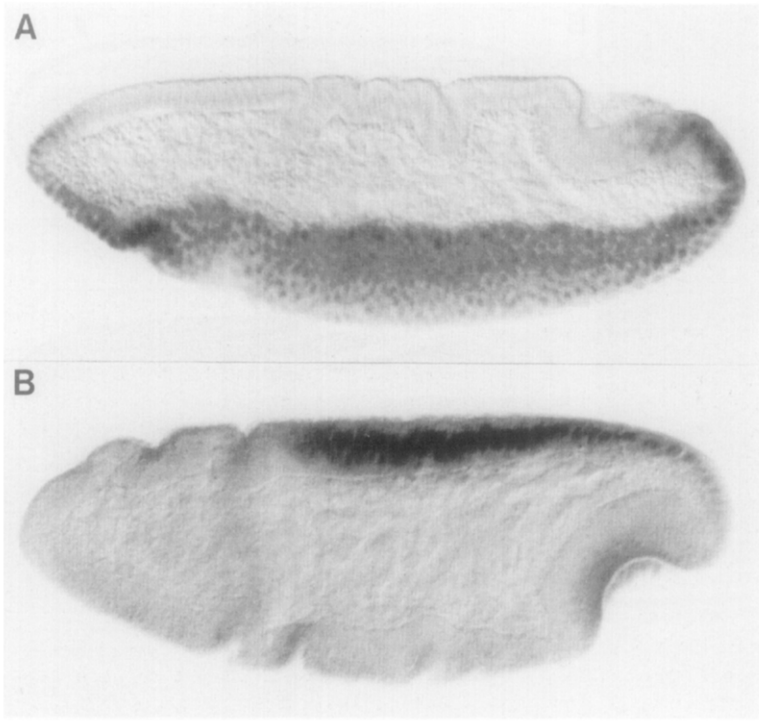

Figure 4. Expression of the Mesoderm-Specific Gene *twist* at the Site of Perivitelline Fluid Injection Containing the *Tl* Ligand

Embryos were fixed and stained using an antibody directed against the product of the *twist* gene (Roth et al., 1989). Embryos are oriented as they were in the unmanipulated egg with the anterior to the right and the dorsal surface upward. (A) shows a stained Oregon R wild-type embryo undergoing germband extension. (B) shows a *pip* mutant embryo that was injected dorsally with perivitelline fluid from a *Tl* mutant embryo and stained during germband extension.

tion that in Tl^+ embryos it might be bound and unavailable for transplantation proved correct, since the activity could not be detected in embryos mutant for *dl*, presumably because such embryos retain wild-type activity of *Tl*. All dorsal group genes whose products act upstream of *Tl* should also be active in *dl* mutant embryos. Table 2 shows that *pip* and *ndl* embryos that are Tl^+ do not produce an activity capable of rescuing embryos of the other genotypes. To

Table 2. Perivitelline Fluid–Mediated Rescue of the *pip*, *ndl*, and *wind* Mutant Embryos

Parental Genotype of Perivitelline Fluid Donors	Parental Genotype of Perivitelline Fluid Recipients	Number of Injected Embryos	Orientation of Gastrulation in Rescued Embryos	Rescued Embryos with Filzkörper	Rescued Embryos with Ventral Denticles
$Tl^{5BRE}/Df(3R)ro^{XB3}$	pip^{366}/pip^{664}	88	Reversed	33	11
$dl^1/Df(2L)TW119$	pip^{366}/pip^{664}	85	No rescue	0	0
pip^{366}/pip^{664}	pip^{366}/pip^{664}	77	No rescue	0	0
ndl^{046}/ndl^{093}	pip^{366}/pip^{664}	27	No rescue	0	0
snk^{073}/snk^{229}	pip^{366}/pip^{664}	77	No rescue	0	0
Tl^{rm9}/Tl^{9QRE}	pip^{366}/pip^{664}	68	Reversed	59	6
$pip^{664}Tl^{rm9}/pip^{338}Tl^{9QRE}$	pip^{366}/pip^{664}	76	No rescue	0	0
$ndl^{260}Tl^{rm9}/ndl^{093}Tl^{9QRE}$	pip^{366}/pip^{664}	88	No rescue	0	0
$tub^{118}Tl^{rm9}/tub^{238}Tl^{9QRE}$	pip^{366}/pip^{664}	73	Reversed	50	15
$Tl^{5BRE}/Df(3R)ro^{XB3}$	ndl^{046}/ndl^{093}	64	Reversed	25	8
$dl^1/Df(2L)TW119$	ndl^{046}/ndl^{093}	65	No rescue	3[a]	0
Tl^{rm9}/Tl^{9QRE}	ndl^{046}/ndl^{093}	55	Reversed	37	3
$ndl^{260}Tl^{rm9}/ndl^{093}Tl^{9QRE}$	ndl^{046}/ndl^{093}	37	No rescue	0	0
$pip^{664}Tl^{rm9}/pip^{338}Tl^{9QRE}$	ndl^{046}/ndl^{093}	57	No rescue	1[a]	0
$Tl^{5BRE}/Df(3R)ro^{XB3}$	$wind^{RP}/wind^{T6}$	76	Reversed	39	6
$dl^1/Df(2L)TW119$	$wind^{RP}/wind^{T6}$	79	No rescue	0	0

Perivitelline fluid taken from embryos derived from the denoted maternal genotypes was transplanted dorsally into the perivitelline fluid of embryos lacking *pip*, *ndl*, or *wind* activity. Isolation and transplantation of perivitelline fluid and analysis of the effects of perivitelline fluid transplantation upon the gastrulation movements and cuticular rescue of recipients are as described in the Experimental Procedures. The orientation of the cephalic fold and the extension of the germband in pip^- embryos after the introduction of *Tl*-derived perivitelline fluid indicate that gastrulation movements occur in reverse orientation, with respect to the orientation of the eggshell. Maternal genotypes are given only with respect to the dorsal group genotypes. The availability of recombinant chromosomes carrying Tl^{rm9} and other dorsal group mutant alleles dictated the use of Tl^{rm9}/Tl^{9QR} combinations. Embryos derived from $Tl^{rm9}Tl^{9QR}$ females show an apolar lateralized phenotype, generally exhibiting laterally derived denticles around their circumferences. The additional removal of *ea*, *snk*, or *gd* activity results in completely dorsalized embryos. Tl^{9QR} does not express any RNA and therefore contributes no *Tl* product. This indicates that the Tl^{rm9} gene product responds to ligand. Nevertheless, these embryos can serve as a source of diffusible ligand. These observations suggest that Tl^{rm9} encodes a protein with an impaired ability to bind to the ligand.

[a] We have observed that after prolonged storage at 18°C females of the genotype ndl^{046}/ndl^{093} begin to produce embryos that are not completely dorsalized. Therefore this allelic combination is temperature sensitive. We presume that the 3 embryos producing filzkörper after injection of perivitelline fluid from *dl* donor embryos resulted from residual *ndl* activity at the low temperature.

Table 3. Temporal Parameters of Perivitelline Fluid–Mediated Rescue

Age of Recipient Embryos	Number of Embryos Injected	Number of Embryos Exhibiting Rescue
2	67	0
3	51	11
4	71	52
5	66	2
6	56	0

Perivitelline fluid taken from *Tl* mutant embryos was transplanted dorsally into the perivitelline space of *pip* mutant embryos at the noted stages of development (Campos-Ortega and Hartenstein, 1985). Isolation and transplantation of perivitelline fluid and analysis of the effects of perivitelline fluid transplantation upon cuticular rescue of recipients are as described in the Experimental Procedures. Rescued embryos were those showing the production of filzkörper or ventral denticles.

test whether this was the consequence of removal of active ligand by the *Tl* gene product, or the absence of ligand altogether, because *ndl* or *pip* is required for its production, we also tested embryos from females doubly mutant for both *Tl* and one of the somatically required genes. Rescuing activity could not be detected in the perivitelline fluid of *pip Tl* or *ndl Tl* embryos. In contrast, perivitelline fluid from *tub Tl* embryos could rescue *pip* mutant embryos. These experiments indicate that the production of polarizing activity depends on the wild-type function of *pip* and *ndl*, but not on the activity of genes acting downstream of *Tl* such as *tub*. (For technical reasons, *wind* was not tested, although we assume that the wild-type activity of *wind* is also required for ligand formation.)

To investigate the relationships between the somatically required dorsal group genes, we transplanted perivitelline fluid from *pip Tl* embryos into *ndl* recipients as well as from *ndl Tl* donors into *pip* recipients. In neither case was any restoration of dorsoventral pattern observed in the recipient embryos (Table 2). From this we conclude that the restoration of pattern after perivitelline fluid transplantation is not the simple result of complementation of the mutant phenotypes by introduction of the wild-type gene products.

Temporal and Spatial Parameters of Perivitelline Fluid–Mediated Rescue

To determine the time at which recipient embryos are competent to be rescued, perivitelline fluid from Tl^- embryos was transplanted into *pip* mutant embryos at various stages of development (Table 3). Syncytial blastoderm (stage 4 of Campos-Ortega and Hartenstein, 1985) embryos are most responsive to injection, while embryos in the early cleavage stages only rarely respond to transplanted perivitelline fluid. This might indicate that the *Tl* receptor is not present or capable of binding to the ligand prior to stage 4. The transplanted activity might thus diffuse within the perivitelline space of the recipient embryo, perhaps becoming equally distributed by stage 4 in a concentration incapable of influencing the dorsoventral axis. The latest time at which rescue could be consistently observed was shortly before cellularization (stage 4d). Without exception, the site of injection determined the ventral side of the rescued pattern in the mutant recipient embryos. For technical reasons, abundant quantities of perivitelline fluid could not be recovered from donor embryos before stage 5, a time long after the actions of the dorsal group have accomplished the graded nuclear uptake of *dl* protein in normal development. For this reason, it was not possible to determine the time at which the diffusible ligand first becomes detectable in the donor embryos. However, our experiments indicate that the polarizing activity is relatively stable. Indeed, the activity was still detectable in donor embryos at 24 hr of embryogenesis, long after the activity would act during normal development.

We have shown that the positioning of perivitelline fluid determines the ventral side of the restored pattern in recipient embryos. This suggests that during normal development, the active compound is present at the ventral side of the egg. By removing perivitelline fluid from either the dorsal or ventral side of donor eggs and testing its polarizing activity we investigated whether the activity is asymmetrically distributed. Table 4 shows that perivitelline fluid taken from the dorsal side of Tl^- mutant embryos is as active as that taken from the ventral side. Therefore, at this time, at least in embryos that lack the receptor, no localization of polarizing activity is detectable. The polarizing activity appears to be evenly distributed within the perivitelline fluid. However, since molecules as large as BSA diffuse rapidly in the perivitelline fluid, it is likely that in Tl^- mutant embryos, the ligand might display a distribution not found in normal development.

Discussion

Like the embryo and the eggshell enclosing it, the egg chamber, within which the egg is produced, shows conspicuous dorsoventral polarity. The cells of the egg chamber are derived from two distinct populations (for review see King, 1970; Mahowald and Kambysellis, 1980). The oocyte and 15 associated nurse cells are derived from germline precursor cells. The nurse cells form a cluster of cells at the anterior end of the developing oocyte, which provide the majority of material required for growth during oogenesis and for subsequent embryonic pattern forma-

Table 4. Spatial Parameters of Perivitelline Fluid–Mediated Rescue

Position of Fluid Removal	Number of Embryos Injected	Number of Embryos Exhibiting Rescue
Ventral	74	40
Dorsal	82	45

Perivitelline fluid was taken either dorsally or ventrally from *Tl* mutant embryos and transplanted dorsally into the perivitelline space of *pip* mutant embryos. Isolation and transplantation of perivitelline fluid and analysis of the effects of perivitelline fluid transplantation upon cuticular rescue of recipients are as described in the Experimental Procedures. Rescued embryos were those showing the production of filzkörper or ventral denticles.

tion and morphogenesis. The oocyte and nurse cells are surrounded by an epithelium of somatically derived follicle cells that synthesize the vitelline coat and the chorion, the layers of the eggshell. The eggshell produced by these somatic cells has a characteristic, dorsoventrally asymmetric shape. During the development of the wild-type embryo, the nuclear uptake of *dl* protein is maximal on the side of the embryo adjacent to the ventral side of the egg. Consequently, gastrulation and subsequent morphogenetic movements occur in an invariant orientation with respect to the eggshell. This suggests that an intrinsic polarity within the egg is transduced to the embryo during the determination of dorsoventral polarity.

The results of our mosaic analysis demonstrate the requirement for the expression of the genes *ndl, pip*, and *wind* in the somatic tissue (presumably in the ovarian follicle cells) of the adult female, where they may function in the initial process that defines the polarity of the embryonic dorsoventral axis. Using perivitelline fluid transplantations, we have identified an activity capable of defining the orientation of the dorsoventral axis. This activity can only be detected in the perivitelline fluid of embryos that do not express the *Tl* receptor, suggesting that the active factor corresponds to the *Tl* ligand and that this ligand is limited in amount. Presumably, when the ligand is produced in an embryo that expresses *Tl*, it is rapidly bound and sequestered from the perivitelline fluid. In the absence of the receptor, the ligand diffuses within the perivitelline fluid and becomes evenly distributed. Our observations that perivitelline fluid from *pip Tl* and from *ndl Tl* embryos could not restore pattern to *ndl* or *pip* recipient embryos, respectively, support a model in which the active principle is a single end product of a reaction requiring all three of the somatically required genes. In contrast, they do not support a model in which the distinct products of each of the genes are present in the perivitelline fluid, where they complement one another. A simple interpretation of these findings is that the products of the somatically required genes cooperate in a process that leads to the ventral formation of the ligand for the *Tl* receptor. Additional observations (unpublished data) indicate that the products of the dorsal group genes *ea, snk*, and *spz* are also secreted into the perivitelline fluid where they are required for the formation of the active ligand.

A simple hypothesis explaining our observations is that the *Tl* ligand is present in the newly laid egg in an anchored or inactive state, perhaps deposited in the vitelline membrane, and that active ligand is produced during stage 4 (syncytial blastoderm). While it was not possible to test perivitelline fluid from donors younger than stage 5 for activity, our demonstration that stage 2 and 3 recipient embryos are only weakly responsive to ligand implies that at this time during normal development the *Tl* receptor is not present at sufficient levels or in a form capable of binding ligand productively and transmitting the signal. Alternatively, the inability to restore pattern elements to embryos prior to stage 3 might indicate the absence of another necessary determinant (i.e., *tub* or *pll*). Ligand production during wild-type development may occur at the time at which sufficient levels of receptor are present. If so, it seems likely that active ligand is produced at stage 4, the time at which the embryo is most competent to respond to it. Histochemical methods employing antibodies directed against the *Tl* receptor (C. Hashimoto, unpublished data; Keith and Gay, 1990) should allow the observation of the kinetics of *Tl* protein synthesis and localization to the embryonic membrane.

We propose that a population of ventrally located follicle cells in the egg chamber direct the ventral formation of the *Tl* ligand in the egg and that the genes *ndl, pip*, and *wind* are involved in this process. In this way, the ventral follicle cells act to induce the ventrally situated cells of the embryo to adopt a ventral developmental fate. Interestingly, this inductive interaction has a time delay, since the follicle cells are no longer present during the time at which the ventral cells are determined. Therefore, the relationship between the products of the somatically required dorsal group genes and the polarizing activity is not necessarily a simple one.

One possible mechanism for the activation of *Tl* is that 1 of the somatically required dorsal group genes encodes the *Tl* ligand and is expressed only in a ventral population of follicle cells. At the time of egg deposition, the ligand would be present, in an inactive state, only on the ventral side of the egg. Alternatively, in a more complex mechanism, the somatically required dorsal group genes could give rise to an asymmetry in the egg leading to the ventral assembly or activation of a ligand that is not itself encoded by any of the somatically required dorsal group genes. Currently we cannot distinguish between these possibilities. However, both hypotheses require the asymmetric expression or activity of 1 or more of the somatically required dorsal group genes in the follicle cells. The existence of spatially restricted gene expression in subpopulations of follicle cells has been observed directly (Fasano and Kerridge, 1988; Grossniklaus et al., 1989), while the expression of the *tsl* gene in polar subpopulations of follicle cells has been shown to be required for the formation of terminal pattern elements in the embryo (Stevens et al., 1990).

The spatially restricted expression of follicle-specific genes is presumably under the control of the two genes, *top* and *gurken* (*grk*) (Schüpbach, 1987; Manseau and Schüpbach, 1989), that take part in a signaling pathway whose function is to repress the ventralization of follicle cell populations. Females mutant for *top* or *grk* give rise to eggs and embryos that are ventralized. The absence of these gene products disrupts a signaling process between the oocyte and follicle cells, causing follicle cells at inappropriate positions to be directed toward a ventral fate. The ventralization of the embryo could then be explained as resulting from abnormally high levels or an expanded spatial domain of *Tl* ligand production under the influence of the follicle cell–specific gene products. *top*, whose expression is required in the somatic tissue of the ovary, has been cloned and encodes the Drosophila homolog of the epidermal growth factor receptor (Price et al., 1989; Schejter and Shilo, 1989). This is consistent with its predicted activity in a signaling mechanism between the oocyte and the follicle cells.

As described above, it has been observed that after cytoplasmic rescue into dorsal group mutants, only *Tl* mutant embryos could be polarized by the site of injection (Anderson et al., 1985b). The ventral formation of a *Tl* ligand in limited amounts provides an explanation for this finding. Our results suggest that in mutant embryos lacking the *Tl* receptor, the ligand is evenly distributed within the perivitelline fluid. After injection of wild-type cytoplasm into *Tl* mutant embryos, *Tl* protein would be present only near the site of injection. Therefore, the evenly distributed ligand present in the perivitelline fluid would mediate *Tl* activation at the site of cytoplasmic injection.

The mechanism by which the nuclear concentration of *dl* protein becomes graded remains unclear. It is possible that the graded activation of *Tl* is a direct consequence of the graded activity of 1 of the somatically required dorsal group genes within the sheet of follicle cells that enclose the developing egg. In such a mechanism *Tl* ligand would be produced in a gradient. Alternatively, a stripe of *Tl* ligand could be produced, with the graded activation of *Tl* resulting from the effects of diffusion of the ligand in the perivitelline fluid after its release. A third possibility would be the formation of a gradient via the cytoplasmic diffusion of the factors that act downstream of *Tl* but upstream of *dl*.

In comparison with the other three systems that determine the anteroposterior axes of the embryo, the establishment of dorsoventral polarity is most similar in its mechanism to the formation of the terminal structures. The two systems determining anterior and posterior pattern rely on prelocalized positional determinants present within the cytoplasm of the newly laid egg (Frohnhöfer and Nüsslein-Volhard, 1986; Berleth et al., 1988; Driever and Nüsslein-Volhard, 1988a, 1988b; Lehmann and Nüsslein-Volhard, 1986). In contrast, neither the formation of terminal structures (Klingler et al., 1988) nor the determination of the dorsoventral axis of the embryo uses a prelocalized determinant within the oocyte. In both the dorsal (*Tl*) and the terminal (*tor*) systems, an evenly distributed membrane protein has been identified in which gain-of-function alleles have been observed (Anderson et al., 1985a; Klingler et al., 1988). Within the terminal system the gene *tor* encodes a receptor tyrosine kinase (Sprenger et al., 1989) that is presumably activated at the poles of the embryo, ultimately resulting in the regulation of zygotic gene activity at the two termini. It has been suggested that a ligand for *tor* is expressed in the follicle cells adjacent to the two poles of the embryo (Stevens et al., 1990). Similarly, our results suggest that the release of an active determinant, formed under the influence of a spatially restricted subset of follicle cells on the ventral side of the egg, causes the ventral activation of the *Tl* rceptor. This initiates a signal transduction pathway that controls the graded nuclear uptake of the *dl* morphogen into the nuclei of the syncytial blastoderm. The *dl* morphogen then controls the differential expression of the zygotic genes involved in pattern formation along the dorsoventral axis.

Experimental Procedures

Fly Stocks and Maintenance

All stocks were maintained and eggs were collected employing standard conditions and procedures (Roberts, 1986; Wieschaus and Nüsslein-Volhard, 1986). Staging of embryos was according to Campos-Ortega and Hartenstein, 1985.

The wild-type stock used was Oregon R. dl^1 was described in Nüsslein-Volhard, 1979 and Nüsslein-Volhard et al., 1980. *Df(2L)TW119* is in Lindsley and Zimm, 1987. gd^7 is from Konrad et al., 1988a. The dorsal group gene alleles pll^{rm8}, spz^{rm7}, Tl^{rm9} (designated as *mel(3)8, mel(3)7,* and *mel(3)9* by T. Rice, 1973), ea^1, ea^2, ndl^{046}, ndl^{093}, pip^{386}, pip^{864}, pll^{078}, snk^{073}, snk^{229}, spz^{197}, $Df(3L)ro^{XB3}$ (uncovering *Tl*), Tl^{5BRE}, tub^{118}, and tub^{238} have been described in Anderson and Nüsslein-Volhard, 1984a, 1986 and Anderson et al., 1985a. $wind^{RP}$ (Schüpbach and Wieschaus, 1989) was obtained from Trudi Schüpbach. The $wind^{T6}$ allele was obtained in a screen for alleles of maternal effect mutants on the second chromosome carried out by U. Mayer, R. Lehmann, and C. Nüsslein-Volhard (unpublished data). K. Anderson constructed the recombinant third chromosomes carrying the following dorsal group allele combinations: ndl^{093} Tl^{9QRE}, ndl^{280} Tl^{rm9}, pip^{386} Tl^{9QRE}, pip^{864} Tl^{rm9}, tub^{238} Tl^{9QRE}, and tub^{118} Tl^{rm9}. The dominant female sterile allele $Fs(1)ovo^{D1}$ is described in Busson et al., 1983. The strain *Tf(3;2)4304,ry⁺βgal*^{ftzP}, carrying the *ftz* promoter–*lacZ* transgene, was obtained from Yash Hiromi (Hiromi et al., 1985).

A complete description of the mutant stocks used can be found in Tearle and Nüsslein-Volhard, 1987. All chromosomes carried visible markers allowing identification of genotypes of interest. For the description of these visible markers and balancer chromosomes used see Lindsley and Grell, 1968 and Lindsley and Zimm, 1990.

Pole Cell Transplantations

Pole cells were transplanted according to standard procedures (see Lehmann and Nüsslein-Volhard, 1987). In tests of germline requirement for gene expression, transheterozygous mutant virgin females were mated to transheterozygous mutant males of the same genotype. Pole cells were removed from the mutant embryos at stage 3 or 4 and transplanted into the progeny of a cross between ovo^{D1} carrying males and wild-type Oregon R females at stage 2 or 3. All of the female progeny of this cross carry the ovo^{D1} allele and are sterile (Busson et al., 1983), unless pole cell transplantation has been successful. The hatching female progeny putatively carrying transplanted pole cells were mated with transheterozygous mutant males of the same genotype as the donors. Fertile females producing eggs, which resulted from successful pole cell transplantation, were scored for whether the embryos were dorsalized or not. Normal hatching progeny were raised to adulthood. In the three cases in which the fertile females produced hatching offspring, (*ndl*, *pip*, and *wind*), appropriate visible genetic markers allowed unambiguous determination of the genotypes of the progeny, ensuring that they were derived from transplanted transheterozygous mutant pole cells.

In tests of the somatic dependence of the genes *ndl* and *pip*, pole cells were taken from embryos of the genotype *Tf(3;2)4304ry⁺βgal*^{ftzP}, which carry a *ftz* promoter–*lacZ* transgene (Hiromi et al., 1985), but are otherwise wild type. Pole cells were then transplanted into the progeny of the two crosses:

males ovo^{D1};ndl^{093}/TM3 × females ndl^{046}/TM3 (testing *ndl*)

males ovo^{D1};pip^{386}/TM3 × females pip^{864}/TM3 (testing *pip*)

For each of the two experiments, the hatching female progeny that were transheterozygous for *ndl* or *pip* were crossed to Oregon R males. The dorsalized progeny of the fertile females were then stained for *lacZ* expression to ensure that they were derived from transplanted pole cells rather than fertile ovo^{D1+} escapers.

Determination of β-Galactosidase Expression

β-Galactosidase expression by the progeny of pole cell recipients was determined either by staining for enzyme activity according to the protocol of Hiromi et al., 1985 or by immunological staining of the protein using an antibody directed against the *lacZ* gene product. For immunological staining, fixed devitellinized embryos were first incubated with rabbit anti-β-galactosidase antibody (a gift of Ulrike Gaul). Embryos were then incubated with biotinylated horseradish peroxidase–avidin complex bound to biotinylated second antibody (Vector Systems, Avidin/Biotin ABC system). This procedure was carried out

as described by Macdonald and Struhl (1986) with the modification that, during the washes, 100 mM NaCl was included in the solutions.

Perivitelline Fluid Manipulations and Scoring of Injected Embryos

For the collection of perivitelline fluid donor embryos were staged under Voltalef 3S oil. Dorsalized embryos exhibiting the formation of folds all around their circumferences (corresponding in age to wild-type embryos undergoing germband extension, stage 7/8) (Figure 2A) were dechorionated in 50% Chlorox and mounted on a coverslip as described in Frohnhöfer and Nüsslein-Volhard, 1986. Using an unbroken needle, under Voltalef 10S oil, the vitelline membrane of each embryo was punctured at a point along its length where the embryo had detached from the vitelline membrane. The embryos were punctured at random positions along the dorsoventral axis, except where otherwise noted. Following puncture, a drop of perivitelline fluid of about 0.2 nl was observed to leak out (Figure 2A). Perivitelline fluid from several punctured embryos was then collected into one needle for transplantation.

Dechorionated, dried, and mounted late stage 3 or stage 4 embryos served as the recipients for perivitelline fluid transplantation except where otherwise noted. Approximately 0.1 nl of perivitelline fluid was injected dorsally into the perivitelline space of each recipient under Voltalef 15S oil. To accomplish this, the needle carrying donor perivitelline fluid was introduced into the ventral side of the recipient at a point between 30% and 50% of the egg length. The tip of the needle was extended to the inner surface of the vitelline membrane on the dorsal side of the egg. It was then drawn back a small distance, and a small volume of perivitelline fluid was injected into the space produced by this manipulation. After successful introduction of perivitelline fluid, the space between the oolemn and the inner surface of the vitelline membrane on the dorsal side of the embryo could be seen to enlarge.

At least 50 embryos at the appropriate stage were injected in most experiments. Injected embryos were observed at a time corresponding to the onset of gastrulation. The polarity of gastrulation with respect to the eggshell could be determined by observation of embryos displaying conspicuous polarization of the cephalic furrow or of the extension of the germband. Injected embryos were allowed to develop under oil for at least 4 days at 18°C. Voltalef oil covering the injected embryos was removed in heptane, and the coverslip carrying the embryos was inverted and mounted on a slide with a drop of Hoyer's mounting medium and lactic acid (1:1) (van der Meer, 1977). After they had cleared, injected embryos were scored for the presence of filzkörper material and ventral denticles using Nomarski or dark-field optics.

Determination of *twist* Expression in Injected Embryos

Injected embryos were allowed to develop under oil until they had begun to gastrulate. At this point the oil was removed using heptane, and the embryos were fixed, still attached to the coverslip, in a 1:1 mixture of heptane and 4% formaldehyde in PEMS buffer (Macdonald and Struhl, 1986). Following fixation, the coverslip carrying the embryos was placed briefly in methanol and then into the PBS buffer containing 0.1% Tween 20. The fixed embryos were then hand peeled from their vitelline membranes and placed in methanol prior to antibody staining. For immunological staining, embryos were first incubated with rabbit anti-*twist* antibody (Roth et al., 1989). Embryos were then incubated with biotinylated horseradish peroxidase–avidin complex bound to biotinylated second antibody (Vector Systems, Avidin/Biotin ABC system). As before, this procedure was carried out as described by Macdonald and Struhl (1986) with the modification that, during the washes, 100 mM NaCl was included in the solutions.

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References

Anderson, K. V. (1987). Dorsal-ventral embryonic pattern genes of *Drosophila*. Trends Genet. *3*, 91–97.

Anderson, K. V., and Nüsslein-Volhard, C. (1984). Information for the dorsal-ventral pattern of the *Drosophila* embryo is stored as maternal mRNA. Nature *311*, 223–227.

Anderson, K. V., and Nüsslein-Volhard, C. (1986). Dorsal-group genes of *Drosophila*. In Gametogenesis and the Early Embryo, J. Gall, ed. (New York: Alan R. Liss, Inc.), pp. 177–194.

Anderson, K. V., Jürgens, G., and Nüsslein-Volhard, C. (1985a). Establishment of dorsal–ventral polarity in the Drosophila embryo: genetic studies on the role of the *Toll* gene product. Cell *42*, 779–789.

Anderson, K. V., Bokla, L., and Nüsslein-Volhard, C. (1985b). Establishment of dorsal–ventral polarity in the Drosophila embryo: the induction of polarity by the *Toll* gene product. Cell *42*, 791–798.

Berleth, T., Burri, M., Thoma, G., Bopp, D., Richstein, S., Frigerio, G., Noll, M., and Nüsslein-Volhard, C. (1988). The role of localization of *bicoid* RNA in organizing the anterior pattern of the *Drosophila* embryo. EMBO J. *7*, 1749–1756.

Bours, V., Villalobos, J., Burd, P. R., Kelly, K., and Siebenlist, U. (1990). Cloning of a mitogen-inducible gene encoding a κB DNA-binding protein with homology to the *rel* oncogene and to cell-cycle motifs. Nature *348*, 76–80.

Busson, B., Gans, M., Komitopoulou, K., and Masson, M. (1983). Genetic analysis of three dominant female sterile mutations on the X-chromosome of *Drosophila melanogaster*. Genetics *105*, 309–325.

Campos-Ortega, J. A., and Hartenstein, V. (1985). The Embryonic Development of *Drosophila melanogaster* (Berlin: Springer-Verlag), pp. 9–84.

Casanova, J., and Struhl, G. (1989). Localized surface activity of *torso*, a receptor tyrosine kinase, specifies terminal body pattern in *Drosophila*. Genes Dev. *3*, 2025–2038.

Driever, W., and Nüsslein-Volhard, C. (1988a). A gradient of *bicoid* protein in Drosophila embryos. Cell *54*, 83–93.

Driever, W., and Nüsslein-Volhard, C. (1988b). The *bicoid* protein determines position in the Drosophila embryo in a concentration-dependent manner. Cell *54*, 95–104.

Fasano, L., and Kerridge, S. (1988). Monitoring positional information during oogenesis in adult *Drosophila*. Development *104*, 245–253.

Frohnhöfer, H. G., and Nüsslein-Volhard, C. (1986). Organisation of anterior pattern in the *Drosophila* embryo by the maternal gene *bicoid*. Nature *324*, 120–125.

Gelinas, C., and Temin, H. M. (1988). The *v-rel* oncogene encodes a cell-specific transcriptional activator of certain promoters. Oncogene *3*, 349–355.

Ghosh, S., Gifford, A. M., Riviere, L. R., Tempst, P., Nolan, G. P., and Baltimore, D. (1990). Cloning of the p50 DNA binding subunit of Nf-κB: homology to *rel* and *dorsal*. Cell *62*, 1019–1029.

Grossniklaus, U., Bellen, H. J., Wilson, C., and Gehring, W. J. (1989). P-element-mediated enhancer detection applied to the study of oogenesis in *Drosophila*. Development *107*, 189–200.

Hashimoto, C., Hudson, K. L., and Anderson, K. V. (1988). The *Toll* gene of Drosophila , required for dorsal-ventral embryonic polarity, appears to encode a transmembrane protein. Cell *52*, 269–279.

Hiromi, Y., Kuroiwa, A., and Gehring, W. J. (1985). Control elements of the Drosophila segmentation gene *fushi tarazu*. Cell *43*, 603–613.

Ip, Y. T., Kraut, R., Levine, M., and Rushlow, C. A. (1991). The *dorsal* morphogen is a sequence-specific DNA-binding protein that interacts with a long-range repression element in Drosophila . Cell *64*, 439–446.

Keith, F. J., and Gay, N. J. (1990). The *Drosophila* membrane receptor Toll can function to promote cellular adhesion. EMBO J. *9*, 4299–4306.

Kieran, M., Blank, V., Logeat, F., Vandekerckhove, J., Lottspeich, F., Le Bail, O., Urban, M. B., Kourilsky, P., Baeuerle, P. A., and Israël, A. (1990). The DNA binding subunit of Nf-κB is identical to factor KBF1 and homologous to the *rel* oncogene product. Cell *62*, 1007–1018.

King, R. C. (1970). Ovarian Development in *Drosophila melanogaster*. (New York: Academic Press).

Klingler, M., Erdelyi, M., Szabad, J., and Nüsslein-Volhard, C. (1988). Function of *torso* in determining the terminal anlagen of the *Drosophila* embryo. Nature *335*, 275–277.

Konrad, K. D., Goralski, T. J., and Mahowald, A. (1988a). Developmental analysis of *fs(1)gastrulation defective*, a dorsal-group gene of *Drosophila melanogaster*. Roux's Arch. Dev. Biol. *197*, 75–91.

Konrad, K. D., Goralski, T. J., and Mahowald, A. P. (1988b). Developmental genetics of the gastrulation defective locus in *Drosophila melanogaster*. Dev. Biol. *127*, 133–142.

Lehmann, R., and Nüsslein-Volhard, C. (1986). Abdominal segmentation, pole cell formation, and embryonic polarity require the localized activity of *oskar*, a maternal gene in Drosophila. Cell *47*, 141–152.

Lehmann, R., and Nüsslein-Volhard, C. (1987). *hunchback*, a gene required for segmentation of an anterior and posterior region of the *Drosophila* embryo. Dev. Biol. *119*, 402–417.

Lindsley, D. L., and Grell, E. H. (1968). Genetic variations of *Drosophila*, Carnegie Inst. Wash. Publ. 627.

Lindsley, D. L., and Zimm, G. (1987). The genome of *Drosophila melanogaster*, part 3: rearrangements. Dros. Inf. Serv. *65*, 40.

Lindsley, D. L., and Zimm, G. (1990). The genome of *Drosophila melanogaster*, part 4: genes L-Z, balancers, transposable elements. Dros. Inf. Serv. *68*, 1–382.

Macdonald, P. M., and Struhl, G. (1986). A molecular gradient in early *Drosophila* embryos and its role in specifying the body pattern. Nature *324*, 537–545.

Mahowald, A. P., and Kambysellis, M. P. (1980). Oogenesis. In The Genetics and Biology of *Drosophila*. Vol. 2d, M. Ashburner and T. R. F. Wright, eds. (London: Academic Press), pp. 141–224.

Manseau, L., and Schüpbach, T. (1989). *cappuccino* and *spire*: two unique maternal-effect loci required for both the anteroposterior and dorsoventral patterns of the *Drosophila* embryo. Genes Dev. *3*, 1437–1452.

Nüsslein-Volhard, C. (1979). Maternal effect mutations that alter the spatial coordinates of the embryo of *Drosophila melanogaster*. In Determinants of Spatial Organization, I. Koenigsberg and S. Subtelney, eds. (New York: Academic Press), pp. 185–211.

Nüsslein-Volhard, C., and Roth, S. (1989). Axis determination in insect embryos. In Cellular Basis of Morphogenesis. Ciba Foundation Symp. *144*, 37–55.

Nüsslein-Volhard, C., Lohs-Schardin, M., Sander, K., and Cremer, C. (1980). A dorsoventral shift of embryonic primordia in a new maternal-effect mutant of *Drosophila*. Nature *283*, 474–476.

Nüsslein-Volhard, C., Frohnhöfer, H. G., and Lehmann, R. (1987). Determination of anteroposterior polarity in *Drosophila*. Science *238*, 1675–1681.

Price, J. V., Clifford, R. J., and Schüpbach, T. (1989). The maternal ventralizing locus *torpedo* is allelic to *faint little ball*, an embryonic lethal, and encodes the Drosophila EGF receptor homolog. Cell *56*, 1085–1092.

Rice, T. (1973). Isolation and characterisation of maternal effect mutants: an approach to the study of early determination in *Drosophila melanogaster*. Ph.D. thesis, Yale University, New Haven, Connecticut.

Roberts, D. B. (1986). Basic *Drosophila* care and techniques. In *Drosophila*, A Practical Approach, D. B. Roberts, ed. (Oxford: IRL Press), pp. 1–39.

Roth, S., Stein, D., and Nüsslein-Volhard, C. (1989). A gradient of nuclear localization of the *dorsal* protein determines dorsoventral pattern in the Drosophila embryo. Cell *59*, 1189–1202.

Rushlow, C. A., Han, K., Manley, J. L., and Levine, M. (1989). The graded distribution of the *dorsal* morphogen is initiated by selective nuclear transport in Drosophila. Cell *59*, 1165–1177.

Santamaria, P., and Nüsslein-Volhard, C. (1983). Partial rescue of *dorsal*, a maternal effect mutation affecting the dorsoventral pattern of the *Drosophila* embryo, by the injection of wild-type cytoplasm. EMBO J. *2*, 1695–1699.

Schejter, E. D., and Shilo, B.-Z. (1989). The Drosophila EGF receptor homolog (DER) gene is allelic to *faint little ball*, a locus essential for embryonic development. Cell *56*, 1093–1104.

Schüpbach, T. (1987). Germ line and soma cooperative during oogenesis to establish the dorsoventral pattern of egg shell and embryo in Drosophila melanogaster. Cell *49*, 699–707.

Schüpbach, T., and Wieschaus, E. (1986). Germline autonomy of maternal-effect mutations altering the embryonic body pattern of *Drosophila*. Dev. Biol. *113*, 443–448.

Schüpbach, T., and Wieschaus, E. (1989). Female sterile mutations on the second chromosome of *Drosophila melanogaster*. I. Maternal effect mutations. Genetics *121*, 101–117.

Seifert, E., Müller-Holtkamp, F., Marcey, D., and Jäckle, H. (1987). Injection of wild-type cytoplasm and poly(A)+ RNA provokes phenotype rescue in *spätzle* mutant *Drosophila* embryos. Roux's Arch. Dev. Biol. *196*, 78–82.

Sprenger, F., Stevens, M. L., and Nüsslein-Volhard, C. (1989). The *Drosophila* gene *torso* encodes a putative receptor tyrosine kinase. Nature *338*, 478–483.

Stevens, L. M., and Nüsslein-Volhard, C. (1991). Development of the terminal anlagen of the *Drosophila* embryo depends upon interactions between the germline and the somatic follicle cells. In Cell-Cell Interactions in Early Development, J. Gerhart, ed. (New York: Wiley-Liss, Inc.), in press.

Stevens, L., Frohnhöfer, H., Klingler, M., and Nüsslein-Volhard, C. (1990). Localized requirement for *torsolike* expression in follicle cells for the development of terminal anlagen of the *Drosophila* embryo. Nature *346*, 660–663.

Steward, R. (1987). *Dorsal*, an embryonic polarity gene in *Drosophila*, is homologous to the vertebrate proto-oncogene, *c-rel*. Science *238*, 692–694.

Steward, R. (1989). Relocalization of the *dorsal* protein from the cytoplasm to the nucleus correlates with its function. Cell *59*, 1179–1188.

Tearle, R., and Nüsslein-Volhard, C. (1987). Tübingen mutants and stocklist. Dros. Inf. Serv. *66*, 209–269.

Thisse, B., Stoetzel, C., Gorostiza-Thisse, C., and Perrin-Schmidt, F. (1988). Sequence of the *twist* gene and nuclear localization of its protein in endomesodermal cells of early *Drosophila* embryos. EMBO J. *7*, 2175–2183.

van der Meer, J. M. (1977). Optical clean and permanent whole mount preparations for phase-contrast microscopy of cuticular structures of insect larvae. Dros. Inf. Serv. *52*, 160.

Warn, R. M., and Magrath, R. (1982). Observations by a novel method of surface changes during the syncytial blastoderm stage of the Drosophila embryo. Dev. Biol. *89*, 540–548.

Wieschaus, E., and Nüsslein-Volhard, C. (1986). Looking at embryos In *Drosophila*. A Practical Approach, D. B. Roberts, ed. (Oxford: IRL Press), pp. 199–228.