Establishment of Dorsal-Ventral Polarity in the Drosophila Embryo: The Induction of Polarity by the Toll Gene Product

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Summary

Drosophila females that lack Toll gene activity produce dorsalized embryos, in which all embryonic cells behave like the dorsal cells of the wild-type embryo. Injection of wild-type cytoplasm into young Toll embryos restores their ability to produce a normal dorsal-ventral pattern in a position-dependent manner. No matter where the cytoplasm is injected relative to the dorsal-ventral axis of the egg shell, the position of the injected cytoplasm defines the ventralmost part of the rescued pattern. Although injection of wild-type cytoplasm into mutants at six other dorsal-group loci also restores the ability to produce lateral and ventral structures, only Toll embryos lack any residual dorsal-ventral polarity. Experiments suggest that the activity of the Toll product is normally regulated by other dorsal-group genes and that the function of the Toll product is to provide the source for a morphogen gradient in the dorsal-ventral axis of the wild-type embryo.

Introduction

Embryonic development unfolds in a series of pattern-forming processes. Genetic analysis has made it clear that the first step in the establishment of the spatial pattern of the Drosophila embryo is the definition of the primary body axes, the anterior-posterior axis and the dorsal-ventral axis (Nüsslein-Volhard, 1979). In Drosophila, the orientation, polarity, and basic features of these two patterns are directed by maternal effect genes. This maternal information provides the framework for subsequent regional differentiation in the embryo, directed by genes active in the embryo itself.

The dorsal group of maternal effect genes is a set of ten genes distributed throughout the Drosophila genome, each of which is necessary for the establishment of the dorsal-ventral pattern of the Drosophila larva (Anderson and Nüsslein-Volhard, 1984a, 1984b). All ten are pure maternal effect genes: the embryonic phenotype depends exclusively on the genotype of the mother, and not on that of the embryo itself. All ten produce the same lack of function mutant phenotypes: the absence of any one of these genes in the mother results in the absence of all ventral and lateral pattern elements in the embryo, and all embryonic cells behave and differentiate like the dorsal cells of the wild-type embryo. In order to understand how positional information in the dorsal-ventral axis of the Drosophila embryo is established, it is necessary to distinguish among these superficially indistinguishable gene activities and to define the function of individual components of the system.

Genetic studies of both recessive and dominant alleles suggest that within this group of genes, the Toll gene product plays a central role in coupling the production of ventral structures with their distribution in space (Anderson et al., 1985). The cytoplasmic injection experiments described here provide an independent test of the activity of the Toll gene, and they also single out the Toll gene function as being directly responsible for the polarity of the dorsal-ventral pattern.

Injection of wild-type cytoplasm normalizes the embryonic mutant phenotype for seven of the dorsal-group loci, so that the embryos differentiate lateral or ventral pattern elements never seen in uninjected embryos (Anderson and Nüsslein-Volhard, 1984b). The rescue assay has made it possible to define some molecular aspects of how the information from the different genes is stored in the egg (Anderson and Nüsslein-Volhard, 1984b). In addition, the rescue assay provides a means of analyzing the spatial distribution of, and requirement for, the products of the different dorsal-group genes.

The dorsal-ventral pattern of the Drosophila embryo has a defined orientation relative to the surrounding egg shell. The egg shell is curved on one side, the presumptive ventral side of the embryo, and has specialized chorionic appendages dorsally. For six of the seven dorsal-group genes in which the mutant phenotype is modified by the injection of wild-type cytoplasm, the normal dorsal-ventral polarity is always seen in the rescued embryos. For tube, snape, easter, spätzle, and pelle, no matter where the cytoplasm is removed from the wild-type donors and no matter where it is injected, the rescued pattern has normal dorsal-ventral polarity: the ventralmost structures derive from those cells on the curved side of the egg (Anderson, unpublished; F. Müller-Holtkamp and H. Jäckle, personal communication). The rescue of dorsal mutant embryos has more strict spatial limitations; a rescue response is seen only if the wild-type cytoplasm is delivered to the ventral region of the mutant embryos (Santamaria and Nüsslein-Volhard, 1983). But this again indicates that these mutant embryos retain a subliminal dorsal-ventral polarity.

The dorsalized embryos produced by females that lack Toll gene activity respond to the injection of wild-type cytoplasm by differentiating lateral or ventral structures. Here we describe that, in contrast to the six other rescuable mutants, the polarity of the rescued pattern is determined by the site at which the Toll rescuing activity is introduced into the mutant embryo. The local injection of the Toll rescuing activity can result in the organization of a complete dorsal-ventral pattern in the Toll embryo in any

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of a small volume of cytoplasm from wild-type cleavage stage dorsalized embryos (Anderson et al., 1985). Injection of a small volume of cytoplasm from wild-type cleavage stage embryos (approximately 1/9 of total egg contents) into cleavage stage embryos produced by Toll- females restores lateral and ventral structures, in a pattern defined by the site of injection. For instance, when the wild-type cytoplasm is deposited in the mutant embryos at 30% egg length (measured from the posterior pole) on the curved, presumptive ventral side of the egg (see Figure 1), the embryos differentiate cuticle that frequently has a normal dorsal-ventral pattern posteriorly, with up to eight abdominal ventral denticle bands of normal width, a normal dorsal hair field, and a normal posterior end including the dorsolaterally derived posterior tracheal specializations, the filzkörper (Figure 2). These embryos show coordinated muscular contractions posteriorly, and therefore have differentiated even the most ventrally derived pattern element, mesoderm. While the part of the embryo near the injection site has differentiated a full dorsal-ventral pattern, the anterior remains totally dorsalized, not even differentiating the dorsolaterally derived antennal and maxillary sense organs of the head.

As with the other rescuable dorsal-group mutants (Anderson and Nüsslein-Volhard, 1984a), the phenotypic rescue can be observed in the pattern of gastrulation (Figure 3). The first indication of the rescue response is seen when the newly completed blastoderm cells change shape, precisely at the site where the wild-type cytoplasm was injected. These cells first form a local indentation, and then invaginate into the interior of the embryo. This invagination of cells probably represents a highly localized ventral furrow, since it forms at the same time, and with roughly the same morphology, as a normal ventral furrow. After these cells have invaginated, the pole cells are pushed to the dorsal side, as in the normal extension of the germ band. The germ band extends only to about 30%-40% egg length (rather than the normal 70% egg length), again indicating that the extent of the anterior-posterior rescue response is limited. Injection into a prospective ventral site, but more anteriorly at 70% egg length, rescues the dorsoventral pattern anteriorly (Figures 1, 2). These embryos differentiate many head structures including antennal and maxillary sense organs, mouth hooks, and head skeleton parts, as well as thoracic denticle bands, but remain totally dorsalized posteriorly.

If the wild-type cytoplasm, instead of being delivered to the ventral side, is injected into the flat, presumptive dorsal side of the egg at 30% egg length (Figure 4), then the final differentiated pattern also frequently shows a full range of dorsoventral pattern elements arranged in their normal sequence: the embryos move; abdominal ventral denticle bands are of the normal width; and filzkörper and dorsal hairs are present (Figure 2). When observed within the egg shell, however, the embryos often appear to be reversed in the dorsal-ventral axis, with their ventral denticles on the dorsal side of the egg case. Since these embryos do move inside the egg shell and are frequently twisted, this apparent reversal is difficult to score.

At the time of gastrulation, the dorsally injected Toll- embryos show a clear reversal of the normal dorsal-ventral axis (Figure 3). As with the ventrally injected embryos, there is first a local indentation of the blastoderm and then a ventral furrow-like invagination of cells at the injection site. After the invagination is completed, the pole cells are
Induction of Dorsal-Ventral Polarity

Figure 2. Phase Contrast Views of the Cuticle Produced by Injected Toll Embryos

Cleavage stage Toll embryos were injected with 1% of the total egg contents of a wild-type cleavage stage embryo at defined positions and were allowed to differentiate. Cuticle preparations show the nature and distribution of the pattern elements rescued. (a) Uninjected Toll embryo, with dorsal hairs around the entire dorsal-ventral circumference. (b) Wild-type cytoplasm injected ventrally, 30% egg length. The abdomen has a normal dorsal-ventral pattern, with eight abdominal ventral denticle bands of normal morphology, dorsal hairs on the dorsal side, and the dorsolaterally derived filzkörper (fk) posteriorly. Anterior to A1, the embryo is still dorsalized. (c) Ventral injection, 70% egg length. Ventral and lateral pattern elements are rescued anteriorly. Although head involution has not occurred, head structures are present, including the antennal and maxillary sense organs (am) and the floor of the pharynx (ph). Three thoracic and the first two abdominal ventral denticle bands are present, but the embryo remains dorsalized more posteriorly. (d) Dorsal injection, 30% egg length. As with ventral injections, a normally proportioned pattern is seen, with ventral denticle bands extending anteriorly as far as T3, as well as dorsal hairs and filzkörper. (e) Injection into the central yolk, 30% egg length. Several bands of abdominal ventral denticle bands are present, but they encircle the dorsal-ventral circumference. In the region where ventral denticles are present, there are no dorsal hairs, nor are the dorsolaterally derived filzkörper present. Again the anterior of the embryo remains dorsalized.
carried ventrally, as the germ band extends 180° opposite to its normal direction.

As soon at gastrulation, polarity can be induced in any orientation relative to the dorsal-ventral axis of the egg shell, always defined by the site of injection. If the wild-type cytoplasm is delivered near the egg cortex, the cells at that site invaginate and the germ band extends away from that site, whether the injection site is ventral, dorsal, or lateral. Thus, by the criteria of gastrulation, the site of injection defines the ventral pole of the pattern, independent of the axis of the egg shell.

In the differentiated cuticle, the position dependence of the rescue response is most evident in those embryos in which the wild-type cytoplasm is injected into the central yolky region of the egg, again at 30% egg length (Figure 2). More than half of these embryos differentiate the ventrolaterally derived ventral denticles without differentiating the more dorsally derived filzkörper. The ventral denticles frequently ring the entire dorsal-ventral circumference, so that in the rescued region there are no dorsal hairs. The wild-type cytoplasm in this case has promoted the differentiation of ventrolateral pattern elements, but these elements are incorrectly distributed in space, and the pattern lacks polarity. The pattern of gastrulation in these embryos is variable. Occasionally cells invaginate dorsally, occasionally ventrally, and frequently not at all. In most cases, the pattern of gastrulation lacks a clearly defined polarity.

The phenotypic responses that have been described are the typical responses shown by more than half the embryos injected at a particular site. As shown in Figure 4, there is some variability in the response. Of the embryos injected near the ventral or dorsal periphery 10%–15% show the response usually seen with central injection, differentiating ventral denticles but not filzkörper; of those
Table 1. Age Dependence of the Rescue of Toll- Embryos

<table>
<thead>
<tr>
<th>Zalokar Stage of Embryos at Time of Injection</th>
<th>Invagination at Injection Site</th>
<th>Cuticular Pattern of Developed Embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Embryos</td>
<td>No Response (%)</td>
</tr>
<tr>
<td>9</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>10-11</td>
<td>52</td>
<td>0</td>
</tr>
<tr>
<td>12-13</td>
<td>60</td>
<td>70</td>
</tr>
<tr>
<td>14 (Cellularization)</td>
<td>75</td>
<td>100</td>
</tr>
</tbody>
</table>

All embryos were injected at 30% egg length; approximately half were injected dorsally and half ventrally at each stage. The same extent of response was seen in those embryos injected ventrally and those injected dorsally. Donor cytoplasm was always total egg contents from cleavage stage Oregon R embryos.

Table 2. The Autonomous Activity of Toll[Q]: Percentage of Differentiated Embryos with Flikkörper after Injection with Cytoplasm from Toll[Q] Embryos (No. of Embryos)

<table>
<thead>
<tr>
<th>Donors</th>
<th>Recipients</th>
<th>Wild Type</th>
<th>Toll[Q]</th>
<th>Toll[Q]</th>
<th>ank Toll[Q]</th>
<th>ank Toll[Q]</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gastrulation defective</td>
<td>0 (73)</td>
<td>0 (145)</td>
<td>40 (402)</td>
<td>49 (76)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pipe</td>
<td>0 (180)</td>
<td>29 (90)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>snake</td>
<td>0 (36)</td>
<td>37 (71)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Donor embryos were produced by females of the following genotypes: Oregon R; Toll[Q]/TM6; Toll[Q]/TM2; gdl[Q]/gdl[Q]; Toll[Q]/+; snk[Q]/snk[Q]; snk[Q]/snk[Q]; Toll[Q]/snk[Q]; Toll[Q]. Recipient embryos were produced by females of the genotypes gd7/Df(l)KA10; pip[Q]/pip[Q]; and snk[Q]/snk[Q]. Recipients and donors were both at cleavage stage at the time of injection (before pole cell formation).

injected in the middle, 10%-15% make both filzkörper and ventral denticles, as in the normal pattern. This variability probably indicates that in 10%-15% of the injected embryos the cytoplasm was not accurately delivered to the desired region.

The mutant phenotype of Toll- embryos is first visible at the onset of gastrulation, 3.5 hr (at 25°C) after fertilization. The injection of wild-type cytoplasm can induce polarity and lead to the local formation of a complete dorsal-ventral pattern in Toll- embryos up until midsyncytial blastoderm (cycle 11 of Zalokar [Zalokar and Erk, 1976]; 1.75 hr, at 25%, after fertilization). After that time both the strength of the response and the fraction of embryos showing any response fall off rapidly (Table 1). Toll- embryos injected after cellularization of the blastoderm has begun (early in cycle 14; 3 hr after fertilization) show no phenotypic response either in the final cuticular pattern or in the pattern of gastrulation.

The injected wild-type cytoplasm apparently defines the ventral pole of the dorsal-ventral pattern in the Toll- embryos. In the experiments described above, approximately 1% of total egg cytoplasm was injected, so only a small fraction of the Toll rescuing activity normally present in the wild-type egg is necessary to establish a complete pattern. The Toll rescuing activity is not detectably localized in the wild-type embryo, since even cytoplasm removed exclusively from the dorsal cells of the wild-type blastoderm elicits the same response as total egg cytoplasm (data not shown). So although the Toll rescuing activity can define the ventral pole in the apolar environment of the Toll- hosts, it is apparently present but inactive in the dorsal regions of the wild-type embryo.

The Autonomous Activity of the Product of the Dominant Ventralizing Allele Toll[Q]

The experiments described above show that the Toll rescuing activity can define the polarity of the dorsal-ventral pattern. The relationship between Toll gene activity and the functions of the other dorsal-group genes has also been investigated in cytoplasmic injection experiments. Dominant gain of function alleles of Toll cause a ventralization of the embryonic pattern: the region of ventral pattern elements is expanded at the expense of dorsal pattern elements. This ventralized phenotype is not the result of transcriptional overproduction, but rather reflects the presence of a product with abnormal activity (Anderson et al., 1985). One explanation for the ventralization of the embryonic pattern is that the Toll[Q] product interacts with the product(s) of other dorsal-group gene(s) such that their effective concentration is increased.

In order to assay the concentration of the products of other genes, cytoplasm from embryos produced by Toll[Q] females was used as a donor in the injection of mutant embryos resulting from mutations at other dorsal-group loci. Cytoplasm taken from the ventralized embryos produced by Toll[Q] females and injected into easter and dorsal embryos appeared to rescue both mutants at a slightly higher frequency than wild-type cytoplasm (data not shown). A clearer effect was seen when cytoplasm from Toll[Q] embryos was injected into gastrulation defect-
injected snake embryos differentiate filzkörper, the same as males is injected into snake-embryos, about 40% of the embryos produced by snk TlgQ/snk T/+ double mutant females are not rescued by wild-type cytoplasm, gd and pip. Cytoplasm is injected. When cytoplasm taken from embryos produced by TolP0/+ females was injected into gd embryos, it elicits the differentiation of filzkörper in about 40% of the injected embryos. The phenotypic rescue can be seen in the pattern of gastrulation (a), where the pole cells have migrated dorsally as in normal germ band extension, and in the differentiated cuticle (b), where filzkörper are produced.

The rescue of gd and pip was obtained only with the TolPQ allele and not with the three other dominant ventralizing alleles of Toll, indicating that the observed rescue reflects the particular properties of the TollPQ product. To test whether the rescue of gd by TolP0Q actually represents the increased concentration of the gd product in TollPQ embryos, cytoplasm from the embryos produced by gd/gd, TollPQ/+ females was injected into gd embryos. The double mutant cytoplasm elicited filzkörper production just as effectively as TollPQ cytoplasm alone (Table 2). Thus, it is not an increased amount of the gd product in the TollPQ donors that rescues the gd mutant phenotype, but rather the TollPQ product.

This activity of TollPQ in the absence of other dorsal-group gene activities can be detected not only in the two mutants not rescued by wild-type cytoplasm, gd and pip, but also in other dorsalized embryos, when double mutant cytoplasm is injected. When cytoplasm taken from embryos produced by snk TollPQ/snk TollP/+ double mutant females is injected into snaker embryos, about 40% of the injected snake embryos differentiate filzkörper, the same response seen with gd and pip. As most clearly seen with gd, where the presence or absence of the gd° gene in the donor TollPQ/+ females does not affect either the frequency or the nature of the response, the rescue obtained with TollPQ cytoplasm does not reflect an increase in the products of other dorsal-group genes in the TollPQ embryos. Instead it reprosents the abnormal ability of TollPQ cytoplasm to direct the production of the dorsolaterally derived filzkörper (despite the fact that TollPQ embryos do not themselves make filzkörper even in the absence of the activity of gd°, pip°, and snk°.

Discussion

The cytoplasmic injection experiments described here show that in the absence of the Toll gene product, the Drosophila embryo has no inherent polarity in the dorsal-ventral axis. The local microinjection of cytoplasm from young wild-type embryos can organize a complete dorsal-ventral pattern in any orientation relative to the dorsal-ventral axis of the egg shell. Toll is the only one of the rescuable dorsal-group mutants in which the lack of function results in this loss of polarity. The cytoplasmic rescuing activity for Toll that has the ability to define a new dorsal-ventral polarity is either the Toll gene product itself or a substance that is the direct result of Toll gene activity, since although it is absent in Toll embryos, it is present in mutants derived from all other known dorsal-group loci (K. A., data not shown). The Toll gene product plays a unique role among the dorsal-group maternal effect genes; it appears to be the only gene whose product is necessary both for the production of ventral pattern elements and for the definition of the polarity of the dorsal-ventral axis.

The injection experiments reveal other parameters of the Toll rescuing activity that shed some light on its probable mode of action. The rescue response of the Toll/ embryos differs from the other rescuable dorsal-group mutants in its very strong yet local character. Toll/ embryos that are injected ventrally and posteriorly can differentiate a complete range of dorsal-ventral pattern elements posteriorly, but remain totally dorsalized anteriorly. Mutant embryos from all other loci show a more diffuse response. For example, when posteriorly injected easter embryos make narrow bands of abdominal ventral denticles, they also make the dorsolaterally derived antennal and maxillary sense organs of the head. The lack of diffusion of the Toll rescuing activity is also apparent in the pattern of gastrulation: at the precise site where the wild-type cytoplasm was injected during cleavage stages, cells invaginate at the beginning of gastrulation, more than 2 hr later.

In the region near the injection site, a complete normal dorsal-ventral pattern can be obtained, with functional muscles, ventral denticle bands of the correct width, and a normal dorsal hair field. This strong response, seen after the injection of only 1% of the total contents of the wild-type embryo, indicates that the rescuing activity for Toll is present in the wild-type embryo in vast excess of the amount needed.

The maximal rescue response, including the ability to
Induction of Dorsal-Ventral Polarity

induce locally a complete dorsal-ventral pattern in any orientation perpendicular to the anterior-posterior axis, as seen late as the middle of the syncytial blastoderm stage. This is only 1.75 hr (at 25°C) before the phenotype is visible in the pattern of gastrulation, so the necessary redistribution of positional information in the complete dorsal-ventral axis can be accomplished in this rather short time. Thirty minutes later it is too late to generate a complete dorsal-ventral pattern. Some of the embryos injected at this later stage appear to make ventral pattern elements without making laterally derived structures. This suggests that the tol rescuing activity rapidly creates a local high point of positional information and that intermediate positional values between this high point and the dorsal ground state are filled in by a separate, slower process.

The Tol gene product cannot be a simple determinant of ventral pattern elements, since it is found in vast excess in the wild-type embryo and is present not only ventrally, but also dorsally, in the cytoplasm of the wild-type embryo. We would not have been able to detect a difference between the concentration of Tol rescuing activity on the ventral side of the embryo and that on the dorsal side, since the injected material gives the same complete rescue of dorsal-ventral pattern over a severalfold difference in volume injected (K. A., data not shown). It is nonetheless paradoxical that the Tol product present on the dorsal side of wild type does not induce ventrality in situ, but does when injected into Tol- embryos. One way to explain this difference is to hypothesize that the rescuing activity is a precursor form of the active Tol product. This precursor form would be activated only ventrally in wild type, but in the absence of any other activated region, would become active on its own.

An initially even distribution of morphogenetic information that can autonomously generate a polarized stable state has been observed in the establishment of the polarity of the basic body axes in several other organisms. In Xenopus, for example, the dorsal-ventral axis can arise in any orientation and is normally fixed by the point of sperm entry (Gerhart et al., 1981). In the alga Fucus, it is also the point of sperm entry that defines a basic body axis, the apical-basal axis (Jaffe, 1968). In both Xenopus and Fucus minor physical stimuli such as gravity, light, and electrical fields can override the effect of the point of sperm entry and redefine the polarity of the axis (Jaffe, 1968; Gerhart et al., 1981). This initial trigger must then be amplified by components homogeneously available within the egg to give rise to the definitive body axis. The dorsal-ventral axis of the Drosophila embryo appears to be established by a similar process. The Tol precursor form is distributed approximately evenly within the egg and normally responds to some (undefined) localized trigger to become active on the curved side of the egg. Under the appropriate experimental conditions, as shown here, Tol can become active at other positions and thereby define a different polarity of the dorsal-ventral axis. This dramatic regulative behavior of the Drosophila embryo is at odds with the traditional classification of the Drosophila embryo as rigidly mosaic in its early development and, together with other embryological experiments (Schubiger and Wood, 1977), suggests that the apparently mosaic behavior of Drosophila is a function of the impossibility of performing cell transplantation experiments with pregastrulation embryos and not a reflection of the underlying pattern-forming mechanisms.

The Tol gene product itself appears to be directly responsible for the generation of the dorsal-ventral pattern. Cytoplasmic injection experiments show that the dominant allele of Tol, TLR-1, bypasses the normal requirement for several other dorsal-group genes for the production of lateral pattern elements. The ability of cytoplasm from the ventralized TOL-1 embryos to promote autonomously the production of filzkörper in the absence of gd+, pip+, or snk+ is consistent with the production of ventral denticles in the embryos produced by gds; TLR-1+, pip TLR-1+/pip Ti', and snk TLR-1+/snk Ti' double mutant females (Anderson et al., 1985). In addition, the injection experiments rule out the possibility that the production of laterally derived pattern elements in the double mutants is the result of the amplification of residual gene activity in the gd, pip, or snk mutant allele used. The injection experiments also reinforce the genetic observations which indicate that the dominance of this allele is a reflection of a product with altered activity, rather than the result of transcriptional overproduction. The double mutant phenotypes and the injection experiments both suggest that the TOL-1 mutation has a dominant ventralizing effect because its product is insensitive to a system of regulation that involves the gd+, pip+, and snk+ products. The weak response seen in the injection experiments suggests that although TOL-1 does not absolutely require gd+, pip+, or snk+ for its activity, it functions inefficiently in the absence of these gene products. Both the double mutant phenotypes and these injection experiments suggest that gd, pip, and snk exert their effects on the embryonic pattern by regulating the activity of Tol+, and it is the activity of the Tol gene product that directly controls the embryonic phenotype.

The Tol gene product has striking similarities with the source of a diffusible morphogen gradient (the "activator") in the reaction-diffusion model proposed by Gierer and Meinhardt (1972). In their lateral inhibition model a stable morphogen gradient in space can be generated from an initially nearly homogeneous distribution of the morphogen source as the result of the interactions between the poorly diffusible "activator" and a more rapidly diffusible "inhibitor." The activator is initially present in an inactive precursor or storage form. The production of a small amount of the active form of the activator autocatalytically promotes the conversion of the precursor to the active form. The activator also promotes the synthesis of the diffusible inhibitor, which in turn limits the activation of the activator. With the appropriate diffusion and kinetic constants, these interactions result in a high local activator concentration and a gradient of the inhibitor across a field. Like the autocatalytic activator, the activity of the Tol gene product appears to define a source for a morphogen gradient. Also like the activator, it diffuses poorly, and appears to be present in a precursor form that is activated ventrally and whose activation is normally blocked dor-
sally. The trans interactions between alleles of \( T_{ol} \) can also be explained in terms of autocatalytic activation (Anderson et al., 1985).

Despite these provocative similarities, it is unlikely that the classic lateral inhibition model accurately describes the process of establishing the dorsal-ventral pattern, since such a system would be unstable in the short axis of the embryo (Meinhardt, 1982). Nevertheless, it is an attractive hypothesis to explain the generation of spatial inhomogeneity in the dorsal-ventral axis to propose that the \( T_{ol} \) gene product acts via autocatalysis to provide a local source for a morphogen gradient. Further studies on \( T_{ol} \) and its interaction with other gene products should make it possible to define the actual pattern-generating mechanisms involved.

**Experimental Procedures**

**Injection Procedure**

Embryos were prepared for injection as described (Anderson and Nüsslein-Volhard, 1984b), except that embryos were dried by being placed under a Zeiss air stream incubator for 7 min at 29°C. Embryos in a given series were oriented on double-stick tape so that the wild-type cytoplasm could be delivered to a defined dorsal-ventral and anterior-posterior position. Unless otherwise stated, both donors and hosts were used during cleavage stages (prior to pole cell formation). Injected embryos were observed with transmitted light at gastrulation, and then were allowed to develop at 29°C. Culture preparations were made as described (Santamaria and Nüsslein-Volhard, 1983).

**Mutant Strains**

The dorsalized embryos produced by females carrying several combinations of \( T_{ol} \) alleles and deficiencies were tested for response to injected cytoplasm. All combinations tested that are amorphic by genetic criteria showed the position-dependent response described in Results. Females carrying the following allele combinations were tested: \( T_{ol}(D3R)7/7 ~ T_{ol}(D3R)7/7 ~ T_{ol}(D3R)7/7 ~ T_{ol}(D3R)7/7 ~ T_{ol}(D3R)7/7 \). The origin and genetic nature of the \( T_{ol} \) alleles and deficiencies used are described in the accompanying article (Anderson et al., 1985). The crosses used to obtain these females yielded fewer of the desired trans heterozygotes than expected (approximately 7% of expected), indicating a semilethality common to the \( T_{ol} \) revertant and deficiency alleles. In subsequent experiments, this semilethality was mapped to the left of the \( T_{ol} \) gene itself; the \( T_{ol} \) revertant alleles show nearly normal (40% of expected) viability in trans to \( D(D3R)7/7 ~ D(D3R)7/7 ~ D(D3R)7/7 ~ D(D3R)7/7 ~ D(D3R)7/7 \). The origin of the \( T_{ol} \) gene in these experiments, the \( T_{ol} \) revertant alleles show nearly normal (40% of expected) viability in trans to \( D(D3R)7/7 \) (the gift of Peter Lewis), which breaks in or immediately adjacent to, \( T_{ol} \), destroying \( T_{ol} \) activity, and deletes material distally (Anderson et al., 1985). The alleles of gastrulation defective, \( pipe \), and snake used (Anderson and Nüsslein-Volhard, 1984b) are like null alleles because they result in totally dorsalized embryos, and the \( gd \) and \( ank \) phenotypes are not enhanced in trans to deficiencies.

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