Zygotic Expression and Activity of the Drosophila Toll Gene, a Gene Required Maternally for Embryonic Dorsal-Ventral Pattern Formation

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ABSTRACT

Maternal expression of the Toll gene is required for the production and the correct spatial organization of all lateral and ventral structures of the Drosophila embryo. We show here that the Toll gene is transcribed zygotically in the embryo and that zygotic expression is important for the viability of the larva. Both genetic and molecular data indicate that the zygotic Toll product has the same biochemical activity as the maternal product. The spatial distribution of the Toll transcript in the embryo was analyzed. In contrast to the uniform distribution of the maternal RNA, the zygotic Toll RNA is present in a complex spatial and temporal pattern in the embryo. A striking feature of this pattern is the correlation of the regions of invaginating cells with sites of accumulation of zygotic Toll RNA.

SYSTEMATIC screens for mutations that alter the spatial pattern of the cuticle of the Drosophila larva have been remarkably successful in identifying gene products that play specific roles in embryonic pattern formation (Nusslein-Volhard, Wieschaus and Kluding 1984; Jürgens et al. 1984; Wieschaus, Nusslein-Volhard and Jürgens 1984). Both zygotically and maternally expressed genes have been identified, and in many cases it has been possible to assign the gene a unique function in the generation of embryonic pattern (reviewed in Scott and O'Farrell 1986; Mahowald and Hardy 1985).

The Toll gene is one of these genes that has been shown to play a specific role in defining the spatial organization of the dorsal-ventral pattern in the early embryo. Toll was identified on the basis of a series of maternal effect alleles which cause dramatic changes in the embryonic dorsal-ventral pattern, independent of the genotype of the fertilizing sperm (Anderson, Jürgens and Nusslein-Volhard 1985). Absence of Toll function in the mother causes a complete dorsalization of the embryo, such that cells at all positions of the embryo differentiate like cells in the dorsal regions of the wild-type embryo. Dominant gain-of-function alleles of Toll cause a ventralization of the embryonic pattern, with loss of the dorsal epidermal structures and expansion of the remaining ventrolateral pattern elements. These opposing phenotypes suggest that the Toll product is not only necessary for the development of lateral and ventral structures, but that its activity is also important in defining the position at which those structures are produced. The importance of the Toll product in defining the spatial organization of the dorsal-ventral pattern was most directly shown in phenotypic rescue experiments in which Toll+ cytoplasm was injected into Toll− embryos (Anderson, Bokla and Nusslein-Volhard 1985). In those experiments, the high local concentration of the Toll+ product at the injection site defined the polarity of the entire embryonic dorsal-ventral pattern. These genetic and rescue studies established that the product of the maternally expressed Toll gene plays a specific and central role in early embryonic pattern formation.

For the majority of maternal effect mutations, only single maternal effect alleles at a locus have been isolated, and these represent leaky, partial loss-of-function alleles of essential genes (Perrimon et al. 1986). For those loci, like Toll, where several maternal effect alleles have been isolated, it has been supposed that those genes are expressed only in the female during oogenesis and that their only function is to promote normal embryonic development.

A combination of genetic and molecular data, presented here, clearly demonstrates that the Toll gene is expressed and is functional zygotically as well as maternally. Almost all individuals homozygous for null mutations in Toll die as larvae despite the Toll+ genotype of the mother, indicating the importance of zygotic Toll product. To help define the zygotic function of Toll, we have examined the temporal and spatial pattern of accumulation of zygotic Toll RNA during embryogenesis. The distribution of the zygotic Toll transcript in the embryo is surprisingly complex. Intriguingly, the sites of zygotic Toll RNA accumulation are correlated with regions undergoing morphogenetic movements. This correlation suggests the hypothesis that the Toll protein may be a component of the cellular machinery that promotes morphogenetic movements.
Mutant alleles: The maternal effect phenotypes of Toll alleles are described in Table 1. Most of the Toll alleles designed to identify both lethal and maternal alleles of Toll alleles, TrB1, TrB2 and TlLB', were isolated in a screen of tube, another dorsal group gene. Briefly, rucuca (ru h st cu sr e' ca) males were fed 30 µM EMS according to the procedure of Lewis and Bacher (1968) and then mated with ru st/In(3LR)561, DTS 4 th st Sb e females (Marsh 1978). Single F1 progeny rucuca*/In(3LR)561, DTS4 were mated with tub2't'QR!st and of tube, another dorsal group gene. The crosses were kept at 28° to kill off DTS4-bearing progeny. 5340 mated with tub2'TP'RQ!st with 5340 mated with tub2'TP'RQ!st and of tube or Toll were identified on the basis of the absence of st e ca progeny. Lines with viable rucuca*/tub TI progeny were put in egg-laying blocks, and embryonic phenotypes were scored 24–48 hr after egg laying in living, differentiated embryos (Nüsslein-Volhard 1977). No alleles of tube or Toll were recovered. One allele of Toll, TP'TP', was recovered on the basis of lethality; on retest it was found to be semilethal. Although the maternal effect phenotype of TPIbalancer, the number of TP/TPI progeny relative to TP/Tpbalancer classes was observed; that is, no dominant lethality in the many reciprocal crosses carried out, no difference was possible to distinguish between these two classes, so it was assumed that they were equally viable. In addition, in the many reciprocal crosses carried out, no difference in viability was observed if a particular Toll allele was maternally or paternally contributed.

Viability measurements and temperature-sensitive period determination: The viability of flies carrying heterallelic combinations of Toll alleles was determined relative to the viability of the Toll alleles when heterozygous with a balancer chromosome. That is, in the cross TP'/balancer × TP/balancer, the number of TP/TP' progeny relative to the number of TP/balancer or TP'balancer progeny was defined as the relative viability of TP'/TP. For those crosses in which it was possible to distinguish between the TP'/balancer and the TP/balancer on the basis of markers, no significant differences between the viability of the two balancer classes was observed; that is, no dominant lethality of particular Toll alleles was observed. In other crosses, it was not possible to distinguish between these two classes, so it was assumed that they were equally viable. In addition, in the many reciprocal crosses carried out, no difference in viability was observed if a particular Toll allele was maternally or paternally contributed.

The temperature sensitive period for lethality was determined by letting the parents lay eggs for 12 or 24 hr at the appropriate temperature, and then shifting the progeny in bottles up or down in temperature at the desired time after egg laying. Progeny were not staged by morphological criteria.

RNA analyses: Total embryonic poly (A)+RNA was purified and assayed by blot hybridization using standard procedures (Maniatis, Frisch and Sambrook 1982). The hybridization probe for the Toll RNA was the 1.8-kb EcoRI fragment of the Tp1 clone (see MATERIALS AND METHODS) that hybridizes to the 5.3-kb Toll RNA (upper bands) and with actin DNA (lower bands). The actin signal comes from a reprobing of the blot with actin DNA, followed by a shorter exposure time than that needed to see a strong Toll signal, and was photographically superimposed here, so the relative signal intensities do not reflect the relative abundance of the two RNAs. a: 0–3-hr embryos; b: 3–6 hr; c: 6–9 hr; d: 9–12 hr; e: 12–15 hr; f: 15–18 hr; g: 18–21 hr (age in hours at 22°).

Trichloroacetic acid (TCA) precipitated RNA was prepared as previously described (Anderson and Lengyel 1981), except that in order to prevent pelleting of the polysomes containing Toll RNA it was necessary to add 0.5% Triton X-100 to 0.5% and deoxycytidylate to 0.5% to the homogenate before it was layered on the sucrose gradient.

In situ hybridization to embryonic tissues: In situ hybridizations were carried out essentially according to the method of Mahoney and Lengyel (1987). Briefly, embryos were fixed in paraformaldehyde, and the whole embryos were hybridized with 35S-labeled SP6 antisense transcript from the 2.4-kb EcoRI fragment of the Toll transcription unit (Hashimoto, Hudson and Anderson 1988) cloned into pGEM1 (Promega). After washing to remove unhybridized label, embryos were embedded in methacrylate, 2µm sections were cut, plastic was removed with xylene, and slides were dipped in 1:1 diluted Kodak NTB2 emulsion. Slides were exposed for 7–14 days, developed and stained with Giemsa. No signal was detected in control hybridizations with 35S-labeled sense RNA transcribed from the T7 promoter of pGEM1.

RESULTS

Molecular demonstration of the zygotic expression of Toll: We have recently cloned the Toll gene and identified a 5.3-kb poly(A)+ Toll transcript that carries the biological activity required for the maternally-directed establishment of the dorsal-ventral embryonic axis (Hashimoto, Hudson and Anderson...
Zygotic Expression of the *Toll* Gene

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FIGURE 3.—The zygotic *Toll* transcript. *Df(3R)T194RXIDf(3R)rbYB3(T1-)* virgin females (*T1-*) were mated to males of the same genotype (lane b) or wild type males (lanes a, c and d). Embryonic RNA was prepared, fractionated by electrophoresis and probed with the *Toll* DNA (upper bands) and actin DNA (lower bands in lanes a and b). a, *T1-* females mated to wild type males, 1–24-hr embryonic RNA. b, *T1-* females mated to *T1-* males, 1–24-hr embryonic RNA. c, *T1-* females mated to wild-type males, 0–6-hr embryonic RNA. d, *T1-* females mated to wild-type males, 6–18-hr RNA. Lower band in lane d is a degradation product.}

roughly constant concentration until late embryogenesis, when it dropped in abundance (Figure 1). This transcript was not detected in larvae (HASHIMOTO, HUDSON and ANDERSON 1988). The *Toll* RNA was associated with polysomes both in early embryos and at later embryonic stages (Figure 2). Thus well after the embryonic dorsal-ventral pattern had been defined by the maternal effect activity of *Toll*, a *Toll* transcript was moderately abundant (roughly 50-fold less abundant than actin mRNA) (HASHIMOTO, HUDSON and ANDERSON 1988) and appeared to be actively translated.

It was possible to demonstrate that the late embryonic *Toll* 5.3-kb RNA was the product of transcription from the embryonic genome rather than a stable maternal mRNA. *Df(3R)T194RXIDf(3R)rbYB3(T1-)* females make no detectable ovarian *Toll* transcript (HASHIMOTO, HUDSON and ANDERSON 1988). When the *T1-* females were mated to *T1-* males, their embryos produced no detectable *Toll* transcript (Figure 3b). When *T1-* virgin females were mated to wild type males, the embryos accumulated a significant amount of 5.3-kb *Toll* RNA, which must have been transcribed zygotically from the paternally derived wild type allele (Figure 3a). The zygotically transcribed *Toll* RNA was at least 10 fold more abundant in 6–18 hr embryos than in 0–6-hr embryos (Fig. 3, c and d). Since these embryos were strongly dorsaled

1988). This transcript was present in ovaries and early embryos, as predicted from earlier studies (ANDERSON and NUSSEIN-VOLHARD 1984). A *Toll* transcript of the same apparent size was detectable at a
as a result of the maternal genotype, it is not appropriate to compare this data quantitatively to the level of zygotic Toll expression in the wild type embryo, but the results do demonstrate that Toll is transcribed during embryogenesis.

To test whether the zygotically transcribed Toll RNA has the same biological activity as the maternal Toll transcript, poly(A)^+RNA purified from 1-24-hr embryos from Toll^- mothers and wild type fathers was injected into Toll^- embryos. This RNA rescued the dorsIALIZED Toll^- maternal effect phenotype (Figure 4), and the extent and character of the rescue was similar to that seen with RNA purified from young wild type embryos. Poly(A)^+RNA purified from the same stage embryos from the same mothers mated to Toll^- fathers did not rescue the maternal, dorsIALIZED Toll^- phenotype, although it contained a comparable amount of actin mRNA (Figure 3b).

These results show that the biochemical activities of the Toll maternal and zygotic products are overlapping if not identical, although they do not rule out the existence of slight differences in the structure of the maternal and zygotic products.

The Toll transcript is present in a complex spatial and temporal pattern in the embryo: To help define a possible function for the zygotic Toll transcript, we examined the spatial distribution of Toll RNA in the wild type embryo by in situ hybridization. Both the maternal and zygotic Toll transcripts were analyzed.

During the early stages of cleavage, before the activation of zygotic transcription (Edgar and Schubiger 1986), the maternal Toll transcript was found to be uniformly distributed in the embryo (Figure 5a). During the syncytial blastoderm stage, the Toll RNA moved towards the periphery of the embryo, apparently accompanying the segregation of cytoplasm to the periphery (Foe and Alberts 1983) (Figure 5b). At the end of syncytial blastoderm, most of the Toll RNA was found underneath the nuclei, but no dorsal-ventral asymmetry in its distribution was detected. During cellularization, Toll RNA was still found basal to the nuclei, but its abundance decreased greatly throughout the embryo (Figure 5c). Thus throughout the stages when the maternal Toll activity is required for dorsal-ventral pattern
formation, the *Toll* RNA is present at equal concentration at all dorsal-ventral positions.

At the onset of gastrulation, slight asymmetries in the distribution of the *Toll* RNA in the embryo were detected (Figure 6). Slightly greater amounts of *Toll* RNA were seen at both the anterior and posterior poles relative to other regions of the embryo, in the cells that later contribute to the anterior and posterior midguts. At the same stage, a slight dorsal-ventral asymmetry in the distribution of the *Toll* RNA was seen: immediately after the invagination of the ventral furrow, the intensity of signal in the mesodermal cells of the invaginated ventral furrow was significantly greater than that seen in the adjacent, uninvaginated cells. Since the overall abundance of *Toll* RNA was decreasing at this stage, these local asymmetries may have reflected either local stabilization of the maternal RNA or localized zygotic transcription.

Shortly after the beginning of germ band extension, it became clear that zygotic transcription of *Toll* had begun, due to the increased intensity of signal in the cells of the posterior midgut and at the anterior tip increased, as the anterior cells began invaginating to join the anterior midgut (compare to Technau and Campos-Ortega 1985). Soon after the germ band extended to its maximum extent (stage 9 of Campos-Ortega and Hartenstein 1983), a strong *Toll* signal was seen in a midventral strip along the entire length of the germ band, in the mesectodermal cells (Fig. 7, d and e). Later in the extended germ band stage (late stage 10), additional sites of *Toll* RNA expression became apparent in a segmentally-repeating pattern in the region of the tracheal placodes (Figure 7b) and in the region of the salivary gland placodes (Figure 7c). *Toll* expression in the region of the tracheal pits persisted during their invagination. Thus during the extended germ band stage, *Toll* RNA was found to accumulate in all the regions undergoing invaginations, as well as in the mesectoderm.

After germ band shortening (stages 13 and 14) the *Toll* transcript was seen in many embryonic tissues, including the pharynx, esophagus, hindgut, the Malpighian tubules, throughout the epidermis and in the somatic muscles (Figure 7f). In the epidermis, a greater concentration of *Toll* RNA was seen at the base of the segmental furrows, in what appear to be the muscle attachment sites (Fig. 7, g and h). In the late embryo (stage 16), when most of embryonic differentiation was complete, the level of *Toll* RNA dropped throughout the embryo, and the only site...
FIGURE 7.—Spatial distribution of the zygotic *Toll* transcript. a, Sagittal section of a late stage 8 embryo. Dorsal up, anterior left. *Toll* RNA is found in the posterior midgut, and cells at the anterior tip of the embryo that will contribute to the anterior midgut. No signal is seen in mesoderm at this stage. b, A slightly oblique parasagittal section of a stage 10 embryo. Dorsal up, anterior left. Segmentally repeated expression of *Toll* is seen in the ten tracheal pits. c, Stage 10, a more medial section than in (b) shows hybridization in the region of the salivary gland placode (arrowhead). Dorsal up, anterior left. d, A yet more medial section of a stage 10 embryo, showing expression in the mid-ventral stripe of the mesectoderm (arrows). Dorsal up, anterior left. e, Cross-section of a stage 9 embryo, showing the expression in the mesectoderm. f, Horizontal section, showing labeling of the ectoderm and somatic muscles after germ band shortening. Anterior left. g, Parasagittal section, after germ band shortening. Dorsal left, anterior up. Labeling of the ectoderm is more intense in narrow regions at the base of the segmental furrow that appear to be the muscle attachment sites (arrows). h, An enlargement of (g) with muscle attachment sites indicated by the arrows. a–f, 11-day exposures. g and h, 6-day exposure.
of abundant *Toll* RNA was in the dorsal vessel (not shown).

**Zygotic expression of *Toll* is important for viability:** Since the *Toll* gene was originally characterized on the basis of maternal effect phenotypes shown by mutant females that had survived to the adult stage, the abundance and the complex spatial distribution of the zygotic *Toll* transcript was surprising. However, three criteria indicate that none of the four dominant and six recessive alleles of *Toll* isolated on the basis of a maternal effect phenotype is a null allele: each allele results in an embryonic phenotype distinguishable from that of all other alleles; in heteroallelic combinations each allele can be shown to produce phenotypes different from combinations with deficiencies; none of these alleles produces the complete dorsalization of the embryo characteristic of null alleles at other dorsal group loci.

Apparent null alleles of *Toll* were isolated as phenotypic revertants of the dominant gain of function alleles (Anderson, Jürgens and Nüsslein-Volhard 1985). These alleles have been defined as null alleles because they behave like deficiencies in trans combinations with other alleles and because they cause complete dorsalization of the embryo as a maternal effect in trans to one another or in trans to deficiencies for the locus. In addition, several of these revertant alleles do not make a detectable amount of *Toll* transcript (Hashimoto, Hudson and Anderson 1988). Each of these apparently null revertant alleles was found to be associated with a recessive decrease in viability (Table 1). In each case there were some adult *Toll*− escapers that eclosed 1 to 2 days later than their balancer siblings, but appeared to be of normal morphology. The escaper males were fertile, and the only defect apparent in the females was that they produced completely dorsalized embryos.

Three alleles isolated as recessive maternal effect mutations, *TPR*32, *TPR*26 and *TPR*44, that show a temperature-sensitive maternal effect phenotype were found to be also temperature-sensitive for viability (Table 1). For instance, a female of the genotype *TPR*44/*Df(3R)ro80B* produced embryos that develop a nearly normal pattern at 18°C, and were strongly dorsalized at 29°C. Animals of the genotype *TPR*44/*Df(3R)ro80B* that were raised at 18°C or 22°C all survived to adulthood, but only 7% survived if raised at 29°C.

The temperature-sensitive lethality associated with some alleles made it possible to define the developmental stage when zygotic activity of *Toll* is important for survival. The temperature-sensitive period for zygotic viability was examined for two temperature-sensitive alleles, *TPR*44 and *TPR*26. For both of these alleles, the temperature sensitive period began late in embryogenesis and extended into the second larval instar (data for *TPR*44 is shown in Figure 8). Temperature shifts in early embryogenesis, or in the late third larval instar through pupal stages had no effect on viability.

**TABLE 1**

Viability of *Toll* alleles over *Df(3R)ro80B*

<table>
<thead>
<tr>
<th>Allele*</th>
<th>No. of flies</th>
<th>Percent of expected <em>Toll</em> flies surviving relative to balancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Null alleles</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>TPR</em>1 (D0)</td>
<td>5</td>
<td>841</td>
</tr>
<tr>
<td><em>TPR</em>2 (D0)</td>
<td>10</td>
<td>895</td>
</tr>
<tr>
<td><em>TPR</em>3 (D0)</td>
<td>19</td>
<td>1615</td>
</tr>
<tr>
<td><em>TPR</em>4 (D0)</td>
<td>8</td>
<td>481</td>
</tr>
<tr>
<td><em>TPR</em>5 (D0)</td>
<td>29</td>
<td>663</td>
</tr>
<tr>
<td><em>TPR</em>6 (D0)</td>
<td>34</td>
<td>558</td>
</tr>
<tr>
<td><em>TPR</em>7 (D0)</td>
<td>83</td>
<td>842</td>
</tr>
<tr>
<td>Dominant alleles</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>TPR</em>1 (V)</td>
<td>436</td>
<td>909</td>
</tr>
<tr>
<td><em>TPR</em>2 (V)</td>
<td>249</td>
<td>542</td>
</tr>
<tr>
<td><em>TPR</em>3 (D0)</td>
<td>8</td>
<td>792</td>
</tr>
<tr>
<td><em>TPR</em>4 (D0)</td>
<td>4</td>
<td>1172</td>
</tr>
<tr>
<td>Recessive alleles</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>TPR</em>1 (22°C) (D1)</td>
<td>355</td>
<td>586</td>
</tr>
<tr>
<td><em>TPR</em>2 (29°C) (D0)</td>
<td>88</td>
<td>736</td>
</tr>
<tr>
<td><em>TPR</em>3 (22°C) (D0)</td>
<td>430</td>
<td>926</td>
</tr>
<tr>
<td><em>TPR</em>4 (29°C) (D0)</td>
<td>27</td>
<td>780</td>
</tr>
<tr>
<td><em>TPR</em>5 (18°C) (D1)</td>
<td>290</td>
<td>792</td>
</tr>
<tr>
<td><em>TPR</em>6 (22°C) (D0)</td>
<td>129</td>
<td>542</td>
</tr>
<tr>
<td><em>TPR</em>7 (29°C) (D0)</td>
<td>12</td>
<td>571</td>
</tr>
<tr>
<td><em>TPR</em>1 (22°C) (D2)</td>
<td>12</td>
<td>979</td>
</tr>
<tr>
<td><em>TPR</em>2 (29°C) (L)</td>
<td>383</td>
<td>599</td>
</tr>
<tr>
<td><em>TPR</em>3 (29°C) (D1)</td>
<td>102</td>
<td>276</td>
</tr>
<tr>
<td><em>TPR</em>4 (29°C) (D1)</td>
<td>90</td>
<td>248</td>
</tr>
</tbody>
</table>

Progeny from the crosses *Df(3R)ro80B*/*TM3 × *TUM3* were counted. Unless otherwise noted, all crosses were carried out at 22°C. *Df(3R)ro80B* deletes *97D1-D15*, removing genes both proximal and distal to *Toll*, but not deleting any other dorsal group genes. *TPR*1 and *TPR*2 are inversions; all other alleles are cytologically normal, apparent point mutations.

Maternal effect phenotypes are shown in parentheses for the allele in trans to *Df(3R)ro80B* at the temperature shown. The phenotypic categories are based on the gastrulation pattern and cuticle pattern, as previously described (Anderson, Jürgens and Nüsslein-Volhard 1985): D0 = strongly dorsalized, V = ventralized; D1 = moderately dorsalized; D2 = weakly dorsalized; L = lateralized.
the markers of the surviving adults showed that the laggard larvae represented the Toll class.

The early larval temperature-sensitive period and the early larval lethality suggest that the embryonic transcription of Toll that we have observed is of functional importance. However, no obvious, completely penetrant anatomical defects were seen in the $Tl$ larvae. The dorsal-ventral and segmentation patterns of the larval cuticle appeared normal. The only reproducible abnormalities seen in cuticle preparations were variable discontinuities in the tracheal system in approximately 20% of the dying larvae. Some larvae were missing an entire main tracheal trunk; in others, a tracheal trunk was interrupted. We also examined histological sections of embryos from crosses in which 50% of the embryos should have been $Tl^-/Tl^-$. All the embryos examined appeared to be of normal morphology. In particular, the gut and salivary glands, prominent sites of Toll RNA accumulation in the embryo, appeared normal at the level of light microscopy.

**Maternal and zygotic functions of Toll are genetically distinguishable:** Although the data indicated that there was a single product of the Toll gene, the maternal and zygotic phenotypes were found to have different genetic properties. The most obvious difference was quantitative: only alleles that reduced the maternal activity enough to result in the production of the extreme, strongly dorsalized embryos reduced viability. For instance, the $Tl^{B1}$ and $Tl^{B2}$ alleles were completely penetrant, moderately dorsalizing maternal effect alleles, such that homozygous females produced embryos that differentiated the dorsolaterally derived filzkörper, but not the ventrolaterally derived ventral denticle bands. These alleles were completely viable (Table 1). Similarly, females homozygous for the recessive allele $Tl$ produced lateralized embryos that differentiated the ventrolaterally-derived ventral denticles, and no zygotic lethality was associated with this allele.

The maternal activity depends critically on interactions among the products of the Toll alleles present in the embryo (Anderson, Jürgens and Nüsslein-Volhard 1985), but such interactions were not of obvious importance for the zygotic function of Toll. Several combinations of non-null alleles caused the same strong maternal effect dorsalization seen with null alleles, but allele combinations of this sort were not associated with a decrease in zygotic viability. For instance, the $Tl^{B1}$ allele appeared to act antimorphically in combination with recessive alleles: $Tl^{B1}+/-$ females produced ventralized embryos and $Tl^{m09}/+$ $Df(3R)ro^{50b}$ females produced lateralized embryos, but $Tl^{B1}/Tl^{m09}$ females produced extremely dorsalized embryos, as in the null phenotype. In contrast, $Tl^{B1}/Tl^{m09}$ zygotes were completely viable.

Another difference between the maternal and zygotic Toll functions was that the four dominant maternal effect alleles had no dominant effect on viability. It is worth noting, however, that the dominant alleles did have an incompletely penetrant dominant phenotype: up to 50% of the heterozygous flies had melanotic tumors. Melanotic tumors are not true tumors, and instead appear to represent an immune-like response to foreign substances (Sparrow 1978). Since melanotic tumors were not found in the null phenotypic revertant derivatives of the dominant alleles, this phenotype appeared to be specifically related to the dominant character of these Toll alleles.

In its maternal role in the establishment of the embryonic dorsal-ventral pattern, the Toll gene product must interact, directly or indirectly, with the products of the other dorsal-group genes. To explore the possibility that the products of these other genes are also involved in the same zygotic process as Toll, the viability of Toll alleles was assayed in several...
double mutant combinations. Double mutants with nudel, snake and tube produced no significant change in the survival of Toll mutant zygotes (data not shown). Double mutants of easter alleles with Toll null alleles had no effect on Toll semilethality, but some alleles of easter increased the probability of survival of temperature-sensitive Toll alleles. For instance, 95% of TT444/Df(3R)ro80b zygotes died at 29°, but no lethality was seen in eaTtllea'Df(3R)ro80b zygotes raised at 29° (Table 2). The same double mutant combination that suppressed Toll lethality enhanced the dorsalizing maternal effect. That is, eaTtllea' females produced very weakly dorsalized embryos at 18°, as did TT444/ea'Df(3R)ro80b females, but the double mutant females produced strongly dorsalized embryos. Thus easter can be active in both the maternal and zygotic process that Toll participates in, while the maternal activities of the two genes are required for the same process, the zygotic activities seem to be antagonistic.

DISCUSSION

The activity of the Toll gene is important in at least two processes during Drosophila development. Maternal expression of Toll is required for the production of the embryonic dorsal-ventral pattern, and zygotic expression of Toll in the embryo is required in some process (or processes) important for the viability of the early larva. The zygotic Toll transcript is of the same size as the maternal transcript, and can substitute for the maternal product, as shown by its ability to rescue the maternal effect phenotype. Temperature-sensitive alleles are temperature-sensitive for both the maternal effect phenotype and for zygotic viability. Based on these data, the simplest hypothesis is that the Toll gene encodes a single protein that is used in more than one process.

The maternal, dorsal-ventral pattern forming activity of Toll is not modified by zygotic expression of Toll, since the degree of pattern defect is independent of the paternal genotype. For instance, embryos produced by TT444/Df(3R)ro80b females at 18° show the same pattern of weak dorsalization whether they are mated with Toll- males, wild type males or Dp(Toll)+ males (K. V. ANDERSON, unpublished data).

Zygotic lethality is associated only with those alleles that eliminate nearly all Toll activity, while the maternal process is more sensitive to alterations in the Toll product. Thus, the recessive maternal effect Toll alleles behave as leaky alleles. Toll then falls into the class of essential genes with leaky maternal effect alleles like deep orange or rudimentary (BISCHOFF and LUCCHESI 1973; NORBY 1973; SEGRAVES et al. 1983), important metabolic genes whose products are needed at higher concentrations in the early embryo than at any other point in the life cycle.

The conclusion that zygotic Toll gene activity is important for viability does not diminish the significance of the previous experiments that showed that the Toll gene product plays a specific and central role in the establishment of the embryonic dorsal-ventral pattern. It seems reasonable, as pointed out for the pleiotropic daughterless gene (CRONMILLER and CLINE 1987), that genes central to one developmental process are likely to be involved in other developmental processes.

In order to better understand the functions of the maternal and zygotic Toll transcripts, their spatial distribution was examined by in situ hybridization. The maternal Toll transcript is uniformly distributed in the early embryo, from the earliest stages examined through the cellularization of the blastoderm. These are the stages when the maternal Toll+ product is active, as judged both by the maternal temperature-sensitive period (ANDERSON and NUSSELEIN-VOLHARD 1986) and by the stages when it is possible to rescue the phenotypic rescue experiments by microinjection of wild type cytoplasm (ANDERSON, BOKLA and NUSSELEIN-VOLHARD 1985). The transcript of the one other dorsal group gene that has been examined, dorsal, is also uniformly distributed in the early embryo at the stages when its product must be active, and drops in abundance slightly before the stage when the level of maternal Toll RNA drops (STEWART, AMBROSIO and SCHUPBACH 1987). The results of the in situ hybridizations confirm the conclusions of the phenotypic rescue experiments, in which it was found that the activity that rescues the Toll+ phenotype is present on both the ventral and dorsal sides of the young wild type embryo (ANDERSON, BOKLA and NUSSELEIN-VOLHARD 1985). Any localization of Toll activity must therefore occur at a translational or post-translational level.

At the onset of gastrulation, there is a slightly greater concentration of Toll RNA in the ventrally derived mesodermal cells than in the surrounding, uninvaginated cells. Judging by the time course of the decrease in grain density in the embryo as a whole, the relatively higher amounts of Toll RNA in

TABLE 2

<table>
<thead>
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<th>Percentage of Toll flies surviving relative to balancer</th>
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<tr>
<td>TT444/Df(3R)ro80b</td>
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<tr>
<td>ea7/lea7</td>
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<tr>
<td>ea7/lea7'</td>
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<td>ea7/lea7'</td>
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Crosses between ea TTR33 stocks were carried out at 29°. The total number of progeny from the crosses counted is shown in parenthesis. The easter maternal effect phenotypes are: ea7/lea7 is temperature-sensitive, such that the females produce weakly dor- salized embryos at 18° and strongly dorsalized embryos at 29°; ea7/ ea7' females produce moderately dorsalized embryos at all temper- atures; no zygotic lethality is associated with the easter alleles alone.
the mesodermal cells of the ventral furrow could reflect greater stability of the RNA in that region, rather than new zygotic synthesis of Toll RNA. This uneven RNA distribution is seen after the maternal product has exerted its primary effect on the dorsal-ventral pattern. Therefore, the greater abundance of Toll RNA in the mesodermal cells is an effect, not a cause, of the asymmetry of the dorsal-ventral pattern.

Zygotic expression of Toll begins quite early in embryogenesis. At the end of the cellular blastoderm stage, when the overall level of maternal Toll RNA has fallen substantially, a significantly greater amount of Toll RNA is seen at the anterior and posterior poles of the embryo in the cells that will become incorporated into the anterior midgut and the posterior midgut, respectively. We cannot distinguish whether this pattern reflects a stabilization of the maternal RNA at the two poles or the onset of zygotic Toll transcription in those regions. It is clear, however, that within 30 min after cellularization of the blastoderm zygotic transcription of Toll in the same cells has begun, since an increased level of signal is seen in the cells derived from the anterior and posterior ends of the embryo, as they invaginate into the parts of the midgut.

The zygotic Toll RNA accumulates at many different sites in the embryo in a complex temporal and spatial pattern, but the cells that accumulate the RNA do share some common features. During the extended germ band stage, all cells undergoing invaginations contain a high concentration of Toll transcript (Figure 9). In the posterior midgut, the anterior midgut, the salivary gland placodes and the tracheal placodes, Toll RNA is seen shortly before and during the time of invagination. This correlation suggests that one aspect of the biochemical function of Toll may be to facilitate morphogenetic movements. The expression of Toll is not limited to invaginating cells, however, since the zygotic Toll transcript also accumulates in other regions, including in the mesectoderm and at the muscle attachment sites. Those two regions are both sites of connection between ectodermal and mesodermal cells, suggesting that Toll might be involved in the association of the two germ layers. The nucleotide sequence of the Toll transcript indicates that the Toll protein is an integral membrane protein (Hashimoto, Hudson and Anderson 1988). Thus the spatial pattern of zygotic Toll RNA accumulation, together with the protein sequence, suggest the hypothesis that the Toll protein may have a direct role in cell interactions or cell adhesion.

The lack of zygotic expression of the Toll gene in the TI+/-TI- embryo is not associated with any completely penetrant morphological defects visible by light microscopy in the tissues where we see Toll transcript. The only defects that may be attributable to the absence of embryonic Toll RNA are abnormalities in the tracheal system in about 20% of the TI- larvae. Other regions of the embryo that accumulate Toll RNA, including the gut and salivary glands, appear normal. Thus although Toll is expressed in a complex pattern in the embryo and is important for larval viability, we cannot clearly show that the zygotic Toll product is required in any particular morphogenetic process. One possible explanation for the lack of a clear anatomical phenotype in the absence of zygotic Toll expression is that the maternal Toll- product may partially rescue the Toll- zygotes. Given the observation that the zygotic Toll RNA can be detected within 30 min of the time when the maternal RNA level drops, it seems likely that maternal Toll protein is present in the same cells that transcribe Toll zygotically. Since removal of the maternal Toll product results in the extremely abnormal morphology of the dorsalized embryo and consequent lethality, it is not possible to directly assess whether a Toll- zygote whose mother had failed to contribute Toll- product to the egg would have developmental defects in addition to the maternal effect dorsalization. Thus, on the basis of the current data, we cannot distinguish between the possibility that the Toll product plays a minor role in the development of the embryo after gastrulation and the possibility that it plays a more fundamental role that is phenotypically masked by persisting maternal protein.

These studies on the zygotic expression of Toll provide a new perspective on the nature of this gene product and its maternal role in the establishment of the embryonic dorsal-ventral pattern. Although there are both quantitative and qualitative genetic differences between the maternal and zygotic Toll activities, a single product is made at both times. The spatial pattern of zygotic Toll expression, together with the predicted sequence of the Toll protein (Hashimoto, Hudson and Anderson 1988), suggests that the Toll protein may be a membrane protein directly involved
in morphogenesis. In the generation of the dorsal-ventral pattern of the embryo, there is a tight correlation between the pattern of morphogenetic movements at gastrulation and the determination of cell fate in the dorsal-ventral axis (Anderson and Nusslein-Volhard 1984). These studies raise the possibility that the maternal Toll protein may be involved in coupling early morphogenetic movements with cell determination in the dorsal-ventral axis.

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LITERATURE CITED


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