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Establishment of Dorsal-Ventral Polarity in the Drosophila Embryo: Genetic Studies on the Role of the *Toll* Gene Product

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Summary

Within the group of maternal effect genes necessary for the establishment of the dorsal-ventral pattern of the Drosophila embryo, the *Toll* gene mutates to give a singular variety of embryonic phenotypes. Lack of function alleles produce dorsalized embryos as a recessive maternal effect. Dominant gain of function alleles result in ventralized embryos. Other recessive alleles cause partial dorsalization or lateralization of the embryonic pattern. Gene dosage studies indicate that the dominant ventralized phenotype results from an altered activity of the *Toll* product. Complementation studies show specific *trans* interactions between copies of the *Toll* product. Double mutant phenotypes suggest that the products of several other dorsal-group genes regulate the activity of *Toll*.

Introduction

The two primary axes of the Drosophila larva, anterior-posterior and dorsal-ventral, are established by the action of two independent sets of maternal effect genes (Nüsslein-Volhard, 1979). The establishment of the basic features of the dorsal-ventral embryonic pattern specifically requires the action of a group of ten maternal effect genes, the "dorsal-group" (Anderson and Nüsslein-Volhard, 1984a, 1984b). Females carrying loss of function mutations in any one of these genes produce normally shaped eggs in which there is a coordinated shift of the entire dorsal-ventral pattern in the blastoderm fate map, such that all cells assume a fate normally assigned to cells at more dorsal positions of the embryo. The similarity of the embryonic mutant phenotypes suggests that each gene product is an essential component of a single system of positional information. By studying the interactions between the products of these genes both genetically and molecularly, we hope to understand how positional information in the dorsal-ventral axis is generated.

Although these maternal effect genes are transcribed during oogenesis, their products are required for a process that is not fixed until some time after fertilization, probably just before the cellularization of the blastoderm. The embryonic phenotypes resulting from mutations in seven of the genes can be partially or completely normalized by injection of wild-type cytoplasm into mutant embryos until late in the syncytial blastoderm stage (Anderson and Nüsslein-Volhard, 1984b; Anderson, unpublished). In all cases tested, cytoplasm from dorsalized embryos resulting from mutations at one locus can rescue mutant embryos from other loci as effectively as wild-type cytoplasm, so the products of these genes are independently synthesized and stored in the egg. Since these gene activities are required for a single process, there must be some interaction, either direct or indirect, between their products after fertilization on a posttranscriptional level.

One of the maternal effect dorsal-group genes, *Toll*, is unique because, in addition to recessive dorsalizing *Toll* alleles, other mutant *Toll* alleles cause ventralization or lateralization of the embryonic pattern. In this paper we describe the embryonic phenotypes produced by both dominant and recessive *Toll* alleles, the effect of gene dosage on the dominant phenotypes, and the phenotypes of double mutants of $Toll^{D}$ and other dorsal-group genes. This phenotypic analysis suggests that mutations in *Toll* directly alter both the production and the spatial distribution of positional information in the dorsal-ventral axis. In addition, the appropriate activity of the *Toll* gene product appears to depend on its interaction both with other copies of the *Toll* product and with the products of other dorsal-group genes. From these results, together with the results of the accompanying paper (Anderson et al., 1985), a picture emerges in which the $Toll^{+}$ gene product couples the production of morphogen with its correct spatial distribution, and directly determines the polarity of the dorsal-ventral pattern.

Results

The mutations in the *Toll* gene that are described here (listed in Table 1) are all maternal effect mutations that result in an altered dorsal-ventral embryonic pattern. The embryonic phenotypes can be evaluated both in the pattern of gastrulation and in the cuticular structures produced by differentiated embryos, as illustrated in the phenotypic overview in Figure 1. The results presented here characterize the embryonic mutant phenotypes resulting from mutant *Toll* alleles and attempt to define genetically the nature of the lesions that give rise to these particular changes in the embryonic dorsal-ventral pattern.

The $Toll^{D}$ Phenotype

In the course of a number of mutant screens in which isogenic lines were established (Nüsslein-Volhard et al., 1984; Jürgens et al., 1984, and unpublished), six totally penetrant dominant maternal effect mutations were identified and recovered (see Experimental Procedures). Females heterozygous for each of the mutations produce embryos that develop and differentiate cuticle of characteristically mutant pattern. Four of these dominant maternal effect mutations share a common embryonic phenotype, the *Toll* phenotype. The cuticle pattern of $Toll^{D}$

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Table 1. *Toll* Alleles

Allele Designation	Chromosome Mutagenized	Mutagen	Cytology
Dominant, ventralizing			
Tl^{1}	Wild type	EMS	Normal
Tl^{5B}	*rucuca*	EMS	Normal
Tl^{9Q}	*rucuca*	EMS	Normal
Tl^{84C}	*ru st e ca*	EMS	Normal
Revertants			
Tl^{RXH}	Tl^{1}	X ray	Normal
Tl^{RXZ*}	Tl^{1}	X ray	In (3R) 97D; 99EF
Tl^{RXJ}	Tl^{1}	X ray	In (3R) 97D; 98F
Tl^{RXD}	Tl^{1}	X ray	Normal
Tl^{RXA}	Tl^{1}	X ray	Normal
Tl^{RXE}	Tl^{1}	X ray	T (2; 3) 97D; centric heterochromatin
Tl^{5BRXQ}	Tl^{5B}	X ray	Df (3R) 97A; 97D
Tl^{5BRXV}	Tl^{5B}	X ray	In (3LR) 74A; 97D
Tl^{5BRXI}	Tl^{5B}	X ray	Df (3R) 97B; 97E
Tl^{5BRXP}	Tl^{5B}	X ray	Df (3R) 97A; 98A
Tl^{5BREK}	Tl^{5B}	EMS	Df (3R) 97A, 97DE
Tl^{5BREL}	Tl^{5B}	EMS	Normal
Tl^{5BREM}	Tl^{5B}	EMS	Normal
Tl^{5BREQ}	Tl^{5B}	EMS	Normal
Tl^{5BREY}	Tl^{5B}	EMS	Normal
Tl^{5BREF*}	Tl^{5B}	EMS	Normal
Tl^{5BREW}	Tl^{5B}	EMS	In (3R) 97A; 97D + Df (3R) 97B; 97D
Tl^{5BREH}	Tl^{5B}	EMS	Normal
Tl^{5BREJ}	Tl^{5B}	EMS	ND
Tl^{5BRER*}	Tl^{5B}	EMS	Normal
Tl^{5BRES}	Tl^{5B}	EMS	Normal
Tl^{5BREN}	Tl^{5B}	EMS	In (3R) 97D; 99B
Tl^{9QRE}	Tl^{9Q}	EMS	In (3R) 97D; 98CD
Tl^{9QRX}	Tl^{9Q}	X ray	Df (3R) 97B; 97D
Tl^{84cRXB}	Tl^{84C}	X ray	Normal
Tl^{84cRXD}	Tl^{84C}	X ray	Df (3R) 97B; 97EF
Recessive alleles			
Tl^{r632}	*ru st e ca*	EMS	Normal
Tl^{rm9}†	*mwh red e*	EMS	Normal
Tl^{r444}	*ru st e ca*	EMS	Normal
Tl^{r26}‡	*st e*	EMS	Normal

* Stocks were lost after initial characterization.
† This allele was isolated by T. Rice (Ph.D. thesis, Yale University, 1973) and designated *mel(3)9* by him. His allele *mel(3)10* is also allelic to *Toll*, but behaves phenotypically like *mel(3)9* in all tests and appears to represent the same mutagenic event as *mel(3)9*.
‡ This allele was isolated on the basis of a leaky dominant dorsalized phenotype.

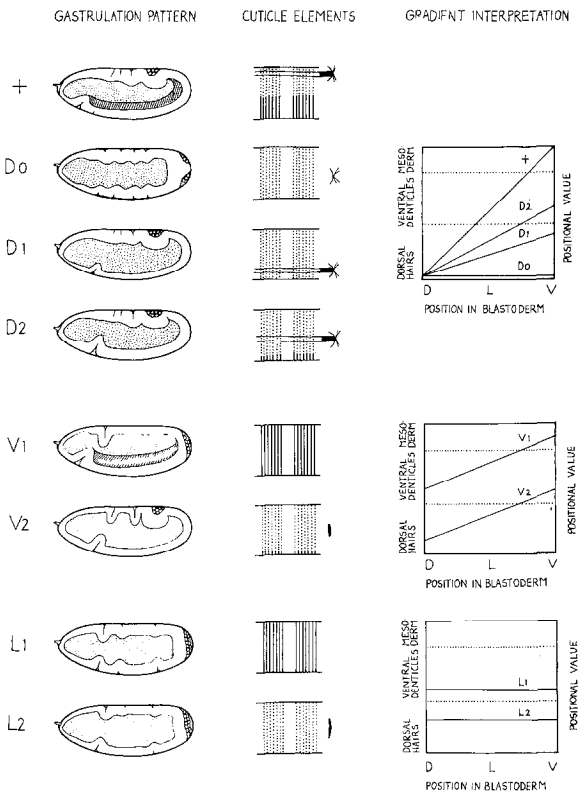

Figure 1. Classification of Embryonic Phenotypes Observed in *Toll* Mutant Embryos

Like mutants in the other dorsal-group loci, mutants in *Toll* produce characteristic changes in the pattern of gastrulation that correlate with the nature and pattern of the cuticular structures in the differentiated embryos. The changes in gastrulation and in the cuticle pattern both can be explained by shifts in the fate map, affecting the fate of cells during the morphogenetic movements of gastrulation and then the final fate of differentiated cells (Anderson and Nüsslein-Volhard, 1984a). For instance in a totally dorsalized embryo (D0), such as those embryos produced by $Toll^{Revertant}$ females, the ventral furrow fails to form at gastrulation and the pattern of transverse folding appears to represent the extension of the normal dorsal folds along the entire dorsal-ventral circumference. This pattern of gastrulation always correlates with the failure to differentiate cuticle elements, except those deriving from the dorsalmost region of the fate map. The characteristics of gastrulation sketched here (anterior to the left; dorsal up) for about 20 min after the completion of cellularization of the blastoderm that are most useful in classifying the phenotypes are the invagination of presumptive mesoderm in the ventral furrow (//////), the dorsal-ventral position of the head fold (seen at 65% egg length from the posterior pole), and the degree to which the germ bands extends dorsally (seen in the position of the pole cells). The cuticle elements used in the classification are the spiracles (⁜), normally derived from the dorsal midline; the dorsal cuticle with its dorsal hairs (⁘); filzkörper (■), normally derived from a dorsolateral position; and the ventral cuticle with its ventral denticle bands (|||||). This phenotypic analysis makes it possible to describe the shift in the dorsal-ventral fate map, as illustrated in the gradient interpretation. The cells in the ventral portion of the wild-type blastoderm are assumed to have a high positional value and as a result invaginate in the ventral furrow and give rise to mesoderm, while cells at more lateral and dorsal positions have lower positional values and differentiate ventral cuticle or dorsal cuticle accordingly. Thus the gradient interpretation for L1 can be considered as description of the blastoderm fate map of embryos showing this phenotype, where all blastoderm cells behave both at gastrulation and in the final cuticle produced like the cells at the lateral positions of the wild-type blastoderm. Some refinements of the phenotypes have been derived from histological analysis: for instance, although in living embryos the ventral furrow of ventralized (Vl) embryos appears larger than in wild type,

embryos differs strikingly from the wild-type pattern (V1 in Figure 2.) Instead of the characteristic array of denticle bands ventrally and fine hairs dorsally, $Toll^{D}$ embryos have rings or patches of ventral denticles along the entire dorsal-ventral circumference and lack dorsal hairs altogether. Other structures normally derived from dorsal and dorsolateral anlagen (Lohs-Schardin et al., 1979) are also missing: filzkörper, spiracles, head sensory organs, and the head skeleton are all absent. Thus the cuticle patterns appears ventralized: ventral pattern elements occupy a larger region than normal, at the expense of dorsal pattern elements. Histological sections of differentiated $Toll^{1}$ embryos also show an expansion of the ventral nervous system, which derives from a ventrolateral anlage (Campos-Ortega, 1983).

Early in the development of $Toll^{D}$ embryos, the pattern

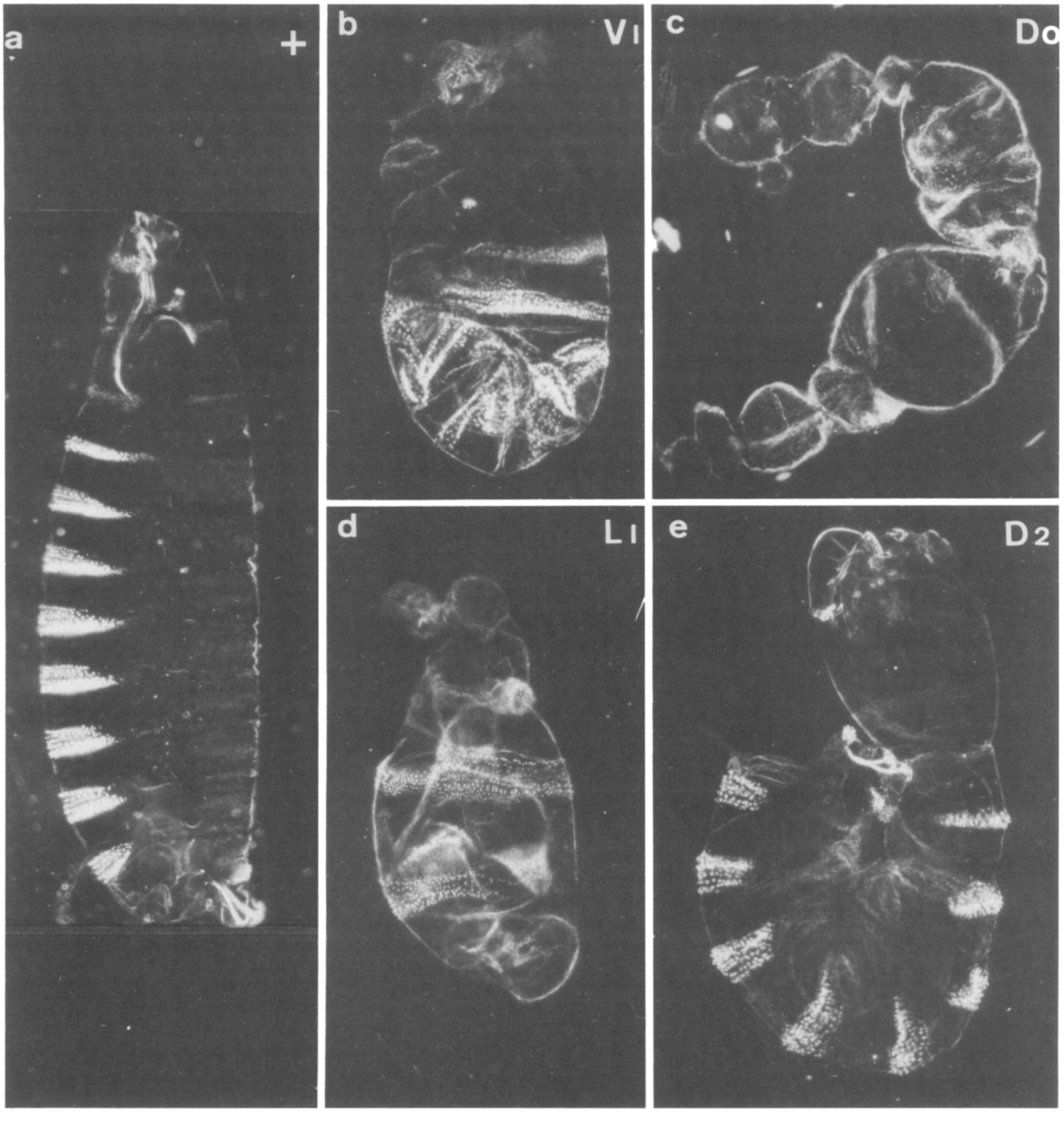

Figure 2. Dark Field Photographs of the Cuticle Produced by *Toll* Mutant Embryos

(a) Wild type (+). (b) Ventralized embryo (V1, according to the nomenclature of Figure 1). Maternal genotype: $Tl^{9Q}/+$. (c) Dorsalized embryo (D0). Maternal genotype: $Tl^{5BREQ}/Df(3R)Tl^{9QRX}$. (d) Lateralized embryo (L1). Maternal genotype: $Tl^{rm10}/Df(3R)ro^{80b}$. (e) Partially dorsalized embryo (D2). Maternal genotype: Tl^{r444}/Tl^{r444} (18°C).

of morphogenetic movements at gastrulation also shows a loss of dorsal, and expansion of ventral, pattern elements (V1 in Figure 3). This is seen in the shift of the normally lateral cephalic fold to the dorsal side and the absence of the normal dorsal folds. Normal polarity is retained, since the ventral furrow forms midventrally. Histological sections of gastrulating $Toll^{9Q}$ embryos show that the ventral furrow is not significantly larger than in wild type (Figure 4), so the shift in cell fate affects primarily more lateral anlagen. Overall, the phenotype of $Toll^{D}$ embryos can be described as the result of a coordinated shift in the fate of blastoderm cells with respect to the dorsal-ventral axis, such that the dorsal cells assume a more ventral fate (Anderson and Nüsslein-Volhard, 1984a), as sketched in Figure 1.

this is apparently an artifact resulting from the failure of germ band extension (compare Figure 4). The phenotypes have been classified as dorsalized (D), ventralized (V), or lateralized (L). The dorsalized embryos all make the most dorsal pattern elements, and the partially dorsalized embryos (D1 and D2) have a clear polarity in the dorsal-ventral pattern. Ventralized embryos fail to make the most dorsal pattern elements with corresponding expansion of the lateral anlage and have a clear polarity. Lateralized embryos fail to make both the most dorsal and the most ventral pattern elements and lack observable polarity in the dorsal-ventral axis.

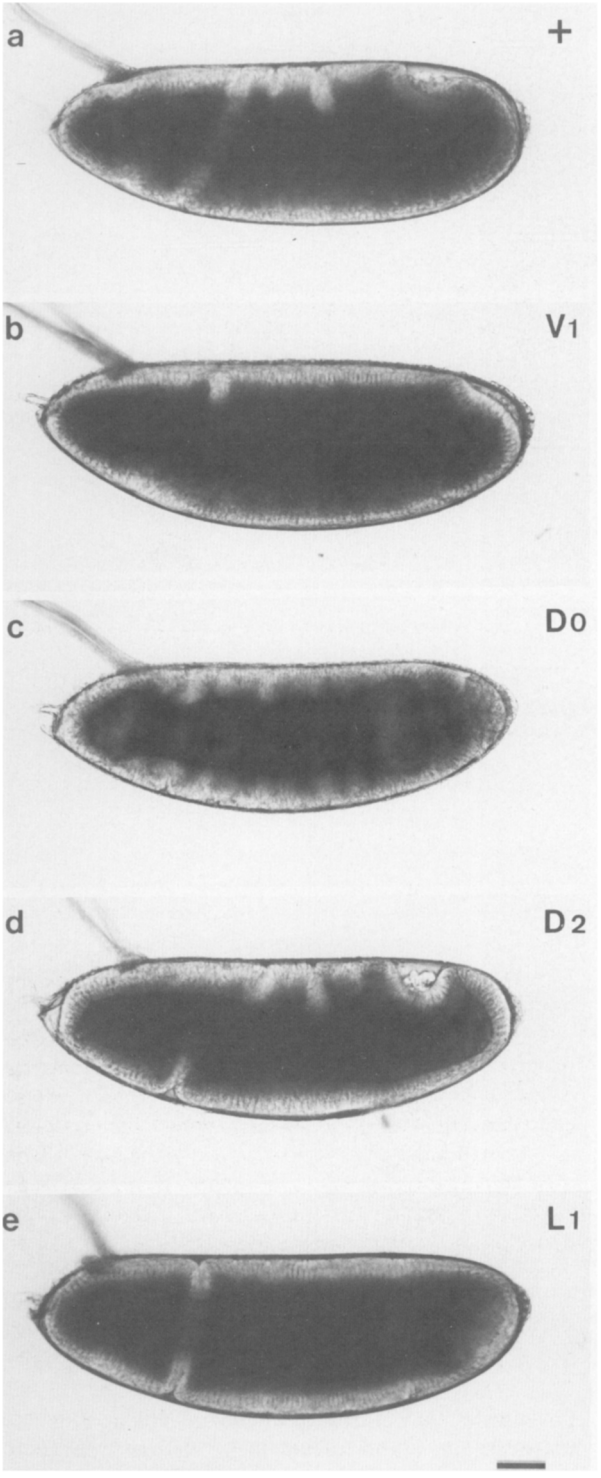

Figure 3. Living Embryos 15–20 Minutes after Completion of Cellularization of the Blastoderm, Showing the Pattern of Gastrulation

(a) Wild type (+). (b) Ventralized (V1). Maternal genotype: $Tl^{9Q}/+$. (c) Dorsalized (D0). Maternal genotype: $Tl^{5BREQ}/Df(3R)Tl^{9QRX}$. (d) Partially dorsalized (D2). Maternal genotype: Tl^{r444}/Tl^{r444} (18°C). (e) Lateralized (L1). Maternal genotype: $snk^{mel(3)4}Tl^{9Q}/snk^{073}Tl^{+}$.

Phenotypic Reversion of *Toll*^{D}

Since dominant mutations frequently represent the gain of an abnormal function, the embryonic *Toll*^{D} phenotype alone does not prove that the *Toll*$^{+}$ product is normally required for establishing the embryonic dorsal-ventral pattern. In addition, despite the similar phenotypes produced by the four dominant ventralizing mutations, the fact that these mutations result in totally penetrant dominant female sterility made it impossible to test allelism of these four mutations. We reasoned that if the *Toll*^{D} mutations reflect altered gene activity rather than insufficient activity of the remaining gene copy, it should be possible to revert the dominant female sterility of these mutations by inactivating the gene(s).

The reversion scheme (Experimental Procedures) is a simple selection for fertility of F1 females carrying a mutagenized *Toll*^{D} chromosome. Since the protocol does not require sorting the F1 flies, a large number of mutagenized *Toll*^{D} chromosomes could be easily tested.

Phenotypic revertants of all four dominant mutations were obtained at high frequency (Table 2). Females heterozygous for the *Toll* revertant chromosomes are fully fertile. Even after recombination to remove other lethals, many of the revertant chromosomes are homozygous lethal, which can be attributed to the fact that many of these are visible chromosomal rearrangements. It was possible to obtain viable *trans* combinations for all revertant chromosomes. These *trans* heterozygous females produced dorsalized embryos as a maternal effect. This recessive phenotype was mapped by recombination for all of the revertants to the *e–ca* interval, and more precisely for several individual revertants to 3–91. This location is consistent with the mapping of the *Toll*^{1} and *Toll*^{9Q} dominant mutations by mitotic recombination in the male germ line to the *e–ca* interval (E. Wieschaus and G. Jürgens, unpublished data). Thirteen of the twenty-six revertant chromosomes are cytologically visible chromosomal aberrations (Table 1). Seven deficiencies in the region 97 were isolated, as well as five inversions and a translocation which share a common breakpoint in 97D1,2. Thus, by complementation and mapping behavior, all four dominants are alleles of a single gene located in polytene bands 97D1,2, and all revertants represent second mutations of that same gene.

Toll Recessive Phenotypes

All of the Tl^{5B} and Tl^{9Q} revertants, and one Tl^{1} revertant (Tl^{1RXH}), appear to be amorphic, in that their phenotypes in *trans* to both dominant and recessive alleles cannot be distinguished from the phenotypes of those alleles in *trans* to a deficiency. The recessive amorphic phenotype, shown by females carrying two revertant chromosomes in *trans*, is the recessive dorsalizing maternal effect phenotype where all embryonic cells behave both at gastrulation and in differentiation like the dorsal cells of wild type (D0 in Figures 2 and 3). Females heterozygous for $Df(3R)Tl^{9QRX}$, which deletes 97A-97D1,2, and $Df(3R)ro^{XB3}$, which deletes 97D1,2-D9, can be obtained and produce dorsalized embryos. Since this combination should represent the physical absence of the *Toll* gene, the only absolute requirement for the *Toll* gene is during oogenesis. This dorsalized amorphic phenotype is indistinguishable from the lack of function phenotypes of the other nine

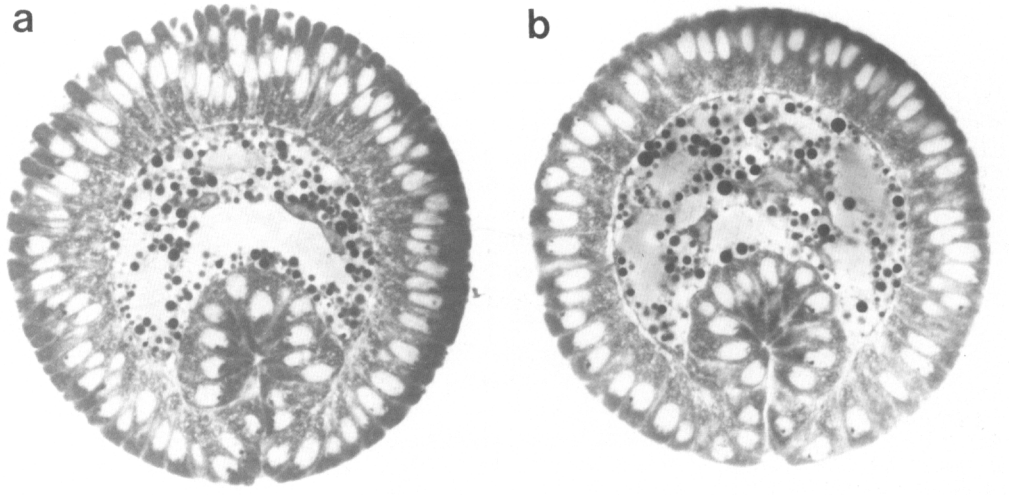

Figure 4. Transverse Section of a Gastrulating Embryo
(a) Wild type. (b) *Toll*^{9Q}/+ at about 50% egg length. Dorsal side up.

Table 2. Yield of Revertants of Dominant *Toll* Mutations

Toll Allele	Mutagen	Chromosomes Tested	Revertants Recovered	Chromosomal Aberrations
Tl^{1}	X-ray	6,000	6	3
Tl^{9Q}	X-ray	1,250	1	1
Tl^{9Q}	EMS	100	1	1
Tl^{5B}	X-ray	4,200	4	4
Tl^{5B}	EMS	3,500	12	3
Tl^{84c}	X-ray	3,050	2	1
Total		18,100	26	13

dorsal-group maternal effect loci. In addition to the 26 revertants, four alleles of *Toll* have been isolated independently on the basis of their recessive maternal effect. All recessive and revertant *Toll* alleles fail to complement, and produce maternal effect phenotypes that alter the dorsal-ventral pattern of the embryo. None of the alleles identified on the basis of its recessive phenotype appears to be amorphic. Females homozygous for any of these alleles produce embryos that have some lateral or ventral pattern elements (Figure 2).

The embryos from *Tl*^{r26}, *Tl*^{r632}, and *Tl*^{r444} females are partially dorsalized, producing some laterally derived structures in addition to dorsal structures. *Tl*^{r632} and *Tl*^{r26} are more strongly dorsalized than *Tl*^{r444}. Both *Tl*^{r632} and *Tl*^{r444} are temperature sensitive, showing a stronger dorsalization of the embryonic pattern at 29°C than at 18°C. At 18°C, the *Tl*^{r444} phenotype is very weakly dorsalized. At gastrulation, only the ventral furrow is absent (D2 in Figure 3); and in the differentiated cuticle, nearly all the normal pattern elements are present (D2 in Figure 2). These embryonic phenotypes are like those described for weak alleles at the *dorsal* locus, where such phenotypes have been shown to result from a shift in the blastoderm fate map (Nüsslein-Volhard et al., 1980). These phenotypes appear to be consistent with the same continuous relationship between degree of ventral development and amount of residual gene activity shown for *dorsal* genetically (Nüsslein-Volhard, 1979) and *snake* biochemically (Anderson and Nüsslein-Volhard, 1984b).

The phenotype of embryos produced by *Tl*^{rm9} homozygotes is unique to the *Toll* locus. In most cases, the cuticle of the differentiated embryos has rings of ventral denticles along the dorsal-ventral axis and no dorsal hairs are present (L1 in Figure 2). This phenotype is reminiscent of the *Toll*^{D} phenotype, but the body shape is frequently an elongated tube rather than the squat *Toll*^{D} shape, and the denticles are finer and less heavily pigmented. The difference between the *Tl*^{rm9} recessive phenotype and the *Tl*^{D} phenotype is clearest in the pattern of gastrulation. In contrast to the dorsoventrally asymmetric pattern of gastrulation of *Tl*^{D}, with a ventral furrow and a head fold dorsally, *Tl*^{rm9} embryos show no polarity in the dorsal-ventral axis (like the L1 pattern in Figure 3). *Tl*^{rm9} embryos do not make a ventral furrow, the cephalic fold is prominent both dorsally and ventrally, and all morphogenetic movements at gastrulation are symmetrical in the dorsal-ventral axis. Since both the dorsalmost structures (the dorsal hairs) and the ventralmost structures (the ventral furrow and mesoderm) are missing, we interpret this phenotype as being lateralized; that is, all cells of the blastoderm assume an intermediate dorsal-ventral positional value (see Figure 1). The gastrulation phenotype of these embryos is quite constant, while the cuticle phenotype shows an inherent variability. Thirty to forty percent of the embryos fail

Table 3. Complementation Behavior of *Toll* Alleles

Recessive Alleles	r444	r632	r26	rm9	9QRE	
r444	D2					
r632	D2	D1				
r26	D2	D1	*			
rm9	D2	D1	D1	L1		
9QRE	D2	D1	D1	L1		
Df(3R)*ro*^{80b}	D2	D1	D1	L1	D0	

Dominant Alleles	+	r444	r632	r26	rm9	Df(3R)*ro*^{80b}
Tl^{1}	V1	V1	V1	V1	V1	V1
9Q	V1	V1	V1	V1	V1	V1
5B	V1	V2	D0	D0	D0	D0
84c	V1	V2	D0	nd	L2	D0

* The r26 chromosome is homozygous lethal.

The embryonic phenotypes produced in the various allele combinations were scored both at gastrulation and in the differentiated cuticle pattern and classified according to the scheme presented in Figure 1. The phenotypes of combinations involving the alleles r444 and r632 are temperature sensitive, and the 18°C phenotypes are presented here. In most cases, the embryonic phenotypes are quite invariant and could be clearly classified. A few allele combinations are characterized by inherent variability. The recessive combinations involving rm9 classified here as L1, for instance, show the lateralized pattern of gastrulation and most embryos make ventral denticles all along the dorsal-ventral circumference. Up to 40% fail to make ventral denticles at all, some having a field of filzkörper material (like the L2 phenotype) and some making dorsal cuticle only (not distinguishable from the D0 cuticle phenotype). Variability is also seen in the r444/5B and r444/84c 18°C phenotypes. Both combinations give rise to some embryos that look like the phenotype described for V2, but many appear more dorsalized than the V2 pattern, making no ventral denticles. Since at gastrulation the head fold is visible dorsally, these embryos are missing the most dorsal regions and therefore do not fall into the dorsalized class of phenotypes; nor do they fall into the lateralized class, since they have a clear dorsal-ventral polarity. The r444/5B phenotype can be distinguished from the r444/84c phenotype because in the combination with 5B fewer embryos (5% cf. 35%) make ventral denticles.

to make vental denticles, and make only dorsal cuticle, with or without a field of filzkörper material at the posterior end (these may represent the L2 phenotype of Figure 1). In all cases, however, no polarity in the dorsal-ventral axis can be detected.

The complementation behavior of the *Tl*^{rm9} allele is of particular interest, since its homozygous phenotype is without precedent. Although the *Tl*^{rm9} homozygous phenotype is indistinguishable from *Tl*^{rm9} in *trans* to deficiency, embryos from females carrying *Tl*^{rm9} in *trans* to the recessive dorsalizing alleles look, in each case, like the homozygous dorsalizing allele. For example, females heterozygous for the strongly dorsalizing allele *Tl*^{r632}, which results in dorsolaterally derived filzkörper but never in ventral denticles, and for *Tl*^{rm9}, which results in ventral denticles, produce embryos that make filzkörper but not ventral denticles. Thus the *Tl*^{rm9} allele clearly does not fit into an allelic series like that described for *dorsal*, where the degree of residual gene activity is proportional to the ventralmost structures produced.

In general, the recessive alleles of *Toll* do not behave as if the residual gene activity of the two alleles is averaged in the final phenotype (Table 3). Rather, they can be ranked in a different sort of allelic series, a hierarchy of dominance. Weakly dorsalizing alleles are dominant over more strongly dorsalizing alleles, and the three recessive partially dorsalizing alleles are dominant over the lateralizing *Tl*^{rm9} allele.

The Dominant *Toll* Phenotype: The Effect of Gene Dosage

The ability to obtain phenotypic revertants of *Toll*^{D} by second mutational events which destroy the *Toll*$^{+}$ activity demonstrates that the *Toll*^{D} mutations represent the gain of abnormal function. As evidenced by the totally dorsalized phenotypes of the *Toll* revertants which represent the lack of function, the *Toll*$^{+}$ product is necessary for the production of ventral embryonic structures. In this light, the *Toll*^{D} phenotype could be interpreted as the result of overproduction: too much ventral-determining substance is made. To test genetically whether a mutation causes overproduction, the number of wild-type copies of the gene can be varied and the phenotype assessed for enhancement or weakening of the dominant effect.

In the absence of a wild-type copy of *Toll*, the four dominant alleles behave in two very different ways. The *Toll*^{1} and *Toll*^{9Q} mutations in *trans* to deficiency retain their ventralized cuticular phenotypes, and cannot be readily distinguished from the dominant alleles in the presence of one wild-type copy. The other two dominant alleles, *Toll*^{5B} and *Toll*^{84C}, in *trans* to deficiency result in the amorphic, completely dorsalized embryonic phenotype (Figure 5). These two alleles are apparently inactive in *cis*; therefore they must exert their dominant effect by modifying the activity of *Toll*$^{+}$ in *trans*.

The behavior of *Tl*^{5B} and *Tl*^{84C} in *trans* to deficiencies makes the idea that the ventralized dominant phenotype is the result of transcriptional overproduction less tenable, but it would still be possible to imagine mechanisms for the mutant gene to enhance the transcription of its homolog. The model of simple transcriptional overproduction is, however, ruled out by the behavior of the dominants in the presence of an extra copy of the wild-type *Toll* gene. Females that carry two copies of the wild-type *Toll* gene in addition to *Toll*^{D} are still completely sterile, but the phenotype of the embryos they produce is normalized compared to the phenotype with only one copy of *Toll*$^{+}$ (Figure 5). All four dominants combined with the duplication result in embryos where the ventral denticle bands, although clearly wider than the wild-type denticle bands, have de-

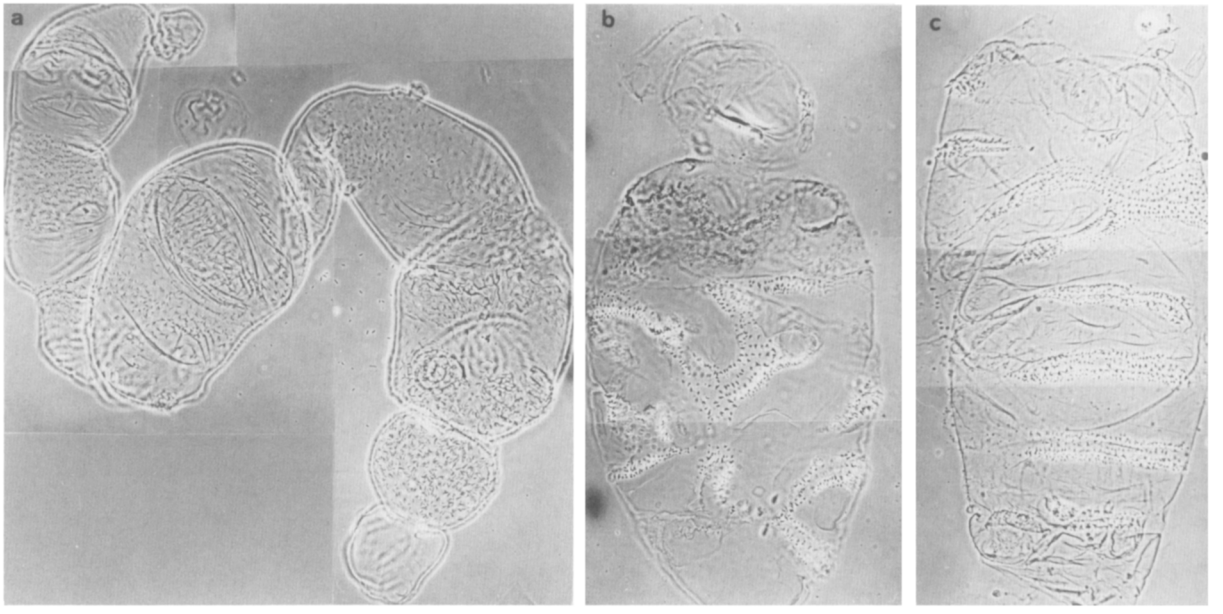

Figure 5. Phase Contrast Photographs of Differentiated Cuticle, Showing the Effect of the Number of Wild-Type Copies of the *Toll* Gene on the $Toll^{5B}$ Phenotype

Maternal genotypes: (a) Tl^{5B}/Df(3R)ro^{80b} (0 wild-type copies); (b) Tl^{5B}/+ (1 wild-type copy); (c) Tl^{5B}/+; XX/Dp(3;Y)B158 (2 wild-type copies). The weakening of the ventralization of Tl^{5B} is seen not only with this large duplication, which duplicates the entire end of 3R distal to 97B, but also with females that are Tl^{5B}/Df(3R)A121; XX/Dp(3;Y)B158, which are duplicated only for 97B-D.

fined edges and are much more orderly than the standard Tl^{D} phenotype. The normalization of the pattern by an extra Tl^{+} copy is most pronounced with Tl^{84C}, where all embryos produced by Tl^{84C}/+/+ females make dorsal hairs and 25% make filzkörper. The partial restoration of the normal pattern in embryos whose mothers should produce more Tl^{+} product shows that the ventralized Tl^{D}/+ phenotype cannot be the result of overproduction of a normal gene product.

The interaction of the two dominant alleles $Toll^{5B}$ and $Toll^{84C}$ with the *trans* allele is seen not only in the phenotypes produced in *trans* to deficiencies, but also in combination with the recessive alleles of *Toll*. For instance, Tl^{rm9}/Df and Tl^{r632}/Df make ventrolateral and dorsolateral structures, respectively, but Tl^{rm9}/Tl^{5B} and Tl^{r632}/Tl^{5B} are totally dorsalized (see Table 3). This *trans* inactivation of mutant alleles is correlated with the dominant *trans* interaction with the wild-type allele, since all revertants of Tl^{5B} behave like the deficiencies and no longer show either kind of *trans* effect. The $Toll^{84C}$ allele also appears to inactivate mutant alleles in *trans*, but less completely than $Toll^{5B}$. Females that are heterozygous for $Toll^{84C}/Tl^{rm9}$, for instance, produce embryos that do not make ventral denticles but do make filzkörper material and so fail to produce the ventrolaterally derived structures made by Tl^{rm9} homozygotes, but are still not like the amorphic, totally dorsalized embryos.

Double Mutants of $Toll^{D}$ and Other Dorsalizing Loci

Among the ten maternal effect loci required for the production of ventral embryonic structures, only *Toll* has mutant alleles that produce the opposing, ventralized phenotype. By analyzing the embryonic phenotypes produced by females simultaneously heterozygous for $Toll^{D}$ and homozygous for recessive dorsalizing mutations, we hoped to uncover differences between the nine remaining loci, as well as to investigate the relationship of *Toll* to the other dorsalizing maternal effect genes.

Double mutants of $Toll^{D}$ with *dorsal* (chromosome II) and *gastrulation defective* (I) were constructed by ordinary crosses. In order to make double mutants of $Toll^{D}$ and the third chromosomal dorsalizing mutants, recombinants had to be isolated between the dominant female sterile chromosome and the recessive dorsalizing mutants. Such recombinants were constructed by inducing mitotic recombination in the male germ line (see Experimental Procedures).

The double mutants of $Toll^{9Q}$ and *gastrulation defective*, *nudel*, *pipe*, *snake*, and *easter* all show a common maternal effect phenotype (Table 4). Like the recessive allele Tl^{rm9}, these embryos lack both the dorsalmost and ventralmost pattern elements, and can be considered lateralized. The cuticle has rings of fine ventral denticles along the dorsal-ventral circumference, without dorsal hairs (like the L1 phenotype in Figure 2). At gastrulation, there is no ventral furrow, the cephalic fold is present both dorsally and ventrally, and the folds of gastrulation show no polarity in the dorsal-ventral axis (L1 in Figure 3). Since by all available criteria the dorsalizing alleles used are amorphic, in the presence of $Toll^{9Q}$ the normal requirement for these five genes for the production of laterally derived structures is overcome. These mutations do modify the $Toll^{9Q}$ phenotype, resulting in the loss of the ventralmost pattern elements and also in the loss of visible polarity.

Only one locus tested, *dorsal*, shows a different double

Table 4. Double Mutant Phenotypes

	Tl^{+}	Tl^{9Q}	Tl^{1}	Tl^{5B}
dl^{1}	D0	D0	D0	D0
dl^{2}	D2	D2	D2	D2
gd	D0	L1	L1	D0
ndl	D0	L1	nd	D0
pip	D0	L1	nd	nd
snk	D0	L1	nd	nd
ea	D0	L1	nd	nd

The embryonic double mutant phenotypes were scored both at gastrulation and in the differentiated cuticle and were classified according to the scheme in Figure 1. The mothers carried one of the dominant *Toll* alleles and were homozygous for a recessive strongly dorsalizing mutant. The recessive allele combinations used were *gd*^{7}/*gd*^{7} (Mohler and Carroll, 1984) or *gd*^{7}/Df(1)KA10 (Craymer and Roy, 1980); *ndl*^{260}/*ndl*^{111}; *pip*^{386}/*pip*^{664}; *snk*$^{mel(3)4}$/*snk*^{073}; *ea*^{IIR65}/*ea*^{IIR65}. For each genotype, the phenotypes observed showed no significant variation from the prototypes of Figure 1.

mutant phenotype with *Toll*^{9Q}: the embryos produced by *Toll*^{9Q}; *dl*^{1}/*dl*^{1} females are totally dorsalized, indistinguishable from those produced by *dl*^{1} homozygotes. In contrast to the other dorsalizing loci tested, the *dorsal*$^{+}$ activity is necessary for the production of ventral structures even in the presence of *Toll*^{9Q}. Double mutants of *Toll*^{9Q} with the hypomorphic, partially dorsalizing allele *dl*^{2} show the same phenotype as with *dl*^{2} alone—the embryos differentiating (at 18°C) filzkörper and narrowed ventral denticle bands.

The double mutant combinations with Tl^{D} alleles, like the behavior of the Tl^{D} alleles in *trans* to deficiencies, reveal that the phenotypically identical dominant alleles fall into two genetic classes. Although all four dominant alleles show the same hypostatic relation to *dorsal*, the phenotype of other double mutants depends on the *Toll*^{D} allele used. Although both Tl^{1}/+; *gd/gd* and Tl^{9Q}/+; *gd/gd* are lateralized, both Tl^{5B}/+; *gd/gd* and Tl^{84C}/+; *gd/gd* appear dorsalized, like *gd* alone. In the only other case tested, *ndl* Tl^{5B}/*ndl* Tl^{+} is also dorsalized, like *ndl* alone. Since the ability of Tl^{5B} or Tl^{84C} to ventralize the embryonic pattern requires a Tl^{+} copy, this indicates that *gd*$^{+}$ and *ndl*$^{+}$ are necessary for the activity of *Toll*$^{+}$ (and therefore of Tl^{5B} and Tl^{84C}), but not for the activity of Tl^{9Q} or Tl^{1}.

Discussion

Females carrying mutations in the *Toll* gene produce embryos that have a variety of alterations in the dorsal-ventral pattern (Figure 1, Table 3). In the absence of any *Toll*$^{+}$ activity, females produce embryos in which all blastoderm cells assume the fate of the dorsalmost cells of the wild-type embryo. Recessive, partial lack of function alleles of *Toll* can result either in embryos in which the ventralmost pattern elements are lacking and the remaining dorsal and lateral elements are proportionally expanded (Tl^{r632}, Tl^{r444}, Tl^{r26}) or in embryos in which both the ventralmost and the dorsalmost pattern elements are lacking and lateral pattern elements are differentiated by all cells (Tl^{rm9}). Dominant alleles, which have been recovered at a frequency comparable to that of the recessive alleles, lack dorsal pattern elements and show an expansion of the ventrolateral anlagen.

All of these phenotypes can be described in terms of a gradient model (Anderson and Nüsslein-Volhard, 1984a; Figure 1), in which there is a graded distribution of a morphogen along the dorsal-ventral axis and blastoderm cells adopt particular developmental pathways according to the local concentration of a morphogen. According to this model, the mutant *Toll* phenotypes result from changes in the total amount of morphogen, in the distribution of morphogen, or in both amount and distribution. Too little morphogen is produced by the recessive dorsalizing alleles. In Tl^{rm9}, morphogen is made but not distributed unequally in space. The dominant ventralizing alleles make too much morphogen and its spatial distribution is more homogeneous than in wild type.

Since phenotypic revertants of the dominant alleles were obtained by making second mutations that inactivate or remove the gene altogether, the dominant alleles must represent the gain of abnormal gene activity. Despite the fact that the ventralized Tl^{D} phenotype suggests an excess of gene activity, the phenotype does not result from the transcriptional overproduction of a normal product. This is most clearly shown by the partial suppression of the ventralization of all four dominant alleles by an additional copy of the wild-type *Toll* gene. The ventralized Tl^{D} phenotype therefore must represent the altered activity of the *Toll* transcript or protein.

Several observations suggest that there is a direct interaction between the copies of the *Toll* gene product. Two of the four dominant alleles, Tl^{5B} and Tl^{84C}, behave like amorphic alleles when placed in *trans* to a deficiency. The products of these alleles are thus inactive on their own, yet in combination with the wild-type product produce an abnormal activity. These two dominant alleles also partially inactivate recessive alleles in *trans*, again demonstrating that the products of the two alleles can interact with one another in specific ways. The complementation behavior of recessive alleles may also reflect interactions between copies of the *Toll* product, since heteroallelic combinations never represent an intermediate between the two input alleles, and instead only one allele appears to be active.

Two classes of models could explain the specific interactions seen between *Toll* alleles. One model is that the *Toll* protein product is present as a dimer or multimer whose activity depends on interactions between subunits. An alternative model is that the active *Toll* product autocatalytically promotes the further activation of other copies of the *Toll* product. For example, the translation of the *Toll* mRNA could be regulated, with the *Toll* protein promoting the translation of the *Toll* mRNA. The autocatalytic mechanism is attractive because autocatalysis is an essential feature of a number of models that define how a stable gradient of a morphogen could be established in space (for example, see Gierer and Meinhardt, 1972). However, the data currently available do not allow us to distinguish between these two classes of models.

The system that establishes dorsal-ventral positional information in the embryo requires the action of nine mater-

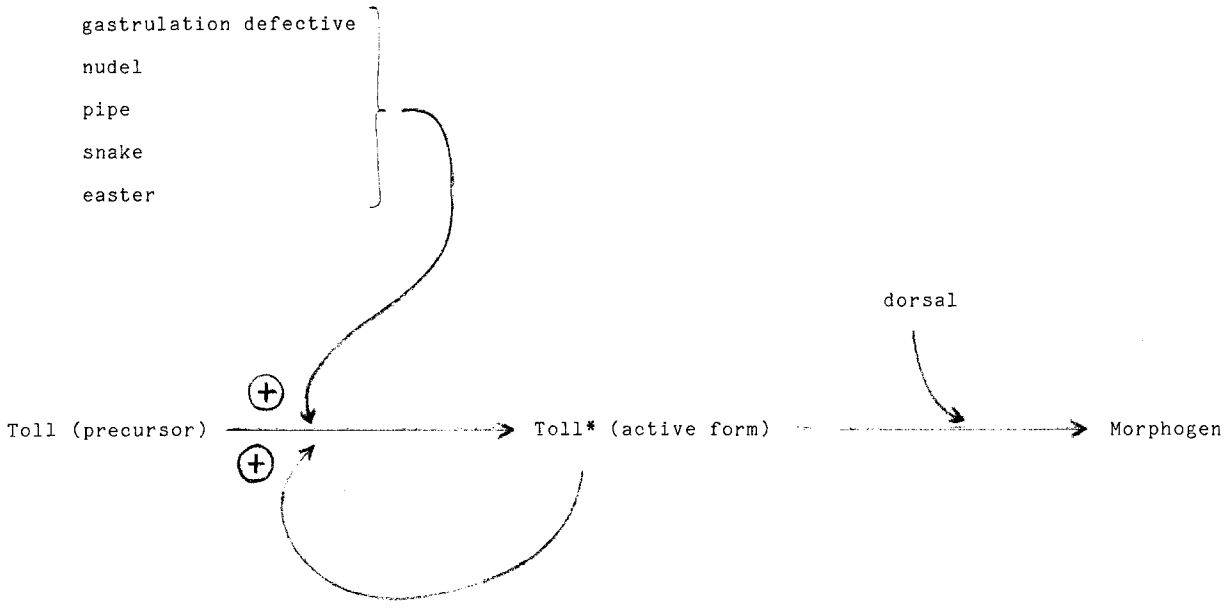

Figure 6. Suggested Flow of Information in the Establishment of the Dorsal-Ventral Pattern

This scheme is designed to show the relative roles of *dorsal* and the *gastrulation defective* group in the system inferred from double mutant studies, and the possible autoactivation of the *Toll* product inferred from the allelic complementation behavior. Both the *gd* group and the *Tl** form are proposed to promote the activation of the precursor *Tl* form to its active form. The active forms of *Toll* and *dorsal* are both required for the production of active morphogen. If this model is to help explain the spatial parameters of the system, then there must also be some substance (as yet undefined) that limits or inhibits the conversion of the *Toll* precursor to its active form.

nal effect dorsal-group genes in addition to *Toll*. In the absence of any one of these components, all cells differentiate according to a dorsal ground state. The simple model in which each of these genes controls one step in a linear biochemical pathway leading to the production of a ventralizing morphogen is ruled out by the double mutants of the recessive alleles of other dorsal-group genes with $Toll^{9Q}$, since in the presence of Tl^{9Q} ventrolaterally derived structures can be produced in the absence of *gastrulation defective*$^+$, *nudel*$^+$, *pipe*$^+$, *snake*$^+$, or *easter*$^+$. This epistasis of Tl^{9Q} over all dorsal-group genes tested, with the exception of *dorsal*, can be explained if *gd*, *ndl*, *pip*, *snk*, and *ea* mutants are normally dorsalized because of their failure to promote the activity of Tl^+, and in the presence of Tl^{9Q} the production of active morphogen becomes independent of those gene products. In the lateralized double mutants with Tl^{9Q}, the production of ventral epidermis in the absence of *gd*$^+$, etc., is accompanied by the loss of polarity in the dorsal-ventral axis. Under normal conditions the synthesis of morphogen activity is coupled with its unequal distribution. The $Toll^{9Q}$ mutation partially uncouples these two steps, indicating that the correct activity of Tl^+ is required for the normal coupling of morphogen synthesis and distribution.

The working model we find most attractive is diagrammed in Figure 6. In this model, the *Tl*$^+$ and *dl*$^+$ activities are directly required for the synthesis of active morphogen. The *Toll* product is initially present in a precursor form, and the conversion from precursor to active *Toll* product is regulated in a position-dependent manner relative to the dorsal-ventral axis. Both the active form of the *Toll* product and the products of the other dorsalizing genes (*gd*$^+$, *ndl*$^+$, *pip*$^+$, *snk*$^+$, *ea*$^+$) are required in a way that we do not yet understand for this spatial regulation. In the absence of this spatial regulation, the *Tl*$^+$ product fails to be activated, and no morphogen is produced. The dominant mutations Tl^1 and Tl^{9Q} disrupt the aspect of the *Tl* product regulated by the activity of *gd*$^+$, etc., and allow production of active morphogen in the absence of *gd*$^+$ (etc.), but the morphogen is no longer correctly distributed in space.

Experimental Procedures

Strains and Nomenclature

All marker mutants and balancer stocks are described in Lindsley and Grell (1968). The *rough* deficiencies, $Df(3R)ro^{80b}$ and $Df(3R)ro^{XB3}$, were a gift from Peter Lewis. $Df(3R)ro^{80b}$ deletes complementation groups both proximal and distal to *Toll* (Anderson, unpublished), while $Df(3R)ro^{XB3}$ breaks in, or immediately adjacent to, *Toll* and deletes material distally. The translocations T(Y;3)B158 and T(Y;3)A121 were used to construct the duplication for *Toll* (Lindsley, Sandler, et al., 1972). The duplication used combines the distal portion of 3R from *B158* (breakpoint in 97B) with the proximal portion of 3R from *A121* (breakpoint in 97D) and results in a duplication of 97B-D. Dominant alleles of *Toll*, collectively referred to as Tl^D, are denoted simply by allele number. Recessive alleles are written as small r followed by allele numbers. The recessive alleles isolated by T. Rice (Ph.D. thesis, Yale University, 1973), *mel(3)9* and *mel(3)10*, are referred to as Tl^{rm9} and Tl^{rm10}. Revertants are designated by the dominant allele reverted, followed by R, either X for X-ray-induced or E for EMS-induced, and then a letter.

Origin of the *Toll* Mutations

The four $Toll^D$ chromosomes were isolated on the basis of the phenotype of embryos produced by females from isogenic lines established from single EMS-treated F1 males. The total number of lines tested is equivalent to approximately 30,000 lethal hits on the third chromosome, or a frequency of recovery of Tl^D alleles of 1 in 7500 lethal hits. The first three $Toll^D$ mutations were identified in the course of screens

for zygotic lethal mutations. *Toll*^{1} was induced in a wild-type third chromosome in the second chromosomal zygotic lethal screen (Nüsslein-Volhard et al., 1984). *Toll*^{9Q} and *Toll*^{5B} were induced in *rucuca* in the third chromosomal zygotic lethal screen (Jürgens et al., 1984). *Toll*^{84C} was induced in a *ru st e ca* chromosome, and found fortuitously in an allele screen for other loci. The *Toll*^{D} chromosomes are carried in stocks in which the males carry *Toll*^{D}/*TM3* and the fertile females carry either the dominant male sterile R24 (Jürgens, unpublished) or the male-lethal translocation T(1;3)OR60 (Lindsley and Grell, 1968) over *TM3*.

The recessive mutations *Tl*^{r632} and *Tl*^{r444}, both EMS-induced in *ru st e ca* chromosomes, were recovered in systematic screens for maternal effect mutations. *Tl*^{r632} was identified on the basis of its homozygous phenotype in a systematic search for third chromosomal maternal effect mutations (C. Nüsslein-Volhard, G. Jürgens, K. Anderson, and R. Lehmann, unpublished). *Tl*^{r444} was recovered on the basis of the embryonic phenotype produced by females carrying the mutagenized chromosome in *trans* to a large deficiency for the *Toll* region, Df(3R)*Tl*^{5BRXP}. *Tl*^{r26}, EMS-induced in *st e*, was fortuitously isolated in another screen on the basis of its leaky dominant maternal effect phenotype (F. Müller-Holtkamp, unpublished data). The partial dominance of this allele results from abnormal gene activity, since deficiencies for the region do not show a haploinsufficient dominant phenotype. Two alleles were isolated by T. Rice (Ph.D. thesis, Yale University, 1973) in his screen for third chromosomal maternal effect mutations: *mel(3)9* and *mel(3)10* (our designation: *Tl*^{rm9} and *Tl*^{rm10}). However, because these mutations both arose in a single small mutagenesis experiment, Rice suspected that they do not represent independent mutational events. Our phenotypic analysis supports the hypothesis that these two chromosomes represent a single mutation, and we treat them as such in our presentation. The three alleles isolated as recessives by Rice and in our laboratory were recovered in screens of approximately 13,500 third chromosome lethal hits, giving a frequency of recovery of *Tl*^{r} alleles of 1 in 4500 lethal hits.

Reversion of *Toll*^{D}

To obtain phenotypic revertants of *Toll*^{D} in which heterozygous females are no longer sterile, *Toll*^{D}/*TM3,Sb* males were mutagenized either with 4000 rad X-rays or 30 mM EMS (Lewis and Bacher, 1968) and mated to virgin *TM1*/*TM3,Ser* females, Males were removed after 5 days. Since *TM1* carries a *sbd* allele that is lethal over *Sb*, and the *TM3* chromosomes share lethal breakpoints, all F1 females carried a mutagenized *Toll*^{D} chromosome over either *TM1* or *TM3,Ser*, making it unnecessary to sort the F1. Instead, F1 males and females were shaken over into new tubes, with roughly 50 females per bottle. If (4–7 days later) larvae were detected in the bottle, the females in that bottle were set up individually in egg-laying blocks (Nüsslein-Volhard, 1977) and their living progeny were examined under oil. In most cases it was possible to identify a female producing wild-type progeny, and she was used to establish the revertant line. In cases where no living fertile female could be identified, the line was established from the larvae in the tube. Isogenic lines were established from F2 females. Cytological analysis of revertant chromosomes was carried out in outcrosses of the revertant stock to Oregon R wild type.

Three of the deficiencies isolated as revertants, Df(3R)*Tl*^{5BRXQ} and Df(3R)*Tl*^{9QRX} and Df(3R)*Tl*^{5BREW}, share a common distal breakpoint in 97D1,2, the same position as the common breakpoint of five inversions isolated as *Toll* revertants (Table 1). These three deficiencies delete material proximal to 97D1,2 and apparently break within, or immediately adjacent to, the *Toll* gene, since they are viable in *trans* to Df(3R)*ro*^{XB3}, which deletes material distal to 97D1,2 and which is also *Tl*$^{-}$. Thus nine of the 26 revertants, these three deficiencies, five inversions, and a translocation have cytologically visible breaks within the *Toll* gene. This observation, coupled with the high overall frequency of revertants, suggests some inherent instability in the *Toll* gene in the *Toll*^{D} chromosomes. Such an instability could also explain the high frequency of EMS-induced revertants (approximately 1 in 600 third chromosomal lethal hits) compared to the frequency of EMS-induced alleles isolated as recessives (approximately 1 in 4500 third chromosomal lethal hits).

Mitotic Recombination in the Male Germ Line

The fully penetrant sterility of females heterozygous for *Tl*^{D} made it impossible to recover recombinants by normal meiotic recombination. Instead, recombinants between *Tl*^{D} and the third chromosomal recessive dorsalizing loci were made by inducing mitotic recombination in the male germ line (Hannah-Alava, 1965). First instar larvae from the cross (dorsalized mutant/*TM3* ♀♀ × *Toll*^{D}/*TM3* ♂♂) were irradiated with 1200 rad X-rays. When they emerged, adult males heterozygous for *Toll*^{D} and the dorsalizing mutation were mated to marker virgin females; in the next generation recombinant males were isolated on the basis of marker exchange. These males were used to establish single lines that were tested for both presence of *Toll*^{D} and the recessive maternal effect mutation.

Analysis of Embryonic Phenotypes

Eggs were collected on yeasted agar plates as described (Nüsslein-Volhard, 1977). The gastrulation pattern was observed in living embryos after the eggs had been covered with Voltalef 3S oil to render the opaque chorion transparent. For photography, undechorionated embryos were mounted in a drop of Voltalef 3S oil on a slide and covered with a thin stripe of coverslip. Semithin (3 μm) sections of glutaraldehyde-fixed embryos were performed according to Zalokar and Erk (1976). For cuticle preparations, differentiated embryos were dissected out of the vitelline membrane, fixed in glycerol-acetic acid for 15 min at 60°C, and mounted in Hoyer's medium (Van der Meer, 1977).

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