

Dominant and recessive mutations define functional domains of *Toll*, a transmembrane protein required for dorsal–ventral polarity in the *Drosophila* embryo

David S. Schneider, Kathy L. Hudson, Ting-Yi Lin, and Kathryn V. Anderson

Genetics Division, Department of Molecular and Cell Biology, University of California, Berkeley, California 94720 USA

The asymmetry of the dorsal–ventral pattern of the *Drosophila* embryo appears to depend on the ventral activation of the transmembrane *Toll* protein. The *Toll* protein is found around the entire dorsal–ventral circumference of the embryo, and it appears to act as a receptor for a ventral, extracellular signal and to then relay that signal to the cytoplasm in ventral regions of the embryo. Three of five recessive loss-of-function alleles of *Toll* are caused by point mutations in the region of the cytoplasmic domain of *Toll* that is similar to the mammalian interleukin-1 receptor, supporting the hypothesis that *Toll* acts as a signal-transducing receptor. Nine dominant gain-of-function alleles that cause *Toll* to be active in dorsal, as well as ventral, regions of the embryo are caused by mutations in the extracellular domain. Three of the dominant alleles appear to cause the protein to be constitutively active and are caused by cysteine-to-tyrosine changes immediately outside the transmembrane domain. All six of the remaining dominant alleles require the presence of a wild-type transmembrane *Toll* protein for their ventralizing effect and all encode truncated proteins that lack the transmembrane and cytoplasmic domains.

[Key Words: Dorsal–ventral; *Drosophila* embryo; *Toll* interleukin-1 receptor]

Received January 7, 1991; revised version accepted February 8, 1991.

Twelve maternally transcribed genes are required for dorsal–ventral pattern formation in the *Drosophila* embryo (Anderson and Nüsslein-Volhard 1984; Schüpbach and Wieschaus 1989). The products of these genes create a ventral-to-dorsal morphogen gradient by regulating the nuclear translocation of the *dorsal* protein in a spatially asymmetric manner (Steward et al. 1988; Roth et al. 1989; Rushlow et al. 1989; Steward 1989). In the nuclei, *dorsal* protein presumably acts as a concentration-dependent transcriptional regulator that activates or represses distinct zygotic target genes in specific regions of the embryo (Rushlow et al. 1987; Roth et al. 1989). To understand the generation of dorsal–ventral asymmetry in the embryo and the mechanisms leading to the production of the nuclear *dorsal* protein gradient, we are studying the biochemical functions of, and interactions among, the maternal gene products in this pathway.

Among these maternal effect genes, the *Toll* gene plays a crucial role in defining the spatial organization of the pattern. Embryos produced by females that lack *Toll* activity are dorsalized, lacking all lateral and ventral pattern elements. Dominant gain-of-function alleles of *Toll* that result in expansion of ventral pattern elements have been recovered at high frequency, pointing to the impor-

tance of *Toll* in determining the organization of the dorsal–ventral pattern (Anderson et al. 1985b). In addition, among the seven genes for which injection of wild-type cytoplasm can rescue the dorsalized mutant phenotype, only *Toll*[−] mutant embryos show no residual dorsal–ventral asymmetry, with the site of ventral structures defined by the site of deposition of the wild-type gene product (Anderson et al. 1985a). Thus, in these injection experiments, a high local concentration of the *Toll* product can define the polarity of the dorsal–ventral pattern. In the wild-type embryo, the *Toll* protein is evenly distributed at the syncytial blastoderm stage, the time of *Toll* activity (Anderson and Nüsslein-Volhard 1986; Hashimoto et al. 1991), suggesting that the *Toll* protein is spatially regulated such that it becomes active on the ventral side of the embryo.

The 12 maternal gene products required for dorsal–ventral pattern formation appear to define a pathway through which information passes from outside the embryo to the nuclei. Two sequenced dorsal-group genes that are known to act genetically upstream of *Toll*, *east-er* and *snake*, encode extracellular-type serine proteases that are present in the extracellular perivitelline space between the plasma membrane and the eggshell (De-

Lotto and Spierer 1986; Chasan and Anderson 1989; D. Stein and C. Nüsslein-Volhard, pers. comm.; Y. Jin, R. Chasan, and K.V. Anderson, unpubl.). The *Toll* gene encodes a transmembrane protein found in the plasma membrane (Hashimoto et al. 1988, 1991). Together, both molecular and genetic evidence suggests that *Toll* occupies a position in the pathway where it could transmit a signal from extracellular upstream gene products to intracellular, downstream gene products, finally giving rise to the nuclear gradient of *dorsal* protein.

The *Toll* gene encodes a 1097-amino acid protein that contains a single, membrane-spanning domain (Hashimoto et al. 1988) and has some features of known signal-transducing receptors. The cytoplasmic domain of *Toll* is similar to the intracellular domain of the human and mouse interleukin-1 receptors (IL-1Rs), (see Fig. 2, below); (Sims et al. 1988, 1989). The 803-amino acid residue extracellular domain, of *Toll* is composed largely of two blocks of leucine-rich (L-rich) repeats. L-rich repeats of this type have been found in a diverse group of proteins including the transmembrane protein platelet protein glycoprotein (gp) 1b α and β , gp IX, and the lutropin-choriogonadotropin receptor (Lopez et al. 1987, 1988; Hickey et al. 1989; McFarland et al. 1989). The *Drosophila* protein chaoptin, which is composed almost exclusively of L-rich repeats, can mediate homotypic cell adhesion (Krantz and Zipursky 1990), and *Toll* itself can promote heterotypic cell adhesion (Keith and Gay 1990), providing evidence that this motif defines a protein-protein interaction domain.

An array of ethylmethane sulfonate (EMS)-induced recessive and dominant alleles of *Toll* that cause a variety of changes in the size and spatial distribution of ventral and lateral structures in the embryo has been isolated (Anderson et al. 1985b; Gerttula et al. 1988; Erdélyi and Szabad 1989). Here we describe the mutations responsible for nine dominant and five recessive alleles of *Toll*. The mutant sequences define domains of the *Toll* protein that are essential for its normal activity. In addition, the mutations may define extracellular sites of interaction with proteins that spatially regulate the activity of *Toll* and intracellular regions that are required for *Toll* to transmit the signal that controls nuclear localization of the *dorsal* protein.

Results

Recessive mutations in Toll alter either the intracellular or extracellular domain of the protein

Each of the recessive *Toll* alleles analyzed has some residual activity, with a distinctive phenotype and complementation behavior. Four of the five recessive alleles sequenced contained a single base change from the parental chromosome that would cause a single amino acid change (Fig. 1). Three alleles were found to have a mutation in the cytoplasmic domain, and two were found to have mutations in the extracellular domain of the *Toll* protein.

Females homozygous for *Tl^{r26}* produce strongly dorsal-

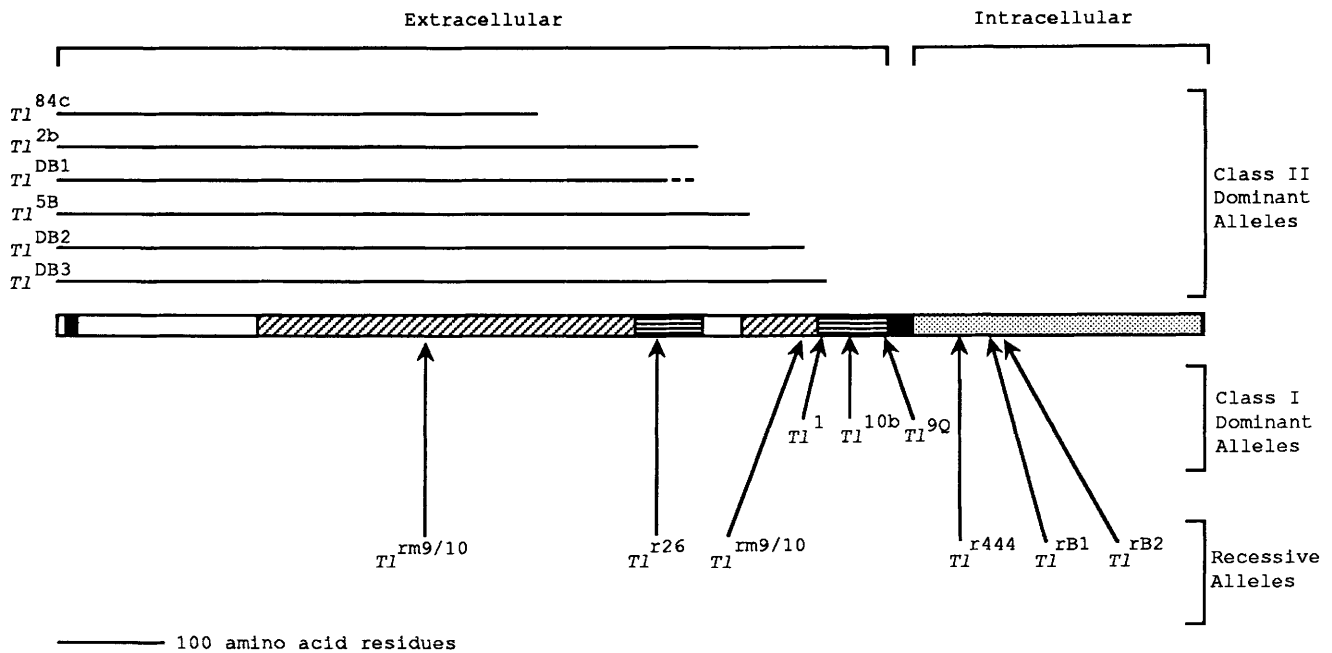
ized embryos (Anderson et al. 1985b). This allele is slightly temperature sensitive and in some genetic backgrounds causes a dominant dorsalizing phenotype, indicating that it has some antimorphic character. An extracellular mutation was found in *Tl^{r26}* in the cysteine-containing motif following the first block of L-rich repeats (Fig. 1).

The *Tl^{rm9}* mutation causes an unusual embryonic phenotype. The embryos are lateralized; both dorsal and ventral structures are absent, and all cells adopt lateral fates (Anderson et al. 1985b). Two nonconservative amino acid substitutions were found in the extracellular domain of *Tl^{rm9}* in the first and second blocks of leucine repeats (Fig. 1). We have not determined whether one or both changes are required to generate the mutant phenotype.

Three recessive dorsalizing alleles, *Tl^{r444}*, *Tl^{rB1}*, and *Tl^{rB2}*, have mutations in the cytoplasmic domain of the *Toll* molecule (Fig. 1). The cytoplasmic domain mutations are particularly interesting in light of the sequence similarity between the *Toll* protein and the IL-1R (Fig. 2) (Hashimoto et al. 1988; Sims et al. 1988, 1989). The extracellular domains of these two proteins are completely different, but the cytoplasmic domains are 26% identical and 43% similar in sequence. The two cytoplasmic domains can be aligned from the first residue after the transmembrane domain through the entire 217-amino-acid-residue length of the IL-1R cytoplasmic domain without the introduction of large gaps. The *Toll* cytoplasmic domain has an additional 68-amino-acid residue tail that is not present in the IL-1R. The three cytoplasmic *Toll* mutations are located in the region of similarity between the two molecules (Fig. 2). The strongest allele, *Tl^{rB2}*, changes a valine found in both *Toll* and the IL-1 receptor to a methionine. The temperature-sensitive allele *Tl^{r444}* changes a phenylalanine (tyrosine in the IL-1R) to isoleucine, and the weakest allele, *Tl^{rB1}*, changes a histidine to a tyrosine, the amino acid residue found in that position in the IL-1R.

Two classes of dominant alleles of Toll change the extracellular domain

Females carrying any of the 12 dominant alleles of *Toll* in *trans* to a wild-type allele produce ventralized embryos in which the ventral and lateral pattern elements are expanded at the expense of dorsal structures. The dominant ventralizing alleles of *Toll* fall into two genetic classes that differ in their requirement for a wild-type allele of *Toll*. Females carrying a class I allele (*Tl¹*, *Tl^{9Q}*, *Tl^{10b}*) in *trans* to a null allele of *Toll* produce ventralized embryos. In contrast, females carrying a class II allele (*Tl^{84c}*, *Tl^{5B}*, *Tl^{DB1}*, *Tl^{DB2}*, *Tl^{DB3}*, *Tl^{2b}*, *Tl^{3c}*, *Tl²⁰ⁱ*, *Tl^{18a}*) in *trans* to a null allele of *Toll* produce dorsalized embryos, like females that lack all *Toll* activity (Anderson et al. 1985b; Hudson 1989). Class II alleles also resemble null alleles in that they lack the *Toll* activity required for zygotic viability (Gerttula et al. 1988; Hudson 1989). None of the class I alleles are temperature sensitive, but two of the class II alleles (*Tl^{DB1}* and *Tl^{2b}*)

A**B**

Allele	Base Change	Coding Change	Phenotype (29°C)
Class I			
1	G2838 → A	Cys755 → Tyr	Strongly ventralized
10b	G2916 → A	Cys781 → Tyr	Extremely ventralized
9Q	G2970 → A	Cys799 → Tyr	Strongly ventralized
Class II			
84c	C1964 → T	Gln464 → STOP	Strongly ventralized
DB1	Δ2332 to 2345	Deletion and Frameshift	Weakly ventralized (18°C moderate)
2b	C2414 → T	Gln614 → STOP	Weakly ventralized (18°C moderate)
5B	C2579 → T	Gln669 → STOP	Strongly ventralized
DB2	G2743 → A	Trp723 → STOP	Moderately ventralized
DB3	G2832 → T	Trp753 → STOP	Moderately ventralized
Recessive			
rm9/10	AA1639 → GC	Lys355 → Ser	Lateralized
rm9/10	C2715 → A	Thr714 → Asn	Lateralized
r26	T2379 → C	Val576 → Ala	Strongly dorsalized
r444	T3149 → A	Phe859 → Ile	Strongly dorsalized (18°C weak)
rB1	C3251 → T	His893 → Tyr	Weakly dorsalized
rB2	G3305 → A	Val911 → Met	Moderately dorsalized

Figure 1. Summary of dominant and recessive *Toll* mutations. (A) The open reading frames of the class II dominant alleles are depicted by solid lines. The dotted line in *Tl*^{DB1} indicates the 33-amino acid residue tail that follows the 11-bp deletion in this allele. The locations of the mutations in the class I dominant alleles and the recessive alleles are indicated by arrows. Hatched areas indicate L-rich repeats; solid areas indicate signal sequence and the transmembrane domain; horizontal lined areas denote terminal flanking domains; the stippled area denotes the cytoplasmic domain. (B) The mutations listed are the changes seen in the coding strand of the *Toll* gene. Expanded mesoderm is the most extreme ventralized phenotype, with most cells contributing to the most ventral pattern element, the mesoderm; if any cuticle is present, it is ventral cuticle. Strongly ventralized embryos have circumferential ventral denticle bands and no dorsal hairs. Moderately ventralized embryos have expanded ventral denticle bands but retain some dorsal hairs; weakly ventralized embryos have normal ventral denticle belts and slight head and tail defects. Strongly dorsalized (D0) (Anderson et al. 1985b) embryos have circumferential dorsal hairs and no ventral denticles or filzkörper. Moderately dorsalized (D1) embryos have expanded fields of dorsal hairs and filzkörper but no ventral denticles. Weakly dorsalized (D2) embryos have narrowed ventral denticle belts, a field of dorsal hairs, and filzkörper. Lateralized embryos have circumferential ventral denticles but no mesoderm or dorsal hairs. The allele *Tl*^{rm10} has the same phenotype as *Tl*^{rm9} and was isolated in the same screen (Rice 1973), which led Rice et al. to propose they could represent duplicates of a single mutation. We found that *Tl*^{rm10} contained both changes found in *Tl*^{rm9} and conclude that these two alleles arose as a single event.

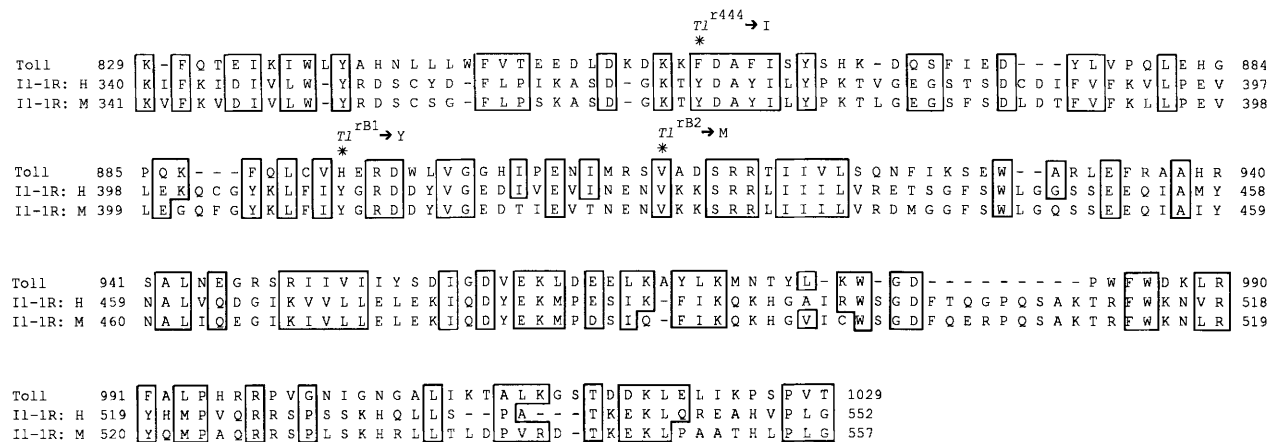


Figure 2. *Toll* and IL-1R sequence similarity. The sequence of the cytoplasmic domain of *Toll* is aligned with the cytoplasmic domains of the human and mouse IL-1Rs (IL-1R H and IL-1R M, respectively). Similarities between the cytoplasmic domain of *Toll* and the IL-1R are boxed. *Toll* has an additional 68 amino acids in its cytoplasmic domain following the region of similarity. Amino acid similarities were defined according to Miyata, where $I \approx V \approx L \approx M$, $D \approx E \approx Q \approx N$, $F \approx Y \approx W$, $H \approx K \approx R$, and $G \approx A \approx S \approx T \approx P$ (Miyata et al. 1979). The amino acid changes found in the cytoplasmic domain of *Toll* alleles are marked.

are cold sensitive, producing more severe phenotypes at 18°C than at 29°C (Fig. 3C,D). Class I and class II alleles also differ in their dependence on upstream genes. Double-mutant females that lack the activity of genes upstream of *Toll* and carry a class I *Toll* allele produce embryos that make ventral or lateral structures in a dorsoventrally symmetric pattern, whereas double-mutant flies carrying class II alleles of *Toll* and upstream mutations produce dorsalized embryos (Anderson et al. 1985b; K.V. Anderson, unpubl.). In summary, class I alleles of *Toll* are *cis* active and partially independent of the activity of upstream genes, whereas class II alleles are *cis* inactive, can alter the activity of the wild-type *Toll* product in *trans*, and require upstream genes for activity.

In general, the class I alleles produce more strongly ventralized phenotypes than most class II alleles, although the range of phenotypes overlaps. The most extreme ventralized phenotype is seen in embryos laid by mothers carrying the class I allele $Tl^{1^{ob}}$ (Erdélyi and Szabad 1989). In these embryos the most ventrally derived tissue, the mesoderm, is expanded and laterally derived epidermal structures are either absent or greatly reduced (Hudson 1989). The ventralized phenotypes of embryos produced by mothers carrying the class I alleles Tl^1 or $Tl^{8^{Q}}$ or the class II alleles $Tl^{8^{4c}}$ or Tl^{5B} are similar (Fig. 3B) (Anderson et al. 1985b). Females carrying one of these four alleles produce ventralized embryos with expanded ventral epidermis and the complete loss of dorsal epidermis, but no expansion of the mesoderm. The other class II alleles cause a weaker ventralization of the embryonic pattern, with some dorsal pattern elements still present. The weakest phenotype is caused by the cold-sensitive allele Tl^{DB1} ; at 29°C, 50% of the embryos hatch into larvae and the remainder do not undergo head involution and may not shorten the germ band (Fig. 3D).

Nine of the dominant alleles were sequenced, and all contained mutations in the extracellular domain of the

Toll molecule (Fig. 1). The sequence changes found in the dominant alleles fall into two molecular classes that coincide with the two genetically defined classes.

Each of the class I alleles changed a cysteine to a tyrosine in the sequence after the second block of L-rich repeat domain, immediately adjacent to the transmembrane domain (Fig. 1). This clustering of mutations prompted us to examine the carboxy-terminal flanking sequences of other L-rich repeat-containing proteins more closely. In *Toll*, both blocks of L-rich repeats are followed by a cysteine-containing motif, which is also found in a subset of proteins that have extracellular L-rich repeats including L-rich glycoprotein (Takahashi et al. 1985), the platelet transmembrane proteins gp 1b α and β , gp IX (Lopez et al. 1987, 1988; Hickey et al. 1989), and the lutropin–choriogonadotropin receptor (McFarland et al. 1989). We found that the similarity between these motifs extends further than described previously (Hickey et al. 1989; Keith and Gay 1990) and that the motifs contain four, rather than only two, conserved cysteine residues (Fig. 4). Each of the three class I alleles replaced one of the four cysteine residues in this motif with a tyrosine residue (Fig. 4).

In each of the six class II alleles sequenced, premature stop codons or small deletions that introduced premature stop codons were found (Fig. 1). The truncated polypeptides produced by these alleles would be from 464 to 753 amino acid residues in length and would include one or both blocks of L-rich repeats. None of the truncated proteins would contain the transmembrane domain. No simple relationship between the length of a truncated class II polypeptide and its phenotypic strength has emerged. For instance, the two strongest class II *Toll* alleles, Tl^{5B} and $Tl^{8^{4c}}$, encoded truncated polypeptides of very different lengths.

Protein blots confirmed that $Tl^{classII}/Tl^+$ heterozygotes synthesized both full-length and truncated *Toll*

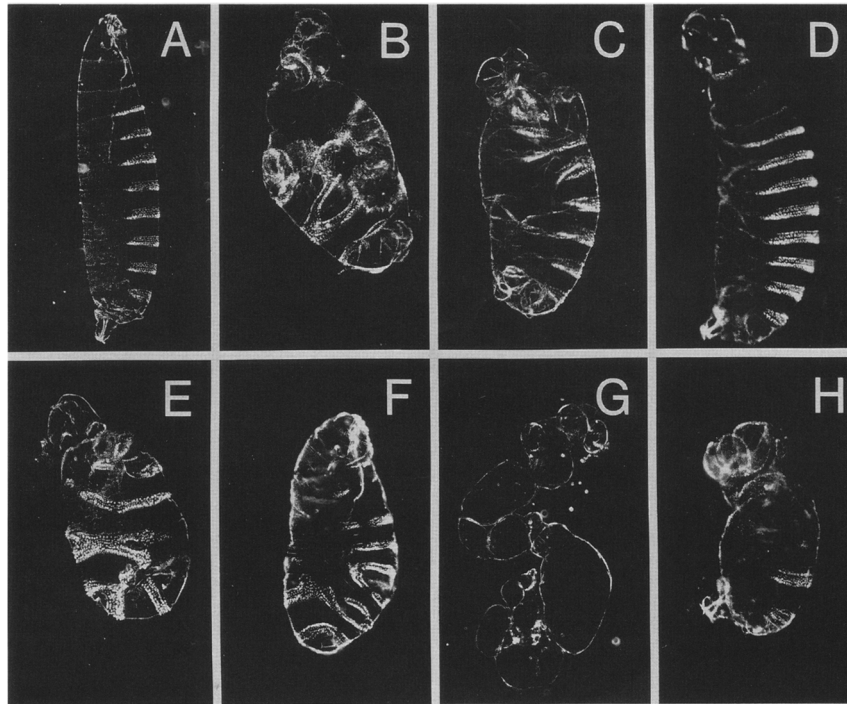


Figure 3. Cuticular phenotypes produced by dominant ventralizing *Toll* alleles and by injection of dominant allele transcripts. (A) Wild-type, first-instar larva. (B) The cuticle of a strongly ventralized embryo produced by a *Tl*^{84c}/TM3 female. (C) The cuticle of a moderately ventralized embryo produced by a *Tl*^{DB1}/TM3 female at 18°C. (D) The cuticle of a weakly ventralized embryo produced by a *Tl*^{DB1}/TM3 female at 29°C. (E) The cuticle of a wild-type embryo injected dorsally at 25% egg length with a transcript encoding a *Tl*^{trunc1} (66 µg/ml). Both head and tail structures are ventralized. (F) The cuticle of a wild-type embryo injected at 25% egg length on the dorsal surface with the class I *Tl*^{10b} cDNA transcript (66 µg/ml). Only the posterior end of the embryo was ventralized in these injections. (G) The cuticle of a strongly dorsalized embryo produced by a *ea*⁴/*ea*^{5022rX1} female. (H) The cuticle pattern of an embryo produced by an *ea*⁴/*ea*^{5022rX1} female that was injected with a *Tl*^{10b} transcript at 25% egg length. This embryo differentiated both the dorsolaterally derived filzkörper and the ventrolaterally derived ventral denticle belts of the sixth, seventh, and eighth abdominal segments.

proteins (Fig. 5). The sizes of the observed proteins were consistent with the sizes predicted from their sequences. The truncated proteins examined were all ~25% as abundant as the full-length *Toll* protein, perhaps because they are not as stable as the transmembrane protein.

Injection of dominant allele transcripts can ventralize the embryonic pattern

To test whether the coding changes found in the dominant alleles were responsible for the mutant phenotypes, we assayed the activity of both class I and class II mutant transcripts injected into wild-type embryos. This assay relied on the ability of injected transcripts of a full-length *Toll* cDNA to rescue the dorsalized phenotype of embryos laid by *Tl*⁻ females (C. Hashimoto, unpubl.). Injection of high concentrations of wild-type transcripts into wild-type embryos never produced a ventralizing phenotype (Table 1).

We replaced segments of the *Toll* cDNA construct with restriction fragments containing the point mutations of class I dominant alleles and assayed the activity of transcripts of mutant alleles by injection into cleavage-stage, wild-type embryos (Table 1). Transcripts con-

taining the *Tl*¹ or *Tl*^{10b} cysteine-to-tyrosine changes caused ventralization of the embryonic pattern, confirming that these changes are sufficient to produce a dominant ventralizing phenotype (Fig. 3F).

The extent of ventralization produced by the class I transcripts depended on the concentration of the injected transcripts. Injection of high concentrations (>66 µg/ml) of *Tl*^{10b} and *Tl*¹ (Table 1) transcripts into wild-type embryos induced the formation of local cup-shaped invaginations at the time of gastrulation at the site of deposition (Table 1). We believe that these local furrows correspond to local mesodermal invaginations. Embryos that had large local invaginations differentiated to have large holes in their cuticles, with only ventral cuticular structures present in the surrounding cuticle. We interpreted these embryos as having expanded mesoderm, similar to embryos produced by females carrying the *Tl*^{10b} allele. Injections of lower concentrations (13 µg/ml) of *Tl*^{10b} and *Tl*¹ transcripts produced more weakly ventralized embryos that resembled those produced by *Tl*¹ females, with ventral denticle bands encircling the circumference of the larval cuticle. Thus, although *Tl*^{10b}/+ females produce more strongly ventralized embryos than *Tl*¹/+ females, the products of both *Tl*^{10b} and *Tl*¹ caused an

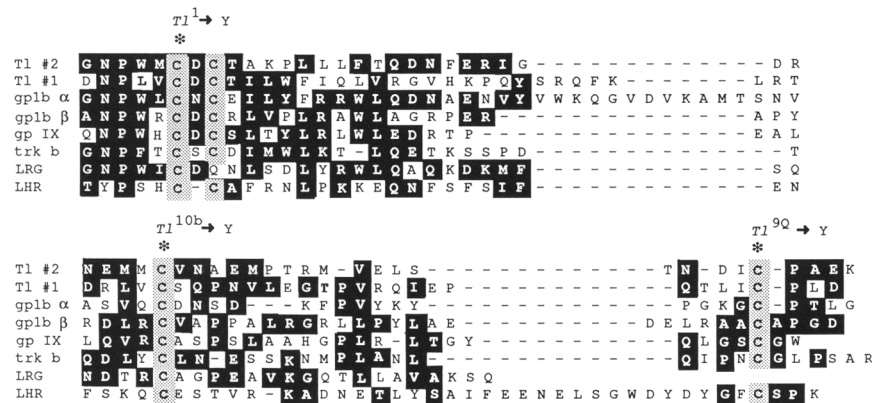


Figure 4. L-rich repeat terminal-flanking sequence similarity. The sequences of the regions carboxy-terminal to the blocks of leucine repeats in the extracellular domain in the *Toll* protein are aligned with leucine repeat carboxy-terminal-flanking sequences found in human blood platelet glycoprotein 1b α and β (gp1b α and β) (Lopez et al. 1987, 1988), human blood platelet glycoprotein IX (gp IX) (Hickey et al. 1989), the protein tyrosine kinase receptor trk b (Klein et al. 1989), L-rich glycoprotein (LRG) (Takahashi et al. 1985), and the lutropin receptor (LHR) (McFarland et al. 1989). Amino acid residues are grouped on the basis of similarities between their physical and chemical properties, which were defined according to Miyata, $I \approx V \approx L \approx M$, $D \approx E \approx Q \approx N$, $F \approx Y \approx W$, $H \approx K \approx R$, and $G \approx A \approx S \approx T \approx P$ (Miyata et al. 1979). Similar amino acids are shown in white with a black background. The four cysteine residues found in these repeats are highlighted with a dotted background. The mutations responsible for the dominant phenotypes of class I *Toll* alleles are in the second *Toll* terminal repeat at the positions shown.

expansion of the ventrally derived mesoderm at high concentrations and at lower concentrations expanded the laterally derived ventral epidermis without detectably expanding the mesoderm.

When wild-type *Toll* mRNA is injected into *Tl⁻* embryos the dorsal–ventral pattern is rescued along one-third to one-half of the anterior–posterior length of the embryo (Anderson et al. 1985a; C. Hashimoto, unpubl.). The ventralizing effects produced by injection of *Tl¹* or *Tl^{10b}* transcripts into wild-type embryos were also spatially restricted. Local invaginations at the site of injection were seen when the transcripts were deposited either dorsally or ventrally at the periphery of the embryo. When the transcripts were placed dorsally, a furrow in-

vaginated at that site ~ 2 min before the invagination of the ventral furrow. No interference with the invagination of the normal ventral furrow was detected. When the transcripts were injected ventrally, a cup-shaped invagination at the injection site slightly preceded, and then appeared to be superimposed on, normal ventral furrow invagination. Injection at 25% egg length (0% egg length = posterior pole) prevented germ-band extension but did not affect the dorsal–ventral position of the cephalic fold (67% egg length). Larval cuticles of embryos injected at 25% egg length frequently had wild-type head structures but ventralized posteriors (Fig. 3F). No clear differences were seen in the cuticular patterns between embryos injected ventrally and those injected dorsally.

To test the activity of transcripts encoding truncated products like the class II alleles, we synthesized a *Toll* cDNA (*Tl^{trunc1}*) that has a stop codon 5 amino acid residues amino-terminal to that found in *Tl^{5B}*. Injection of high concentrations (330 μ g/ml) of a *Tl^{trunc1}* transcript into wild-type embryos at any dorsal–ventral position caused a moderate ventralization of the embryonic pattern, with expanded ventral denticle bands and the loss of some dorsal pattern elements, but some dorsal structures were always differentiated (Fig. 3E). No local ventral furrow was ever induced in these injections (Table 1). In contrast with the local effects seen with class I transcript injections, when the *Tl^{trunc1}* transcript was injected at 25% egg length, the cephalic fold was shifted to the dorsal side of the embryo and germ-band extension was blocked. Both head and tail cuticle patterns were ventralized in these embryos (Fig. 3E). The global ventralization produced by class II, but not class I, transcripts suggests that the class II, but not the class I, proteins diffuse throughout the extracellular perivitelline space of the embryo.

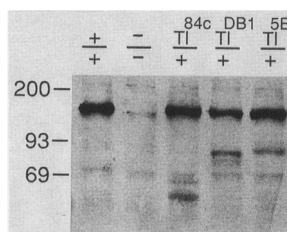


Figure 5. Class II allele protein expression. Extracts of 0- to 4-hr embryos were separated on 7.5% polyacrylamide gels and transferred to nitrocellulose. Blots were probed with a rabbit polyclonal antibody raised against the amino-terminal domain of *Toll* (Materials and methods). The product of *Tl^{84c}* migrates at 63 kD, the product of *Tl^{DB1}* at 82 kD, and the product of *Tl^{5B}* at 84 kD, the same order of size predicted from the sequences. Each truncated product migrates ~ 10 kD larger than predicted from its sequence, probably due to glycosylation. Wild-type *Toll* protein is glycosylated and therefore migrates at 135 kD rather than the 125 kD predicted from the open reading frame (Hashimoto et al. 1991).

Table 1. Phenotypes of embryos injected with transcripts encoding dominant Toll alleles

Transcript	Recipient	Concentration (μg/ml)	Injection position	Local furrow (%)	n_g	Differentiation pattern (%)					n_c
						hatch	FK + VD	VD	mes. exp.		
<i>TI^{10b}</i>	wild type	330	dorsal	82	49	0	2	10	88	21	
<i>TI^{10b}</i>	wild type	330	ventral	90	52	0	5		95	47	
<i>TI^{10b}</i>	wild type	66	dorsal	21	68	4	13	34	49	63	
<i>TI^{10b}</i>	wild type	66	ventral	78	81	20	17	17	46	81	
<i>TI^{10b}</i>	wild type	13	dorsal	0	47	48	21	21	12	18	
<i>TI^{10b}</i>	wild type	13	ventral	11	18	50	28	11	11	44	
<i>TI¹</i>	wild type	200	dorsal	76	46		30	23	47	47	
<i>TI¹</i>	wild type	200	ventral	52	46		45	12	43	51	
<i>TI¹</i>	wild type	40	dorsal	10	10		73	22	5	40	
<i>TI¹</i>	wild type	40	ventral	0	11		92	4	4	26	
<i>TI^{trunc1}</i>	wild type	330	dorsal	0	20	0	18	82		25	
<i>TI^{trunc1}</i>	wild type	330	ventral	0	25	4	35	61		29	
<i>TI⁺</i>	wild type	1000	dorsal	0	42	100				33	
<i>TI⁺</i>	wild type	1000	ventral	0	37	100				21	
Differentiation pattern (%)											
						no FK	FK	FK + VD	VD	mes. exp.	
<i>TI^{10b}</i>	<i>TI⁻</i>	330		80	35	9			91	11	
<i>TI^{10b}</i>	<i>TI⁻</i>	66		64	44		12		88	16	
<i>TI^{10b}</i>	<i>TI⁻</i>	13		71	51	2	38	6	54	48	
<i>TI^{10b}</i>	<i>TI⁻</i>	2		0	46	15	70	15		27	
<i>TI^{10b}</i>	<i>TI⁻</i>	0.4				100				17	
<i>TI^{10b}</i>	<i>ea⁻</i>	330		92	25		4	54	42	24	
<i>TI^{10b}</i>	<i>ea⁻</i>	66		72	75	4	51	20	25	59	
<i>TI^{10b}</i>	<i>ea⁻</i>	13		0	58	6	50	38	6	18	
<i>TI¹</i>	<i>TI⁻</i>	200		87	137					0	
<i>TI^{trunc1}</i>	<i>TI⁻</i>	2000		0	41	100				8	
<i>TI^{trunc1}</i>	<i>ea⁻</i>	330		0	65	100				11	

Local furrow refers to the formation of a local mesoderm invagination during gastrulation at the site of injection. The columns headed n_g and n_c record the number of embryos observed at gastrulation or the number of larval cuticles counted, respectively. Embryos that did not hatch but did not have defects in dorsal-ventral pattern formation are not included here. Hatching was not scored for *Tl¹* injections. Cuticles recorded in the FK column were moderately dorsalized and contained only dorsolaterally derived filzkörper but not the more ventrally derived ventral denticles. Cuticles recorded in the FK + VD column contained filzkörper material and some ventral denticles. In injections into wild-type embryos the FK + VD column includes cuticles that were moderately ventralized. For injections into dorsalized embryos, the FK + VD column lists both moderately dorsalized, essentially wild-type and moderately ventralized cuticles, which could not be unambiguously distinguished from one another. Cuticles recorded in the VD column contained ventral denticles but no filzkörper material and, therefore, were more strongly ventralized than those in the FK + VD column. Cuticles recorded in the mes. exp. (mesoderm expanded) column contained large holes in their cuticles, which resemble those found in extremely ventralized embryos in which the mesoderm has expanded; only ventral cuticle was seen surrounding the holes. Transcripts were injected onto the dorsal side of *Tl⁻* or *ea⁻* embryos.

We also assayed the activity of the dominant transcripts injected into dorsalized embryos that lacked the activity of *Toll* or the upstream dorsal group gene *easter* (*ea*) (Table 1). At an appropriate concentration, injection of class I transcripts near the periphery of embryos produced by *Tl⁻* females or *ea⁻* females promoted the development of a normal, asymmetric dorsal-ventral pattern (Fig. 3H). In both kinds of recipient, the most ventral structures developed at the site of RNA deposition. The dose-response curve for rescue was very steep, with high

concentrations of transcript ventralizing the embryos, and only a limited concentration range generating a wild-type pattern (Table 1). Five times more dominant *Toll* RNA was required to rescue lateral and ventral structures in *ea⁻* embryos than in *Tl⁻* embryos, suggesting that the *ea⁺* activity present in the *Tl⁻* embryo potentiates the activity of the product of the dominant allele. As expected from the *cis* inactivity and upstream gene dependence of the class II alleles, injection of class II transcripts into embryos produced by *Tl⁻* and *ea⁻*

females did not rescue any lateral or ventral structures (Table 1).

Discussion

Our current hypothesis is that the *Toll* protein either directly transduces a signal across the plasma membrane of the embryo or is part of a signal transduction complex. To investigate this hypothesis, we have sequenced mutant *Toll* alleles that cause well-defined changes in the embryonic dorsal–ventral pattern. The sequence changes define functional domains of the *Toll* protein that are consistent with the idea that it acts as a receptor for an extracellular, spatially asymmetric signal and passes on a signal through its cytoplasmic domain. Recessive mutations that lower the activity of *Toll* alter either the extracellular or cytoplasmic domains of the protein. Dominant gain-of-function alleles, which cause *Toll* to be active in dorsal as well as ventral parts of the embryo, are all caused by mutations in its extracellular domain. The two genetic classes of dominant *Toll* alleles correspond to two distinct classes of molecular lesions, identifying two regions of the extracellular domain that are of particular importance in controlling the activity of the *Toll* protein.

Class I dominant Toll alleles encode constitutively active products

The class I dominant alleles define the juxtamembrane cysteine-containing motif adjacent to the L-rich repeats as a domain that can control the activity of the *Toll* molecule. The 18 extracellular cysteine residues in *Toll* do not form disulfide bonds with other proteins (Hashimoto et al. 1991), suggesting that the cysteines changed in these alleles normally participate in intramolecular disulfide bonds. The L-rich repeat carboxy-terminal flanking sequence of L-rich glycoprotein contains only two cysteine residues that form an intramolecular disulfide bond (Takahashi et al. 1985). The pattern of sequence similarity between the proteins suggests that disulfide bonds form between Cys 1 and Cys 3 and between Cys 2 and Cys 4 in this domain of *Toll*. The conformational changes that lead to abnormal activity of class I *Toll* alleles could be due either to the substitution of bulky tyrosine residues for cysteine residues or to interference with normal disulfide bond formation. Both factors may be important. We predict that the *Tl^{10b}* and *Tl¹* mutations disrupt the same disulfide bond, but they do not cause identical phenotypes.

Like *Toll*, the *Caenorhabditis elegans lin-12* gene encodes a transmembrane protein that is believed to function as a signal transducer (Yochem et al. 1988). Dominant mutations of *lin-12* that increase the activity of the protein change single extracellular amino acids adjacent to the transmembrane domain (Greenwald and Seydoux 1990). This suggests that although these two proteins share no sequence homology, the conformation of the region just outside the transmembrane domain may be important in defining the activity of the cytoplasmic do-

main of both *Toll* and *lin-12*. In addition to producing similar mutant phenotypes, these domains may also be important in regulating the activity of the wild-type proteins. The similar activity of these two domains in *Toll* and *lin-12* suggests that extracellular juxtamembrane domains could have similar functions in other signal-transducing molecules.

Because the class I alleles are active in the absence of upstream genes, it is likely that the class I alleles of *Toll* send a constitutive signal to the cytoplasm at all dorsal–ventral positions. The injection experiments reported here confirm this hypothesis by demonstrating that class I transcripts can induce ventral structures on the dorsal side of the wild-type embryo or in an embryo that lacks the activity of the upstream gene *easter*.

When the class I transcripts are injected locally into *Tl⁻* or *ea⁻* embryos, ventral structures differentiate at the injection site and a complete, normally proportioned dorsal–ventral pattern can develop (Fig. 3H). The asymmetric dorsal–ventral pattern of the injected *ea⁻* embryos contrasts with the dorsoventrally symmetric lateralized embryos produced by *Tl¹-ea⁻* double-mutant females (Anderson et al. 1985b). This difference could be accounted for if in *Tl¹-ea⁻* embryos the *Tl¹* product is constitutively active at all dorsal–ventral positions, producing a lateralized phenotype, while in *ea⁻* embryos injected with a high concentration of *Tl¹* RNA, the constitutively active, nondiffusing *Tl¹* product is confined to one side of the embryo and can then generate a normal, asymmetric pattern.

The *Tl¹-ea⁺* embryo is ventralized and has normal polarity, with a ventral furrow forming on the normal ventral side. The asymmetry seen in the *Tl¹ (ea⁺)* embryo must be the result of an enhancement of the activity of the *Tl¹* product above its baseline constitutive level by a ventral signal that depends on *easter* activity. This kind of enhancement was seen in the *Tl¹* transcript injections, where the activity of the *Tl¹* product was approximately fivefold lower in the absence of *ea⁺* (Table 1). Thus, in the wild-type embryo it is likely that asymmetric activation of the uniformly distributed *Toll* product by an *easter*-dependent upstream ventral signal gives rise to the normal pattern.

It is interesting that two very different means of achieving localized *Toll* activity, local activation by an upstream signal or local injection of a nondiffusible, constitutively active receptor, can both result in a normal dorsal–ventral pattern. It seems likely that the spatial distribution of active *Toll* molecules is different in the two cases, suggesting that mechanisms act downstream of *Toll* to regulate proportioning of the dorsal–ventral pattern.

Truncated class II dominant products activate the wild-type Toll protein in trans

The class II dominant alleles of *Toll* encode stable truncated polypeptides (Fig. 5) that lack both the transmembrane and cytoplasmic domains and are therefore presumably secreted into the extracellular perivitelline

space. The truncated, secreted products of the class II alleles are active only in the presence of both the transmembrane *Toll* protein and the products of the genes upstream of *Toll*. Thus, the amino-terminal 464 amino acids of *Toll* must include a domain that, together with the normal upstream gene products, can control the activity of wild-type *Toll* protein.

It is possible to explain the phenotypes of the class II alleles by three different kinds of interactions: The amino-terminal domain of *Toll* could bind the transmembrane *Toll*, it could bind to an inhibitor of *Toll*, or it could bind to an activator of *Toll*. In the first model, the truncated *Toll* proteins could increase the activity of the wild-type *Toll* gene product by interacting directly with the wild-type transmembrane protein. Many transmembrane signal transducers require ligand-induced multimerization to be activated (Ullrich and Schlessinger 1990). If *Toll* activation also requires multimerization, the truncated molecules may facilitate this process. In a second model, the activity of the full-length *Toll* protein is increased because truncated *Toll* molecules sequester an inhibitor of wild-type *Toll*. This model predicts that there is an upstream inhibitor of *Toll*, but no such molecule has been identified genetically. In a third model, the dominant ventralizing activity of class II alleles depends on the secretion and solubility of their gene products. If the upstream gene products create a diffusible asymmetric activating signal on the ventral side of the embryo, truncated *Toll* molecules could bind to the activator and promote its diffusion to the dorsal side of the embryo, where the activator could be released to activate wild-type *Toll* protein.

Biochemical and molecular genetic evidence indicates that truncated and secreted forms of many cell-surface receptors are produced naturally (Goodwin et al. 1990). The truncated receptors could decrease receptor activity by competing for ligand with the transmembrane form or by interfering with the signal transduction process (Basu et al. 1989; Taira et al. 1989). However, in contrast with these dominant negative interactions, the *Toll* class II alleles indicate that a truncated protein can induce the ectopic activity of a full-length receptor.

The Toll cytoplasmic domain: Homology to the IL-1R

The sequence similarity between the cytoplasmic domains of *Toll* and the IL-1R and the mapping of the loss-of-function alleles of *Toll* to the region of similarity suggest the hypothesis that *Toll* and the IL-1R transmit their signals by similar mechanisms. The mechanism of signal transduction by the IL-1R is not yet clear, although studies have implicated increases in cAMP or diacylglycerol as steps in the pathway (Rosoff et al. 1988; Shirakawa et al. 1988; Zhang et al. 1988). A number of new recessive *Toll* alleles that have been isolated recently (N. Machin, pers. comm.) may help to evaluate the significance of the sequence similarity and to define functional regions within both cytoplasmic domains.

The potential homology between *Toll* and the IL-1R is particularly tantalizing because the proteins that act

downstream of these two transmembrane proteins may act in similar signal transduction pathways. *Toll* indirectly controls the activity of the *dorsal* protein by controlling its nuclear localization (Roth et al. 1989; Rushlow et al. 1989; Steward 1989). Similarly, in some cell lines, IL-1 causes the transcription factor NF- κ B to be translocated from the cytoplasm into the nucleus, where it is then active (Shirakawa et al. 1989). The sequence similarity of *dorsal* to the DNA-binding p50 subunit of NF- κ B (Ghosh et al. 1990; Kieran et al. 1990), the regulation of both transcription factors at the level of nuclear translocation, and the sequence similarity of *Toll* and the IL-1R suggest that the intermediates in the two pathways may be also similar.

Materials and methods

Mutant alleles

Most *Toll* alleles have been described previously (Anderson et al. 1985b; Gerttula et al. 1988; Erdélyi and Szabad 1989). The dominant alleles *Tl^{DB1}* (Dominant Berkeley 1), *Tl^{DB2}*, and *Tl^{DB3}* were fortuitously isolated in F₂ screens for maternal-effect mutations (S. Wasserman, D. Morisato, and K.V. Anderson, unpubl.).

Sequencing of mutant alleles

The sequences of the *Toll* alleles were determined by the dideoxy chain-termination technique (Sanger 1977), using the Sequenase system (U.S. Biochemical Corporation). The sequences of the parental chromosomes of all the *Toll* alleles, with the exception of *Tl^{r26}*, *Tl^l*, *Tl^{10b}*, and *Tl^{2b}*, were also determined. Genomic DNA, including the *Toll* open reading frame and a 106-bp intron between cDNA nucleotides 1793 and 1794 (Hudson 1989), was sequenced by using oligonucleotide primers spaced every 200 bp.

Two mutant *Toll* alleles were cloned from genomic libraries. The *Tl^{84c}* allele was cloned from an EMBL 4 library of partial *Sau3A*-digested genomic DNA from *Tl^{84c}/TM3* flies. The *Tl^{9Q}* allele was cloned from a λ FIX (Stratagene) library of *XhoI*-digested genomic DNA obtained from *Tl^{9Q}/TM3* flies. Alleles of *Toll* cloned from the TM3 balancer chromosome were identified by the presence of a polymorphic *Bam*HI restriction site that is not present in the *Tl^{84c}* and *Tl^{9Q}* alleles. The open reading frames of these two dominant alleles were sequenced completely.

The remaining alleles were sequenced from mutant genomic DNA amplified by the polymerase chain reaction (PCR) (Saiki et al. 1988). The recessive alleles were sequenced from PCR-amplified DNA that was cloned into pBluescript (Stratagene). The 3.4-kb *Toll* open reading frame-containing DNA was amplified in two fragments from DNA obtained from flies homozygous for each recessive allele. The entire open reading frame of each of the recessive alleles was sequenced. Mutant sequences were confirmed by directly sequencing PCR products. The sequences of *Tl^{10b}*, *Tl^l*, *Tl^{DB1}*, *Tl^{5B}*, *Tl^{2B}*, *Tl^{DB2}*, and *Tl^{DB3}* were obtained by directly sequencing DNA amplified from genomic DNA isolated from flies carrying the mutant alleles in *trans* to a deficiency (*Tl^{10b}*, *Tl^l*, and *Tl^{DB1}*) or in *trans* to a balancer (TM3) chromosome (*Tl^{2b}*, *Tl^{5B}*, *Tl^{DB2}*, and *Tl^{DB3}*). *Tl^{10b}* and *Tl^l* were sequenced from nucleotide 2535 to nucleotide 3040, *Tl^{DB1}* and *Tl^{2b}* were sequenced from nucleotide 2285 to nucleotide 2475, *Tl^{DB2}* and *Tl^{DB3}* were sequenced from nucleotide 2555 to 2872.

TI^{5B} was sequenced from nucleotide 1248 to nucleotide 2451 and from nucleotide 2540 to 2930.

Transcripts of mutant alleles

A full-length, wild-type *Toll* cDNA was constructed [C. Hashimoto, unpubl.] in a pGEM-2 vector (Promega). cDNAs including the point mutations in the dominant alleles TI^1 and TI^{10b} were constructed by replacing a *Pfl*MI–*Stu*I restriction fragment in the wild-type cDNA with a fragment (nucleotide 2800–3483 in the cDNA sequence) amplified from mutant DNA by PCR. The reconstructed dominant alleles were sequenced over the *Pfl*MI–*Stu*I restriction fragment to ensure that the DNA did not contain any PCR-derived artifacts.

A *Toll* allele containing a stop codon in the extracellular domain (TI^{trunc1}) was constructed by inserting an *Xba*I linker (New England Biolabs) containing stop codons in all three reading frames into the open reading frame of the *Toll* cDNA. The pGEM-2 vector containing the full-length, wild-type cDNA was linearized with *Stu*I, which cuts in the cytoplasmic domain of *Toll*. To place the linkers in the extracellular domain of *Toll*, *Exo*III deletions were performed on this linearized plasmid essentially as described previously (Henikoff 1987). Nonphosphorylated *Xba*I linkers were ligated to the deleted plasmids, and the ligation reactions were precipitated to remove unligated linkers. The ligated DNA was resuspended in 40 mM Tris-HCl (pH 7.5), 20 mM $MgCl_2$, and 50 mM NaCl, heated to 65°C, and cooled slowly to allow the free ends to anneal. This recircularized DNA was used to transform *Escherichia coli* MC1061 cells. TI^{trunc1} contains an *Xba*I linker after nucleotide 2559 in the *Toll* cDNA sequence and an 1825-bp deletion from nucleotide 2559 to 4384.

Transcript injection

pGEM-2 templates were linearized and SP6 transcripts were generated essentially as described (Krieg and Melton 1987), in the presence of 500 μ M TTP, CTP, ATP (Pharmacia), cap analog (5' GpppG^{3'}; Pharmacia), 50 μ M GTP, and 2.5 nM [α -³²P]CTP, at 800 Ci/mmol (Amersham). After 2 hr, the reaction mixture was diluted with one volume of 100 mM NaCl, 30 mM EDTA, 20 mM Tris (pH 7.5), and 1% SDS and passed over a 1-ml Sephadex G-50 spin column, equilibrated in 0.3 M sodium acetate and 0.1% SDS, to remove unincorporated nucleotides. The reaction was then extracted with phenol and precipitated twice with ethanol to remove residual SDS. Transcripts were resuspended in injection buffer (Anderson and Nüsslein-Volhard 1984). The concentration of the transcribed RNA was determined by calculating the percentage incorporation of the radiolabeled CTP.

Protein analysis

Staged 0- to 4-hr embryos were collected, dechorionated, and homogenized in a solution of 10 mM Tris-HCl (pH 8.0), and 1 mM EDTA. Wild-type embryo extracts were obtained from Oregon-R flies. TI^- embryos were obtained from *Df*(3R) TI^{roXB3} /*Df*(3R) TI^{QRX} females, which make no *Toll* RNA (Hashimoto et al. 1988). Ventralized embryos were collected from $TI^{classII}/TI^+$ flies. Extracts were centrifuged at 10,000g for 10 min to remove insoluble material, and 100 μ g of protein was loaded per lane. Proteins were blotted as described (Hashimoto et al. 1991). Blots were probed with a 1 : 40 dilution of affinity-purified rabbit polyclonal primary antibody raised against the amino-terminal portion of *Toll* (Hashimoto et al. 1991), and 1 : 10,000 dilution of HRP-linked goat-anti-rabbit secondary antibody (Bio-Rad). *Toll* protein was visualized by using an ECL kit (Amersham).

Acknowledgments

We thank Arend Sidow for advice concerning direct sequencing of PCR products and Chris Kaiser for help in sequence analysis. We also thank Becky Chasan, Chip Ferguson, Linda Hicke, Bruce Kimmel, Sylvia Sanders, and Mike Simon and the members of the Anderson laboratory for helpful comments on the manuscript. This work was supported by grants from the National Institutes of Health (GM 35437), the National Science Foundation (DCB 8452030), and the American Cancer Society (NP726) to K.V.A.

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

References

- Anderson, K.V. and C. Nüsslein-Volhard. 1984. Information for the dorsal-ventral pattern of the *Drosophila* embryos is stored as maternal mRNA. *Nature* **311**: 223–227.
- . 1986. Dorsal-group genes of *Drosophila*. In *Gametogenesis and the early embryo* (ed. J. Gall), pp. 177–194. Alan R. Liss, New York.
- Anderson, K.V., L. Bokla, and C. Nüsslein-Volhard. 1985a. Establishment of dorsal-ventral polarity in the *Drosophila* embryo: The induction of polarity by the *Toll* gene product. *Cell* **42**: 791–798.
- Anderson, K.V., G. Jürgens, and C. Nüsslein-Volhard. 1985b. Establishment of dorsal-ventral polarity in the *Drosophila* embryo: Genetic studies on the role of the *Toll* gene product. *Cell* **42**: 779–789.
- Basu, A., M. Raghunath, S. Bishayee, and M. Das. 1989. Inhibition of tyrosine kinase activity of the epidermal growth factor (EGF) receptor by a truncated receptor form that binds to EGF: Role for interreceptor interaction in kinase regulation. *Mol. Cell. Biol.* **9**: 671–677.
- Chasan, R. and K.V. Anderson. 1989. The role of *easter*, an apparent serine protease, in organizing the dorsal-ventral pattern of the *Drosophila* embryo. *Cell* **56**: 391–400.
- DeLotto, R. and P. Spierer. 1986. A gene required for the specification of dorsal-ventral pattern in *Drosophila* appears to encode a serine protease. *Nature* **323**: 688–692.
- Erdélyi, M. and J. Szabad. 1989. Isolation and characterization of dominant female sterile mutations of *Drosophila melanogaster*. I. Mutations on the third chromosome. *Genetics* **122**: 111–127.
- Gertula, S., Y. Jin, and K.V. Anderson. 1988. Zygotic expression and activity of the *Drosophila Toll* gene, a gene required maternally for embryonic dorsal-ventral pattern formation. *Genetics* **119**: 123–133.
- Ghosh, S., A.M. Gifford, L.R. Riviere, P. Tempst, G.P. Nolan, and D. Baltimore. 1990. Cloning of the p50 DNA binding subunit of NF- κ B: Homology to *rel* and *dorsal*. *Cell* **62**: 1019–1029.
- Goodwin, R.G., D. Friend, S.F. Ziegler, R. Jerzy, B.A. Falk, S. Gimpel, D. Cosman, S.K. Dower, C.J. March, A.E. Namen, and L.S. Park. 1990. Cloning of the human and murine interleukin-7 receptors: Demonstration of a soluble form and homology to a new receptor superfamily. *Cell* **60**: 941–951.
- Greenwald, I. and G. Seydoux. 1990. Analysis of gain-of-function mutations of the *lin-12* gene of *Caenorhabditis elegans*. *Nature* **346**: 197–199.
- Hashimoto, C., K.L. Hudson, and K.V. Anderson. 1988. The *Toll* gene of *Drosophila*, required for dorsal-ventral embryonic polarity, appears to encode a transmembrane protein. *Cell*

- 52: 269–279.
- Hashimoto, C., S. Gerttula, and K.V. Anderson. 1991. Plasma membrane localization of the *Toll* protein in the syncytial *Drosophila* embryo: Importance of transmembrane signaling for dorsal-ventral pattern formation. *Development* (in press).
- Henikoff, S. 1987. Unidirectional digestion with exonuclease III in DNA sequence analysis. *Methods Enzymol.* **155**: 156–165.
- Hickey, M.J., S.A. Williams, and G.J. Roth. 1989. Human platelet glycoprotein IX: An adhesive prototype of leucine-rich glycoproteins with flank-center-flank structures. *Proc. Natl. Acad. Sci.* **86**: 6773–6777.
- Hudson, K.L. 1989. Ph.D. thesis. University of California, Berkeley.
- Keith, F.J. and N.J. Gay. 1990. The *Drosophila* membrane receptor *Toll* can function to promote cellular adhesion. *EMBO J.* **9**: 4299–4306.
- Kieran, M., V. Blank, F. Logeat, J. Vanderkerckhove, F. Lottspeich, O. Le Bail, M.B. Urban, P. Kourilsky, P.A. Baeuerle, and A. Israël. 1990. The DNA binding subunit of NF- κ B is identical to factor KBF1 and homologous to the *rel* oncogene product. *Cell* **62**: 1007–1018.
- Klein, R., L.F. Parada, F. Coulier, and M. Babacid. 1989. *trkB*, a novel tyrosine protein kinase receptor expressed during mouse neural development. *EMBO J.* **8**: 3701–3709.
- Krantz, D.E. and S.C. Zipursky. 1990. *Drosophila* chaoptin, a member of the leucine-rich repeat family, is a photoreceptor cell-specific adhesion molecule. *EMBO J.* **9**: 1969–1977.
- Krieg, P.A. and D.A. Melton. 1987. In vitro RNA synthesis with SP6 RNA polymerase. *Methods Enzymol.* **155**: 397–415.
- Lopez, J.A., D.W. Chung, K. Fujikawa, F.S. Hagen, T. Papayanopoulou, and G.J. Roth. 1987. Cloning of the α chain of human platelet glycoprotein Ib: A transmembrane protein with homology to leucine-rich α_2 -glycoprotein. *Proc. Natl. Acad. Sci.* **84**: 5615–5619.
- Lopez, J.A., D.W. Chung, K. Fujikawa, F.S. Hagen, E.W. Davie, and G.J. Roth. 1988. The α and β chains of human platelet glycoprotein Ib are both transmembrane proteins containing a leucine-rich amino acid sequence. *Proc. Natl. Acad. Sci.* **85**: 2135–2139.
- McFarland, K.C., R. Sprengel, H.S. Phillips, M. Köhler, N. Rosenthal, K. Nikolics, D.L. Segaloff, P.H. Seeburg. 1989. Lutropin-choriogonadotropin receptor: An unusual member of the G protein-coupled receptor family. *Science* **245**: 494–499.
- Miyata, T., S. Miyazawa, and T. Yasonaga. 1979. Two types of amino acid substitution in protein evolution. *J. Mol. Evol.* **12**: 219–236.
- Rice, T.B. 1973. Ph.D. thesis. Yale University, New Haven, Ct.
- Rosoff, P.M., N. Savage, and C.A. Dinarello. 1988. Interleukin-1 stimulates diacylglycerol production in T lymphocytes by a novel mechanism. *Cell* **54**: 73–81.
- Roth, S., D. Stein, and C. Nüsslein-Volhard. 1989. A gradient of nuclear localization of the *dorsal* protein determines dorsoventral pattern in the *Drosophila* embryo. *Cell* **59**: 1189–1202.
- Rushlow, C., M. Frasch, H. Doyle, and M. Levine. 1987. Maternal regulation of *zerknüllt*: A homoeobox gene controlling differentiation of *dorsal* tissues in *Drosophila*. *Nature* **330**: 583–586.
- Rushlow, C.A., K. Han, J.L. Manley, and M. Levine. 1989. The graded distribution of the *dorsal* morphogen is initiated by selective nuclear transport in *Drosophila*. *Cell* **59**: 1165–1177.
- Saiki, R.K., U.B. Gyllenstein, and H.A. Erlich. 1988. The polymerase chain reaction. In *Genome analysis: A practical approach* (ed. K.E. Davies), pp. 141–152. IRL Press, Oxford.
- Sanger, F., S. Nicklen, and A.R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci.* **74**: 5463–5467.
- Schüpbach, T. and E. Wieschaus. 1989. Female sterile mutations on the second chromosome of *Drosophila melanogaster*. I. Maternal effect mutations. *Genetics*. **121**: 101–117.
- Shirakawa, F., U. Yamashita, M. Chedid, and S.B. Mizel. 1988. Cyclic AMP—An intracellular second messenger for interleukin 1. *Proc. Natl. Acad. Sci.* **85**: 8201–8205.
- Shirakawa, F., M. Chedid, J. Suttles, B.A. Pollok, and S.B. Mizel. 1989. Interleukin 1 and cyclic AMP induce κ immunoglobulin light-chain expression via activation of an NF- κ B-like DNA-binding protein. *Mol. Cell. Biol.* **9**: 959–964.
- Sims, J.E., R. Acres, C.E. Grubin, C.J. McMahan, J.M. Wignall, C.J. March, and S.K. Dower. 1989. Cloning of the interleukin 1 receptor from human T cells. *Proc. Natl. Acad. Sci.* **86**: 8946–8950.
- Sims, J.E., C.J. March, D. Cosman, M.B. Widmer, H.R. MacDonald, C.J. McMahan, C.E. Grubin, J.M. Wignall, J.L. Jackson, S.M. Call, D. Friend, A.R. Alpert, S. Gillis, D.L. Urdal, and S.K. Dower. 1988. cDNA expression cloning of the IL-1 receptor, a member of the immunoglobulin superfamily. *Science* **241**: 585–589.
- Steward, R. 1989. Relocalization of the *dorsal* protein from the cytoplasm to the nucleus correlates with its function. *Cell* **59**: 1179–1188.
- Steward, R., S.B. Zusman, L.H. Huang, and P. Schedl. 1988. The *dorsal* protein is distributed in a gradient in early *Drosophila* embryos. *Cell* **55**: 487–495.
- Taira, M., M. Taira, N. Hashimoto, F. Shimada, Y. Suzuki, A. Kanatsuka, F. Nakamura, Y. Ebina, M. Tatibana, H. Makino, and S. Yoshida. 1989. Human diabetes associated with a deletion of the tyrosine kinase domain of the insulin receptor. *Science* **245**: 63–68.
- Takahashi, N., Y. Takahashi, and F.W. Putnam. 1985. Periodicity of leucine and tandem repetition of 24-amino acid segment in the primary structure of leucine-rich α_2 -glycoprotein of human serum. *Proc. Natl. Acad. Sci.* **82**: 1906–1910.
- Ullrich, A. and J. Schlessinger. 1990. Signal transduction by receptors with tyrosine kinase activity. *Cell* **61**: 203–212.
- Yochem, J., K. Weston, and I. Greenwald. 1988. The *Caenorhabditis elegans lin-12* gene encodes a transmembrane protein with overall similarity to *Drosophila* Notch. *Nature* **335**: 547–550.
- Zhang, Y., J.-X. Lin, Y.K. Yip, and J. Vilček. 1988. Enhancement of cAMP levels and of protein kinase activity by tumor necrosis factor and interleukin 1 in human fibroblasts: Role in the induction of interleukin 6. *Proc. Natl. Acad. Sci.* **85**: 6802–6805.



Dominant and recessive mutations define functional domains of Toll, a transmembrane protein required for dorsal-ventral polarity in the *Drosophila* embryo.

D S Schneider, K L Hudson, T Y Lin, et al.

Genes Dev. 1991, **5**:

Access the most recent version at doi:[10.1101/gad.5.5.797](https://doi.org/10.1101/gad.5.5.797)

References

This article cites 40 articles, 16 of which can be accessed free at:
<http://genesdev.cshlp.org/content/5/5/797.full.html#ref-list-1>

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).

