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Recruitment of Tube and Pelle to signaling sites at the surface of the *Drosophila* embryo

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SUMMARY

A signaling pathway initiated by activation of the transmembrane receptor Toll defines dorsoventral polarity in the *Drosophila* embryo. Toll, which is present over the entire surface of the embryo, is activated ventrally by interaction with a spatially restricted, extracellular ligand. Tube and Pelle then transduce the signal from activated Toll to a complex of Dorsal and Cactus. Here we demonstrate by an mRNA microinjection assay that targeting of either Tube or Pelle to the plasma membrane by myristylation is sufficient to activate the signal transduction pathway that leads to Dorsal nuclear translocation. Using confocal immunofluorescence microscopy we also show that activated Toll induces a localized recruitment of Tube and Pelle to the plasma membrane. Together, these results strongly support the hypothesis that intracellular signaling requires the Toll-mediated formation of a membrane-associated complex containing both Tube and Pelle.

Key words: Dorsal, NF-κB, IL-1 Receptor, Dorsoventral polarity, plasma membrane, Signal transduction, *Drosophila*

INTRODUCTION

The dorsoventral axis of the *Drosophila* embryo is defined by a nuclear concentration gradient of the transcription factor Dorsal (reviewed by Chasan and Anderson, 1993). Dorsal is initially present throughout the embryonic cytoplasm, where it is retained by an inhibitory protein, Cactus. Upon localized activation of the transmembrane receptor Toll, a signal is transduced in ventral and ventrolateral regions of the embryo that induces Cactus proteolysis, thereby freeing Dorsal (Belvin et al., 1995; Bergmann et al., 1996; Reach et al., 1996). Free Dorsal translocates into nuclei, where it directs expression of ventral-specific genes and repression of dorsal-specific genes.

Signal transduction from Toll to the Dorsal/Cactus complex requires the Tube and Pelle proteins (Hecht and Anderson, 1993). Tube, like Toll, lacks a recognizable catalytic domain (Letsou et al., 1991; Hashimoto et al., 1988); Pelle is a protein kinase (Shelton and Wasserman, 1993). Mutations that block Toll activation or that eliminate Tube or Pelle function result in embryos in which all of the transcription factor Dorsal remains cytoplasmic (Anderson et al., 1985). Such embryos lack any ventral tissues and die as hollow tubes of dorsal epidermis. Tube and Pelle bind to each other, as well as to Dorsal (Großhans et al., 1994; Galindo et al., 1995; Edwards et al., 1997; Yang and Steward, 1997).

Although the Toll pathway was identified on the basis of its role in dorsoventral patterning, this signaling cascade also participates in the *Drosophila* immune response (Hultmark, 1994; Lemaitre et al., 1996). Furthermore, similar pathways mediate immune and defensive responses in organisms ranging from plants to mammals (Wilson et al., 1997; Wasserman, 1993; Belvin and Anderson, 1996; Verma et al., 1995). In humans, for example, the type I Interleukin-1 receptor, which shares sequence similarity with Toll in its intracellular domain, initiates an acute phase response mediated in part by IRAK (Interleukin-1 receptor associated kinase), NF-κB, and IκB, vertebrate counterparts to Pelle, Dorsal, and Cactus (Schneider et al., 1991; Gay and Keith, 1991; Cao et al., 1996; Steward, 1987; Geisler et al., 1992; Kidd, 1992). Since the acute phase response is a prerequisite for activation of the adaptive immune response, signaling through the Toll pathway appears to be essential for both innate and adaptive immunity (Medzhitov and Janeway, 1997).

Evidence suggests that critical interactions among components of the Toll pathway occur at the plasma membrane of the embryo. First, most of the Tube protein in embryos is localized to the surface (Galindo et al., 1995). Second, substitution of Tube or Pelle for the intracellular domain of the Torso transmembrane receptor generates a chimera that drives signaling in the absence of Toll activation (Großhans et al., 1994; Galindo et al., 1995). Epistasis analysis is consistent with a model in which Tube targets Pelle to the plasma membrane upon Toll receptor activation (Galindo et al., 1995).

Here we investigate the mechanism of Toll-mediated signaling through an analysis of Tube and Pelle localization and function. Using myristylation to target Tube and Pelle to the plasma membrane, we demonstrate that membrane

association of Tube or Pelle is sufficient to activate signal transduction. We further show that activation of Toll leads to a recruitment of Tube and Pelle to cell surface sites of signal transduction. These results provide strong support for the hypothesis that activation of Toll drives formation of membrane associated signaling complexes containing both Tube and Pelle.

MATERIALS AND METHODS

Fly stocks

Drosophila melanogaster stocks were maintained on standard cornmeal-yeast-agar medium (Ashburner, 1989) at 18, 22 or 25°C. Oregon R was used as the wild-type stock. The P[Tl^{10b}-*bcd*] transgenic fly strain (Huang et al., 1997) was kindly provided by A. Huang and M. Levine. All mutations are described in FlyBase (http://flybase.bio.indiana.edu).

Immunocytochemistry, immunoprecipitation and immunoblotting

The anti-Dorsal and anti-Tube sera have been described previously (Reach et al., 1996; Letsou et al., 1993). The anti-Pelle serum (Großhans et al., 1994) was a generous gift from A. Bergmann. The anti-Actin serum was purchased from Boehringer Mannheim (monoclonal C4). Embryos were collected, fixed, devitellinized and stained using standard protocols (Galindo et al., 1995). Double-labeling experiments, immunoprecipitations, and immunoblotting were carried out as described previously (Reach et al., 1996; Edwards et al., 1997).

Microscopy and digital image processing

Image acquisition and analysis was carried out as described previously (Galindo et al., 1995) using either an MRC-1024 (Bio-Rad) or an LSM-410 (Zeiss) laser scanning confocal unit. Fluorescein, Cy3 and Cy5 images were collected separately, using the 488, 568, and 633 (Zeiss) or 647 (Bio-Rad) nm lines of the lasers, respectively, to excite the fluorophores. The fluorescein, Cy3, and Cy5 signals were viewed with the BP510-525, the BP575-640 and the RG665 (Zeiss) filters, respectively. Quantitation of pixel intensities was performed with either the Bio-Rad LaserSharp software or the Carl Zeiss LSM 3.95 software.

RNA synthesis, embryo injection and cuticle preparations

RNA synthesis and cuticle preparation was performed as described previously (Shelton and Wasserman, 1993). Cuticle phenotypes were scored at the posterior end, the site of RNA injection.

DNA manipulations

For the *src-pll* and *src-tube* constructs, exonic sequence encoding the first 90 amino acids of Src was amplified by PCR from the *pKB-csw*^{src90} construct (Allard et al., 1996), kindly provided by John Allard. The amplified DNA, flanked by a 5′ *Nco*I site and a 3′ *Bst*EII site, was then inserted into the *Nco*I and *Bst*EII sites in the previously described *tor-pll* and *tor-tube* constructs (Galindo et al., 1995). The result was the substitution of *src*^{90} for the *tor*-encoding portion of the constructs, generating *src-pll* and *src-tube* fusion cDNAs. The *src*^{G2A} construct differs from *src*^{90} in that the 5' primer used for the PCR amplification of *src*^{G2A} converts the second codon from GGC (glycine) to GCC (alanine).

RESULTS

Tube and Pelle signal in the absence of Toll activation when targeted to the plasma membrane

Tube and Pelle have been shown to signal constitutively when fused to the extracellular and transmembrane domains of the *Drosophila* Torso receptor (Großhans et al., 1994; Galindo et al., 1995). It was proposed that constitutive activation of these chimeras was the direct result of membrane localization (Galindo et al., 1995). However, the Torso sequences could also have activated the chimeras by, for example, mediating an ectopic extracellular protein-protein interaction.

To determine whether membrane association is in fact sufficient to constitutively activate Tube and Pelle, we fused Src^{90}, the amino-terminal 90 amino acids of the *Drosophila* Src tyrosine kinase (Simon et al., 1985), to full-length Tube and to the catalytic domain of Pelle. The Src^{90} domain contains a myristylation signal that directs association with the plasma membrane (Pellman et al., 1985; Allard et al., 1996). We transcribed the *src*^{90}*-pelle* and *src*^{90}*-tube* fusion constructs in vitro and then assayed their activity by microinjecting the resulting RNAs into syncytial blastoderm embryos.

The ability of microinjected RNA transcripts to stimulate dorsoventral signaling was analyzed by examining cell fate markers in the cuticle secreted by the developing embryo. In wild-type embryos, Toll-mediated signaling to Dorsal leads to

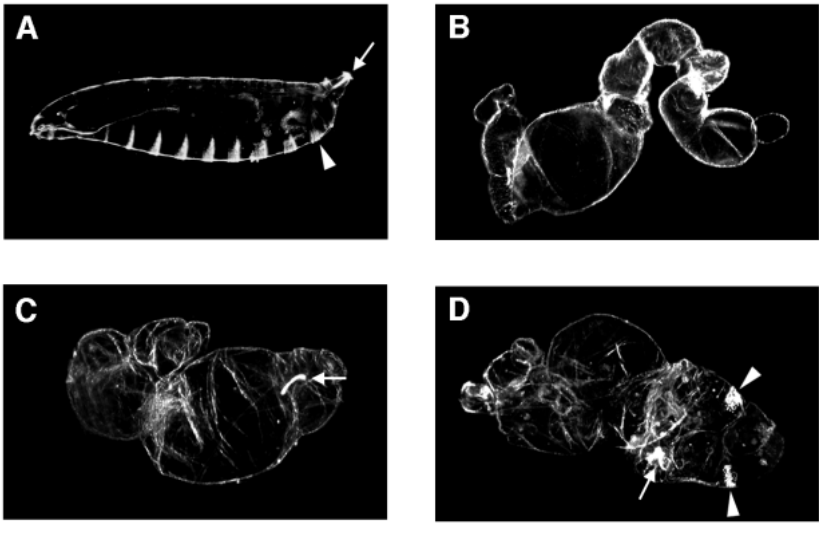

Fig. 1. Myristylated Tube and Pelle chimeras signal constitutively. Cuticles are oriented with anterior to the left. (A) Cuticle of a wild-type embryo. The dorsolateral filzkörper (arrow) and the ventral denticles (arrowhead) form in response to low and high levels of Toll activation, respectively. (B) Cuticle of an embryo derived from a gd^2/gd^2 female. Filzkörper and ventral denticles are absent. (C) Cuticle of a gd^2/gd^2 embryo injected with the Src^{90}-Tube construct at 2 mg/ml concentration. The arrow points to filzkörper material formed near the site of injection. (D) Cuticle of a gd^2/gd^2 embryo injected with the Src^{90}-Pelle construct at 2 mg/ml concentration. The arrow points to filzkörper material and the arrowheads to ventral denticles formed near the posterior site of injection.

Table 1. Constitutive activity of *src-pelle* and *src-tube* constructs

RNA injected	No. cuticles scored	% Lacking FK and VD	% FK	% VD
None	50	100	0	0
src^{90}-tube	31	71	29	0
src^{90}-pelle	42	48	38	14
src^{G2A}-tube	37	100	0	0
src^{G2A}-pelle	41	100	0	0

RNA transcripts were produced in vitro and injected at a concentration of 2 mg/ml into stage 2 embryos derived from gd^2/gd^2 females. Embryos were scored either as having only dorsal cuticle, as having filzkörper (FK) only, and as having ventral denticles (VD) with or without filzkörper.

the formation of filzkörper and ventral denticle belts (Fig. 1A), cuticle structures representative of dorsolateral and ventral ectodermal fates, respectively (Lohs-Schardin et al., 1979). Mutations exist in which intracellular signaling is abolished due to a block in the extracellular pathway required for Toll ligand activation. For example, in embryos generated by females lacking function at the *gastrulation defective* locus (gd^2 embryos), Toll is inactive and Dorsal remains exclusively cytoplasmic (Konrad et al., 1988; Stein and Nüsslein-Volhard, 1992). Such embryos fail to form filzkörper and ventral denticles (Table 1, row 1; Fig. 1B).

Injection of either src^{90}-tube or src^{90}-pelle RNA into gd^2 embryos directed signaling at the posterior site of microinjection, as evidenced by the appearance of filzkörper (Table 1, rows 2,3; Fig. 1C,D). Src^{90}-pelle RNA also induced the ectopic expression of ventral denticle belts (Table 1, row 3; Fig. 1D). These results demonstrate the ability of both Src^{90}-Tube and Src^{90}-Pelle to direct Dorsal nuclear translocation in the absence of Toll activation. We note that the signaling induced by Src^{90}-Tube and Src^{90}-Pelle is weaker than that seen with Torso fusion constructs, perhaps as a result of less efficient or less intimate membrane contact.

To confirm that the constitutive activity of Src-Tube and Src-Pelle is myristylation dependent, we mutated the second amino acid in the Src portion of each chimera from glycine to alanine; such a *G2A* mutation destroys the Src myristylation signal (Allard et al., 1996). Unlike src-tube RNA, src^{G2A}-tube RNA failed to induce signaling in microinjected gd^2 embryos (Table 1, row 4). The fact that the *G2A* mutation eliminated the constitutive activity of Src-Tube did not, however, reflect a general disruption in protein structure or stability; src^{G2A}-tube RNA rescued *tube* mutant embryos to hatching (data not shown) and thus retains wild-type Tube activity. Introduction of the *G2A* mutation also eliminated the constitutive activity of the Src-Pelle chimera (Table 1, row 5).

Based on these results, we conclude that Pelle and Tube are activated upon targeting to the plasma membrane.

Tube localizes asymmetrically along the dorsoventral axis

We have shown previously that the majority of the Tube protein molecules in embryos associate with the cell surface and that this association occurs over the entire circumference of the embryo (Galindo et al., 1995). Since this localization is not restricted to ventral regions, it cannot represent an activated state of Tube. However, based on the Src^{90} fusion experiments, we envisioned that Toll activates Tube by driving association of Tube with the plasma membrane. To detect any such signal-related membrane association, we carried out a detailed examination of Tube localization, using the nuclear concentration gradient of Dorsal as a marker for the dorsoventral axis (Figs 2, 3).

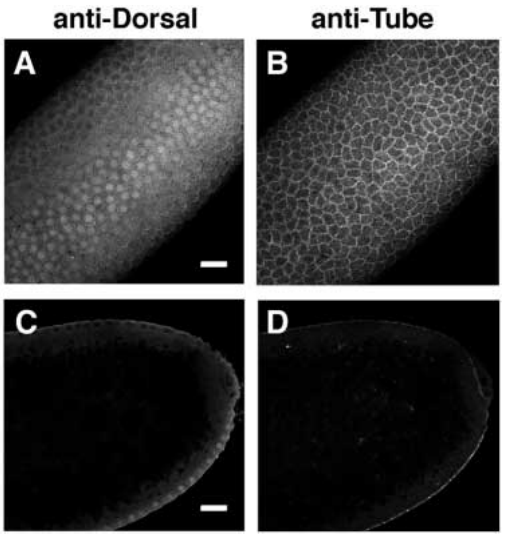

Fig. 2. Tube distributes asymmetrically along the dorsoventral axis in wild-type embryos. All photographs are of embryos from wild-type females examined by confocal immunofluorescence microscopy. The images are paired, showing staining with either fluorescein-conjugated antibodies to Dorsal (A,C) or antibodies to Tube detected with a Cy3-conjugated secondary antibody (B,D). The two pairs represent surface views of the ventral aspect (A,B; scale bar, 25 μm) and longitudinal optical sections in the plane of the dorsoventral axis (C,D; scale bar, 20 μm).

Surface views of wild-type embryos reveal that Tube forms a mesh-like array. Such a localization pattern is characteristic of proteins associated with the membranes and cytoskeleton that cap and surround each blastoderm nucleus (Hashimoto et al., 1991; Warn et al., 1984). For Tube, the mesh-like pattern is most intense along the ventral midline, suggesting a concentration of Tube in this region (Fig. 2B). Confocal cross-sectional views confirm the existence of a dorsoventral asymmetry in the distribution of Tube at the embryo surface (Fig. 2D). Tube is more highly concentrated along the ventral surface than the dorsal surface; the difference in concentration, as assayed by quantitation of staining intensity, is two-fold.

Higher magnification ventral views reveal that Tube clusters along the embryo surface (Fig. 3D, arrows). These clusters or aggregates are approximately 2 μm in diameter and are located between adjacent ventral nuclei at sites of membrane invagination. On the dorsal side of the embryo, clusters of Tube are less readily detectable (Fig. 3C). Another membrane-associated protein, Actin, does not show any clustering on either the dorsal or ventral side of the embryo (Fig. 3E,F).

Redistribution of Tube is a consequence of Toll activation

To determine whether the asymmetric distribution of Tube depends on Toll-mediated signaling, we examined Tube localization in genetic backgrounds that either inactivate or

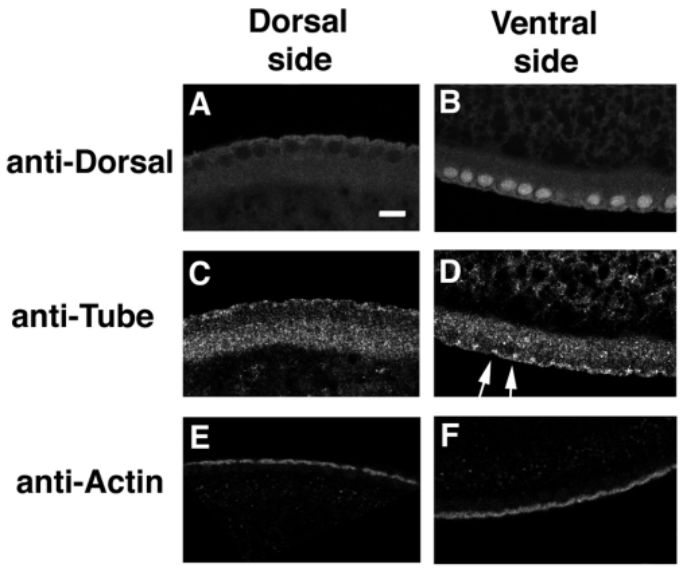

Fig. 3. Tube clusters at signaling sites on the ventral surface of wild-type embryos. All photographs are high magnification longitudinal optical sections of embryos from wild-type females examined by confocal immunofluorescence microscopy (scale bar, 10 μm). The first four images are paired, showing staining with either fluorescein-conjugated antibodies to Dorsal (A,B) or antibodies to Tube detected with a Cy3-conjugated secondary antibody (C,D). A separate embryo was stained with antibodies to Actin which were detected with a Cy5-conjugated secondary antibody (E,F). (A,C,E) Optical sections of the dorsal side. (B,D,F) Optical sections of the ventral side. Arrows in D point to bright foci of Tube staining.

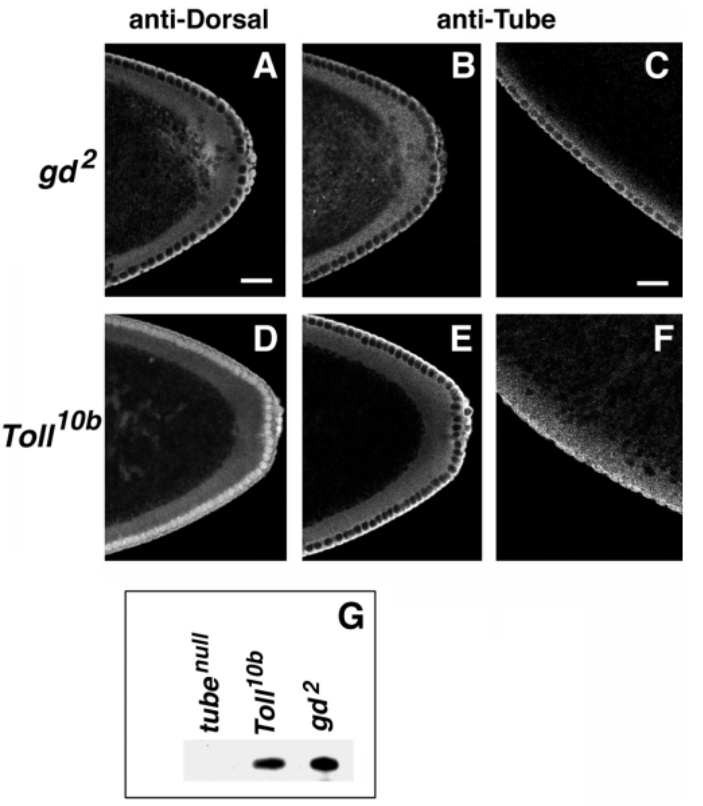

Fig. 4. Toll activation governs Tube localization. (A,B,D,E) Longitudinal optical cross sections of embryos oriented with the anterior to the left; scale bar, 20 μm. (C,F) High magnification optical cross sections of embryos; scale bar, 16 μm. Embryos from either gd^2/gd^2 females (A,B,C) or $Toll^{10b}$/+ females (D,E,F) were analyzed as in Fig. 2. (G) Tube protein was immunoprecipitated from embryos produced by $tube^{null}$ ($tub^{R5.6}/Df(3R)XM3$), $Toll^{10b}$/+, and gd^2/gd^2 females and analyzed by immunoblotting.

constitutively activate the signaling pathway. The gd^2 mutation, as mentioned earlier, blocks Toll activation, whereas the $Toll^{10b}$ mutation alters the Toll extracellular domain so as to constitutively activate the receptor throughout the embryo (Schneider et al., 1991). Both mutations eliminate the dorsoventral asymmetry in Tube staining observed in the wild type (Fig. 4B,E; compare to Fig. 2D). However, Tube was more highly concentrated at the periphery of $Toll^{10b}$ embryos than gd^2 embryos (compare Fig. 4B,C with 4E,F). This difference likely reflects a change in the distribution, and not the amount, of Tube within the embryo, since immunoblot analysis of immunoprecipitated Tube protein revealed comparable levels of Tube in $Toll^{10b}$ and gd^2 embryos (Fig. 4G).

The $Toll^{10b}$ and gd^2 results support the model that activated Toll directs recruitment of Tube to the embryo surface. The asymmetry in Tube localization in the wild type was not, however, as striking as that observed for Cactus or Dorsal (Bergmann et al., 1996; Reach et al., 1996; Roth et al., 1989; Rushlow et al., 1989; Steward, 1989). We therefore also explored the distribution of Tube in P[$Toll^{10b}$-*bcd*] embryos, in which Toll-mediated signaling is ectopically oriented along the anteroposterior axis (Huang et al., 1997). Females carrying the P[$Toll^{10b}$-*bcd*] transgene express high levels of the $Toll^{10b}$ cDNA fused to the *bicoid* (*bcd*) 3′UTR and consequently produce embryos in which the *bcd* sequences localize the $Toll^{10b}$ mRNA to the anterior pole (Berleth et al., 1988; Macdonald and Struhl, 1988).

Tube concentrates at the plasma membrane in and near the anterior pole of P[$Toll^{10b}$-*bcd*] embryos (Fig. 5A,B). Moreover, clusters of Tube are prominent anteriorly on both the dorsal and ventral surfaces of such embryos (Fig. 5B, arrows). As noted previously (Huang et al., 1997), the $Toll^{10b}$ protein expressed from the P[$Toll^{10b}$-*bcd*] construct also forms cell surface aggregates at the anterior pole (Fig. 5C,D). Comparison of the patterns of Tube and Toll localization in P[$Toll^{10b}$-*bcd*] embryos indicates that the Toll aggregates colocalize with the Tube aggregates (Fig. 5E, arrows).

Pelle localizes to regions expressing high levels of activated Toll

Since Tube and Pelle are thought to interact in embryos, we reasoned that Pelle, like Tube, might localize to sites of Toll activation. Using a polyclonal anti-Pelle serum to stain wild-type embryos, we found that Pelle is distributed throughout the embryo. A fraction of Pelle localized to the surface of embryos, but there was no significant asymmetry in this distribution of Pelle across the dorsoventral axis (Fig. 6C,D; compare with Fig. 2B,D).

If Pelle undergoes a signal-dependent membrane association in wild-type embryos, this association is too short-lived or involves too few of the Pelle molecules in the embryo to be readily detectable. However, our localization studies had

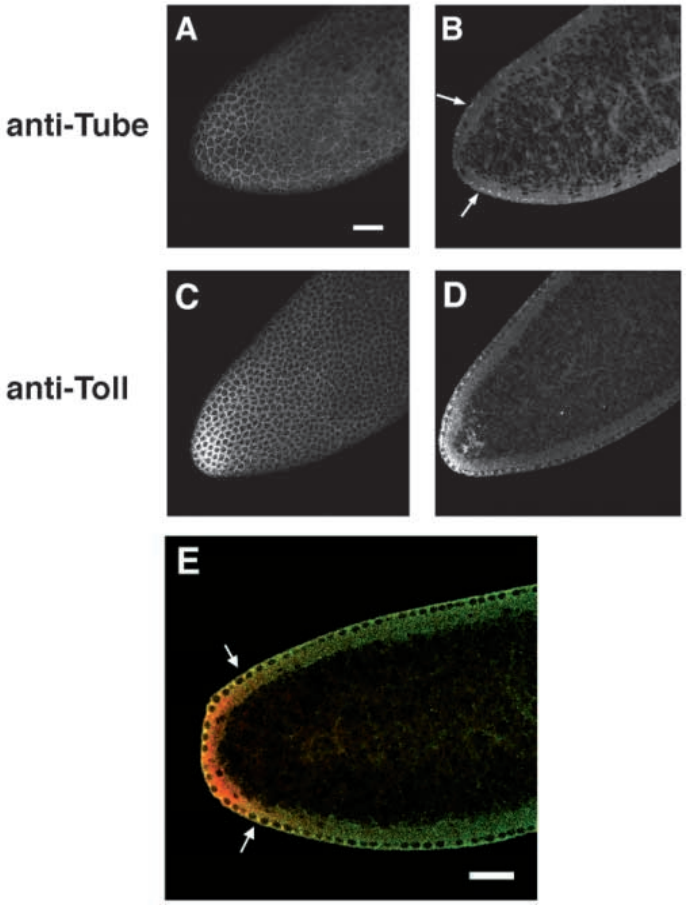

Fig. 5. The *P[Toll^{10b}-bcd]* transgene induces anterior localization of Tube. All photographs are of embryos from females carrying the *P[Toll^{10b}-bcd, w+]* transgene analyzed by confocal microscopy. (A) Surface view and (B) longitudinal optical cross section of the anterior end of an early syncytial blastoderm embryo stained with anti-Tube sera detected with a Cy3-conjugated secondary antibody. Note the bright clusters of Tube at the anterior pole (arrows). (C) Surface view and (D) longitudinal optical cross section of an early syncytial blastoderm embryo stained with anti-Toll sera detected with a Cy3-conjugated secondary antibody. Scale bar, 40 μm for A-D. (E) Longitudinal optical cross section of a syncytial blastoderm embryo stained with anti-Tube conjugated to fluorescein (green) and anti-Toll detected with a Cy5-conjugated secondary antibody (red). Co-localizing clusters of Tube and Toll appear as yellow spots (arrows). Scale bar, 45 μm.

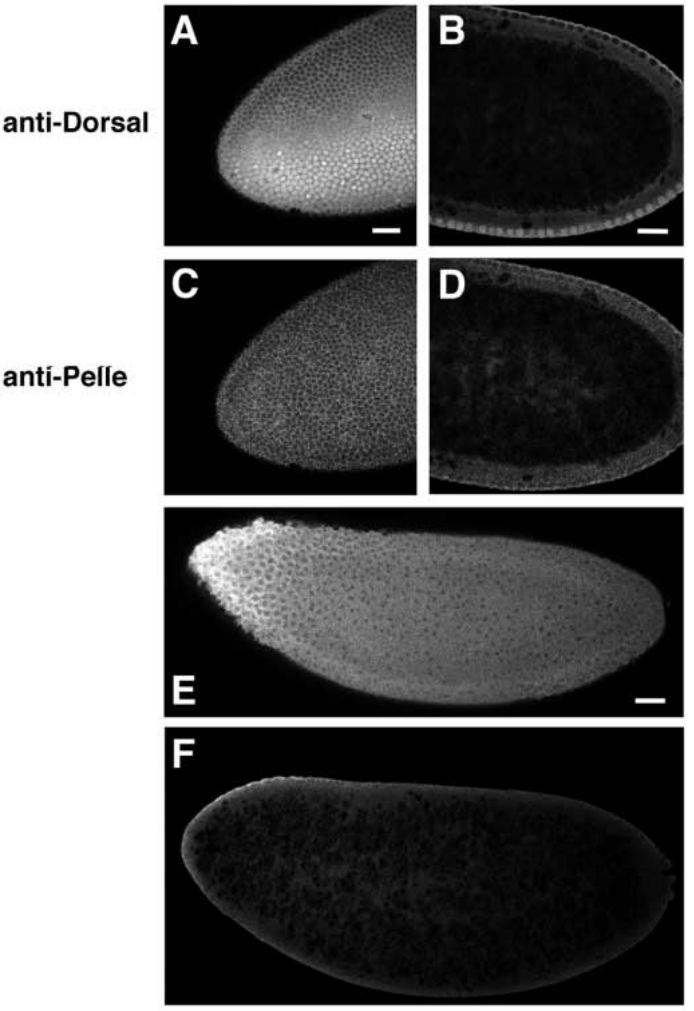

Fig. 6. Activated Toll recruits Pelle to the plasma membrane. All images are of embryos oriented with anterior to the left and analyzed by confocal microscopy. (A-D) Paired images of embryos from wild-type females labeled with fluorescein-conjugated anti-Dorsal sera (A,B) and anti-Pelle sera detected with a Cy3-conjugated secondary antibody (C,D). (A,C) Surface view of the anterior end of a mid-stage 4 (syncytial blastoderm) embryo; scale bar, 30 μm. (B,D) Longitudinal optical cross section of a syncytial blastoderm embryo; scale bar, 27 μm. (E,F) Embryos generated by females carrying the *P[Toll^{10b}-bcd, w+]* transgene were labeled with anti-Pelle sera as described above; scale bar, 30 μm. (E) Surface view of an early syncytial blastoderm embryo. (F) Longitudinal optical cross section of an early syncytial blastoderm embryo.

indicated that signal-dependent protein relocalization is enhanced in embryos expressing high levels of Toll^{10b} protein at the anterior pole. We therefore also examined Pelle localization in embryos generated by P[*Toll^{10b}-bcd*] females.

A large fraction of the Pelle protein localized to the anterior end of P[*Toll^{10b}-bcd*] embryos (Fig. 6E), indicating a significant recruitment or stabilization of Pelle protein at this pole. Confocal cross sections reveal that the anterior-localized Pelle is predominantly at the embryonic periphery, possibly in association with the plasma membrane (Fig. 6F). High levels of activated Toll thus promote localization of both Tube and Pelle to the surface of the embryo. Since Tube localization at the anterior end of P[*Toll^{10b}-bcd*] embryos mirrors that in the ventral portion of wild-type embryos, we propose that the localization of Pelle in P[*Toll^{10b}-bcd*] embryos similarly reflects a relocalization occurring in wild-type embryos in response to Toll activation.

DISCUSSION

Tube and Pelle transmit signaling information from activated Toll receptors to the Dorsal-Cactus complex. We find that both Tube and Pelle undergo translocation to sites of Toll activation.

Previous studies have demonstrated that Toll activation also alters the subcellular distribution of Dorsal and Cactus (Roth et al., 1989; Rushlow et al., 1989; Steward, 1989; Bergmann et al., 1996; Reach et al., 1996). Toll-mediated signaling thus brings about a redistribution of each of the genetically defined, downstream pathway components. We consider below the implications of these results for the mechanism of signal transduction and for the patterning of the syncytial embryo.

Tube exhibits two modes of membrane association

We previously found that much of the Tube protein in embryos associates with the embryo surface independent of the activity or presence of the Toll receptor (Galindo et al., 1995). Here we show that a fraction of the Tube protein in embryos concentrates at the cell surface in response to Toll activation. Furthermore, we demonstrate that direct association of Tube with the plasma membrane, via a membrane anchor, is sufficient to induce signaling. Together, these results indicate that Tube associates with the embryo surface in both a presignaling state and an activated/signaling state.

The presignaling state of Tube might reflect an association with the submembranous cytoskeleton. Binding of Tube to a cytoskeletal component could position Tube near the intracellular domain of Toll without bringing about a membrane-induced activation of Tube. In this regard, we have recently demonstrated that both Tube and Toll interact with the actin-binding protein Filamin in a yeast two-hybrid assay (Edwards et al., 1997).

The association of Tube at or near the plasma membrane in a presignaling state might provide a pool of Tube protein for rapid and efficient recruitment upon binding of ligand to Toll. Since Tube binds to Dorsal in embryos (Edwards et al., 1997), membrane associated Tube could also bring the Dorsal/Cactus complex close to sites where signaling will occur.

Our studies reveal that Toll activation induces the transition to a signaling state marked by the localized clustering of Tube. As reported previously (Huang et al., 1997), the constitutively active Toll^{10b} protein forms clusters of similar appearance and location when expressed at high levels. It may be, therefore, that ligand-induced oligomerization of Toll induces Tube oligomerization. Tube self-association could in turn bring about a conformational change that activates signaling by, for example, unmasking a Pelle binding site. Tube aggregation could also underlie the activity of Src-Tube, since the direct association of Tube with the plasma membrane might be sufficient to induce oligomerization in the absence of Toll activation.

Activated Toll promotes recruitment of Pelle to the plasma membrane

The constitutive activity of Src-Pelle and the membrane association of Pelle at the anterior pole of P[*Toll^{10b}-bcd*] embryos provide substantial evidence that Pelle acts at the plasma membrane to transduce signals from Toll to the Dorsal/Cactus complex. Nevertheless, we do not detect a dorsoventral asymmetry in the wild-type localization of Pelle. We suggest that Pelle relocalizes in the wild type in response to signaling, but that this relocalization involves only a subpopulation of the Pelle protein in embryos. The high levels of Pelle in the anterior half of P[*Toll^{10b}-bcd*] embryos might also reflect a stabilization of Pelle within the signaling complex. Since the essential activity of Pelle in signaling is catalytic (Shelton and Wasserman, 1993), a small number of activated Pelle molecules may be sufficient to mediate signaling to many effector molecules. There is in fact good evidence that the amount of Pelle in embryos greatly exceeds the level required to mediate wild-type signaling (see, e.g. Muller-Holtkamp et al., 1985).

Pelle might differ from Tube not only in the amount of protein that associates with the plasma membrane, but also in the duration of this association. Although Pelle binds specifically to Tube in vitro and in yeast cells (Großhans et al., 1994; Galindo et al., 1995), complexes of Tube and Pelle have not been detected in embryos (Bergmann, 1996; Edwards et al., 1997). Moreover, Pelle can phosphorylate Tube in vitro (Großhans et al., 1994) and catalytically inactive Pelle exhibits an enhanced affinity for Tube in the two-hybrid system (Edwards et al., 1997). We suggest, therefore, that as a consequence of Toll activation Pelle binds to Tube at the plasma membrane, but that Pelle rapidly dissociates upon phosphorylating Tube.

Restriction of signaling to plasma membrane domains

Many signal transduction pathways use relocalization of one or more components to initiate intracellular signaling (Carraway and Carraway, 1995; Mochly-Rosen, 1995). The β-adrenergic receptor kinase is recruited from the cytoplasm to the β-adrenergic receptor upon interaction of the receptor with its ligand (Benovic et al., 1986; Strasser et al., 1986). Raf, a protein kinase in the same sequence subfamily as Pelle, is also activated when localized to the membrane (Leevers et al., 1994; Stokoe et al., 1994). This activation is the result of Raf phosphorylation by a kinase tightly associated with the plasma membrane (Jelinek et al., 1996; Marais et al., 1995). Moreover, IRAK, a vertebrate Pelle homolog, becomes phosphorylated upon formation of a complex with the activated IL-1 receptor (Cao et al., 1996). Comparable phosphorylation of Pelle upon its association with the plasma membrane could activate Pelle and thereby trigger downstream signaling.

Signaling efficiency is often increased by targeting a kinase to the plasma membrane, as is illustrated by phosphoinositol 3-kinase (PI-3K). PI-3K signals in the absence of growth factor stimulus when targeted to the membrane by lipid modification. PI-3K also signals without receptor stimulation if a high specific activity form of PI-3K is expressed, and signaling from PI-3K is maximal when both the high specific activity and lipid modification mutations are combined in one protein (Klippel et al., 1996). These results suggest that targeting to a membrane subdomain sometimes sequesters a kinase near its physiological substrates rather than activating the kinase. In this regard, the interactions of Dorsal with Pelle and Tube (Edwards et al., 1997; Yang and Steward, 1997) might for the most part serve to increase the efficiency of signaling by maintaining proximity among the various signaling components.

In the *Drosophila* embryo the maintenance of optimal signaling efficiency may be critical, since the dorsoventral positional cue is produced as a gradient of ligand in the eggshell (Stein et al., 1991; Morisato and Anderson, 1994; Schneider et al., 1994). This graded information must be accurately transmitted into the embryo to form a gradient of

nuclear Dorsal protein. The interactions among Tube, Pelle and Dorsal, as well as the restriction of signaling to specific submembranous domains, might prevent diffusion of activated signaling components, and thereby maintain the shape of the external gradient.

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