Relocalization of *Drosophila* dorsal protein can be induced by a rise in cytoplasmic calcium concentration and the expression of constitutively active but not wild-type Toll receptors

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The generation of dorso–ventral polarity in *Drosophila* relies on the formation of a nuclear gradient of the rel/nuclear factor κ B transcription factor dorsal in the pre-cellular syncytial embryo by a process of differential nuclear localization. It is thought that the gradient is formed by activation at ventral positions of the membrane receptor Toll that in turn causes the local dissociation of dorsal from the cytoplasmic anchor protein cactus. Although Toll is related in its cytoplasmic domain to the interleukin-1 receptor little is known about the signal transduction pathways that lead from Toll to the relocalization of dorsal. In this paper we have used immunofluorescence microscopy as a direct assay of dorsal protein nuclear localization in the *Drosophila* cell line

Schneider 2. We find that increased cytoplasmic calcium concentration and the expression of constitutively active Toll receptors can induce the relocalization of dorsal. By contrast, we find that activation of endogenous protein kinase A and expression of wild-type Toll receptors, which activate *zen*–chloramphenicol acetyltransferase reporter genes in this system, have only a marginal effect on the cellular distribution of the dorsal protein. Treatment of cells with activators of protein kinase C and radical oxygen intermediates, both of which activate nuclear factor κ B, also has little effect on dorsal protein localization. We propose that different threshold levels of dorsal activation can be established by distinctly regulated signal transduction pathways.

INTRODUCTION

The dorsal protein is the terminal member of the morphogenetic pathway that leads to dorso–ventral pattern formation in the early embryo of *Drosophila* (for a review, see Govind and Steward, 1991). Mutations of *dorsal* and of ten other maternal effect genes (the dorsal group) result in a common phenotype in which the blastoderm embryo fails to elaborate the normal dorso–ventral pattern elements, with all cells developing along a pathway that is normally taken by the dorsal-most cells. The mRNA encoding dorsal protein is distributed uniformly and translated in the cytoplasm, but at the syncytial blastoderm stage of development the protein forms into a dorso–ventral nuclear gradient by a process of differential nuclear localization (Roth et al., 1989; Rushlow et al., 1989; Steward, 1989). It is now thought that such position-dependent localization of dorsal is caused by the activation at ventral positions of a uniformly distributed receptor encoded by another dorsal group gene, *Toll*. The Toll receptor is related in its cytoplasmic sequence to mammalian T-cell interleukin-1 receptors, and in its extracytoplasmic domain to the human platelet receptor glycoprotein 1b (Hashimoto et al., 1988; Keith and Gay, 1990; Gay and Keith, 1991; Schneider et al., 1991). At least two other dorsal group genes (*snake* and *easter*) (Delotto and Spierer, 1986; Chasan and Anderson, 1989) encode secreted serine proteinases, while expression of three other genes (*pipe*, *windbeutel* and *nudel*) is required somatically (Stein et al., 1991; Stein and Nüsslein-Volhard, 1992). The current view of ventrally restricted activation is that a proteolytic cascade is activated in response to a signal that is present or available only at ventral positions, and that an activated proteinase processes a precursor ligand to an active form (Gay

and Keith, 1992; Hecht and Anderson, 1992; Stein and Nüsslein-Volhard, 1992).

At present, little is known of the biochemical nature of the signal transduction pathway that is mediated through Toll receptor, although it would appear to rely on the function of two further intracellular dorsal group products (tube and pelle) (Letsou et al., 1991; Shelton and Wasserman, 1993) and is very probably analogous to that of the interleukin-1 receptor (Heguy et al., 1992). The dorsal protein is known to be a transcription factor that activates the zygotic genes that are directly responsible for dorso–ventral differentiation (Ip et al., 1991). It is related in sequence to the *v-rel* oncogene and to the activator of κ immunoglobulin light chain gene expression, nuclear factor- κ B (NF- κ B), both of which also function as transcription factors (for a review, see Schmitz et al., 1991). NF- κ B binds DNA as a heterodimer of two related subunits and is held in the cytoplasm by association with a third inhibitory subunit inhibitor- κ B (I κ B) (Baeuerle and Baltimore, 1988). NF- κ B can be activated by the treatment of cells with a variety of different agents, including calcium ionophores, phorbol esters and cytokines (Baeuerle, 1991), and the NF- κ B–I κ B complex can be activated *in vitro* (as judged by the conversion to a DNA-binding form) by phosphorylation of I κ B, possibly by protein kinase C (PKC) (Ghosh and Baltimore, 1990). NF- κ B can also be activated by radical oxygen intermediates, perhaps by a separate pathway involving proteolysis (Schreck et al., 1991). In this regard, recent results indicate that the activation of NF- κ B is accompanied by the rapid degradation and subsequent resynthesis of I κ B, findings that suggest that activation may involve the direct proteolysis of I κ B or modification followed by dissociation and degradation (Brown et al., 1993). It has also been shown recently that the p50

Abbreviations used: I κ B, inhibitor- κ B; IL1, interleukin 1; NF- κ B, nuclear factor- κ B; PKA, cyclic-AMP-dependent protein kinase; PKC, protein kinase C; SL2, Schneider 2; DAPI, 4',6'-diamidino-2-phenylindole.

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subunit of NF- κ B is synthesized as a 105 kDa precursor that contains an ankyrin repeat domain. The precursor is thought to be retained in the cytoplasm by intramolecular interaction of the ankyrin repeat sequences and the rel homology region (Naumann et al., 1993).

The dorsal protein forms an oligomeric complex *in vivo* (Govind et al., 1992), and is anchored in the cytoplasm by interaction with the *Drosophila* I κ B homologue, cactus (Roth et al., 1991; Geisler et al., 1992; Kidd, 1992). Previous work has shown that full-length dorsal protein, when expressed in the *Drosophila* cell line Schneider 2 (SL2), is anchored in the cytoplasm (Rushlow et al., 1989) probably by interaction with cactus, which is known to be expressed in these cells. More recent work has indicated a role in signal transduction for cyclic-AMP-dependent protein kinase (PKA) and for wild-type and dominant Toll receptors (Norris and Manley, 1992). In this paper, we report that activation of the endogenous PKA of SL2 cells does not cause translocation of dorsal, but increased cytoplasmic Ca²⁺ is effective in relocating the protein. Furthermore, we find that induced expression of constitutively active but not of wild-type Toll receptors will cause the nuclear localization of dorsal.

MATERIALS AND METHODS

Tissue culture techniques

SL2 (Schneider, 1972) was cultured in M3 medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Shields and Sang, 1977) at 26 °C as described (DiNocera and Dawid, 1983). Plasmids pACdlWT and pACdl560 were a gift of C. Rushlow (Rushlow et al., 1989). Large-scale preparation of plasmids for transfection was by caesium chloride centrifugation, followed by spermine precipitation (Hoopes and McClure, 1981; Maniatis et al., 1982). Transient transfection of SL2 cells was by the calcium phosphate precipitation method (DiNocera and Dawid, 1983).

Assay of cyclic AMP in culture cells

The binding protein assay described by Farndale et al. (1992) was used.

Assay of PKA activity

Logarithmically growing SL2 cells (1–4 × 10⁶ cells/ml in M3 medium) were collected and washed with PBS. Cells were suspended at 2–5 × 10⁶ cells/ml in BSS (55 mM NaCl, 40 mM KCl, 15 mM MgSO₄, 1 mM CaCl₂, 20 mM glucose, 50 mM sucrose, 2 mg/ml BSA, 10 mM Tricine, pH 6.95). Cells were collected and resuspended in lysis buffer (60 mM Mes pH 6.5, 15 mM magnesium acetate, 1 mM EGTA, 100 mM NaCl, 1 mM dithiothreitol, 50 μ M phenylmethanesulphonyl fluoride, 10 μ g/ml leupeptin, 0.1% Triton X-100), then lysed on ice by being squeezed out through a needle. Lysates were cleared by centrifugation at 15000 *g* for 10 min at 4 °C. The assays were carried out as described by Kemp et al. (1977). The reaction mixture contained 60 mM Mes (pH 6.5), 15 mM magnesium acetate, 1 mM EGTA, 100 μ M [γ -³²P]ATP (550 c.p.m./pmol), 100 μ M Kemptide and 20–40 μ g of lysate in a final volume of 40 μ l. For measurement of fully induced activity, reactions were carried out in the presence of 10 μ M cyclic AMP. For inhibition studies, PKA inhibitor peptide (Sigma) was added to a final concentration of 10 nM (*K*_i = 2.3 nM). After incubation at room temperature for 10 min, reactions were terminated by adding 10 μ l of 25% (w/v) trichloroacetic acid and placed on ice for 10 min. Supernatant was recovered and spotted on to phosphocellulose paper

(Whatman P81). The phosphocellulose pieces were washed with 75 mM phosphoric acid five times and counted as described by Casnellie (1991).

Production and purification of anti-dorsal antibody

The dorsal expression plasmid was a gift from C. Rushlow (Rushlow et al. 1989). The production of protein for inoculation was as described previously (Keith and Gay, 1990). Antibody production was in rats. Anti-dorsal antibodies were purified by affinity chromatography as described previously (Keith and Gay, 1990).

Immunofluorescent microscopy

This was performed as described previously (Gay et al., 1988), except that cells were attached to coverslips coated in poly(L-lysine) (Harlow and Lane, 1983). The secondary antibody used was fluorescein-conjugated sheep anti-rat whole antibody (Amersham) for dorsal staining, and rhodamine-conjugated goat anti-rabbit whole antibody for Toll staining. The nucleus was stained with the DNA-specific dye 4',6'-diamidino-2-phenylindole (DAPI) (0.5 μ g · ml⁻¹).

Ionomycin assay

Transiently transfected cells were first washed in PBS (7 mM Na₂HPO₄, 3 mM NaH₂PO₄ and 150 mM NaCl) then resuspended at 2 × 10⁶ cells per ml in PBS, 1 mM MgCl₂ and 1 mM CaCl₂. Samples were then stimulated with reagents at 26 °C and analysed by immunofluorescence. The results were quantified by counting the localization of dorsal protein in at least 100 expressing cells. Dorsal-expressing cells were judged 'nuclear' if the immunofluorescent signal was located totally or predominantly within the nucleus, and 'cytoplasmic' if the staining was excluded from the nucleus (see Figure 5a compared with Figure 5d).

Monitoring intracellular calcium in SL2 cells

Cells were assayed as described previously (Tsien et al., 1982), except that loading was achieved by incubation with 40 mM quin 2 AM ester in PBS, 1 mM MgCl₂ and 1 mM CaCl₂ at 26 °C for 1 h with continuous mixing. Cells were then washed with PBS, 1 mM MgCl₂ and 1 mM CaCl₂, and assays were performed in this buffer. It was found that incubation with lower concentrations of quin 2 AM ester did not result in sufficient loading and hydrolysis of the ester for measurement. The saturation of quin 2 with Ca²⁺ was achieved by the addition of 1 μ M ionomycin in ethanol; although this is a relatively high value, it was found that lower concentrations did not saturate the quin 2 (see Figure 3). The background signal from the cells (autofluorescence and scattering) was determined by the addition of 0.5 mM MnCl₂. Calculation of the proportion of Ca²⁺–quin 2 was achieved using the method of Hesketh et al. (1983) using the equation

$$\text{Ca}^{2+}\text{-quin 2} = \frac{(I - 0.16\Delta I)}{0.84\Delta I} \%,$$

where *I* is the measured fluorescence corrected for background and ΔI is the corrected saturation fluorescence.

Construction of expression plasmid F9-Toll¹⁰⁸

A 3.2 kb *Sna*BI–*Ssp*I fragment from p18NH (Keith and Gay, 1990) was cloned into M13mp10 to produce SNS5. A G-to-A

base change was introduced into the SNS5 DNA at position 2918 in the Toll sequence (Hashimoto et al., 1988) by oligonucleotide-directed mutagenesis. This causes the Cys⁷⁸¹→Tyr mutation found in the class 1 dominant Toll^{10B} receptor (Schneider et al., 1991). The mutant DNA was prepared from replicative form SNS5^{10B} by digestion with *SacI* and *HindIII*, flush-ended and ligated with F9 DNA as described previously (Keith and Gay, 1990).

RESULTS

Forskolin but not ionomycin causes a rise in intracellular cyclic AMP in SL2 cells

The diterpene forskolin elicits large rises in cyclic AMP concentration in most cell types by stimulating the activity of adenylate cyclase enzymes (Seamon and Daly, 1986). We have measured the effect of forskolin on the intracellular cyclic AMP concentration of SL2 cells, and find that treatment results in a greater than 40-fold increase in [cyclic AMP]_i. The increase is induced rapidly, and is enhanced by the presence of phosphodiesterase inhibitor isobutylmethylxanthine to levels that indicate a minimum [cyclic AMP]_i of 4 μ M and 6 μ M in the presence of isobutylmethylxanthine (Figure 1). The K_m for cyclic AMP of the *Drosophila* PKA is in the region of 0.1 μ M (Foster et al., 1984), and thus this kinase is likely to be fully activated on treatment

Figure 1 Effect of forskolin and ionomycin on intracellular cyclic AMP in SL2 cells

Cells were treated with forskolin (100 μ M) ($\square$), forskolin and isobutylmethylxanthine (IBMX) (10 μ M) ($\bullet$) or ionomycin (10 μ M) and IBMX ($\diamond$). Samples were taken and cyclic AMP assayed at the indicated times, as described in the Materials and methods section.

Table 1 PKA activities in SL2 cells

Abbreviation used: PKI, peptide inhibitor of protein kinase A.

Extract	Phosphorylation activity to Kemptide (%)
No addition	33.9
+100 nM PKI	25.8
+10 μ M cyclic AMP	100.0
+100 nM PKI +10 μ M cyclic AMP	29.6

with forskolin. By contrast, treatment of cells with ionomycin, which is shown below to induce a rapid and saturating rise in $[Ca^{2+}]_i$, has no detectable effect on the [cyclic AMP]_i of SL2 cells.

Cyclic AMP-dependent and -independent protein kinase activities present in SL2 cells

To confirm that SL2 cells contain a PKA activity, we have assayed cell extracts using the PKA peptide substrate, Kemptide. The results of such experiments are summarized in Table 1, and show that untreated SL2 cells contain a significant kinase activity for Kemptide. The peptide inhibitor of PKA, PKI, inhibits about 25% of this basal activity, while addition of cyclic AMP stimulates the basal activity by 3-fold. These experiments show that SL2 cells contain a cyclic-AMP-inducible PKA activity, and also additional activities with similar substrate specificity but with distinct regulation to PKA.

Forskolin, phorbol esters and radical oxygen intermediates do not cause a significant alteration in the localization of the dorsal protein

To investigate the involvement of the various characterized signal transduction pathways in the regulated nuclear localization of dorsal, we have examined the distribution of dorsal in transfected SL2 cells after treatment with agents that activate PKA and PKC, with calcium ionophores and with radical oxygen intermediates. Treatment of cells with an activator of PKC (phorbol 12-myristate 13-acetate 1–10 μ M) or with radical oxygen intermediates (hydrogen peroxide, 250 μ M) has no effect upon dorsal protein localization (Keith, 1992). Treatment of cells with forskolin (50 μ M and 100 μ M), shown above to activate endogenous PKA, has only a marginal effect, causing an around 5% increase in cells scored as nuclear (Table 2).

Ionomycin induces relocation of dorsal protein to the nucleus

In contrast to the marginal effect on the cellular localization of dorsal elicited by forskolin, treatment of the cells with the calcium ionophore ionomycin (10 μ M) causes the protein to redistribute and to become predominantly nuclear in localization in approx. 40% of cells (Table 2, Figure 2). Treatment of cells with phorbol ester phorbol 12-myristate 13-acetate in addition to the ionophore marginally enhanced its effect (Table 2). The presence in the assay of the calcium chelator EGTA prevents the relocation of dorsal (Table 2), a result that shows that the observed effect is dependent on calcium and is not an artefact of ionomycin treatment. The electroneutral calcium ionophore A23187 (1 μ M) is also effective in the assay (Keith, 1992). Since much lower doses of ionomycin can induce rapid saturating rises of $[Ca^{2+}]_i$ in other cell types (Hesketh et al., 1983), the effect of the ionophore treatment on $[Ca^{2+}]_i$ of SL2 cells has been

Table 2 Effect of ionomycin and forskolin on cellular localization of dorsal protein

Treatment	Nuclear localization (%)
Control	3.5
Ionomycin	35.8
Ionomycin + EGTA	7.2
Ionomycin + phorbol 12-myristate 13-acetate	40.1
Forskolin	8.3

Figure 2 Localization of dorsal protein expressed in SL2 cells

Cells transiently transfected with pACdIWT were stimulated with 10 μ M ionomycin (**c, d**) or ethanol (**a, b**), and examined by DAPI fluorescence (**a, c**) or immunofluorescence (**b, d**) microscopy.

Figure 3 Effect of ionomycin on $[Ca^{2+}]_i$ of SL2 cells

Cells (2.8×10^6 cells per assay) were loaded with quin 2 AM ester, as detailed in the Materials and methods section. The cells were stimulated with the concentrations of ionomycin indicated. The cells were then permeabilized with Triton X-100 (TX100).

monitored directly (Figure 3) (see the Materials and methods section). Treatment of the cells with concentrations of ionomycin greater than 1 μ M elicits a rapid saturating rise in $[Ca^{2+}]_i$. However, at lower concentrations ionomycin has little effect, although a slow non-saturating rise is detected. An analysis of the relocalization of dorsal induced by various doses of ionomycin (Figure 4) shows that only concentrations causing a saturating rise induce the response fully. Additional experiments designed to examine the kinetics of relocalization show that dorsal protein migrates rapidly to the nucleus after the stimulation of the cells, a process that appears to be complete after 5–10 min, which indicates that *de novo* transcription or translation is unlikely to be involved in the activation process (Keith, 1992).

Figure 4 Effect of ionomycin concentration on cellular distribution of dorsal protein

Cells were treated with the doses of ionomycin indicated and assayed for dorsal localization as described in the Materials and methods section.

Induced expression of dominant but not wild-type Toll receptors causes nuclear translocation of dorsal protein

Genetic experiments indicate that Class 1 dominant alleles of *Toll* encode constitutively activated receptors that are able to induce relocalization of dorsal in the absence of stimulation by ligand (Roth et al., 1989; Schneider et al., 1991). If dominant receptors can exert such an effect in SL2 cells, it would indicate that all the components of the embryonic signal transduction pathway are present. Accordingly, we have co-transfected SL2 cells with dorsal and either wild-type or Toll^{10B} expression plasmids. The Toll receptor genes were cloned into F9 (Keith and Gay, 1990), which places them under the control of the inducible heat-shock promoter. As shown in Figure 5, the expression of Toll^{10B} causes dorsal to become predominantly localized in the nucleus, whereas the expression of wild-type

Figure 5 Effect on dorsal protein of induced expression of dominant and wild-type Toll receptors

Cells were transfected with 0.5 μ g of pACdIWT and 10 μ g either of F9-Toll (a, b, c) or of F9-Toll^{10B} (d, e, f) plasmid and grown for 48 h. Toll expression was induced by heat shock for 30 min, and the cells were allowed to recover for 90 min. The cells were then stained for immunofluorescence with anti-dorsal (a, d) and anti-Toll (b, e) antibodies (see the Materials and methods section) and with DAPI (c, f).

receptors has no effect. Titration of transfecting receptor DNA indicates that even high doses of wild-type Toll-receptor-encoding plasmid have little effect on dorsal (Figure 6a), whereas low levels of transfecting Toll^{10B}-encoding DNA elicit a near maximal response (Figure 6b). Furthermore, at higher doses of Toll^{10B}-encoding plasmid, an effect is detectable in the absence of heat-shock induction, perhaps due to leaky expression of the receptor.

DISCUSSION

The results presented above show that increased $[Ca^{2+}]_i$ can act as a second messenger in the signal transduction pathway leading to the nuclear localization of dorsal. Although calcium- or calmodulin-dependent protein kinases have not been described in *Drosophila*, at least three such activities are known in other systems, one of which has a very similar substrate specificity to

PKA (Hanks and Quinn, 1991). Thus, it is possible that calcium may act through *Drosophila* homologues of this group of kinases. Alternatively, ionomycin may cause the activation of calcium-dependent proteinases and a consequent proteolytic degradation of cactus protein. Recent results that show that, in T lymphocyte cell lines, I κ B is rapidly degraded in response to stimulation with ionomycin and phorbol ester are consistent with such a hypothesis (Brown et al., 1993). We also find that phorbol esters, non-physiological activators of PKC, do not cause the relocation of dorsal. PKC has been implicated in the activation of NF- κ B *in vitro* (Ghosh and Baltimore, 1990), and it is possible that phorbol esters fail to activate insect PKC. However, recent work provides convincing evidence that endogenous PKC activities are induced in SL2 cells by the addition of the concentrations of phorbol esters used in this study (Gurker et al., 1991).

In contrast to a previous report (Norris and Manley, 1992), we observe only a marginal effect on the cellular distribution of

Figure 6 Relocalization of dorsal protein induced by transfection of SL2 cells with wild-type and dominant Toll receptors

SL2 cells were transfected with pActDlwt, and the amounts indicated either of F9-Toll or of F9-Toll^{10B}. The cells were stained for dorsal and for Toll as described in the Materials and methods section and scored for nuclear localization. (a) Toll^{10B} receptor. (●) heat-shock induced; (□) uninduced. Cells scored: 0 μg: 439(–), 358(+); 0.5 μg: 314(–), 372(+); 1.0 μg: 180(–), 333(+); 5.0 μg: 326(–), 328(+); 10.0 μg: 286(–), 256(+). (b) Wild-type receptor. (●) heat-shock induced; (□) uninduced. Cells scored: 0 μg: 437(–), 358(+); 0.5 μg: 357(–), 335(+); 1.0 μg: 148(–), 342(+); 5.0 μg: 311(–), 226(+); 10.0 μg: 250(–), 260(+).

dorsal when the endogenous PKA activity of SL2 cells is induced by treatment with forskolin. Although a small increase in the number of transfected cells scored as nuclear is observed, the effect on dorsal protein localization of forskolin is clearly distinct from that induced by ionomycin treatment or the expression of Toll^{10B} molecules. However, our observations would be consistent with the hypothesis that a subpopulation of dorsal molecules is sensitive to activation by PKA, or that PKA activation is slow and in equilibrium with dephosphorylation mediated by protein phosphatases. In this regard, it should be noted that the experiments of Norris and Manley involved overexpression of the PKA catalytic subunit, a situation that could alter the equilibrium between phosphorylation and dephosphorylation and cause phosphorylation of sites that are not natural substrates of the endogenous PKA. These workers also showed that the activation by wild-type Toll receptors could be inhibited *in vivo* by a PKA inhibitor peptide. However, we cannot demonstrate any effect of wild-type Toll receptor (see below) on dorsal. Furthermore, although PKA inhibitor peptides are very specific for PKA ($K_i = 2.3$ nM), they can inhibit other protein kinases of related specificity (Walsh and Glass, 1992). In view of this fact and because the *in vivo* concentration of the peptide in these experiments is unknown, this result should be treated with caution. Our finding that SL2 cells contain cyclic-AMP-independent PKA-like kinase activities suggests that the

PKA consensus phosphorylation sites present in the sequences both of dorsal and of cactus proteins might be substrates for protein kinases of related specificity but of distinct regulation to PKA. In view of the distinct effects of forskolin and of mutant dominant Toll receptors on the localization of dorsal, we suggest that PKA is unlikely to be involved directly in the signal transduction pathway mediated by Toll^{10B} receptors. However, it is possible that activation of dorsal complexes is hierarchical, and that distinct levels of activation can be achieved by different signal transduction pathways. The experiments of Norris and Manley used a *zen*-CAT reporter construct to assay the relocalization of dorsal. In the embryo, dorsal represses the transcription of *zen*, even at dorso-lateral positions where the concentration of dorsal is relatively low (Ray et al., 1991). Thus it may be that *zen*-CAT assays represent a hypersensitive measure of dorsal relocalization compared with our analysis. This proposal is reinforced by the recent report that the dorsal group gene *pelle* encodes a ubiquitous serine/threonine protein kinase related to the raf and mos oncogene products. This finding suggests that a kinase activity that is distinct from PKA is required to establish ventralizing and lateralizing levels of the nuclear dorsal protein (Shelton and Wasserman, 1993), but does not exclude a role for PKA at some other point in the signal transduction pathway.

Our finding that the induced expression of the class I dominant receptor Toll^{10B} causes the relocalization of dorsal confirms the findings of Norris and Manley (1992), who observed a 40-fold induction of a *zen*-CAT reporter construct when Toll^{10B} was cotransfected with dorsal. These workers also reported a 10-fold stimulation when wild-type Toll was cotransfected with dorsal. However, in our experiments, we could not detect an effect on dorsal localization that was a consequence of the induced synthesis of wild-type Toll receptor even at high doses of transfecting DNA. Titration of Toll^{10B} receptors indicates that, even at high doses of transfecting DNA, only approx. 50% of cells display a nuclear localization of the dorsal protein, a result that contrasts with the near 100% nuclear localization observed with the constitutively nuclear truncated forms of dorsal (Rushlow et al., 1989; Keith, 1992). This effect is not due to variable levels of expression of the receptor, as failure to relocalize dorsal does not correlate with low levels of expression of Toll, as judged by immunofluorescence. This finding suggests that a fraction of the cells cannot transduce the Toll receptor signal, and could be explained if the pathway is regulated in a cell-cycle-dependent manner. It is interesting to note that interaction between cactus and dorsal is mediated by a repetitive motif that is also found in proteins that are known to be regulated in a cell-cycle-dependent manner (Breedon and Nasmyth, 1987; Kidd, 1992). This hypothesis could be tested by inducing the expression of Toll^{10B} in synchronized cell cultures and examining the distribution of dorsal as the cells progress through the cell cycle.

Our results indicate strongly that all necessary components of the embryonic signal transduction pathway between Toll and dorsal are also present in SL2 cells, and that these cells can serve as a model system for the study of this pathway. However, it is possible that the differences between SL2 cells and the native environment for the effect, the syncytial blastoderm embryo, may cause some aspects of this model system to be inappropriate. As noted above, the pathway is likely to be analogous to that mediated by the interleukin 1 (IL1) receptor. The nature of the signals induced by IL1 remains controversial. In the murine T lymphoma cell line LBRM-33, activation requires stimulation with IL1 and phytohaemagglutinin or with calcium ionophore and phorbol ester, although IL1 alone does not cause elevated Ca²⁺ in these cells (Abraham et al., 1987). By contrast, recent reports provide evidence that IL1 causes an accumulation of

cyclic AMP and the activation of a tyrosine protein kinase in D10A cells (Munoz et al., 1992). This conflicting evidence could also be accounted for if different signal transduction pathways can mediate distinct levels of activation of NF- κ B.

The ability to regulate the Toll pathway specifically in SL2 cells by the induction of constitutively active receptors and to assay directly the redistribution of dorsal should facilitate further molecular studies of this important signal transduction pathway. In particular, changes in the phosphorylation states of dorsal and cactus on induction of the pathway may be assayed more readily than in the embryo.

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