Selective nuclear transport of the *Drosophila* morphogen *dorsal* can be established by a signaling pathway involving the transmembrane protein *Toll* and protein kinase A

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Establishment of dorsal–ventral polarity in the early *Drosophila* embryo requires a concentration gradient of the maternal morphogen *dorsal* (*dl*). This concentration gradient is established by selective nuclear transport of *dl* so that *dl* protein is present only in ventral nuclei. The activity of 11 genes is required for *dl* nuclear localization. One of these genes, *Toll*, encodes a transmembrane protein that appears to play the most direct role in regulating *dl* localization. We have examined the effects of *Toll* on *dl* in cotransfected Schneider cells to gain insight into the nature of the interaction between these proteins. We have found that *Toll* can enhance the nuclear localization of *dl* and, independently, the ability of *dl* to activate transcription once in the nucleus. We present evidence that the signaling pathway from *Toll* to *dl* involves protein kinase A (PKA) and that nuclear transport and activation of *dl* results from phosphorylation of *dl* by PKA. We discuss the significance of these results with respect both to *Drosophila* embryogenesis and to the regulation of the mammalian transcription factor NF- κ B.

[Key Words: *Drosophila* morphogen; *dorsal*; *Toll*; protein kinase A]

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Establishment of dorsal–ventral (D/V) polarity in the *Drosophila* embryo requires the activities of several maternal genes, including 11 that comprise the dorsal group (for review, see Anderson 1989; Rushlow and Arora 1990). One member of this group is the D/V morphogen *dorsal* (*dl*), a protein that shares homology, over the amino-terminal 300 amino acids, with the proto-oncogene *c-rel* and the mammalian transcription factor NF- κ B. This region includes a putative DNA-binding domain, a nuclear localization sequence (NLS), and a potential protein kinase A (PKA) phosphorylation site (Steward 1987; Ghosh et al. 1990; Kieran et al. 1990). The *dl* protein is distributed in a concentration gradient over the embryo, with the highest levels present in ventral regions and progressively decreasing levels in dorsal regions (Steward et al. 1988). This gradient results from the selective nuclear transport of the *dl* protein, which is uniformly cytoplasmic in the early embryo until cleavage cycle 10 when it is transported into ventral but not dorsal nuclei (Roth et al. 1989; Rushlow et al. 1989; Steward 1989). Following its accumulation in the nucleus, *dl* functions to influence the expression of several zygotic genes required for D/V polarity (for review, see Anderson 1987; Rushlow and Arora 1990).

The activity of the other dorsal group genes is essential

for the proper localization of the *dl* protein (Anderson and Nüsslein-Volhard 1986). In embryos that carry null mutations in any of these genes, ventral cells follow the developmental pathway of dorsal cells. The dorsalization of these embryos is the result of the absence of the *dl* protein in ventral nuclei, which results in the improper expression of zygotic genes. The function of the dorsal group gene products is to establish the *dl* nuclear protein gradient. Two of these genes, *easter* and *snake*, encode serine proteases (DeLotto and Spierer 1986; Chasan and Anderson 1989), suggesting that a cascade of interactions involving post-translational processing could result in the formation of the signal for *dl* movement into ventral nuclei. This signal is most likely localized in the perivitelline space on the ventral side of the embryo and is transmitted to *dl* through the product of the *Toll* gene (Hashimoto et al. 1988; Stein et al. 1991), which is localized uniformly in the embryo plasma membrane (Hashimoto et al. 1991). Genetic studies have shown that *Toll* is one of the last genes in the D/V cascade, acting upstream of *dl*, and cytoplasmic injection experiments have shown that *Toll* is responsible for establishing polarity in the embryo by defining the position of the D/V axis (Anderson et al. 1985a,b). These observations, along with the isolation of both dominant-ventralizing

as well as recessive-dorsalizing alleles of *Toll* (Anderson et al. 1985a; Schneider et al. 1991), suggest that the *Toll* gene product plays a particularly critical role in regulating *dl* localization. However, nothing is known about the mechanism by which *Toll* enhances nuclear transport of *dl*.

The *Toll* protein has a large extracellular domain that contains two blocks of leucine-rich repeats with adjacent cysteine-containing motifs (Hashimoto et al. 1988; Schneider et al. 1991) and a small cytoplasmic domain that is similar to the cytoplasmic domain of the interleukin-1 receptor (IL-1R) (Schneider et al. 1991). The overall structure of the *Toll* protein is most similar to the α chain of human platelet glycoprotein 1b (GP1b), a receptor for von Willebrand factor and thrombin (Lopez et al. 1987; Hashimoto 1988). The leucine-rich repeat and cysteine-containing motif in GP1b are required for thrombin binding, suggesting that these regions in the *Toll* protein may serve as ligand-binding sites (Keith and Gay 1990). The importance of these regions in *Toll* is indicated by the finding that three of the strongest *Toll*-dominant alleles contain point mutations in one of the cysteine-containing motifs (Schneider et al. 1991).

To study the mechanism of *dl* nuclear transport we have used transient cotransfection assays in *Drosophila* Schneider cells. These cells contain no detectable endogenous *dl* protein so the localization and activity of *dl* produced from transfected templates can be readily observed (Rushlow et al. 1989). In this system, exogenously produced *dl* protein was shown previously to be local-

ized primarily in the cytoplasm at low concentrations of expression vector, but increasingly in the nucleus at higher concentrations. The *dl* protein was also shown to activate expression of several reporter genes in a highly concentration-dependent manner (Rushlow et al. 1989). Here, we have used the cotransfection system as an assay to show that *Toll* can bring about selective nuclear transport of *dl* in Schneider cells, ruling out an absolute requirement of factors unique to the early embryo. Additional experiments indicate that the mechanism appears to involve phosphorylation of *dl* by activated PKA.

Results

We have used transient cotransfection assays in *Drosophila* Schneider cells to study the activity and nuclear transport of the *dl* protein. Figure 1 describes the principal plasmid constructs used in these studies. The cDNAs encoding *dl* and *Toll*, as well as PKA, were each cloned into an actin 5C expression vector so that their expression was driven by the actin 5C promoter, a strong promoter in Schneider cells (Han et al. 1989). The activity of the *dl* protein was measured by its ability to activate expression of the chloramphenicol acetyltransferase (CAT) gene from a 200-bp fragment (–196 to +39) of the *Drosophila zen* promoter (*zen*-CAT200). The *dl* protein was shown previously to activate CAT expression from this promoter in a strongly concentration-dependent manner, from 2- to >1000-fold (Rushlow et al. 1989). This promoter fragment contains no detectable *dl*-bind-

Figure 1. Structures of plasmid constructs. The *dl* protein shares homology with the *rel* and NF- κ B proteins over the amino-terminal 300 amino acids (solid area). This region contains a conserved PKA phosphorylation site, a NLS, and a putative DNA-binding domain. The carboxyl terminus contains proline (PRO)- and glutamine (GLN)-rich regions. Numbers refer to amino acid residues. The *Toll* protein has a large extracellular domain of 804 amino acids, with two blocks of leucine-rich repeats (LEU), adjacent cysteine motifs (CYS), and a small (269-amino-acid) cytoplasmic domain. Solid vertical rectangles indicate the locations of signal sequence and membrane-spanning domain. Numbers refer to amino acid residues. The *zen*-CAT200 reporter plasmid contains ~200 bp from the *zen* promoter (–196 to +39) controlling expression of the CAT gene. The pUC vector also contains SV40 poly(A) sequences.

ing sites, and the mechanism by which *dl* mediates activation is not known (see Discussion). However, for the purposes of the current study, this reporter construct provided an excellent assay for *dl* activity.

Activity of the *dl* protein in the presence of Toll

Genetic studies have identified the *Toll* protein as the most likely candidate for receiving and transmitting the signal that results in movement of the *dl* protein into ventral nuclei (for review, see Anderson 1989). To investigate the underlying mechanism, we wished to determine whether a functional interaction between these two proteins could be observed in a simplified system, that is, cultured cells. To this end, we cotransfected different concentrations of expression vectors containing *Toll* (Act-*Tl*) or *dl* (Act-*dl*) cDNAs, along with *zen*-CAT200, into Schneider cells and assayed the resultant CAT activities. All increases in CAT activity are presented relative to that measured from transfections that contained an equal amount of the actin 5C expression vector without a cDNA insert and were also normalized to an internal-control plasmid (see Materials and methods). Transfections containing the *dl* expression vector alone showed that, as observed previously (Rushlow et al. 1989), *dl* activates CAT expression in a concentration-dependent manner, with strong activations observed at concentrations >1.0 μ g and weaker, more gradually increasing activations at concentrations from 0.1 to 1.0 μ g (Fig. 2, and results not shown).

Cotransfection of Act-*Tl* with Act-*dl* resulted in significant enhancement of CAT expression (Fig. 2). The greatest effects were seen at moderate *dl* concentrations, where CAT activities were correspondingly modest. Thus, an activation of approximately ninefold was observed with 0.3 μ g of Act-*dl*, and activation was approximately sixfold with 0.4 μ g. In numerous independent experiments, we have always detected activations between 6- and 12-fold with optimal concentration of Act-*dl*. At lower amounts of Act-*dl*, activations induced by the expression of *Toll* were reproducibly weaker, perhaps reflecting a subthreshold concentration of *dl* protein in the transfected cells. At high *dl* concentrations (>1.0 μ g), no significant activation was observed with cotransfection of Act-*Tl* (results not shown), perhaps reflecting in part an inability to introduce enough Act-*Tl* to further enhance the strong activations brought about by Act-*dl* alone.

Several controls indicated that the observed increases resulted from a specific interaction involving *dl* and *Toll*. For example, cotransfection of Act-*Tl* with *zen*-CAT200 alone did not affect CAT activity (Fig. 2). Likewise, cotransfections of Act-*Tl* along with expression vectors encoding other *Drosophila* transcriptional activators (*prd*, *z2*, *zen*), together with their appropriate promoter-CAT reporter constructs (Han et al. 1989), did not alter the CAT expression observed with these expression vectors alone (results not shown). Thus, the enhancement of CAT activity induced by *Toll* appears to reflect a specific interaction with *dl*. Also, analysis of *dl* protein concen-

<i>dorsal</i>	- <i>Toll</i>	+ <i>Toll</i>
-	1	1.1
0.1	2.2	3.3
0.2	4.1	16
0.3	3.6	32
0.4	5.6	34
0.6	16	54
1.0	50	168

Figure 2. Activity of wild-type *dl* in the presence of *Toll*. Schneider cells were cotransfected with the indicated amount of *dl* expression vector (μ g), 3.0 μ g of the *zen*-CAT200 reporter plasmid, and either pAct5C (-*Toll*) or *Toll* expression vector (+*Toll*) to bring the final concentration of expression vector to 5.0 μ g. All transfections were normalized for differences in transfection efficiency by using *copia* β -gal as an internal control. The CAT activities shown are expressed relative to cotransfections containing 5.0 μ g of actin 5C expression vector without an insert.

tration by Western blotting showed that cotransfection of *Toll* did not affect the accumulation of *dl* protein (data not shown), ruling out the possibility that the effects of *Toll* were the result of increased levels of *dl*.

Expression of *Toll* results in increased nuclear localization of the *dl* protein

To determine whether the increased activation of CAT expression by *dl* in the presence of *Toll* reflected increased nuclear localization of the *dl* protein, cells were transfected with 0.4 μ g of Act-*dl* alone or 0.4 μ g of Act-*dl* plus 4.6 μ g of Act-*Tl*, fixed, and stained with anti-*dl* antibodies. Representative cells from each transfection are shown in Figure 3a. Cells that received Act-*dl* alone produced the *dl* protein that was localized predominantly in the cytoplasm (Fig. 3a,A). In contrast, *dl* protein was found in both the nucleus and cytoplasm, or in some cells predominantly in the nucleus, in cells cotransfected with Act-*dl* and Act-*Tl* (Fig. 3a,B). A population of stained cells that were transfected with 0.4 μ g of Act-*dl* and increasing amounts of Act-*Tl* were counted for cytoplasmic, nuclear and cytoplasmic, or nuclear localization, and the results are presented in Figure 3b. As the concentration of Act-*Tl* was increased, the number of cells with protein in both the cytoplasm and nucleus, or predominantly in the nucleus, increased by almost

Figure 3. Subcellular localization of *dl* in the presence of *Toll*. (a) Schneider cells were transfected with 0.3 μ g of *dl* (A) or 0.3 μ g of *dl* plus 4.7 μ g of *Toll* (B) and stained with anti-*dl* antibodies and TRITC-conjugated secondary antibodies. The *dl* protein accumulates predominantly in the cytoplasm of transfected cells in the absence of *Toll*. The *dl* protein is found in the nucleus as well as the cytoplasm of cells transfected with *Toll*. (b) Schneider cells were cotransfected with 0.3 μ g of *dl* and the indicated amounts of *Toll* expression vector (μ g) and stained with anti-*dl* and TRITC-conjugated secondary antibodies. At least 50 *dl*-expressing cells from each transfection were counted and scored for staining of the cytoplasm, the nucleus, or both the cytoplasm and nucleus. The percentage of cells in each category is shown.

threefold. These findings suggest that *Toll* functions to aid in relocalization of the *dl* protein from the cytoplasm to the nucleus of Schneider cells, analogous to its role in the early embryo.

The effect of *Toll* on *dl* mutants

Several *dl* mutants were analyzed to identify regions of the protein required for activity and regulation of nuclear localization. Figure 4a describes these mutants and lists the activity and localization observed in transfections containing 1.0 μ g of Act-*dl*, but in the absence of *Toll*. One of these mutants, *dl3*, has been described previously as *dl*-561, and transfections with this mutant showed that the carboxyl terminus is essential for cytoplasmic retention of *dl* (Rushlow et al. 1989). Deletion of (*dl7*), or point mutations in (*dl8*), the nuclear localization signal (NLS), resulted in restriction of *dl* to the cytoplasm, showing that the NLS is required for nuclear localization. Deletion of the carboxy-terminal unique region (*dl4*), or part of the *rel* homology region (*dl6*), resulted in a loss of *dl* activity even though these proteins were constitutively localized in the nucleus, indicating that these regions are required both for activity and for cytoplasmic retention. In contrast, the subcellular localization of *dl5*, which lacks the DNA-binding region (Ip et al. 1991) and is essentially inactive, is similar to wild type,

indicating that these sequences are not necessary for cytoplasmic retention.

These mutants were then used to examine regions of *dl* required for response to *Toll*. Figure 4b presents the results of cotransfection of several of the *dl* mutant expression vectors with Act-*Tl*. In cotransfections containing any of the *dl* mutants that were inactive by themselves, no increase in CAT activity was seen when *Toll* was present (*dl4*, *dl5*, *dl6*, *dl7*, *dl8*). These results show, for example, that the NLS is necessary for *Toll* responsiveness. Unexpected results were obtained with the *dl3* mutant, which lacks 117 carboxy-terminal amino acids but retains some activity ($\sim 30\%$) and is constitutively nuclear (Rushlow et al. 1989). Cotransfections of 0.4 μ g of Act-*dl3* with 4.6 μ g of Act-*Tl* resulted in enhancement of CAT expression by ~ 15 -fold, an activation significantly greater than that observed for wild-type *dl*. As *dl3* is tightly localized in the nucleus in the absence of *Toll*, this finding indicates that *Toll* can affect *dl* in a manner independent of its ability to direct *dl* to the nucleus, and raises the possibility that *Toll* expression can result in direct modification of *dl*. As mentioned above, further deletion of the carboxyl terminus (*dl4*) resulted in a protein that, by itself, was totally nuclear, inactive, and not influenced by the addition of *Toll*. However, when *dl4* was fused to the activation domain from the *Drosophila* *z2* homeo box protein (Han et al. 1989), a

Figure 4. Subcellular localization and activity of *dl* mutants alone and in the presence of *Toll*. (a) The top line indicates the region of *rel* homology (solid area), the NLS, the conserved PKA phosphorylation site, and the approximate position of proline (PRO) and glutamine (GLN) regions of the wild-type *dl* protein. The regions deleted in the mutants are shown. Numbers refer to amino acid residues. Schneider cells were cotransfected with 1.0 μ g of the indicated *dl* expression vector, 4.0 μ g of the actin 5C expression vector without the *dl*-coding region, and 3.0 μ g of the *zen*-CAT200 reporter plasmid. The predominant location of the protein in the cytoplasm (CYTO), the nucleus (NUC), or both is shown in column A. The ability of the protein to activate CAT expression is shown in column B. The CAT activities are expressed relative to cotransfections containing 5.0 μ g of actin 5C expression vector without the *dl*-coding region. (b) Schneider cells were cotransfected with the indicated amount of *dl* expression vector (μ g), 3.0 μ g of the *zen*-CAT200 reporter plasmid, and either pAct5C (- *Toll*) or *Toll* expression vector (+ *Toll*) to bring the final concentration of expression vector to 5.0 μ g. The *dl* mutants are referred to by the numbers used in a. Mutants 7 and 8 behave essentially identically, and representative values are shown.

protein displaying low but significant activity was produced (*dl9*; see Fig. 4a). Cotransfection of Act-*dl9* with Act-*Tl* enhanced the activity of the fusion protein (which is constitutively nuclear), but only by approximately twofold, suggesting that sequences in the *dl* unique region are in some way required for maximal activation by *Toll*.

Effect of *Toll* mutations on activation of *dl*

To begin to determine regions of *Toll* that are necessary for its function, we constructed and analyzed several *Toll* deletion mutants. Figure 5a details the structures of these mutants, and Figure 5b displays the results of cotransfections with Act-*dl*. A deletion of all but the amino-terminal 274 amino acids (RV) of *Toll* resulted in a total loss of *Toll* activity as measured by its ability to enhance the activation of CAT expression of *dl* from *zen*-CAT200. A small deletion (122 amino acids) into the carboxyl terminus (*StuI*) also abolished *Toll* activity, indicating that the intracytoplasmic domain is essential for activity. Because some receptors can be activated by

deletion of their extracellular domains (see Discussion), we deleted most of the extracellular domain of *Toll* (*Tl520*) to determine whether this might activate *Toll*. However, this deletion had no effect on *Toll* activity, as approximately equal CAT activities were detected in cotransfections with Act-*Tl520* and Act-*Tl*. This finding suggests that ligand binding is not required for *Toll* activation in Schneider cells (see Discussion).

Both dominant-ventralizing and recessive-dorsalizing alleles of *Toll* have been identified (Anderson et al. 1985a), and several of these alleles have been cloned and grouped into three classes (Schneider et al. 1991). One class consists of recessive alleles that result from point mutations in the cytoplasmic domain. This is consistent with our observation that an intact cytoplasmic domain is required for *Toll* activity. Another class contains dominant alleles that are amino-terminal truncations, although these ventralize only in the presence of wild-type *Toll*. The final class are dominant alleles that ventralize the embryo by facilitating nuclear transport of *dl* throughout the embryo (Roth et al. 1989; Steward 1989). The strongest of these is *Tl^{10b}* (Erdélyi and Szabad 1989),

Figure 5. The effect of *Toll* mutants on *dl* activity. (a) The extracellular and cytoplasmic domains, as well as the leucine (LEU)- and cysteine (CYS)-rich regions are indicated on wt *Toll*, and regions deleted in the mutants are shown. Numbers refer to amino acid residues. The site of the *Tl^{10b}* mutation is at residue 779, a change of cysteine to tyrosine. (b) Schneider cells were cotransfected with 0.4 μ g of *dl* expression vector, 3.0 μ g of the *zen*-CAT200 reporter plasmid, and either 4.6 μ g of pAct5C (– *Toll*) or 4.6 μ g of the indicated *Toll* expression vector (+ *Toll*). Fold activation is presented as the increase in CAT activity relative to the value from the cotransfection lacking a *Toll* expression vector.

which contains a single amino acid change of a cysteine residue to tyrosine at position 779 (Schneider et al. 1991). Although the mechanism by which this single residue change results in constitutive nuclear localization of *dl* in the embryo is not known, its existence offers a test of the physiological relevance of the cell culture assay that we have used to study *Toll* function. To address this, we cotransfected a construct expressing *Tl^{10b}* together with Act-*dl* and *zen*-CAT200. Expression of *Tl^{10b}* resulted in a 40-fold increase in *dl* activity, or approximately five-fold greater than that obtained with wild-type *Toll* (Fig. 5b), providing strong evidence that the mechanism by which *Toll* activates *dl* in Schneider cells is similar to the mechanism operative in the early embryo.

Expression of PKA results in the enhancement of *dl* activity and nuclear localization

The *dl*, NF- κ B, and *rel* proteins all contain a conserved PKA phosphorylation site directly upstream of their NLSs (Steward 1987; Ghosh et al. 1990; Kieran et al. 1990). Mutations in the *v-rel* PKA site can reduce or abolish the ability of the protein to transform chicken spleen cells while mutations in the *c-rel* PKA site can alter the subcellular localization of the protein (Mosialos 1991). A mutational analysis of the NF- κ B PKA site has not been reported, but the effects of several protein kinases on NF- κ B activity in vitro has been tested (Ghosh and Baltimore 1990). Protein kinase C (PKC) was shown to be most effective in activating NF- κ B DNA-binding, by disrupting NF- κ B/I κ B complexes, and PKA was also shown to activate NF- κ B, although not as effectively as PKC. While the mechanism of PKC activation was shown to involve direct phosphorylation of I κ B, inhibiting its ability to bind NF- κ B, PKA did not phosphorylate

I κ B, suggesting that it activates NF- κ B by a different mechanism. Given these observations, the conservation of the PKA site in all *rel* family members, and our finding that *Toll* enhances *dl* activity independent of subcellular localization, we wished to determine whether PKA plays a role in *dl* activation by *Toll*, perhaps by direct phosphorylation of *dl*.

We first asked whether *dl* activity could be influenced by the expression of exogenous PKA. To this end, a cDNA encoding the *Drosophila* PKA catalytic subunit (Kalderon and Rubin 1988) was cloned into the actin 5C expression vector (Act-PKA), and this construct was cotransfected into Schneider cells together with Act-*dl* and the *zen*-CAT200 reporter. Figure 6a displays the CAT activities obtained for Act-*dl* concentrations from 0.1 to 1.0 μ g with Act-PKA. As with Act-*Tl*, expression of Act-PKA enhanced the ability of *dl* to activate CAT expression. Although the responses obtained with both proteins were similar, PKA activated *dl* somewhat more efficiently than *Toll*. Activations >15-fold were obtained with PKA, compared with ~8-fold with *Toll*.

To investigate the regions of *dl* required for PKA activation, we tested the *dl* mutants shown in Figure 4a in cotransfections with Act-PKA and the results are presented in Figure 6b. As with Act-*Tl*, those mutants that lacked activity by themselves were not affected by the expression of PKA. However, the constitutively nuclear *dl3* was activated by PKA, showing that PKA, like *Toll*, affects both *dl* activity in the nucleus and *dl* nuclear localization (see below). Act-PKA was also cotransfected with Act-*prd*, Act-*z2*, and Act-*zen*, along with their appropriate promoter-CAT constructs, and found not to affect the activations detected with these proteins alone (results not shown). Therefore, the effect of PKA is specific for *dl* and requires a *dl* protein that retains some

a

<i>dorsal</i>	-PKA	+PKA
-	1	1.3
0.1	1.6	16
0.2	2.4	20
0.3	2.7	47
0.4	3.5	37
0.6	18	65
1.0	35	140

b

<i>dorsal</i>	-PKA	+PKA
wt	35	140
3	8.7	71
4	2.5	2.1
5	0.5	1.5
7,8	1.1	1.2
9	12	13

Figure 6. The effect of PKA on wild-type and mutant *dl* activity. (a) Schneider cells were cotransfected with the indicated amount of *dl* expression vector, 3.0 μ g of the *zen*-CAT200 reporter plasmid, and either pAct5C (-PKA) or PKA expression vector (+PKA) to bring the final concentration of expression vector to 5.0 μ g. The activation values are expressed relative to cotransfections containing the actin 5C expression vector without an insert. (b) Schneider cells were transfected with 1.0 μ g of *dl*, 3.0 μ g of the *zen*-CAT200 reporter plasmid, and either 4.0 μ g of pAct5C (-PKA) or 4.0 μ g of PKA expression vector (+PKA). The *dl* mutants are referred to by the numbers used in Fig. 4a.

activity and has an intact *rel* homology region, that is, the requirements for *dl* activation by PKA and *Toll* appear to be identical.

To determine whether expression of PKA also increased the nuclear localization of *dl*, cells were transfected with 0.4 μ g of Act-*dl* alone (Fig. 7, panel A) or 0.4 μ g of Act-*dl* plus 4.6 μ g of Act-PKA (panel B), fixed, and stained with anti-*dl* antibodies. Figure 7 presents pictures of representative cells from each transfection and shows that the expression of PKA resulted in an increased nuclear localization of *dl*. *dl* nuclear localization induced by PKA was more substantial than that observed

with *Toll*. In the presence of PKA, ~50% of the transfected cells showed strong nuclear staining compared with 25% showing predominantly nuclear staining in the presence of *Toll*.

Expression of a protein kinase inhibitor prevents activation of dl by Toll

The above results support the hypothesis that *Toll* activates *dl* by a mechanism involving PKA. To address this idea further, we tested the effect of the inhibitor protein of PKA (PKi) on *dl* activity in the presence of *Toll*. PKi has been analyzed in some detail, and peptide fragments with inhibitory activity have been isolated (Cheng et al. 1985; for review, see Kemp et al. 1988). The PKi peptides specifically inhibit cAMP-dependent protein kinase by binding to the catalytic subunit (for review, see Walsh and Glass 1991). An actin 5C construct containing 26 amino acid residues of PKi fused to *lacZ*-coding sequences, Act-*lacZ*PKi, was cotransfected with Act-*dl* and Act-*Tl*, and the resultant CAT activities were measured (see Materials and methods). This PKi construct encodes a fusion protein containing the 20-amino-acid peptide that has been shown to be the most potent inhibitor of PKA (Cheng et al. 1985). Coexpression of Act-*lacZ*PKi nearly abolished the enhancement of *dl* activity brought about by *Toll* (Fig. 8). This inhibition was specific for *dl* as the CAT activities induced by *z2* or *prd* were not altered by the addition of PKi (results not shown). Inhibition was the result of the PKi moiety in the *lacZ* fusion protein, as identical amounts of an Act-*lacZ* expression vector did not affect *dl* activity (Fig. 8). These results provide strong evidence that enhancement of *dl* activity induced by *Toll* involves activation of PKA.

Mutations in the PKA phosphorylation site of dl affect dl protein localization and activity in the presence of Toll and PKA

To determine whether activation of *dl* by *Toll* and PKA is dependent on the *dl* PKA site, we mutated the presumed site of phosphorylation, the serine (S) residue at position 312. We first changed this S residue to glutamine (Q) to create a site that should not be phosphorylated by PKA. This mutant, Act-*dl*Q, was then cotransfected with Act-*Tl* or Act-PKA, and the resulting CAT activities are shown in Figure 9a. Mutation of the PKA phosphorylation site resulted in only a slight reduction (less than twofold) in the activity of *dl* alone. However, the activation of CAT expression by *dl*Q in the presence of either *Toll* or PKA was reduced significantly relative to wild-type *dl*, in each case by as much as a factor of 10, providing strong evidence that the *dl* PKA site plays an important role in *Toll*-mediated regulation of *dl* activity.

To determine whether the decreased activity of *dl*Q in the presence of PKA or *Toll* reflected an inability of the mutant protein to be translocated to the nucleus, cells transfected with Act-*dl*Q alone (Fig. 9b, panel A), or with Act-*Tl* (not shown) or Act-PKA (panel B) were fixed

Figure 7. Subcellular localization of *dl* in the presence of PKA. Schneider cells were transfected with 0.3 μ g of *dl* (A) or 0.3 μ g of *dl* plus 4.7 μ g of PKA (B) and stained with anti-*dl* and TRITC-conjugated secondary antibodies. The *dl* protein accumulates predominantly in the cytoplasm of transfected cells in the absence of PKA (A). The *dl* protein is found predominantly in the nucleus of cells transfected with PKA (B).

and stained with anti-*dl* antibodies, and pictures of representative cells are shown. These experiments show that *dl*^Q, unlike wild-type *dl*, was not transported effectively to the nucleus in the presence of *Toll* or PKA (panel B), indicating that the *dl* PKA site is critical for regulated nuclear transport of the *dl* protein.

We then wished to determine the effect of placing a

Figure 8. Activity of *dl* in the presence of *Toll* and PKi. Schneider cells were cotransfected with the indicated amounts of *dl* and *Toll* expression vectors (μ g), 3.0 μ g of the *zen*-CAT200 reporter plasmid, and either 3.6 μ g of *lacZ*PKi or 3.6 μ g of actin *lacZ*. The final concentration of expression vector was adjusted to 7.0 μ g with the actin 5C expression vector. Fold activation is presented as the increase in CAT activity relative to a cotransfection containing 7.0 μ g of actin 5C expression vector without an insert.

negative charge at residue 312. Might this be sufficient to enhance nuclear localization in the absence of *Toll*? To address this, the S residue was changed to aspartic acid (D), creating the mutant Act-*dl*D. A similar mutation has been tested in the *rel* protein and was found to increase nuclear localization (Mosialos et al. 1991). Schneider cells were transfected with 0.4 μ g of Act-*dl*D, fixed, and stained with anti-*dl* antibodies as described above. In contrast to cells transfected with Act-*dl*, which had primarily cytoplasmic staining, cells transfected with Act-*dl*D displayed diffuse staining of both the cytoplasm and the nucleus (Fig. 9b, panel C). This pattern was unaffected by cotransfection with PKA or *Toll* (results not shown). We also assayed the activity of the *dl*D protein both alone and in the presence of *Toll* or PKA. The CAT activities presented in Figure 9a show that this mutation results in a protein that was not activated by *Toll* or PKA and whose activity alone was substantially reduced. This latter result is consistent with that observed with *v-rel*, where the corresponding S $\rightarrow$ D change also reduced the activity of the protein in a CAT assay (Mosialos et al. 1991). These findings suggest that a negative charge at position 312 can enhance nuclear localization, and strengthen the view that *Toll* (and PKA) function through serine-312.

Finally, to rule out the possibility that any change made in this region might alter *dl* activity, the proline (P) residue at position 311 was mutated to lysine (K). In the PKA consensus sequence this position can be any amino

Figure 9. Activity and subcellular localization of a *dl* phosphorylation site mutant in the presence of *Toll* and PKA. (a) Schneider cells were cotransfected with the indicated amount of wild-type and mutant *dl* expression vectors (μ g), 3.0 μ g of the *zen*-CAT200 reporter plasmid, and pAct5C, *Toll* expression vector (+*Toll*), or PKA expression vector (+PKA) to bring the final concentration of expression vector to 5.0 μ g. The activation values are expressed relative to cotransfections containing the actin 5C vector without an insert. (b) Schneider cells were transfected with 0.4 μ g of *dl*Q (A), 0.4 μ g of *dl*Q plus 4.6 μ g of PKA (B), or 0.4 μ g of *dl*D (C) and stained with anti-*dl* antibodies and TRITC-conjugated secondary antibodies. The *dl*Q protein accumulates predominantly in the cytoplasm of transfected cells in the presence or absence of *Toll* or PKA; the *dl*D protein accumulates in both the cytoplasm and nucleus.

acid (Kemp and Pearson 1990) so changing it should not affect the localization or activity of *dl* if phosphorylation is the underlying mechanism. This mutant, Act-*dl*RRKS, was cotransfected with *zen*-CAT200 with or without Act-*Tl* or Act-PKA, and the resulting CAT ac-

tivities were determined. These results (not shown) revealed that the activity of the *dl*RRKS protein was identical to wild-type *dl* protein, providing additional support for the idea that phosphorylation of serine-312 by PKA regulates *dl*.

Discussion

We have described a cell culture system in which the regulated nuclear transport and activity of the *dl* protein can be examined. We found that expression of either *Toll* or PKA together with *dl* resulted in an increased nuclear localization of *dl* and an enhancement of the ability of *dl* to activate CAT expression. We also observed that *dl* is somewhat more active, and more cells have strong nuclear *dl* staining when cotransfected with *dl* plus PKA than when cotransfected with *dl* plus *Toll*. This observation supports the notion, discussed below, that PKA is a downstream step in a signal transduction pathway that requires *Toll* to transmit the signal for *dl* nuclear localization to *dl* through PKA.

A model for nuclear localization and activation of *dl*

Our results support the model shown in Figure 10 for *dl* nuclear localization and activation. In this model *Toll* receives an extracellular signal that is ventrally localized in the perivitelline space of the embryo (Stein et al. 1991). Although the identity of this signal is unknown, it most likely functions by binding to or cleavage of the extracellular domain. Deletion of extracellular domains is a mechanism that can activate some receptors. The epidermal growth factor (EGF) receptor (*c-erbB*) can be activated by deletion of its extracellular domain and the oncogene *v-erbB* encodes only the transmembrane and cytoplasmic domain of *c-erbB* (Downward et al. 1984; Nilsen et al. 1985). Two of the *Drosophila* dorsal group genes, *snake* and *easter*, encode serine proteases that act upstream of *Toll* (DeLotto and Spierer 1986; Chasan and Anderson 1989), and either could activate *Toll* by cleaving the extracellular domain. However, the fact that many *Toll* dominant alleles contain point mutations in the extracellular domain (Schneider et al. 1991) perhaps supports ligand binding as the mechanism for *Toll* activation. Point mutations may mimic ligand binding by inducing the aggregation of receptors, resulting in their

activation. For example, some oncogenic forms of the *neu* (*erbB-2*) proto-oncogene contain point mutations in the extracellular domain, and at least one has been shown to lead to an increased aggregation of *neu* receptors (Weiner et al. 1989). Overexpression has also been shown to activate *erbB-2* (DiFiore et al. 1987). We suggest that in Schneider cells, *Toll* is also activated by overexpression and subsequent aggregation.

Once activated, *Toll* signals the nuclear localization and activation of *dl* through PKA. *Toll* most likely uses cAMP as a second messenger to activate PKA. Although we have no direct evidence for this, work with IL-1R supports this view. The IL-1R protein is a receptor for the interleukin 1 (IL-1) hormone, which is involved in mediating immune and inflammatory responses (Sims et al. 1989). The IL-1R and *Toll* cytoplasmic domains share extensive sequence similarity (Schneider et al. 1991), suggesting that they could transmit their signals by a similar mechanism. IL-1 has been shown to induce the expression of interleukin-2 receptors (IL-2R) and to induce thymocyte proliferation, in cells that express IL-1R, through stimulation of cAMP production (Shirakawa et al. 1988). IL-1, as well as cAMP and cAMP analogs, has also been shown to activate κ light-chain expression by activation of a NF- κ B like DNA-binding protein (Shirakawa et al. 1989). Together, these observations suggest that the signaling pathway from *Toll* to *dl* also involves stimulation of cAMP production, leading to the activation of PKA and the phosphorylation of *dl*. Phosphorylated *dl* is free to move into the nucleus and has an enhanced ability to activate transcription.

Genetic studies have placed two dorsal group genes, *tube* and *pelle*, downstream of *Toll* (Govind and Steward 1991). The proteins encoded by these two genes may be used to help transmit the signal from *Toll* to *dl*. The *tube* gene has been cloned, but its sequence reveals nothing about its possible function (Letsou et al. 1991). Because we do not know whether *tube* and/or *pelle* are expressed in Schneider cells, we cannot say whether they are absolutely required for this signaling pathway. No genetic

Figure 10. A model for the activation of *dl* by *Toll*. Before activation, *dl* is held in the cytoplasm, and is therefore inactive, through an association with *cactus*. After *Toll* receives an extracellular signal, *dl* is freed from the cytoplasm and activated as a result of phosphorylation by PKA.

evidence exists implicating PKA in D/V patterning, although this is not surprising given the multiple functions of this protein.

Anchoring of dl in the cytoplasm

Like *dl*, NF- κ B is regulated by its subcellular localization, inactive when in the cytoplasm and active when localized in the nucleus (Baeuerle and Baltimore 1988a). NF- κ B is retained in the cytoplasm by association with I κ B; and upon disruption of the complex, NF- κ B moves into the nucleus (Baeuerle and Baltimore 1988b). Proteins that belong to the I κ B family contain ankyrin repeats (Haskill et al. 1991), and members of the ankyrin family can regulate interactions between membrane and cytoskeletal elements (Lux et al. 1990). Therefore, NF- κ B could be held in the cytoplasm owing to its ability to interact with I κ B, which could be anchored in the cytoplasm through association with the cytoskeleton. The NF- κ B/I κ B complex can be destabilized by the phosphorylation of I κ B by PKC (Ghosh and Baltimore 1990), indicating that phosphorylation can disrupt this protein-protein interaction, resulting in the activation of NF- κ B in vitro. Such an interaction with a protein anchored in the cytoplasm may also be used to retain *dl* in the cytoplasm.

NF- κ B can be activated by a variety of agents including viruses, T-cell mitogens, cytokines, bacterial lipopolysaccharides, and DNA-damaging agents (for review, see Baeuerle and Baltimore 1990). NF- κ B is involved in activating the expression of a variety of genes that are required for immune, infection, inflammatory, and acute phase responses in a variety of cell types (Lenardo and Baltimore 1989; Baeuerle and Baltimore 1990; Libermann and Baltimore 1990). Therefore, NF- κ B should be responsive to a number of signaling pathways, including PKC. In contrast, *dl* activity is only required once, for the establishment of polarity in the early embryo; therefore, *dl* activation probably depends on only one signaling pathway, and the data presented here suggest that this involves phosphorylation of *dl* by activated PKA. We suggest that a similar activation pathway can be used to activate NF- κ B under certain conditions, for example, in response to IL-1. For *dl*, phosphorylation of *dl* itself is required and alone may be responsible for activating *dl* by leading to its release from its inhibitor, presumably the *cactus* protein (see below). However, we cannot rule out that modification of *cactus* also occurs and plays a role, as we found that the *dlQ* mutant was still weakly activated by PKA or *Toll*. The exact mechanism of activation of *dl* by PKA will not be known until the interaction between *dl* and *cactus* is better understood.

The *cactus* gene is one that is required for establishment of D/V polarity in the early embryo (Anderson 1987). Unlike the dorsal group genes that give rise to dorsalized embryos when mutated, *cactus* mutations result in partially ventralized embryos, suggesting that *cactus* is in some way responsible for inhibiting *dl* activity (Roth et al. 1989, 1991; Steward 1989). Therefore, *cactus* has been proposed to perform a function analo-

gous to I κ B, anchoring *dl* in the cytoplasm by forming a *dl-cactus* complex. This idea is further supported by the finding that *cactus*, like I κ B, contains ankyrin repeats (S. Kidd, pers. comm.).

In Schneider cells, *dl* protein is localized in the cytoplasm at low concentrations but is increasingly found in the nucleus as the amount of transfected *dl* is increased (Rushlow et al. 1989). This observation can be explained in terms of an interaction between *dl* and *cactus* because *cactus* is known to be expressed in Schneider cells (S. Kidd, pers. comm.). Increasing the amount of *dl* protein expressed in these cells could saturate the endogenous *cactus* so that at low *dl* concentrations all *dl* is bound by *cactus*. As the amount of *dl* is increased, there is no free *cactus* to interact with *dl*, so *dl* is free to move into the nucleus. In our model for *Toll*-mediated activation of *dl*, phosphorylation of *dl* by PKA disrupts the *dl-cactus* complexes so that *dl* is free to move into the nucleus even at low concentrations.

Activation of transcription by dl

In the early embryo, *dl* influences the expression of several zygotic genes (for review, see Anderson 1987; Rushlow and Arora 1990). Functional binding sites, similar to NF- κ B consensus sites, have been identified upstream of *zen* (Ip et al. 1991) and *twist* (Jiang et al. 1991; Thisse et al. 1991) genes. Expression of *zen* is repressed in ventral regions of the embryo (Rushlow et al. 1987) while *twist* is activated in the ventral-most regions of the embryo (Thisse et al. 1987), suggesting that *dl* can act as both an activator and repressor. In Schneider cells *dl* can activate expression from a variety of promoters that do not appear to contain *dl*-binding sites, including, paradoxically, the *zen*-CAT200 reporter used here [note that the *dl* sites in *zen* are located far upstream of the basal promoter (Ip et al. 1991)]. One reason for this could be the presence of *dl*-binding sites in the CAT reporter plasmid. A possible *dl*-binding site has been found in the pUC vector used here (Thisse et al. 1991). However, a reporter construct containing a deletion of this region is still strongly activated by *dl* (J.L. Norris and J.L. Manley, unpubl.). A possible explanation for the ability of *dl* to activate a wide variety of promoters is that the function of *dl* involves a strong interaction with its target, perhaps a component of the general transcription machinery. This could conceivably allow *dl* to activate transcription in the absence of *dl*-binding sites, especially when expressed at high levels by transfection. In support of this idea, we have observed a strong and specific functional interaction involving TFIID and *dl* in cotransfection experiments (J.L. Norris, J. Colgan, and J.L. Manley, unpubl.).

Whatever the mechanism by which *dl* activates transcription in cultured cells, it is intriguing that both PKA and *Toll* can enhance this activity independent of their effects on localization. This suggests that phosphorylation of *dl* increases the ability of the protein to interact either with DNA or with other factors required to activate transcription. Determining the precise effects of

phosphorylation on *dl* will require further investigation into the proteins with which *dl* interacts, in the cytoplasm as well as the nucleus.

Materials and methods

Recombinant plasmids

All expression vectors were derived from a plasmid that contains the *Drosophila* actin 5C promoter and poly(A) site, pAct5CSRS (pAct5C), which has been described in detail (Han et al. 1989). The *zen*-CAT200 reporter plasmid, pAct-*dl*, and pAct-*dl3* (*dl*-561) have been described previously by Rushlow et al. (1989). The above constructs and pAct-*dl4*, pAct-*dl5*, pAct-*dl6*, and pAct-*dl9* were provided by K. Han (Columbia University, New York). The *dl* NTS mutants, pAct-*dl7* and pAct-*dl8*, were provided by S. Small. The *dlQ*, *dlD*, and *dlRRKS* mutants were constructed by site-directed mutagenesis. A 0.65-kb *SacI*-*EcoRI* fragment was isolated from pAct-*dl* and cloned into pBluescript SK(+) (Stratagene), which had been cleaved with *SacI* and *EcoRI*. The following oligonucleotides were made: 5'-GC-GACGTCCCCAGGATGGAG-3' for *dlQ*; 5'-CGACGTCCCGATGATGGAGTTACC-3' for *dlD*; 5'-CTGCGACGTAAATCGGATGGA-3' for *dlRRKS*. The oligonucleotides were annealed to uracil containing single-stranded DNA, and synthesis of the second strand was done with T4 DNA polymerase. The resulting clones were screened for sequence that encoded the appropriate amino acid change. Clones containing the desired mutations were isolated and digested with *StuI* and *BstXI* to generate a fragment, containing the mutated region, that was used to replace the corresponding wild-type fragment in pAct-*dl*.

The pAct-*Tl* expression vector was constructed by isolating a *NsiI*-*KpnI* fragment, containing the *Toll*-coding region, from the *Toll* cDNA clone [kindly provided by K. Anderson (Schneider et al. 1991)]. The 3' overhang that resulted from cleavage with *NsiI* was digested with the Klenow fragment of DNA polymerase (Klenow) before cleavage by *KpnI*. This fragment was inserted into the pAct5C polylinker, adjacent to the actin 5C promoter, by a filled-in *BamHI* site and a *KpnI* site. The pAct-*TlRV* mutant was constructed by digesting pAct-*Tl* with *EcoRV*, which cleaves at one site within *Toll* and another in the pAct5C polylinker, and removing the 3.6-kb *EcoRV*-*EcoRV* fragment to create an in-frame stop after amino acid 274. To construct pAct-*Tl520*, pAct-*TlRV* was digested with *EcoRV* and *BglII* and ligated with a 2.1-kb *SalI*-*BglII* fragment from pAct-*Tl* to restore the *Toll* membrane-spanning and cytoplasmic domains. The 5' overhang generated by digestion with *SalI* was filled in with Klenow to generate an in-frame deletion of amino acids 274-793. The pAct-*TlStuI* mutant was constructed by digesting pAct-*Tl* with *BglII*, *NcoI*, and *StuI* to generate a 1.4-kb *NcoI*-*BglII* fragment, containing actin 5C promoter sequences and *Toll*-coding sequence for amino acids 1-322, and a 1.9-kb *BglII*-*StuI* fragment that contains *Toll*-coding sequence for amino acids 323-970. These two fragments were ligated with a 7.0-kb *BglII*-*NcoI* fragment from pAct5C, containing actin 5C promoter, actin 5C poly(A), and pBR322 sequences, which had been cleaved with *BglII*. The 5' overhang generated by cleavage with *BglII* was filled-in with Klenow before digestion with *NcoI*. The pAct-*Tl^{10b}* mutant was constructed by digesting pAct-*Tl* with *BglII* and *StuI* to generate a 7.3-kb *BglII*-*BglII* fragment, containing actin 5C promoter and poly(A) sequences, as well as *Toll*-coding sequence for amino acids 1-322, and a 1.6-kb *BglII*-*StuI* fragment that contains *Toll*-coding sequence for amino acids 970-1097. These fragments were ligated

with a *BglII*-*StuI* fragment, containing *Toll*-coding sequence for amino acids 323-969, from the *Tl^{10b}* cDNA clone [kindly provided by K. Anderson] to replace the wild-type sequence with the sequence encoding a cysteine to tyrosine change at amino acid 779.

The pActPKA expression vector was constructed by cleaving the PKA cDNA clone [kindly provided by D. Kalderon (Columbia University)] with *XbaI*, filling in the 5' overhang with Klenow, and digesting with *KpnI*. A 1.1-kb *KpnI*-*XbaI* fragment, containing the PKA-coding sequence, was inserted into the pAct5C polylinker by digesting pAct5C with *KpnI* and *EcoRV*. The pAct-*lacZPKi* expression vector was provided by M.E. Lane (Columbia University).

DNA transfection and transient expression assay

Drosophila Schneider L2 cells were grown and transfected as described previously (Han et al. 1989). Each transfection contained 5.0 μ g of expression vector consisting of the indicated amounts of *dl*, *Toll*, or PKA expression vectors and variable amounts of pAct5C to bring the total amount of expression vector to 5.0 μ g. A total of 10 μ g of DNA was used for each transfection, so the remaining 5.0 μ g of DNA consisted of 2.0 μ g *copi*a long terminal repeat (LTR)-*lacZ* as an internal control and 3.0 μ g of the *zen*-CAT200 reporter plasmid. All transfections were performed in duplicate, and β -galactosidase and CAT activities were measured as described previously (Han et al. 1989). The CAT activities presented represent the average of several independent transfections.

Transfections that included the *lacZPKi* expression vector contained 0.4 μ g of *dl* expression vector, 3.0 μ g of *Toll* expression vector, and 2.0-3.6 μ g of *lacZPKi* expression vector. The total amount of expression vector was adjusted to 7.0 μ g with pAct5C. A total of 12.0 μ g of DNA, including 2.0 μ g of *copi*a LTR-*lacZ* and 3.0 μ g of *zen*-CAT200, was used in each transfection. Cells were harvested and protein extracts were prepared as described (Han et al. 1989). The same amount of extract from each sample was used in a CAT assay. A β -galactosidase assay was performed to ensure that protein was being expressed in approximately equal amounts. Three independent transfections were performed in duplicate, and all gave similar results.

Staining of cells

Schneider cells were transfected as described above, and 48 hr after transfection the cells were fixed on plates with formaldehyde. The cells were blocked with 10% BSA, stained with anti-*dl* primary antibodies [provided by C. Rushlow and M. Levine (Rushlow et al. 1989)], washed twice with washing and dilution buffer (1% BSA, 0.5 M NaCl, and 0.1% Tween 80 in PBS), and stained with TRITC-conjugated secondary antibodies.

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References

- Anderson, K.V. 1987. Dorsal-ventral embryonic pattern genes of *Drosophila*. *Trends Genet.* **3**: 91–97.
- . 1989. *Drosophila*: The maternal contribution. In *Genes and embryos* (ed. D.M. Glover and B.D. Hanes), pp. 1–37. IRL Press, Oxford, England.
- Anderson, K.V. and C. Nüsslein-Volhard. 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., G. Jürgens, and C. Nüsslein-Volhard. 1985a. Establishment of dorsal-ventral polarity in the *Drosophila* embryo: Genetic studies on the role of the *Toll* gene product. *Cell* **42**: 779–789.
- Anderson, K.V., L. Bokla, and C. Nüsslein-Volhard. 1985b. Establishment of dorsal-ventral polarity in the *Drosophila* embryo: The induction of polarity by the *Toll* gene product. *Cell* **42**: 791–798.
- Baeuerle, P.A. and D. Baltimore. 1988a. Activation of DNA-binding activity in an apparently cytoplasmic precursor of the NF- κ B transcription factor. *Cell* **53**: 211–217.
- . 1988b. I κ B: A specific inhibitor of the NF- κ B transcription factor. *Science* **242**: 540–546.
- . 1990. The physiology of the NF- κ B transcription factor. Hormonal regulation of transcription. *Mol. Aspects Cell. Regul.* **6**: 409–432.
- 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.
- Cheng, H.C., S.M. Van Pattern, A.J. Smith, and D.A. Walsh. 1985. A active twenty-amino-acid-residue peptide derived from the inhibitor protein of the cyclic AMP-dependent protein kinase. *Biochem. J.* **231**: 655–661.
- 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.
- DiFiore, P.P., J.H. Pierce, M.H. Kraus, O. Segatto, C.R. King, and S.A. Aaronson. 1987. *erbB-2* is a potent oncogene when overexpressed in NIH/3T3 cells. *Science* **237**: 178–182.
- Downward, J., Y. Yorden, E. Mayes, G. Scrace, N. Totly, P. Stockwell, A. Ullrich, J. Schlessinger, and M.D. Waterfield. 1984. Close similarity of epidermal growth factor receptor and *v-erb-B* oncogene protein sequences. *Nature* **307**: 521–527.
- 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.
- Ghosh, S. and D. Baltimore. 1990. Activation *in vitro* of NF- κ B by phosphorylation of its inhibitor I κ B. *Nature* **344**: 678–682.
- 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.
- Govind, S. and R. Steward. 1991. Dorsoventral pattern formation in *Drosophila*: Signal transduction and nuclear targeting. *Trends Genet.* **7**: 119–125.
- Han, K., M.S. Levine, and J.L. Manley. 1989. Synergistic activation and repression of transcription by *Drosophila* homeobox proteins. *Cell* **56**: 573–583.
- 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* **111**: 1021–1028.
- Haskill, S., A.A. Beg, S.M. Tompkins, J.S. Morris, A.D. Yurochko, A. Johannes-Sampson, K. Mondal, P. Ralph, and A.S. Baldwin. 1991. Characterization of an immediate-early gene induced in adherent monocytes that encodes I κ B-like activity. *Cell* **65**: 1281–1289.
- Ip, Y.T., R. Kraut, M. Levine, and C.A. Rushlow. 1991. The *dorsal* morphogen is a sequence-specific DNA-binding protein that interacts with a long-range repression element in *Drosophila*. *Cell* **64**: 439–446.
- Jiang, J., D. Kosman, Y.T. Ip, and M. Levine. 1991. The *dorsal* morphogen gradient regulates the mesoderm determinant *twist* in early *Drosophila* embryos. *Genes & Dev.* **5**: 1881–1891.
- Kalderon, D. and G.M. Rubin. 1988. Isolation and characterization of *Drosophila* cAMP-dependent protein kinase genes. *Genes & Dev.* **2**: 1539–1556.
- Keith, F.J. and N.J. Gay. 1990. The *Drosophila* membrane receptor *Toll* can function to promote cellular adhesion. *EMBO J.* **9**: 4299–4306.
- Kemp, B.E. and R.B. Pearson. 1990. Protein kinase recognition sequence motifs. *Trends Biochem. Sci.* **15**: 342–346.
- Kemp, B.E., H. Cheng, and D.A. Walsh. 1988. Peptide inhibitors of cAMP-dependent protein kinase. *Methods Enzymol.* **159**: 173–185.
- Kieran, M., V. Blank, F. Logeat, J. Vandekerckhove, 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.
- Lenardo, M.J. and D. Baltimore. 1989. NF- κ B: A pleiotropic mediator of inducible and tissue-specific gene control. *Cell* **58**: 227–229.
- Lettsou, A., S. Alexander, K. Orth, and S.A. Wasserman. 1991. Genetic and molecular characterization of tube, a *Drosophila* gene maternally required for embryonic dorsoventral polarity. *Proc. Natl. Acad. Sci.* **88**: 810–814.
- Libermann, T.A. and D. Baltimore. 1990. Activation of interleukin-6 gene expression through the NF- κ B transcription factor. *Mol. Cell. Biol.* **10**: 2327–2334.
- 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.
- Lux, S.E., K.M. John, and V. Bennett. 1990. Analysis of cDNA for human erythrocyte ankyrin indicates a repeated structure with homology to tissue-differentiation and cell-cycle control proteins. *Nature* **344**: 36–42.
- Mosialos, G., P. Hamer, A.J. Capobianco, R.A. Laursen, and T.D. Gilmore. 1991. A protein kinase-A recognition sequence is structurally linked to transformation by p59^{v-rel} and cytoplasmic retention of p68^{c-rel}. *Mol. Cell. Biol.* **11**: 5867–5877.
- Nilsen, T.W., P.A. Maroney, R.G. Goodwin, F.W. Rottman, L.B. Crittenden, M.A. Raines, and H. Kung. 1985. *c-erbB* activation in ALV-induced erythroblastosis: Novel RNA processing and promotor insertion result in expression of an amino-truncated EGF receptor. *Cell* **41**: 719–726.

- 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.
- Roth, S., Y. Hiromi, D. Godt, and C. Nüsslein-Volhard. 1991. *cactus* a maternal gene required for proper formation of the dorsoventral morphogen gradient in *Drosophila* embryos. *Development* **112**: 371–388.
- Rushlow, C. and K. Arora. 1990. Dorsal-ventral polarity and pattern formation in *Drosophila*. *Sem. Cell Biol.* **1**: 173–184.
- Rushlow, C., M. Frasch, H. Doyle, and M. Levine. 1987. Maternal regulation of *zenknüllt*: A homeobox 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.
- Schneider, D.S., K.L. Hudson, T. Lin, and K.V. Anderson. 1991. Dominant and recessive mutations define functional domains of *Toll*, a transmembrane protein required for dorsal-ventral polarity in the *Drosophila* embryo. *Genes & Dev.* **5**: 797–807.
- 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. Suttelo, 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., B. Acres, C.E. Grubin, C.J. McMahan, J.M. Wignall, C.J. March, and S.K. Dower. 1989. Cloning the interleukin 1 receptor from human T cells. *Proc. Natl. Acad. Sci.* **86**: 8946–8950.
- Stein, D., R. Siegfried, E. Vogelsang, and C. Nüsslein-Volhard. 1991. The polarity of the dorsoventral axis in the *Drosophila* embryo is defined by an extracellular signal. *Cell* **65**: 725–735.
- Steward, R. 1987. *Dorsal*, an embryonic polarity gene in *Drosophila*, is homologous to the vertebrate proto-oncogene, *c-rel*. *Science* **238**: 692–694.
- . 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.
- Thisse, B., C. Stoetzel, M. El Messal, and F. Perrin-Schmitt. 1987. Genes of the *Drosophila* maternal dorsal group control the specific expression of the zygotic gene *twist* in presumptive mesodermal cells. *Genes & Dev.* **1**: 709–715.
- Thisse, C., F. Perrin-Schmitt, C. Stoetzel, and B. Thisse. 1991. Sequence-specific transactivation of the *Drosophila twist* gene by the *dorsal* gene product. *Cell* **65**: 1191–1201.
- Walsh, D.A. and D.B. Glass. 1991. Utilization of the inhibitor protein of adenosine cyclic monophosphate-dependent protein kinase, and peptides derived from it, as tools to study adenosine cyclic monophosphate mediated cellular processes. *Methods Enzymol.* **201**: 304–317.
- Weiner, D.B., J. Liu, J.A. Cohen, W.V. Williams, and M.I. Green. 1989. A point mutation in the *neu* oncogene mimics ligand induction of receptor aggregation. *Nature* **339**: 230–231.

Selective nuclear transport of the *Drosophila* morphogen dorsal can be established by a signaling pathway involving the transmembrane protein Toll and protein kinase A.

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References

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