Cell, Vol. 72, 515–525, February 26, 1993, Copyright © 1993 by Cell Press

pelle Encodes a Protein Kinase Required to Establish Dorsoventral Polarity in the Drosophila Embryo

Christopher A. Shelton and Steven A. Wasserman
Biochemistry Department
University of Texas Southwestern Medical Center
Dallas, Texas 75235-9038

Summary

The *pelle* gene is required for the nuclear import of dorsal protein that establishes dorsoventral polarity in Drosophila embryos. We report here the genetic mapping and molecular characterization of *pelle*. DNA sequence analysis revealed that *pelle* encodes a protein of 501 amino acids, the last 292 of which comprise a protein kinase catalytic domain. Microinjection of in vitro synthesized transcripts containing site-directed mutations indicates that the kinase catalytic domain is required for biological activity. This domain is most similar to that of the raf and mos protein kinases and is predicted to have a serine and threonine specificity. These results provide direct evidence for the role of phosphorylation in the in vivo regulation of a rel-like transcription factor.

Introduction

Development of an organism requires precise and reproducible changes in gene expression. Subcellular localization of regulatory proteins has become recognized as an important mechanism by which developmental cues can control gene expression (Hunt, 1989; Silver, 1991). A variety of transcription factors, including the glucocorticoid receptor, rel-related proteins, the Xenopus nuclear factor xnf7, and v-jun all appear to undergo regulated nuclear import (Picard et al., 1990; Gilmore, 1990; Blank et al., 1991; Miller et al., 1991; Chida and Vogt, 1992). Of these, the proteins of the rel family, including c-rel and v-rel, NF-κB, and the product of the Drosophila *dorsal* gene, are among the best understood.

The *dorsal* gene is maternally required in Drosophila for the establishment of dorsoventral polarity in early embryogenesis. The dorsal protein is initially uniformly distributed in egg cytoplasm; prior to gastrulation, 11 genes function to direct its spatially regulated nuclear import (reviewed in Govind and Steward, 1991; St Johnston and Nüsslein-Volhard, 1992). On the ventral side of the embryo, dorsal protein is translocated into nuclei, whereas on the dorsal side it remains in the cytoplasm. Inactivating alleles of *cactus* cause dorsal to translocate into nuclei throughout the embryo, resulting in all cells adopting a more ventral fate. A loss-of-function mutation in any of the 10 other genes results in embryos in which all dorsal protein remains cytoplasmic and all cells adopt a dorsal fate (Anderson, 1987). In wild-type embryos, the gradient of nuclear localization of dorsal protein established by these genes directs the formation of the dorsoventral axis by activating some downstream genes and repressing others (Ip et al., 1991; Pan et al., 1991; Ray et al., 1991; Thisse et al., 1991).

Regulation of subcellular localization of rel family proteins was first explored biochemically for NF-κB. NF-κB is comprised of two subunits, p50 and p65 (RelA), each of which contains a 300 amino acid domain with a high degree of sequence similarity to the proteins encoded by *v-rel* and *c-rel*, as well as to the Drosophila dorsal protein (Stephens et al., 1983; Wilhelmsen et al., 1984; Steward, 1987; Ghosh et al., 1990; Nolan et al., 1991). Originally identified as an immunoglobulin enhancer binding factor, NF-κB is broadly expressed, but is active in only a subset of cell types, including B lymphocytes and mature macrophages.

In unactivated cells, an inhibitor, IκB, binds NF-κB in the cytoplasm. Upon activation by any of a variety of cytokines and mitogens, the NF-κB–IκB complex breaks down and NF-κB translocates into the nucleus (Baeuerle and Baltimore, 1988a, 1988b). The mechanism by which agents such as IL-1 or TNF-α cause dissociation of the NF-κB–IκB complex is not clear, although there is evidence that phosphorylation of IκB abolishes its inhibitory activity (Shirakawa and Mizel, 1989; Ghosh and Baltimore, 1990; Kerr et al., 1991). Once activated, NF-κB promotes the expression of a range of cytokines and cytokine receptors, as well as the induction of HIV and other viruses (reviewed in Lenardo and Baltimore, 1989).

There are a number of similarities between the NF-κB and dorsoventral pathways. The roles of cactus and dorsal in regulating gene expression are analogous to those of IκB and NF-κB. Indeed, sequence analysis has revealed that the cactus protein bears substantial sequence similarity to proteins of the IκB family, including MAD-3 (IκBα) and bcl-3 (Geisler et al., 1992; Kidd, 1992). These proteins all contain multiple copies of ankyrin-like repeats that mediate the protein–protein interactions required for inhibition (Hatada et al., 1992; Kidd, 1992).

An additional parallel between the NF-κB and dorsoventral pathways comes from characterization of the Drosophila *Toll* gene, which acts upstream of *cactus* and *dorsal* in the genetic hierarchy. The intracellular domain of the transmembrane receptor encoded by *Toll* (Hashimoto et al., 1988) shows sequence similarity to the intracellular domain of the IL-1 receptor, a known positive activator of NF-κB function (Gay and Keith, 1991; Schneider et al., 1991). Furthermore, there is a significant correspondence between residues conserved between the two proteins and residues required for IL-1 receptor function (Heguy et al., 1992). However, the mechanism by which the intracellular domains of these proteins transmit signals remains unclear.

The parallels between the dorsal and NF-κB pathways suggest that characterization of genes involved in the Drosophila system will provide general insight into the regulation of rel proteins. Epistasis analysis has shown that two genes, *tube* and *pelle* (*pll*), function downstream of *Toll* but upstream of *dorsal* and upstream of or in parallel to *cactus*

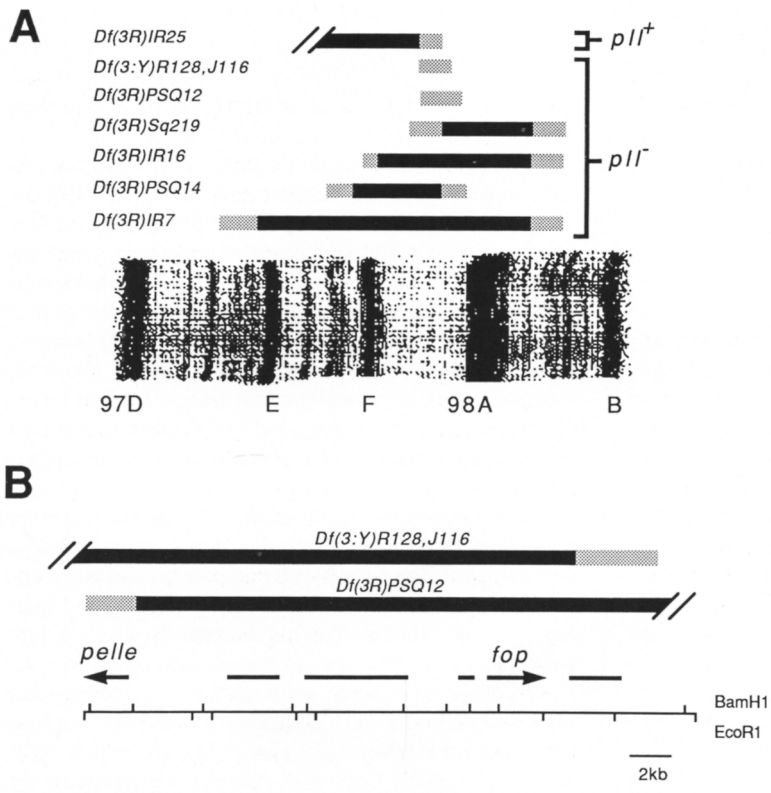

Figure 1. Genetic and Molecular Mapping of *pll*

(A) Depiction of the 97D to 98A cytological interval of the Drosophila polytene chromosome map. DNA absent in polytene preparations of the deletions listed is represented by solid bars; stippling at the ends of the bars indicates uncertainty in the location of the deletion endpoints. *Df(3R)R128,J116* and *Df(3R)PSQ12*, which are cytologically nonvisible, are defined molecularly. Both are mutant for *pll*; together they define the smallest region necessary for *pll* function.

(B) Representation of the portion of 97F necessary for *pll* function (orientation is the same as in [A]). At the top are shown the deletions whose endpoints most closely flank *pll*. At the bottom is a restriction endonuclease map. Above the restriction map, horizontal bars and arrows indicate the approximate positions of embryonic transcripts as deduced from Northern blot and cDNA analysis. Two transcripts, *fop* and *pll*, are maternally expressed; arrows indicate the direction of transcription for each. The distal end of the chromosomal walk performed by Fleming et al. (1990) lies 40 kb to the left of the region displayed.

(Anderson et al., 1985a; P. Hecht and K. Anderson, personal communication; Roth et al., 1991). The *tube* and *pll* gene products, then, are necessary for the intracellular transmission of the signal required for nuclear import of the dorsal protein.

We have previously cloned the *tube* locus (Letsou et al., 1991); we now report the cloning and initial molecular characterization of *pll*. We find that the *pll* gene encodes a protein kinase belonging to the raf–mos subfamily. Using site-directed mutagenesis, we provide evidence that kinase activity is required for pelle function. Based on these experiments and current knowledge of the NF-κB–IκB pathway, we suggest that phosphorylation is a general regulator for nuclear import of the rel family of transcription factors.

Results

Genetic Mapping and Molecular Identification of the *pll* Gene

The *pll* gene was previously localized to 97F in the polytene chromosome map of Drosophila (Anderson and Nüsslein-Volhard, 1984). To refine the mapping of *pll*, we constructed a series of deficiencies in the 97F region (see Experimental Procedures). Cytogenetic and complementation analyses of these deletions allowed us to map *pll* to the central third of 97F (Figure 1A). Using a P element localized to the 97F1 band as an entry point for a chromosomal walk, we isolated 150 kb of DNA from the region. Probing Southern blots with restriction fragments from the walk permitted us to map the endpoints of the deficiencies. These endpoints defined an interval of 30 kb as containing part or all of the *pll* gene (Figure 1B).

To facilitate identification of the *pll* transcript, we used an embryo injection assay to measure gene function. Embryos derived from *pll* females produce a cuticle exhibiting only the dorsalmost pattern elements of wild-type larval cuticle (compare Figures 2A and 2B). Microinjection of RNA from wild-type embryos into embryos from *pll* females can complement this maternal-effect phenotype and thus provides a rapid assay for *pll* gene products (Anderson and Nüsslein-Volhard, 1984; Müller-Holtkamp et al., 1985). The degree of rescue can be determined by scoring larval cuticles; ventral denticles, filzkörper, and dorsal hairs serve as markers for ventral, dorsolateral, and dorsal fates, respectively (Lohs-Schardin et al., 1979; Anderson and Nüsslein-Volhard, 1984).

Using the microinjection assay, we determined the size and developmental expression of the *pll* transcript. When poly(A)$^+$ RNA was separated on a sucrose gradient and individual fractions tested by microinjection into *pll* embryos, rescuing activity was found among RNA species approximately 2 kb in size (data not shown). We also assayed poly(A)$^+$ RNA isolated from different embryonic stages and found that rescuing activity was high in 0–3 hr embryos, low in 3–6 hr embryos, and absent in 6–9 hr embryos. Consistent with our results, H. Jäckle and colleagues have reported that *pll* rescuing activity in poly(A)$^+$ RNA declines rapidly during early embryonic development (Müller-Holtkamp et al., 1985).

When Northern blots of embryonic poly(A)$^+$ RNA were probed with DNA from the 30 kb region necessary for pelle

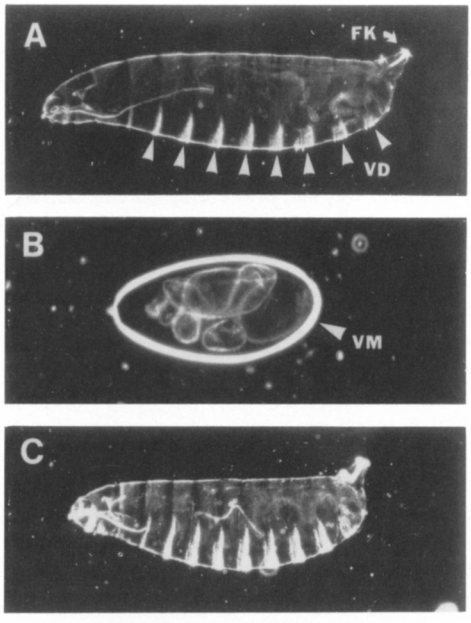

Figure 2. Rescue of the *pll* Maternal Phenotype by Microinjection of RNA

Cuticle preparations of an embryo and larvae are shown with anterior to the left and dorsal at the top.

(A) Uninjected wild-type larva. Filzkörper (FK) and ventral denticles (VD) are indicated; dorsal hairs are present but are not readily visible at this magnification.

(B) Developed embryo laid by a female of the genotype pll^{385}/*Df(3R)PSQ12*. The embryo is composed solely of dorsal epidermis contained within the vitelline membrane (VM).

(C) Hatched larva produced from an embryo of the same maternal genotype as in (B) by injection of ~100 pg in vitro-transcribed RNA from the *pll* cDNA. Complete restoration of the dorsoventral axis is seen.

function, six distinct transcripts were identified. Two of these matched the expected size and developmental profile of *pll*; genomic probes specific for each were used to isolate cDNA clones. To test whether these clones encoded *pll* function, we transcribed the cDNAs in vitro using a linked SP6 promoter and injected the RNA into embryos derived from *pll* females. Transcripts for one of these clones (*fop* in Figure 1B) failed to complement *pll* mutations. In contrast, SP6-generated RNA from the other cDNA fully rescued the *pll* phenotype (Figure 2). At 500 μg/ml, the rescue obtained was stronger than that previously seen for total poly(A)$^+$ RNA or cytoplasmic injections (Müller-Holtkamp et al., 1985), resulting in a high percentage of hatching embryos (Table 1). Both pll^{78} and pll^{385}, two strong loss-of-function alleles, were rescued to hatching. We conclude that we have isolated a functionally full-length *pll* cDNA.

The *pll* Gene Is Developmentally Regulated

In addition to their maternal effect on embryonic dorsoventral polarity, *pll* mutations also have zygotic effects on viability and pupal morphology (P. Hecht and K. Anderson, personal communication; Letsou et al., 1991). These zygotic phenotypes suggest that *pll* may play a role in signaling processes at other points in the Drosophila life cycle. When poly(A)$^+$ RNA from different developmental stages was analyzed on Northern blots, expression of a 1.9 kb *pll* transcript was seen throughout the life cycle (Figure 3). This transcript was expressed most strongly in poly(A)$^+$ RNA from 0–3 hr embryos and adult females. Upon prolonged exposure of some Northern blots, a second transcript of 4.8 kb was faintly visible in poly(A)$^+$ RNA from 2–5 hr embryos.

The *pll* Gene Encodes a Protein with Similarity to Serine/Threonine Kinases

A representative *pll* cDNA, S597, was selected for sequence analysis based on three criteria: the cDNA insert matched the size of the RNA detected by Northern blot analysis of 0–3 hr embryos, cDNAs isolated from four independent cDNA library pools were identical to this clone by restriction mapping, and RNA transcribed in vitro from the

Table 1. Effect of the Injection of Mutant and Wild-Type In Vitro Synthesized RNA on the *pll* Maternal Phenotype

			Cuticular Phenotype				
Injected RNA	Concentration (μg/ml)	Recipient Genotype	Number of Cuticles Scored	Dorsal Epidermis	Filzkörper Material	Ventral Denticles	Hatched Larvae
None	–	pll^{385}/*Df*	103	103	0	0	0
	–	pll^{78}/*Df*	52	52	0	0	0
Wild type	500	pll^{385}/*Df*	47	1	4	10	32
	125	pll^{385}/*Df*	29	0	1	17	11
	30	pll^{385}/*Df*	43	1	5	37	0
	500	pll^{78}/*Df*	35	0	0	8	27
K240R mutant	500	pll^{385}/*Df*	10	10	0	0	0
	500	pll^{78}/*Df*	29	29	0	0	0
D346A mutant	500	pll^{385}/*Df*	10	10	0	0	0
	500	pll^{78}/*Df*	34	31	0	3	0
A350E mutant	500	pll^{385}/*Df*	39	1	2	20	16

RNA transcripts were generated in vitro and injected at the indicated concentrations, as described in Experimental Procedures. *Df* = *Df(3R)PSQ12*. Cuticles of unhatched larvae were scored for the most ventrally derived marker present.

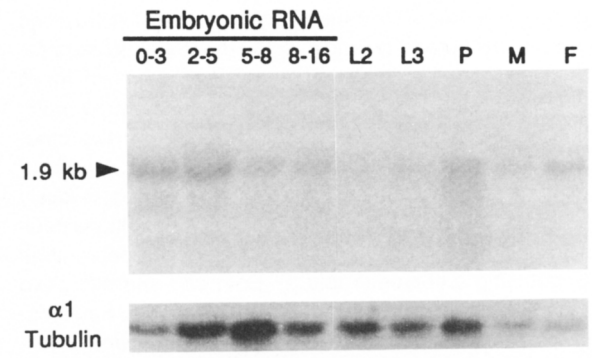

Figure 3. Developmental Regulation of the *pll* Transcript

Northern analysis of *pll* expression during the Drosophila life cycle. 0–3, 2–5, 5–8, and 8–16 represent ages in hr of embryos at 25°C; L2, second-instar larvae; L3, third-instar larvae; P, pupae; M, adult males; and F, adult females. Samples (10 μg) of poly(A)$^+$ RNA were fractionated by denaturing gel electrophoresis and transferred to a nylon membrane. The blot was hybridized with radiolabeled probes generated from the *pll* cDNA (1.4 kb HindIII–EcoRI restriction fragment) and from an α1-tubulin clone (Kalfayan and Wensink, 1982).

cDNA insert provided rescuing activity when injected into embryos from *pll* females.

A single long open reading frame, with a close fit to the Drosophila translational start consensus (Cavener, 1987), was found within the *pll* cDNA clone. Conceptual translation of the open reading frame from the initial methionine to the stop codon yields a protein sequence 501 amino acids long and with a predicted molecular size of 56,124 (Figure 4). No obvious signal sequence is present, consistent with the prediction that pelle acts intracellularly.

The protein sequence was used to search available data bases. The first 209 amino acids were not significantly similar to any other protein sequence. The carboxy-terminal 292 amino acids, in contrast, showed significant similarity to the catalytic domains of protein kinases (Hanks, 1991). In particular, this portion of the predicted pelle protein contains the 12 subdomains defined by blocks of sequence identity and similarity among the more than 150 known protein kinases (Figure 5). Furthermore, the amino acids invariant among all protein kinases are conserved in the pelle protein sequence.

Sequence comparisons predict that the kinase encoded by *pll* is specific for serine and threonine residues. The pelle sequence matches a serine/threonine–specific consensus, but not a tyrosine specific consensus, in two domains that are discriminating for substrate specificity (Table 2). In subdomain VIB, pelle contains the sequence DIKPAN, which fits the serine/threonine but not tyrosine kinase consensus. Similarly, in subdomain VIII, pelle contains the sequence TKIYLPPE, consistent with serine/threonine kinase sequences but divergent from the tyrosine kinase consensus.

The kinase catalytic domain of pelle has greatest similarity to protein kinases of the raf/mos subfamily (Hanks, 1991), but is not the counterpart of any particular member. For example, the degree of identity between pelle and Drosophila raf (Draf), is equivalent to that between pelle and human raf (27.0% and 26.6%, respectively), but is substantially less than the degree of identity between human raf and Draf (66.9%). Among members of the raf/mos subfamily, the protein with the kinase catalytic domain most similar to that of pelle is SRK6 from the kale Brassica oleracea (Stein et al., 1992); the kinase domains from the two proteins, however, share only 30.5% identity. Thus, the pelle kinase represents a new member of the raf/mos subfamily.

Mutations in the Kinase Domain Abolish Pelle Function

To determine the requirement of the pelle kinase catalytic domain for biological activity, we used oligonucleotide-directed mutagenesis to make three mutant *pll* cDNA constructs. In designing these mutant constructs, we took advantage of genetic and crystallographic studies identifying residues essential for activity of cAMP-dependent protein kinase (PKA) (Gibbs and Zoller, 1991a; Knighton et al., 1991). We tested the ability of each construct to rescue the *pll* phenotype by generating transcripts from a linked SP6 promoter and microinjecting the RNA into embryos laid by *pll* females. To ensure that a given mutation was responsible for any phenotypic changes, we reverted each, using an oligonucleotide of wild-type sequence, and confirmed restoration of rescuing activity equivalent to the original *pll*$^+$ construct.

The first mutation, K240R, replaced lysine 240 in the pelle sequence with an arginine. The corresponding residue in mouse PKA, lysine 72, is invariant among all protein kinases and is involved in anchoring the ATP phosphate groups (Knighton et al., 1991). Replacement of the equivalent amino acid with arginine in several other protein kinases abolishes enzymatic and biological activity, despite the conservative nature of the alteration (Booher and Beach, 1986; Singh et al., 1986; Ohno et al., 1990). As predicted, the K240R mutation completely abolished pelle-rescuing activity in the microinjection assay (Table 1).

The second mutation, D346A, converted aspartate 346 into an alanine. The equivalent aspartate residue in other kinases (residue 166 in mouse PKA) is nearly invariant. The biophysical and genetic studies of PKA strongly support the hypothesis that this residue acts as the catalytic base. The D346A mutation in pelle severely reduced rescuing activity; no hatching larvae were produced and only 3 of 44 embryos exhibited any degree of phenotypic rescue (Table 2). Neither the D346A nor the K240R mutation had any phenotypic effect when injected at equivalent concentrations into wild-type embryos (data not shown). Thus, two mutations that should disable protein kinase catalytic function both virtually eliminate pelle biological activity.

In designing the third pelle mutation, we took advantage of information on PKA's interaction with its substrates (Knighton et al., 1991; Gibbs and Zoller, 1991b). The crystal structure of PKA indicates that recognition of the PKA consensus phosphorylation motif (xRRxS) relies, in part, on productive ionic contacts between the two arginine side chains in the substrate and four glutamate residues in the mouse enzyme, E127, E170, E230, and E331. Comparison of the pelle and PKA sequences revealed that pelle

```
   1  GAAGTCATCCACAGGTTTTCATCGAATCGCGTTGGGAATTTACCCAAAAGCGATTTTCGTTTCGCTGCAACAGATTACCAGCGCCGCCAA
  91  CCCCTCATCCAACGGGCACTCTTGCATCCGGAGCCGCATGAATCAGTCGCAACCGGTTGAACCGCCCGCCCATAAGTGAAAGATGAGTGG
   1                                                                                  M  S  G

 181  CGTCCAGACCGCCGAAGCCGAGGCGCAGGCCCAAAACCAAGCGAATGGCAACAGGACCAGGTCGCGTTCCCACTTGGACAACACAATGGC
   4    V  Q  T  A  E  A  E  A  Q  A  Q  N  Q  A  N  G  N  R  T  R  S  R  S  H  L  D  N  T  M  A

 271  CATCCGACTGCTGCCGTTGCCCGTGCGAGCCCAGCTATGTGCCCACTTGGATGCCCTAGACGTATGGCAACAGCTGGCCACGGCCGTAAA
  34    I  R  L  L  P  L  P  V  R  A  Q  L  C  A  H  L  D  A  L  D  V  W  Q  Q  L  A  T  A  V  K

 361  ACTCTATCCCGACCAGGTGGAGCAGATAAGCAGCCAGAAGCAGCGTGGCCGCTCAGCCTCCAATGAGTTCCTCAATATTTGGGGCGGTCA
  64    L  Y  P  D  Q  V  E  Q  I  S  S  Q  K  Q  R  G  R  S  A  S  N  E  F  L  N  I  W  G  G  Q

 451  GTACAATCACACGGTGCAAACATTGTTTGCTTTGTTCAAAAAATTGAAGCTTCATAACGCCATGCGTCTGATCAAAGATTACGTTAGCGA
  94    Y  N  H  T  V  Q  T  L  F  A  L  F  K  K  L  K  L  H  N  A  M  R  L  I  K  D  Y  V  S  E

 541  GGATCTGCACAAGTACATACCGAGGAGCGTGCCCACCATCAGCGAGCTGCGCGCTGCTCCCGATTCCAGTGCCAAAGTAAACAACGGCCC
 124    D  L  H  K  Y  I  P  R  S  V  P  T  I  S  E  L  R  A  A  P  D  S  S  A  K  V  N  N  G  P

 631  GCCGTTTCCCTCCTCCTCGGGCGTCAGCAACTCAAACAACAATCGCACCAGCACAACGGCAACGGAGGAGATACCCAGCCTGGAGTCCCT
 154    P  F  P  S  S  S  G  V  S  N  S  N  N  N  R  T  S  T  T  A  T  E  E  I  P  S  L  E  S  L

 721  GGGCAATATACACATTAGCACCGTACAGCGGGCAGCCGAATCCTTGCTGGAGATCGATTATGCGGAGCTAGAAAACGCCACGGACGGCTG
 184    G  N  I  H  I  S  T  V  Q  R  A  A  E  S  L  L  E  I  D  Y  A  E  L  E  N  A  T  D  G  W

 811  GAGTCCGGATAATCGACTGGGACAGGGCGGATTCGGAGACGTGTACCGCGGCAAATGGAAGCAACTGGACGTGGCCATCAAGGTGATGAA
 214    S  P  D  N  R  L  G  Q  G  G  F  G  D  V  Y  R  G  K  W  K  Q  L  D  V  A  I  K  V  M  N

 901  CTACCGCAGTCCCAACATCGACCAGAAAATGGTGGAGCTGCAGCAGAGCTACAACGAACTCAAGTATTTAAACAGCATCCGGCACGACAA
 244    Y  R  S  P  N  I  D  Q  K  M  V  E  L  Q  Q  S  Y  N  E  L  K  Y  L  N  S  I  R  H  D  N

 991  TATCCTGGCCCTCTACGGATACAGCATCAAAGGTGGTAAGCCGTGCCTCGTCTACCAGCTGATGAAGGGCGGCTCCCTGGAGGCTCGTTT
 274    I  L  A  L  Y  G  Y  S  I  K  G  G  K  P  C  L  V  Y  Q  L  M  K  G  G  S  L  E  A  R  L

1081  ACGAGCGCATAAGGCACAAAACCCACTACCAGCACTCACCTGGCAGCAGCGGTTTAGCATCAGCCTCGGCACGGCTAGAGGCATCTACTT
 304    R  A  H  K  A  Q  N  P  L  P  A  L  T  W  Q  Q  R  F  S  I  S  L  G  T  A  R  G  I  Y  F

1171  TCTGCACACGGCGCGAGGCACACCGCTGATTCATGGAGATATTAAGCCGGCCAACATCCTGCTGGACCAATGTCTGCAGCCAAAAATCGG
 334    L  H  T  A  R  G  T  P  L  I  H  G  D  I  K  P  A  N  I  L  L  D  Q  C  L  Q  P  K  I  G

1261  AGACTTCGGTCTGGTGCGCGAGGGTCCCAAGTCCTTGGACGCTGTGGTGGAAGTGAATAAAGTTTTCGGCACTAAGATCTACCTGCCACC
 364    D  F  G  L  V  R  E  G  P  K  S  L  D  A  V  V  E  V  N  K  V  F  G  T  K  I  Y  L  P  P

1351  GGAGTTCCGCAACTTCAGACAACTCAGCACGGGCGTGGACGTCTACAGCTTCGGCATTGTGCTGTTGGAGGTGTTCACGGGTCGTCAGGT
 394    E  F  R  N  F  R  Q  L  S  T  G  V  D  V  Y  S  F  G  I  V  L  L  E  V  F  T  G  R  Q  V

1441  GACGGATCGCGTGCCGGAAAACGAGACGAAGAAGAATTTGCTGGACTACGTTAAGCAGCAGTGGCGGCAAAACCGGATGGAGCTGCTAGA
 424    T  D  R  V  P  E  N  E  T  K  K  N  L  L  D  Y  V  K  Q  Q  W  R  Q  N  R  M  E  L  L  E

1531  GAAGCACTTAGCAGCACCGATGGGCAAGGAGCTGGACATGTGCATGTGCGCCATCGAGGCGGGCTTGCACTGTACTGCCCTGGATCCGCA
 454    K  H  L  A  A  P  M  G  K  E  L  D  M  C  M  C  A  I  E  A  G  L  H  C  T  A  L  D  P  Q

1621  GGATCGCCCATCCATGAACGCGGTGCTCAAGCGTTTCGAACCGTTTGTTACCGACTAGATGGGCCTTATTATGTTACCATTTTATTGACG
 484    D  R  P  S  M  N  A  V  L  K  R  F  E  P  F  V  T  D  *

1711  AACTTGAAAAGCTCGAAGGACCATTTGTAAACCATTTCCACATTTTGTGATCAATCGCAATATATATTTTTAACTTTAACATTCCAACGA
1801  AAATTTCCCTAAAGACTTATTGTTATGCTTAAATTGTAAATGCTTACCCTATTGTTTATTAAATATTTTTGTACACTTAAAAAAAAAAAA
1891  AAAA
```

Figure 4. The Complete Nucleotide Sequence of the *pll* cDNA S597

Translation of the 501 amino acid pelle open reading frame is shown below the corresponding nucleotides. The nucleotide and amino acid numbering is shown to the left, with the amino acid numbering italicized.

lacks three of the four glutamates. In pelle, serine 298 and alanine 350, respectively, are found in place of glutamate 127 and 170 in PKA; glutamate 331 in mouse PKA lies beyond the pelle stop codon. To test whether this lack of charge was important, we changed the alanine at position 350 in pelle to a glutamate (A350E). Residue 350 in the pelle sequence is closely flanked by invariant residues in subdomain VI and is predicted by the PKA crystal structure to be in contact with the substrate. When assayed by microinjection, the A350E mutation was 4-fold less efficient at rescuing the *pll* phenotype than the wild-type construct (Table 2). The presence of uncharged amino acids at positions 298 and 350 in pelle, coupled with the reduction of biological activity produced by the A350E mutation, indicates that the substrate specificity of the pelle protein is likely to differ from that of PKA.

Time of Action of Pelle

Cytoplasmic injection experiments had shown that pelle function supplied as late as stage 4 of embryonic development (syncytial blastoderm) could rescue the maternal effect *pll* phenotype (Müller-Holtkamp et al., 1985). To determine whether pelle could fulfill its function in establishing dorsoventral polarity prior to this stage, we used the cold-sensitive pll^{122} and pll^{864} alleles to regulate pelle activity during development (Figure 6). Our experiments with embryos from pll^{122}/pll^{864} females, as well as those of Müller-Holtkamp (1985) with embryos from pll^{122} homozy-

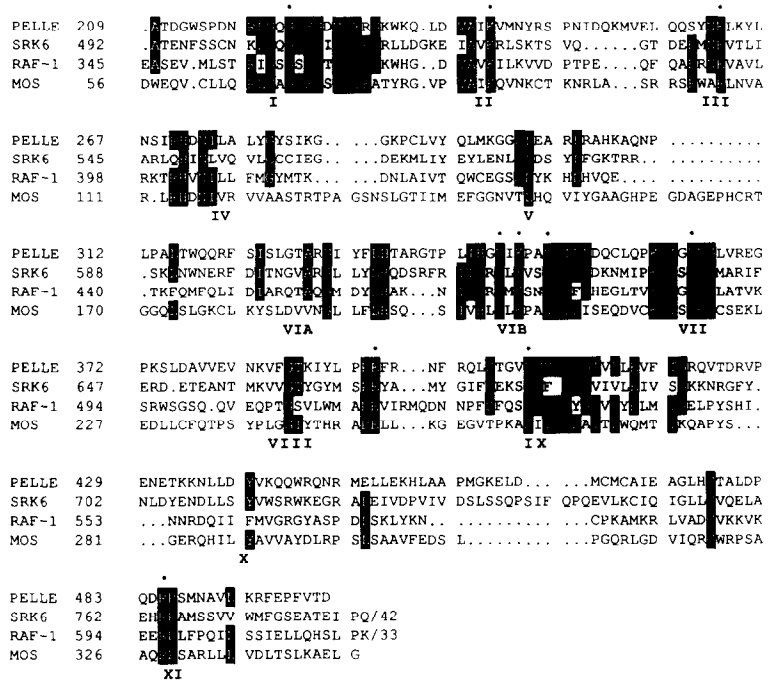

Figure 5. Alignment of the Pelle Kinase Catalytic Domain with Members of the Raf/Mos Protein Kinase Family

Conserved sequence subdomains (Hanks, 1991) are indicated with roman numerals. Sequences represented are the pelle protein sequence, the kale receptor kinase SRK6 (Stein et al., 1992), and the human c-raf (Bonner et al., 1986) and c-mos (Watson et al., 1982) sequences. Amino acid numbering for each sequence appears to the left. Amino acids identical in at least three out of four sequences are highlighted. Amino acids that are strictly conserved among all serine/threonine specific protein kinase sequences are indicated with a dot above the residue. For SRK6 and RAF-1, a number following a slash indicates the number of carboxy-terminal residues omitted.

gotes, revealed that the cold-sensitive period for pelle function centers on stage 4 of development. This stage correlates with the time of nuclear import of the dorsal protein and with the time during which the signaling pathway can be activated by an exogenously supplied, extracellular ligand (Rushlow et al., 1989; Steward, 1989; Roth et al., 1989; Stein et al., 1991).

Discussion

Using deletion mapping to delimit the locus, Northern analysis to detect candidate transcripts, and RNA microinjections both to predict a pelle expression profile and to assay cDNA function, we have identified the *pll* gene. Sequence analysis of a *pll* cDNA clone revealed an open reading frame encoding 501 amino acids. The carboxy-terminal 292 amino acids fit a consensus for protein kinase domains and establish the pelle protein as a new member of the raf/mos subfamily of protein kinases. By analogy to raf (Heidecker et al., 1990), the amino-terminal domain of pelle, which does not bear substantial sequence similarity to other proteins, is predicted to play a role in the regulation of catalytic domain activity. Alterations in this domain might, therefore, lead to constitutive activation of pelle, though no dominant pelle mutations have been identified to date.

Three predicted enzymatic properties of the pelle protein were tested by site-directed mutagenesis: ATP binding, catalytic activity, and enzyme–substrate interaction. Based on the mouse PKA crystal structure and detailed mutagenic studies of yeast PKA, we made specific predictions for the in vivo complementation behavior of mutant *pll* RNA. If kinase activity were important to pelle function, mutations that severely reduced catalytic activity in other kinases should significantly reduce or abolish the biological activity of pelle. As expected, both K240R and D346A lacked significant pelle-rescuing activity. We also predicted that mutations that disrupt substrate interactions should reduce pelle function. The A350E mutation was 4-fold less efficient at rescuing the *pll* phenotype than the wild-type construct. Together, the sequence analysis and mutagenesis experiments indicate that *pll* encodes a kinase whose catalytic activity is required for its maternal gene function.

Role of Phosphorylation by the Pelle Protein Kinase in Signal Transduction

The pelle protein kinase is required for transmission of positional information from the Toll receptor to the dorsal morphogen. The pelle kinase might act in a signal-dependent manner and therefore be instructive, phosphorylating substrates in a spatially restricted manner. If so, its activation must be mediated directly or indirectly by Toll, since the ligand-dependent activation of Toll is sufficient to determine the dorsoventral axis (Anderson et al., 1985b; Stein et al., 1991). Alternatively, phosphorylа-

Table 2. Phosphorylation Specificitiy of Pelle

Kinase Sequence	Subdomain VIB	Subdomain VIII
Ser/Thr consensus	D o K x x N	x x x x x x P E
Pelle kinase	D I K P A N	T K I Y L P P E
Tyr consensus	D L A A R N	P o b W x A P E

The pelle protein sequence was aligned and compared with the consensus determined by Hanks (1991). Hydrophobic consensus is indicated by o, basic consensus by b, and positions lacking any consensus by x.

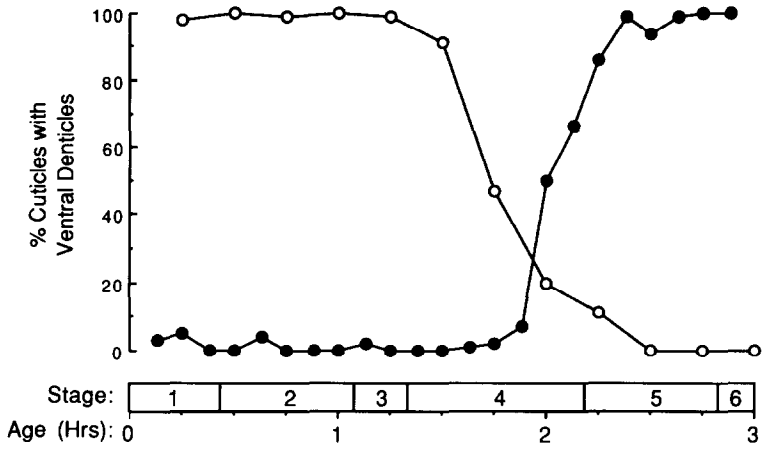

Figure 6. The Temperature-Sensitive Period of *pll*^{122}/*pll*^{R64}

The graph indicates the percentage of embryos producing ventral denticles following a temperature shift at the indicated developmental stage (see Experimental Procedures). Shift from permissive to nonpermissive conditions (closed circles); shift from nonpermissive to permissive conditions (open circles). Each shift was performed twice; the total number of embryos scored averaged 100 embryos for each time point. Developmental stages, according to Campos-Ortega and Hartenstein (1985), are as follows: newly fertilized egg, stage 1; early cleavage, stage 2; pole cell formation, stage 3; syncytial blastoderm, stage 4; cellularization, stage 5. Hours after fertilization are indicated below the developmental stages.

tion by pelle might be signal independent and therefore permissive, modifying a protein as a prerequisite to that molecule's participation in the signal transduction pathway. However, since temperature shift experiments show that pelle is required at the time the pathway is activated, such a modification must either be labile or occur concurrently with signaling. Given the role of phosphorylation in modulating protein activity in a variety of signaling pathways, we favor an instructive role for pelle.

In addition to pelle, the products of at least three genes, *cactus*, *dorsal*, and *tube*, are required downstream of *Toll* in the intracellular signaling pathway (Figure 7). Each represents a potential target for phosphorylation by pelle. We will consider them in turn.

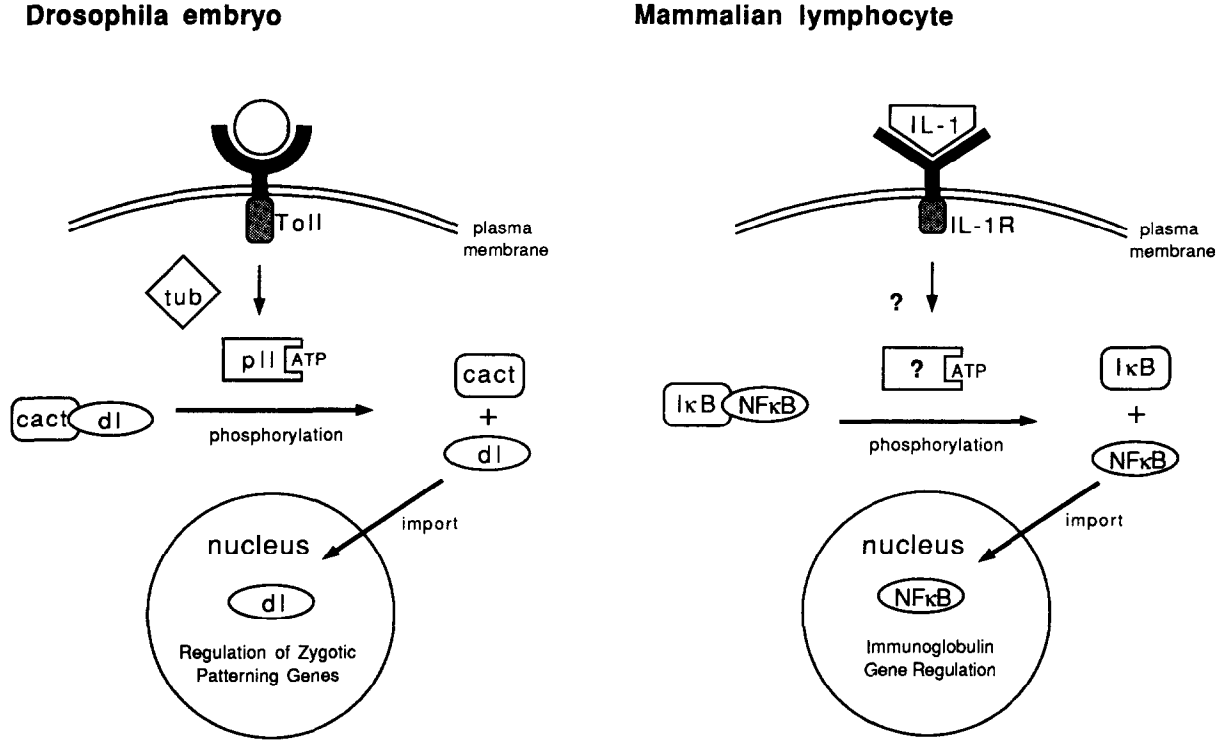

Figure 7. Model of a Conserved Pathway for Regulated Nuclear Import of the Dorsal Protein and NF-κB

(Left) Signal transduction is believed to be triggered on the ventral side of the Drosophila embryo by binding of an extracellular ligand to the Toll receptor (Stein et al., 1991; Stein and Nüsslein-Volhard, 1992; Hecht and Anderson, 1992). Subsequent signal-dependent phosphorylation by the pelle kinase leads to dissociation of the dorsal–cactus complex and dorsal nuclear import. The tube protein plays an essential but as yet poorly understood role in this pathway.

(Right) Binding of the cytokine interleukin-1 (IL-1) to its receptor on the surface of human T cells leads to a parallel pathway of signal-dependent phosphorylation and dissociation of the IκB–NF-κB complex (Blank et al., 1991). Although both protein kinase C and protein kinase A can affect interactions between IκB and NF-κB in vitro, the identity and possible role of any tube or pelle homologs in the human T cell pathway is not known.

The pelle kinase, activated directly or indirectly by Toll, could phosphorylate the cactus protein, causing dissociation of a cactus–dorsal complex and subsequent nuclear import of dorsal. It is known that gain-of-function mutations in *cactus* can prevent dorsal nuclear import (Roth et al., 1991). One such mutation represents an amino-terminal deletion in cactus (Geisler et al., 1992), suggesting that the deleted portion of cactus is normally responsible for receiving a regulatory signal. That the signal is likely to take the form of phosphorylation of the cactus protein by the pelle protein is suggested by the following observations: phosphorylation in vitro of the vertebrate cactus homolog IκB relieves inhibition of NF-κB DNA binding (Ghosh and Baltimore, 1990). Further, the cold-sensitive period for pelle activity matches the temperature-sensitive period obtained for cactus function (Roth et al., 1991). Finally, the cactus protein is phosphorylated in vivo (Kidd, 1992).

The dorsal protein could also be a target of phosphorylation by the pelle protein. Indeed, there is evidence for phosphorylation of dorsal in vivo (Kidd, 1992; S. Gillespie and S. A. W., unpublished data). Phosphorylation of dorsal, and perhaps of cactus, could disrupt the interaction of these two proteins and allow nuclear import of dorsal. Alternatively, the activation of dorsal could require both its release from cactus and its modification by phosphorylation. If so, phosphorylation of dorsal by pelle would of necessity be signal dependent, since embryos from *cactus* mutant females retain dorsoventral polarity, while embryos from females mutant for both *cactus* and *pll* are apolar.

Several laboratories have investigated the possible regulatory role of a conserved PKA phosphorylation site in the dorsal protein that lies just in front of a nuclear localization signal (Steward, 1987; Rushlow et al., 1989; Norris and Manley, 1992). Indeed, site-directed mutation studies of the homologous PKA site in c-rel suggested that phosphorylation at this site may induce nuclear localization (Mosialos et al., 1991). Regardless of the effect of phosphorylation at this PKA site in the dorsal protein, however, our data suggest that the pelle protein does not act at this site. The pelle protein sequence appears to lack the charged residues shown to be necessary for interaction of PKA with the charged PKA recognition site. Of course, it is possible that residues at other positions, either in the amino-terminal region or in the catalytic domain, are responsible for substrate binding. Alternatively, pelle might regulate dorsal by phosphorylation at a site other than the PKA consensus site, or pelle might indirectly regulate dorsal by phosphorylation of other kinases that, in turn, phosphorylate dorsal.

The tube protein might also undergo phosphorylation by pelle as one step of the signal transduction cascade. The *tube* gene encodes an intracellular protein with multiple copies of an 8 amino acid motif (Letsou et al., 1991). These repeats, which always contain a serine or threonine at position 6, could be targets for phosphorylation. However, deletion experiments with tube indicate that the repeats are not strictly required for signal transduction (A. Letsou and S. A. W., unpublished data). Alternatively, the tube protein might act as an accessory protein for pelle, facilitating pelle's activation or its interaction with substrate.

Relationship of Pelle to Other Signaling Pathways

There are substantial similarities between the processes for establishing the dorsoventral axis and the termini of the Drosophila embryo (reviewed in Stein and Stevens, 1991). Our studies on pelle reveal an additional similarity: both pathways require a kinase downstream of a transmembrane receptor protein. The Draf protein, which acts downstream of the torso receptor tyrosine kinase (Ambrosio et al., 1989), and the pelle protein are members of the same protein kinase subfamily. In light of the similarities between the pathways and their coincident time of action, they might be expected to interact. Indeed, the subcellular localization of dorsal protein at the poles of the embryo is affected by the terminal system (Casanova, 1991). It is unlikely that *pll* or other known dorsoventral genes play roles in terminal specification, however, since none has partial loss-of-function phenotypes suggestive of involvement in terminal group function.

As illustrated in Figure 7 and discussed previously, there are also substantial parallels between the Drosophila dorsoventral pathway and the mammalian NF-κB pathway. In particular, the sequence conservation between functionally equivalent proteins in the two pathways is striking. This conservation indicates that, despite regulating disparate development processes in evolutionarily divergent organisms, the two pathways share a common signal transduction mechanism. This suggests that genes important in the Drosophila dorsoventral pathway will have functional homologs in the mammalian pathway. It is highly likely, therefore, that phosphorylation regulates the nuclear import of NF-κB and other rel-like proteins and that as-yet-unidentified mammalian counterparts to the tube and pelle proteins mediate this regulation.

Experimental Procedures

Drosophila Strains

Balancer chromosomes, *pll* alleles, and marker mutations are described in Lindsley and Zimm (1992). The P element insertion Y488 was generated (unpublished data) by mobilization of the P(*lacW*) element construct (Bier et al., 1989) with the *P[ry⁺Δ2,3]* transposase source (Robertson et al., 1988). The ls(3)Pneo97F insertion stock (Cooley et al., 1988) was obtained from the Bloomington Drosophila stock center.

Generation and Analysis of Deficiencies

Three separate screens were performed to isolate deficiencies in the 97F interval. In the first screen, males from the P element insertion strain Y488, containing a P element localized to the 97F1 interval of the polytene chromosome, were mutagenized with 4000 R γ-radiation from a cesium source and outcrossed to w^- females. F1 progeny were then screened for loss of the dominant w^+ eye color marker carried on the P element and used to establish stocks. Deficiencies IR7, IR16, and IR25 were identified from this screen. In the second screen, we used the recessive zygotic *pll* phenotype to isolate events directly affecting the *pll* locus. As previously described (Letsou et al. 1991), pupae homozygous for loss-of-function *pll* alleles are squat, i.e., they have a reduced axial ratio compared with wild-type or *pll*/+ pupae. Males homozygous for an *st e* chromosome were mutagenized with 4000 R γ-radiation and crossed to females carrying a pll^{385} balanced with *TM3*. F1 progeny were screened at the pupal stage for the squat *pll* phenotype and used to establish stocks. Deficiency Sq219 was

identified from this screen. In the third screen, which also took advantage of the zygotic *pll* phenotype, the ls(3)Pneo97F stock was crossed to a strain homozygous for the *P[ry⁺Δ2,3]* transposase-bearing chromosome. Male flies of the genotype *ls(3)Pneo97F/P[ry⁺Δ2,3]* were then crossed to *pll³⁸⁵/TM3* and the progeny were scored at the pupal stage for the *pll* phenotype. Deficiencies PSQ12 and PSQ14 were identified from this screen. *Df(3R)R128,J116* was generated by crossing stocks carrying the translocations *T(Y;3)R128* and *T(Y;3)J116* and selecting the segmentally aneuploid progeny deficient in 97F (Lindsley et al., 1972)

Temperature Shift Experiments

Eggs were collected from *pll¹²²/pll⁸⁶⁴* females at 18°C (nonpermissive temperature) or 25°C (permissive temperature) on yeasted apple juice agar plates. Temperature shifts, determination of developmental age, and phenotypic scoring were carried out essentially as described by Anderson and Nüsslein-Volhard (1986).

Nucleic Acid Manipulations

DNA manipulations were performed as described by Sambrook et al. (1989). The initial isolation of DNA from the 97F region was performed by plasmid rescue (Pirrotta, 1986) of sequences flanking the Y488 P element insertion event. The chromosomal walk was performed following standard techniques utilizing the cosmid (NotBamNot–pCosPer) and phage (EMBL3) genomic libraries generously supplied by J. Tamkun, as well as one Charon 4 clone, φ19.2, from an overlapping walk independently performed in 97F by Fleming et al. (1990). Total RNA was isolated using the hot phenol technique (Jowett, 1986) and poly(A)⁺ RNA was selected using oligo(dT)–cellulose (Collaborative Research). For Northern blots, 10–20 μg of poly(A)⁺ RNA per lane was run in a 1% agarose, 7% formaldehyde gel using MOPS buffer and transferred to nylon membranes. cDNA clones were isolated from the 0–4 hr embryonic library of Brown and Kafatos (1988).

RNA Microinjection

RNA was produced from plasmid templates containing an SP6 promoter as described (Krieg and Melton, 1987), using the cap analog GpppG (Pharmacia LKB). Transcribed RNA was resuspended in injection buffer containing 25 mM KCl, 2.5 mM PIPES, 0.5 mM EDTA, and 25% glycerol. Eggs were collected and microinjected as previously described (Anderson and Nüsslein-Volhard, 1984), except that collections were generally done at 22°C and eggs were dried 8–12 min using a Zeiss air curtain incubator. For *pll*, injection of 250 pl of a 500 μg/ml solution of synthetic RNA corresponds to introduction of a quantity of RNA estimated to be 1000× the amount of endogenous *pll* message in wild-type embryos. Embryos were allowed to complete development for 2 days at 25°C prior to preparation and scoring of cuticles as decribed (Wieschaus and Nüsslein-Volhard, 1986).

Sequence Analysis

The *pll* cDNA S597 was sequenced on both strands and across all cloning sites using the M13mp18 and M13mp19 vectors. Dideoxychain termination sequencing reactions were carried out using reagents from United States Biochemical. Standard 8% polyacrylamide sequencing gels were used, and products were visualized either by using fluorescently tagged dideoxynucleotides and an Applied Biosystems model 370A DNA sequencer or by the incorporation of radiolabeled deoxynucleotides and autoradiography. Initial sequence analysis was performed using the computer program DNA Strider (Marck, 1988); subsequent data base searches and alignments were performed with the University of Wisconsin GCG software package (Devereux et al., 1984). To align the pelle sequence with that of other kinases, an initial alignment was carried out with the GCG program Bestfit, and adjustments, if necessary, were then made to bring invariant residues into register.

Site-Directed Mutagenesis

Mutagenesis was performed using the Altered Sites system from Promega. The *pll* cDNA sequence was cloned into the pSELECT vector such that sense RNA could be transcribed from the SP6 promoter located in the vector as described above. Mutations were introduced using oligonucleotides containing the mutant sequence following standard protocols (Sambrook et al., 1989).

Acknowledgments

We thank Nick Brown, John Tamkun, Peter Fleming, Dan Lindsley, David Stein, Melanie Cobb, David Russell, the Bloomington Drosophila stock center, members of the Wasserman lab, and especially Sherry Alexander and Jeffry Cormier for assistance with this project. We would also like to thank Susan Gillespie, Peter Hecht, and Kathryn Anderson for helpful discussions and communication of unpublished data. We thank Leon Avery, Flora Katz, and Dennis McKearin for helpful comments on the manuscript. This work was supported by a training grant from the National Institutes of Health (GM07062) to C. A. S. and by grants to S. A. W. from the National Science Foundation and the Robert A. Welch Foundation. S. A. W. is a fellow of the David and Lucille Packard Foundation.

Received November 20, 1992; revised December 14, 1992.

References

Ambrosio, L., Mahowald, A. P., and Perrimon, N. (1989). Requirement of the *Drosophila raf* homologue for *torso* function. Nature *342*, 288–291.

Anderson, K. V. (1987). Dorsal-ventral embryonic pattern genes of *Drosophila*. Trends Genet. *3*, 91–97.

Anderson, K. V., and Nüsslein-Volhard, C. (1984). Information for the dorsal-ventral pattern of the *Drosophila* embryo is stored as maternal mRNA. Nature *311*, 223–227.

Anderson, K. V., and Nüsslein-Volhard. C. (1986). Dorsal-group genes of *Drosophila*. In Gametogenesis and the Early Embryo, J. Gall, ed. (New York: Alan R. Liss), pp. 177–194.

Anderson, K. V., Jürgens, G., and Nüsslein-Volhard, C. (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., Bokla, L., and Nüsslein-Volhard, C. (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 Baltimore, D. (1988a). Activation of DNA-binding activity in an apparently cytoplasmic precursor of the NF-κB transcription factor. Cell *53*, 211–217.

Baeuerle, P. A., and Baltimore, D. (1988b). IκB: a specific inhibitor of the NF-κB transcription factor. Science *242*, 540–546.

Bier, N., Vaessin, H., Shepherd, S., Lee, K., McCall, K., Barbel, S., Ackerman, L., Carretto, R., Uemura, R., Grell, E. Jan, L. Y., and Jan, Y. N. (1989). Searching for pattern and mutation in the *Drosophila* genome with a P-*lacZ* vector. Genes Dev. *3*, 1273–1287.

Blank, V., Kourilsky, P., and Israel, A. (1991). NF-κB and related proteins: Rel/dorsal homologies meet ankyrin-like repeats. Trends Biochem. Sci. *17*, 135–140.

Bonner, T. I., Oppermann, H., Seeburg, P., Kerby, S. B., Gunnell, M. A., Young, A. C., and Rapp, U. R. (1986). The complete coding sequence of the human raf oncogene and the corresponding structure of the c-raf-1 gene. Nucl. Acids Res. *14*, 1009–1015.

Booher, R., and Beach, D. (1986). Site-specific mutagenesis of cdc2+, a cell cycle control gene of the fission yeast *Schizosaccharomyces pombe*. Mol. Cell. Biol. *6*, 3523–3530.

Brown, N. H., and Kafatos, F. C. (1988). Functional cDNA libraries from *Drosophila* embryos. J. Mol. Biol. *203*, 425–437.

Campos-Ortega, J. A., and Hartenstein, V. (1985). The Embryonic Development of *Drosophila melanogaster* (Berlin: Springer Verlag).

Casanova, J. (1991). Interaction between *torso* and *dorsal*, two elements of different transduction pathways in the *Drosophila* embryo. Mech. Dev. *36*, 41–45.

Cavener, D. (1987). Comparison of the consensus sequences flanking translation start sites in *Drosophila* and vertebrates. Nucl. Acids Res. *15*, 1353–1361.

Chida, K., and Vogt, P. K. (1992). Nuclear translocation of viral Jun but not of cellular Jun is cell cycle dependent. Proc. Natl. Acad. Sci. USA *89*, 4290–4294.

Cooley, L., Kelley, R., and Spradling, A. (1988). Insertional mutagene-

sis of the *Drosophila* genome with single P elements. Science *239*, 1121–1127.

Devereux, J., Haeberli, P., and Smithies, O. (1984). A comprehensive set of sequence analysis programs for the VAX. Nucl. Acids Res. *12*, 387–395.

Fleming, R. J., Scottgale-Nelson, T., Diederich, R. J., and Artavanis-Tsakonas, S. (1990). The gene *Serrate* encodes a putative EGF-like transmembrane protein essential for proper ectodermal development in *Drosophila melanogaster*. Genes Dev. *4*, 2188–2201.

Gay, N. J., and Keith, F. J. (1991). *Drosophila Toll* and IL-1 receptor. Nature *351*, 355–356.

Geisler, R., Bergmann, A., Hiromi, Y., and Nüsslein-Volhard, C. (1992). *cactus*, a gene involved in dorsoventral pattern formation of Drosophila, is related to the IκB gene family of vertebrates. Cell *71*, 613–621.

Ghosh, S., and Baltimore, D. (1990). Activation in vitro of NF-κB by phosphorylation of its inhibitor IκB. Nature *344*, 678–682.

Ghosh, S., Gifford, A. M., Riviere, L. R., Tempst, P., Nolan, G. P., and Baltimore, D. (1990). Cloning of the p50 DNA binding subunit of NF-κB: homology to *rel* and *dorsal*. Cell *62*, 1019–1029.

Gibbs, C. S., and Zoller, M. J. (1991a). Rational scanning mutagenesis of a protein kinase identifies functional regions involved in catalysis and substrate interactions. J. Biol. Chem. *266*, 8923–8931.

Gibbs, C. S., and Zoller, M. J. (1991b). Identification of electrostatic interactions that determine the phosphorylation site specificity of the cAMP-dependent protein kinase. Biochemistry *30*, 5329–5334.

Gilmore, T. D. (1990). NF-κB, KBF1, *dorsal*, and *rel*ated matters. Cell *62*, 841–843.

Govind, S., and Steward, R. (1991). Dorsoventral pattern formation in *Drosophila*: signal transduction and nuclear targeting. Trends Genet. *7*, 119–125.

Hanks, S. K. (1991). Eukaryotic protein kinases. Curr. Opin. Struct. Biol. *1*, 369–383.

Hashimoto, C., Hudson, K. L., and Anderson, K. V. (1988). The *Toll* gene of Drosophila, required for dorsal-ventral embryonic polarity, appears to encode a transmembrane protein. Cell *52*, 269–279.

Hatada, E. N., Nieters, A., Wulczyn, F. G., Naumann, M., Meyer, R., Nucifora, G., McKeithan, T. W., and Scheidereit, C. (1992). The ankyrin repeat domains of the NF-κB precursor p105 and the protooncogene *bcl-3* act as specific inhibitors of NF-κB DNA binding. Proc. Natl. Acad. Sci. USA *89*, 2489–2493.

Hecht, P. M., and Anderson, K. V. (1992). Extracellular proteases and embryonic pattern formation. Trends Cell Biol. *2*, 197–202.

Heguy, A., Baldari, C. T., Macchia, G., Telford, J. L., and Melli, M. (1992). Amino acids conserved in interleukin-1 receptors (IL-1Rs) and the *Drosophila* Toll protein are essential for IL-1R signal transduction. J. Biol. Chem. *267*, 2605–2609.

Heidecker, G., Huleihel, M., Cleveland, J. L., Kolch, W., Beck, T. W., Lloyd, P., Pawson, T., and Rapp, U. R. (1990). Mutational activation of c-raf-1 and definition of the minimal transforming sequence. Mol. Cell. Biol. *10*, 2503–2512.

Hunt, T. (1989). Cytoplasmic anchoring proteins and the control of nuclear localization. Cell *59*, 949–951.

Ip, Y. T., Kraut, R., Levine, M., and Rushlow, C. A. (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.

Jowett, T. (1986). Preparation of nucleic acids. In *Drosophila*: A Practical Approach, D. B. Roberts, ed. (Oxford: IRL Press).

Kalfayan, L., and Wensink, P. C. (1982). Developmental regulation of Drosophila α-tubulin genes. Cell *29*, 91–98.

Kerr, L. D., Inoue, J., Davis, N., Link, E., Baeurle, P. A., Bose, H. A., and Verma, I. M. (1991). The rel-associated pp40 protein prevents DNA binding of Rel and NF-κB: relationship with IκB-β and regulation by phosphorylation. Genes Dev. *5*, 1464–1476.

Kidd, S. (1992). Characterization of the Drosophila *cactus* locus and analysis of interactions between cactus and dorsal proteins. Cell *71*, 623–635.

Knighton, D. R., Zheng, J. H., Ten Eyck, L. F., Ashford, V. A., Nguyen-Huu, X., Taylor, S. S., and Sowadski, J. M. (1991). Crystal structure of the catalytic subunit of cyclic adenosine monophosphate-dependent protein kinase. Science *253*, 407–414.

Krieg, P. A., and Melton, D. A. (1987). *In vitro* RNA synthesis with SP6 RNA polymerase. Meth. Enzymol. *155*, 397–415.

Lenardo, M. J., and Baltimore, D. (1989). NF-κB: a pleiotropic mediator of inducible and tissue-specific gene control. Cell *58*, 227–229.

Letsou, A., Alexander, S., Orth, K., and Wasserman, S. A. (1991). Genetic and molecular characterization of tube, a *Drosophila* gene maternally required for embryonic dorsoventral polarity. Proc. Natl. Acad. Sci. USA *88*, 810–814.

Lindsley, D. L., and Zimm, G. G. (1992). The Genome of *Drosophila melanogaster* (San Diego, California: Academic Press).

Lindsley, D. L., Sandler, L., Baker, B. S., Carpenter, A. T. C., Denell, R. E., Hall, J. C., Jacobs, P. A., Miklos, G. L. G., Davis, B. K., Gethmann, R. C., Hardy, R. W., Hessler, A., Miller, S. M., Nozawa, H., Parry, D. M., and Gould-Somero, M. (1972). Segmental aneuploidy and the genetic gross structure of the *Drosophila* genome. Genetics *71*, 157–184.

Lohs-Schardin, M., Cremer, C., and Nüsslein-Volhard, C. (1979). A fate map for the larval epidermis of *Drosophila melanogaster*: localized cuticle defects following irradiation of the blastoderm with an ultraviolet laser microbeam. Dev. Biol. *73*, 239–255.

Marck, C. (1988). 'DNA Strider': a 'C' program for the fast analysis of DNA and protein sequences on the Apple Macintosh family of computers. Nucl. Acids Res. *16*, 1829–1836.

Miller, M., Reddy, B. A., Kloc, M., Li, X. X., Dreyer, C., and Etkin, L. D. (1991). The nuclear-cytoplasmic distribution of the *Xenopus* nuclear factor, xnf7, coincides with its state of phosphorylation during early development. Development *113*, 569–575.

Mosialos, G., Hamer, P., Capobianco, A. J., Laursen, R. A., and Gilmore, T. D. (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.

Müller-Holtkamp, F. (1985). Genetische und molekulare Untersuchungen an den dorsalisierenden Mutanten *easter*, *pelle*, und *spätzle* von *Drosophila melanogaster* PhD thesis. University of Tübingen, Tübingen, Germany.

Müller-Holtkamp, F., Knipple, D. C., Seifert, E., and Jäckle, H. (1985). An early role of maternal mRNA in establishing the dorsoventral pattern in *pelle* mutant *Drosophila* embryos. Dev. Biol. *110*, 238–246.

Nolan, G. P., Ghosh, S., Liou, H.-C., Tempst, P., and Baltimore, D. (1991). DNA binding and IκB inhibition of the cloned p65 subunit of NF-κB, a *rel*-related polypeptide. Cell *64*, 961–969.

Norris, J. L., and Manley, J. L. (1992). Selective nuclear transport of the *Drosophila* morphogen *dorsal* can be established by a signaling pathway involving the transmembrane protein *Toll* and protein kinase A. Genes Dev. *6*, 1654–1667.

Ohno, S., Konno, Y., Akita, Y., Yano, A., and Suzuki, K. (1990). A point mutation at the putative ATP-binding site of protein kinase C a abolishes the kinase activity and renders it down-regulation-insensitive. J. Biol. Chem. *265*, 6296–6300.

Pan, D. J., Huang, J. D., and Courey, A. J. (1991). Functional analysis of the Drosophila twist promoter reveals a dorsal-binding ventral activator region. Genes Dev. *5*, 1892–1901.

Picard, D., Kumar, V., Chambon, P., and Yamamoto, K. R. (1990). Signal transduction by steroid hormones: nuclear localization is differentially regulated in estrogen and glucocorticoid receptors. Cell Reg. *1*, 291–299.

Pirrotta, V. (1986). Cloning *Drosophila* genes. In *Drosophila*: A Practical Approach, D. B. Roberts, ed. (Oxford: IRL Press).

Ray, R. P., Arora, K., Nüsslein-Volhard, C., and Gelbart, W. M. (1991). The control of cell fate along the dorsal-ventral axis of the *Drosophila* embryo. Development *113*, 35–54.

Robertson, H. M., Preston, C. R., Phillis, R. W., Johnson-Schlitz, D. M., Benz, W. K., and Engels, W. R. (1988). A stable genomic source of P element transposase in *Drosophila melanogaster*. Genetics *118*, 461–470.

Roth, S., Stein, D., and Nüsslein-Volhard, C. (1989). A gradient of

nuclear localization of the *dorsal* protein determines dorsoventral pattern in the Drosophila embryo. Cell *59*, 1189–1202.

Roth, S., Hiromi, Y., Godt, D., and Nüsslein-Volhard, C. (1991). *cactus*, a maternal gene required for proper formation of the dorsoventral morphogen gradient in *Drosophila* embryos. Development *112*, 371–388.

Rushlow, C. A., Han, K., Manley, J. L., and Levine, M. (1989). The graded distribution of the *dorsal* morphogen is initiated by selective nuclear transport in Drosophila. Cell *59*, 1165–1177.

Sambrook, E. G., Fritsch, E. F., and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, Second Edition (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press).

Schneider, D. S., Hudson, K. L., Lin, T. Y., and Anderson, K. V. (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., and Mizel, S. B. (1989). *In vitro* activation and nuclear translocation of NF-κB catalyzed by cyclic AMP-dependent protein kinase and protein kinase C. Mol. Cell. Biol. *9*, 2424–2430.

Silver, P. A. (1991). How proteins enter the nucleus. Cell *64*, 489–497.

Singh, B., Hannink, M., Donoghue, D. J., and Arlinghaus, R. B. (1986). p37mos-associated serine/threonine protein kinase activity correlates with the cellular transformation function of v-mos. J. Virol. *60*, 1148–1152.

Stein, D., and Nüsslein-Volhard, C. (1992). Multiple extracellular activities in Drosophila egg perivitelline fluid are required for establishment of embryonic dorsal-ventral polarity. Cell *68*, 429–440.

Stein, D., and Stevens, L. (1991). Establishment of dorsal-ventral and terminal pattern in the *Drosphila* embryo. Curr. Opin. Genet. Dev. *1*, 247–254.

Stein, D., Roth, S., Vogelsang, E., and Nüsslein-Volhard, C. (1991). The polarity of the dorsoventral axis in the Drosophila embryo is defined by an extracellular signal. Cell *65*, 725–735.

Stein, J. C., Howlett, B., Boyes, D. C., Nasrallah, M. E., and Nasrallah, J. B. (1992). Molecular cloning of a putative receptor protein kinase gene encoded at the self-incompatibility locus of *Brassica oleracea*. Proc. Natl. Acad. Sci. USA. *88*, 8816–8820.

Stephens, R. M., Rice, N. R., Hiebsch, R. R., Bose Jr., H. R., and Gilden, R. V. (1983). Nucleotide sequence of *v-rel*: the oncogene of reticuloendotheliosis virus. Proc. Natl. Acad. Sci. USA *80*, 6229–6233.

Steward, R. (1987). *Dorsal*, an embryonic polarity gene in Drosophila, is homologous to the vertebrate proto-oncogene, *c-rel*. Science *238*, 692–694.

Steward, R. (1989). Relocalization of the *dorsal* protein from the cytoplasm to the nucleus correlates with its function. Cell *59*, 1179–1188.

St Johnston, D., and Nüsslein-Volhard, C. (1992). The origin of pattern and polarity in the Drosophila embryo. Cell *68*, 201–219.

Thisse, C., Perrin-Schmitt, F., Stoetzel, C., and Thisse, B. (1991). Sequence-specific transactivation of the Drosophila *twist* gene by the *dorsal* gene product. Cell *65*, 1191–1201.

Watson, R., Oskarsson, M., and Vande Woude, G. F. (1982). Human DNA sequence homologous to the transforming gene (mos) of Moloney murine sarcoma virus. Proc. Natl. Acad. Sci. USA *79*, 4078–4082.

Wieschaus, E., and Nüsslein-Volhard, C. (1986). Looking at embryos. In *Drosophila*: A Practical Approach, D. B. Roberts, ed. (Oxford: IRL Press), pp. 199–227.

Wilhelmsen, K., Eggleton, K., and Temin, H. (1984). Nucleic acid sequence of the oncogene *v-rel* in reticuloendotheliosis virus strain T and its cellular homolog, the proto-oncogene *c-rel*. J. Virol. *52*, 172–182.

GenBank Accession Number

The accession number for the sequence reported in this paper is L08476.