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Essay

A Conserved Signal Transduction Pathway Regulating the Activity of the *rel*-like Proteins Dorsal and NF-κB

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INTRODUCTION

Intracellular signal transduction plays a critical role in a number of invertebrate patterning processes. In recent years it has been shown that many steps in these processes require homologues of proteins that transduce signals in cultured mammalian cells. Knowledge of the biochemical function of the vertebrate proteins has made it easier to define the invertebrate signaling pathways. In turn, studies in *Caenorhabditis elegans* and *Drosophila* have enabled the identification of new pathway components, as well as provided insight into signaling mechanisms. As an example, I consider here the establishment of dorsoventral polarity in the *Drosophila* embryo and its relationship to a mammalian signal transduction pathway.

The establishment of the dorsoventral axis in *Drosophila* depends on the spatially regulated subcellular localization of the transcription factor dorsal (for reviews, see Govind and Steward, 1991; St. Johnston and Nüsslein-Volhard, 1992). During oogenesis, dorsal protein is deposited uniformly in the egg cytoplasm, where it is held through an interaction with the cactus protein. Soon after fertilization, a ventrally localized, extracellular ligand acts through the transmembrane receptor Toll to overcome the inhibitory effect of cactus and thereby direct dorsal protein into nuclei (Hashimoto *et al.*, 1988; Roth *et al.*, 1989; Rushlow *et al.*, 1989; Steward, 1989; Stein *et al.*, 1991). When present at high concentrations within nuclei, dorsal protein activates ventral-specific genes, while at the same time repressing dorsal-specific loci. The graded distribution of the dorsal protein in nuclei across the embryo can thus define the dorsoventral axis (for further discussion, see Jiang and Levine, 1993).

In mammalian cells, the subcellular localization of the transcription factor NF-κB is controlled in a manner very similar to that observed for dorsal. An inhibitory protein, IκB, binds stably to NF-κB (Baeuerle and Baltimore, 1988b). This interaction masks a nuclear localization signal (NLS) in NF-κB; the complex therefore remains cytoplasmic (Beg *et al.*, 1992). Activating signals received at the cell surface cause dissociation of the protein-protein complex, freeing NF-κB for translocation into nuclei (Baeuerle and Baltimore, 1988a). Once nuclear, NF-κB, like dorsal, acts as a regulator of a program of gene expression.

At least three components of the dorsal and NF-κB signaling pathways share structural as well as functional similarity (Table 1). First, dorsal and NF-κB belong to a family of DNA-binding proteins that includes the oncoprotein *v-rel* and its cellular homologue, *c-rel* (for reviews, see Blank *et al.*, 1992; Nolan and Baltimore, 1992; Rushlow and Warrior, 1992). These proteins have in common a conserved 300 amino acid region, the *rel*-homology domain, that contains an NLS, a DNA-binding region, and a conserved, consensus phosphorylation site for the cAMP-dependent protein kinase (PKA). Second, cactus and the IκBα isoform each contain multiple ankyrin repeats that mediate the protein-protein interactions required for their function (Davis *et al.*, 1991; Haskill *et al.*, 1991; Geisler *et al.*, 1992; Kidd, 1992). Third, one of the extracellular signals that can activate NF-κB, interleukin-1 (IL-1), acts through a receptor with structural similarity to the *Drosophila* Toll protein (Osborn *et al.*, 1989; Shirakawa *et al.*, 1989). The type I IL-1 receptor and Toll, each of which has a single membrane-spanning segment, are similar in sequence over 217 amino acids in their intracellular domains (Hashimoto *et al.*, 1988; Sims *et al.*, 1988; Gay and Keith, 1991; Schneider *et al.*, 1991). Although the intracellular domains share only 26% amino-acid identity, conserved residues are the site of inactivating mutations in both receptors, supporting the hypothesis that the IL-1 receptor and Toll carry out homologous functions (Schneider *et al.*, 1991; Heguy *et al.*, 1992).

MECHANISM FOR SIGNAL TRANSDUCTION

The mechanism by which IL-1 activates NF-κB is poorly understood. The cytoplasmic portion of the type I IL-1 receptor lacks any biochemical function that can be readily discerned from amino acid sequence. Although protein kinase C (PKC) can activate NF-κB in vitro (Shirakawa and Mizel, 1989; Ghosh and Baltimore, 1990), PKC inhibitors fail to block IL-1 signal transduction in vivo (Macchia *et al.*, 1990; Bomsztyk *et al.*, 1991; Sty-

Table 1. Components of the intracellular dorsoventral signaling pathway in the *Drosophila* embryo

Drosophila protein	Protein function	Predicted MW	Cellular location	Mammalian counterpart	References
Toll	transmembrane receptor	125 000	plasma membrane	Type I IL1-receptor	Hashimoto *et al.*, 1988, 1991
tube	?	50 000	cortical cytoplasm and plasma membrane	?	Letsou *et al.*, 1991; Gillespie & Wasserman, unpublished data
pelle	protein kinase	56 000	?	?	Shelton and Wasserman, 1993
cactus	cytoplasmic retention factor	53 000	cytoplasm	IκB (MAD-3 or pp40)	Geisler *et al.*, 1992; Kidd, 1992; Haskill *et al.*, 1991; Davis *et al.*, 1991
dorsal	transcription factor	75 000	cytoplasm or nucleus	NF-κB (p50, p65)	Steward, 1987, 1989; Rushlow *et al.*, 1989; Roth *et al.*, 1989; Ghosh *et al.*, 1990; Kieran *et al.*, 1990; Nolan *et al.*, 1991

lianou *et al.*, 1992). It has been reported that cAMP and oxygen radicals are involved in IL-1 signaling, but the data are contradictory (Didier *et al.*, 1988; Shirakawa *et al.*, 1989; Schreck *et al.*, 1991; Ray *et al.*, 1992; Stylianou *et al.*, 1992). Most recently, an elevation of ceramide levels in lymphoid tumor cells in response to IL-1 treatment has been taken as evidence for an involvement of the sphingomyelin pathway in IL-1 signal transduction (Mathias *et al.*, 1993).

Although the route for transduction of the IL-1 signal to NF-κB has remained obscure, progress has been made in mapping out the intracellular signaling pathway leading to dorsal activation. Genetic and molecular analyses in *Drosophila* have shown that two gene products, tube and pelle, are required downstream of Toll to promote the nuclear import of dorsal (Roth *et al.*, 1989; Steward, 1989; Hecht and Anderson, 1993). Tube is composed of two structurally and functionally distinct domains (Letsou *et al.*, 1991, 1993). The biochemical function of this protein is unknown, although its association with the plasma membrane and cortical cytoplasm suggests that it may interact with the cytoplasmic domain of Toll (Gillespie and Wasserman, unpublished data). Pelle is a protein kinase predicted to display specificity for serine and threonine residues (Shelton and Wasserman, 1993). Catalytic function is essential for pelle activity, indicating that protein phosphorylation is an obligate step in the transduction of the axis-determining signal.

Among the known signaling components, cactus is a likely substrate for pelle. Although there is no information available as to any signal-dependent modification of cactus, genetic evidence suggests that some, if not all, of the signal from Toll is transduced to dorsal via cactus (Roth *et al.*, 1991; Geisler *et al.*, 1992; for discussion, see Shelton and Wasserman, 1993). It is also possible that pelle acts directly on dorsal. The phosphorylation state of dorsal does in fact increase in response to signaling. However, the observed increase appears to be the product, rather than cause, of the dissociation of dorsal and cactus (Gillespie and Wasserman, unpublished data). As such, it may serve to modulate the rate of nuclear import or the efficiency of dorsal as a transcriptional activator once it has entered the nucleus.

If phosphorylation of cactus by pelle is sufficient to disrupt the dorsal/cactus complex, and hence direct dorsal into nuclei, this modification of cactus must be restricted to the ventral portion of the embryo. Although pelle might become ventrally localized, it is more probable that the pelle protein becomes locally activated, most likely through interactions involving the presumptive regulatory domain in the amino-terminal half of pelle. Such spatial regulation of pelle must be under the direction of Toll, because localized Toll protein can define the dorsoventral polarity within the embryo (Anderson *et al.*, 1985a). Furthermore, if the genes that have been identified in the pathway constitute a complete set, Toll would have to activate pelle either directly or through the intervention of tube (Figure 1).

The genes for tube, Toll, and the other proteins listed in Table I were identified in experiments designed to saturate the *Drosophila* genome for maternal effect patterning mutations (Anderson and Nüsslein-Volhard, 1984; Schüpbach and Wieschaus, 1989). Because only two alleles of tube were found in these screens, a component that is less readily mutable might have been missed entirely. Other pathway components might have escaped detection because their function is so necessary for zygotic development that homozygous mutant females never survive to produce defective embryos. Thus, although a plausible signaling pathway can be designed with only the known proteins, additional components may exist.

CONSERVATION AND DIVERGENCE IN SIGNALING PATHWAYS

The *Drosophila* embryo within which the localization of dorsal is regulated is quite unlike any mammalian

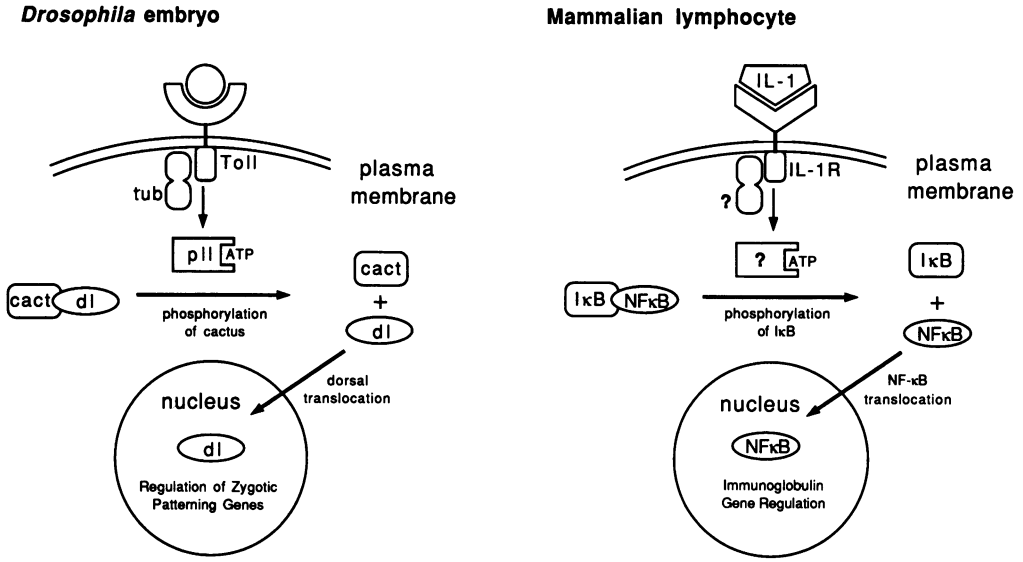

Figure 1. Model of the conserved pathway for regulated nuclear import of the *dorsal* protein and NF-κB (adapted from Shelton and Wasserman, 1993). (Left) Signal transduction is triggered on the ventral side of the *Drosophila* embryo by binding of a ligand to the *Toll* receptor (Stein *et al.*, 1991; Stein and Nüsslein-Volhard, 1992). The *tube* protein, which is found in cortical regions of the embryonic cytoplasm and at the plasma membrane (Gillespie and Wasserman, unpublished data), likely participates in the transfer of the localized signal to the protein kinase, pelle. Subsequent signal-dependent phosphorylation of cactus by pelle leads to dissociation of the dorsal-cactus complex and dorsal nuclear import. (Right) Binding of the cytokine interleukin-1 (IL-1) to its type I receptor on the surface of mammalian cells activates a comparable pathway, culminating in signal-dependent phosphorylation of IκB and dissociation of the IκB-NF-κB complex (Blank *et al.*, 1992; Nolan and Baltimore, 1992). In the example shown, IL-1 interaction with its type I receptor on the surface of mammalian lymphocytes activates NF-κB and thereby regulates the expression of immunoglobulin light chains (Dower *et al.*, 1992; Stylianou *et al.*, 1992).

cell. At the time of signal transduction, the embryo is a syncytium, a single cell that contains about 10^3 nuclei. If tube and pelle were only required to adapt the signaling pathway to this syncytial state, neither would be expected to have a counterpart in the pathway downstream of the IL-1 receptor. This cannot be their sole function, however, because they are active later in *Drosophila* development, after cellularization, when they again appear to act in concert with the other pathway proteins (Letsou *et al.*, 1991). Nevertheless, one or more of the known pathway components may contain sequence elements whose function is dedicated to the syncytial environment and which will not be found in a mammalian homologue. For example, a domain in one of the *Drosophila* proteins might be specifically required within the embryo to regulate diffusion and hence the extent and slope of the signal gradient.

For the three pathway proteins that are known to have vertebrate homologues, the conserved domains have amino acid sequences that have 25–50% identity with the corresponding residues in their mammalian counterparts. The evolutionary conservation of both sequence and function between Toll and the IL-1 receptor, between cactus and IκB, and between dorsal and NF-κB indicates that the two pathways share the same basic mechanism for signal transduction. It is thus easy to imagine that a one-to-one correspondence will be found between each of the proteins in the pathways linking Toll to dorsal and the IL-1 receptor to NF-κB. It is unlikely, however, that each of the branches in the mammalian pathway, both downstream of the IL-1 receptor and upstream of NF-κB (Lenardo and Baltimore, 1989; Blank *et al.*, 1992; Dower *et al.*, 1992), will also be found in *Drosophila*.

PERSPECTIVES

For further studies of the signaling process, the *Drosophila* embryo offers a number of experimental advantages beyond the apparent simplicity of the pathway. First, genetic approaches can be used to screen for new pathway components or for interactions among the known participants. Second, the signaling pathway can be turned on or off by inactive or constitutively active forms of Toll (Anderson *et al.*, 1985b; Schneider *et al.*, 1991). Third, the activity of wild-type or mutated forms of any of the known genes can be readily assayed by microinjecting RNA transcripts generated in vitro into mutant embryos (Hashimoto *et al.*, 1988, Letsou *et al.*, 1991; Geisler *et al.*, 1992; Kidd, 1992; Shelton and Wasserman, 1993; Stein, personal communication). Last, with the identification of the pelle gene product as a protein kinase, in vitro reconstitution of one or more steps in the signaling pathway should soon be possible.

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