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37. We are grateful to N. Xuong for allowing us to use his data collection facilities (University of California at San Diego), P. Tücker for help with data collection at the European Molecular Biology Laboratory, and A. Mitschler for his excellent assistance during data collection. We thank D. Moras and M. H. V. Van Regenmortel for constant interest and critical comments on the manuscript.

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Competition for Overlapping Sites in the Regulatory Region of the *Drosophila* Gene *Krüppel*

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A 730–base pair element regulates expression of the *Drosophila* gap gene *Krüppel* (*Kr*) in response to the fly anterior morphogen *bicoid* (*bcd*). Two hormone receptor–like proteins, encoded by the genes *knirps* (*kni*) and *tailless* (*tll*), bind specifically to the element. In vitro, *kni* protein competes with the homeodomain-containing *bcd* protein in binding to a 16–base pair target sequence. In vivo experiments suggest that both *kni* and *tll* act as competitive repressors of *bcd*-mediated activation of *Kr*. These results suggest a mechanism by which developmental genes can be regulated in response to an activating morphogen gradient antagonized by repressors.

In vertebrates, hormone receptors are transcription factors involved in developmental processes, cell differentiation, and morphogenetic events (*1*). They respond to extracellular ligands, including steroid hormones, vitamin D, or retinoic acid (*1*). In addition to having the common structure of the DNA-binding domain characterized by a pair of Cys-containing zinc fingers (*2*), these transcription factors share a varying degree of sequence similarity in the COOH-terminal ligand-binding domain (*1*). On the basis of sequence similarity, eight *Drosophila* genes have been grouped as putative members of this superfamily of nuclear hormone receptors (*3*). Two of them, *tll* (*4*) and *kni* (*5*, *6*), are gap genes, which are the first genes in the segmentation gene hierarchy to be expressed in *Drosophila* embryonic development (*7*). Consistent with their mutant phenotypes, *kni* and *tll* are expressed in spatially restricted domains along the anterior-posterior axis of the blastoderm embryo, specifying abdominal segments (*4*) and terminal pattern elements (*6*), respectively. Genetic analysis suggests that both genes act as negative regulators of a third gap gene, *Kr* (*8*, *9*), which is required for the formation of thorax and anterior abdominal segments (*7*, *10*). We show that *kni* and *tll* proteins interact with multiple specific sites within the 730-bp regulatory region (*Kr* 730) of the *Kr* gene (*11*). This previously characterized *Kr* 730 element is controlled by the morphogenetic *bcd* protein (BCD) gradient (*12*) and is sufficient to direct correctly localized activation of a reporter gene in the central domain of *Kr* gene expression in early blastoderm embryos (*11*).

Using bacterially expressed *kni* protein (KNI) and the DNA binding region of *tll* protein [TLL(181-517)], we found, with in vitro footprinting (*13*), a single strong KNI binding site and seven TLL(181-517) binding sites on the *Kr* 730 element (Figs. 1 and 2). Palindromic or tandemly repeated sequences, such as those observed with different vertebrate hormone receptors (*14*), were not observed in the KNI and TLL(181-517) consensus sequences (*15*).

The binding sites of TLL and the KNI binding site overlap with previously detected binding sites of the homeodomain *bcd* protein (BCD) (*11*); KNI binds to one region and TLL binds to all regions protected by BCD (Figs. 1 and 2). We analyzed the single BCD site overlapped by a KNI binding site, a 16-bp sequence: 5′-ACTGAACTAAATCCGG-3′. KNI and BCD competed for binding to the 16-bp sequence in vitro. Increasing amounts of BCD competed for the binding of KNI. Conversely, when increasing amounts of KNI were added to a constant amount of BCD, BCD was replaced by KNI binding (*15*). These experiments demonstrate that each of the two proteins can bind but that their binding is mutually exclusive.

To examine a possible functional interaction of KNI and BCD with the 16-bp element in vivo, we transfected tissue culture cells (*16*) with reporter gene constructs containing the bacterial chloramphenicol acetyltransferase (CAT) gene under the control of the 16-bp element (*17*). No effect on the basal level of CAT gene expression was observed when plasmid DNA that contained *kni* was co-transfected. In contrast, plasmid DNA that contained *bcd* caused a dosage-dependent increase in CAT expression (Fig. 3A), indicating that the 16-bp element mediated BCD-dependent activation of gene expression. When KNI was introduced along with BCD (Fig. 3B), BCD-dependent CAT gene activation was suppressed in a manner responsive to the dosage of KNI. KNI did not make the reporter gene inactivable (Fig. 3C), but instead increased the amount of BCD necessary for activation. No repression was observed when the DNA-binding domain of KNI was mutated such that the first Cys in the second zinc finger was replaced by a Leu residue.

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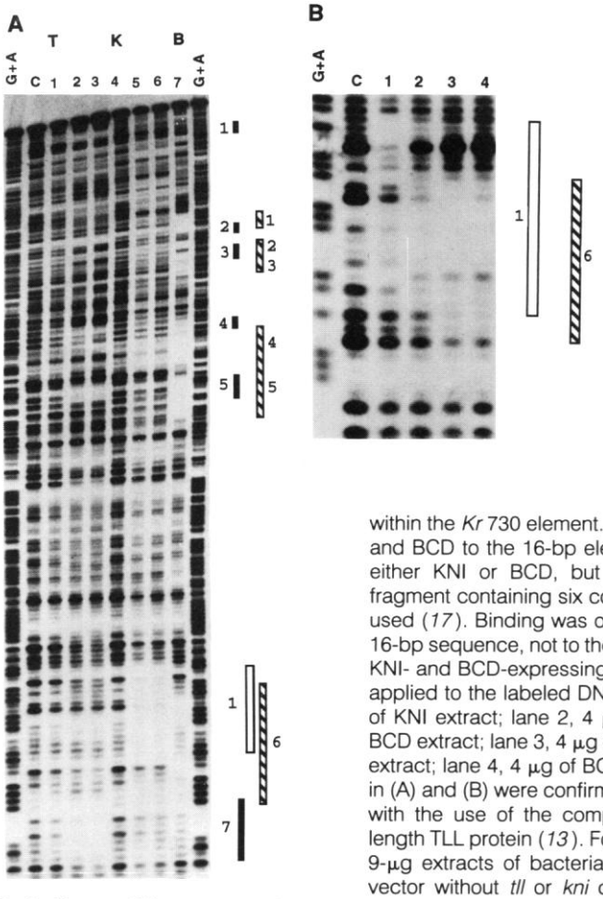

Fig. 1. DNA binding of KNI and TLL(181-517) to the *Kr* 730 element shown with deoxyribonuclease I (DNase I) footprinting (*13*). (**A**) A subfragment of the *Kr* 730 element (*13*) was incubated with 2 μg (lanes 1 and 4), 4 μg (lanes 2 and 5), or 9 μg (lanes 3 and 6) of bacterial extract containing TLL-(181-517) (lanes 1 through 3; T) or KNI (lanes 4 through 6; K). In lane 7 (labeled B), BCD footprints (*11*) are shown for comparison. Further increase of KNI or TLL(181-517) concentrations led to additional, weak protection within the *Kr* 730 element. (**B**) Competitive binding of KNI and BCD to the 16-bp element in vitro. Binding sites for either KNI or BCD, but not both, were protected. A fragment containing six copies of the 16-bp element was used (*17*). Binding was observed only to the core of the 16-bp sequence, not to the multimer junctions. Extracts of KNI- and BCD-expressing bacteria were mixed and then applied to the labeled DNA fragment (*13*). Lane 1, 4 μg of KNI extract; lane 2, 4 μg of KNI extract plus 1 μg of BCD extract; lane 3, 4 μg of KNI extract plus 4 μg of BCD extract; lane 4, 4 μg of BCD extract. Binding sites shown in (A) and (B) were confirmed by footprinting experiments with the use of the complementary strand and of full-length TLL protein (*13*). For control reactions (labeled C), 9-μg extracts of bacteria containing the T7 expression vector without *tll* or *kni* coding sequences were used. G+A, Maxam-Gilbert sequencing reactions. Areas protected by TLL(181-517) (solid bars), KNI (open bars), or BCD (hatched bars) are numbered as in Fig. 2A.

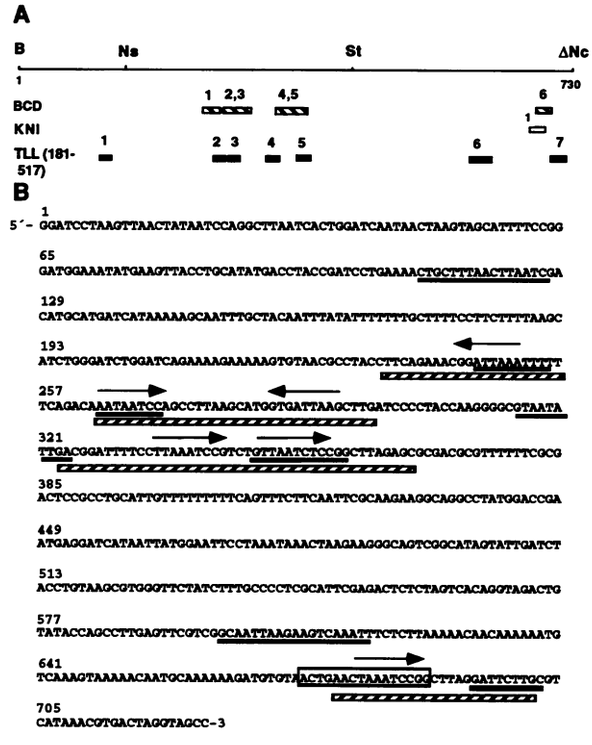

Fig. 2. KNI and TLL overlap of BCD binding sites. (**A**) Physical map of the *Kr* 730 element (*11*), showing diagnostic restriction sites: B, Bam HI; Ns, Nsi I; St, Stu I; and Nc, Nco I. Regions protected by BCD, KNI, and TLL(181-517), as revealed by footprinting experiments (see Fig. 1A), are indicated as bars. One BCD binding site overlaps with the KNI binding site (see also Fig. 1B), whereas TLL binding sites overlap with all regions protected by BCD. (**B**) Sequence of the *Kr* 730 element, including TLL, KNI, and BCD binding sites. Solid lines below the sequence refer to TLL binding sites; the boxed sequence refers to the KNI binding site. Hatched lines underline the reported BCD binding sites (*11*); arrows mark consensus BCD binding sites. For consensus KNI and TLL(181-517) binding sequences, see (*15*).

In order to see whether KNI could also act as a suppressor of BCD-dependent gene activation in the embryo, we placed the 16-bp element upstream of a reporter gene construct that contains the coding sequence of the bacterial *lacZ* gene (*17*). The resulting *Kr* 16–*lacZ* reporter gene construct was inserted into the *Drosophila* genome (*18*), and the *lacZ* expression patterns were monitored in transgenic embryos (*19*) (Fig. 4). No *lacZ* expression was observed in embryos lacking BCD (*20*). Embryos produced by females containing the two normal copies of the *bcd* gene show *lacZ* expression in the anterior cap (Fig. 4A), where BCD is present at its highest concentration (*12*, *21*). When the BCD concentration within the embryo was increased by addition of wild-type copies of the *bcd* gene to the female genome (*21*), the extent of the *lacZ* expression domain correspondingly expanded toward the posterior (Fig. 4, C and D), indicating again that gene expression mediated by the 16-bp element is dependent on *bcd* activity.

To determine whether KNI is also able to interfere with this BCD-dependent gene activation in the embryo, we placed the *Kr* 16–*lacZ* reporter gene in transgenic embryos, where KNI can be expressed under the control of a promoter element responsive to heat shock (*20*). When *kni* gene expression was induced throughout the embryo by heat shock, *lacZ* expression directed by the 16-bp element was eliminated (Fig. 4E). Taken together, these findings indicate that the 16-bp element is a responsive site for the action of KNI and BCD, both in vitro and in vivo. The disruptive effect of mutants in the KNI zinc finger domain and the overlap of KNI and BCD DNA binding sites suggest that KNI represses by competitive DNA binding (*22*) rather than by protein-protein interactions (*23*).

Repression dependent on KNI, due to blocking of BCD-dependent activation, might not be very efficient in regulating *Kr* 730–mediated gene expression because only one out of the six BCD binding sites overlaps a KNI binding site. By contrast, TLL binds to all of the regions protected by BCD on the *Kr* 730 element. These observations correlate well with genetic data indicating that KNI only weakly represses *Kr* (*8*, *24*), whereas TLL can abolish *Kr* expression completely (*9*). The *Kr* 730–mediated expression was only slightly reduced by ectopic expression of KNI, whereas ectopic expression of TLL led to a complete repression in transgenic embryos (Fig. 4, F through H). Both TLL and KNI have domains that substantially resemble the DNA-binding domain of steroid receptors, but while TLL has some weak homology to the ligand-binding domain, KNI has little similarity to that part of the classical steroid

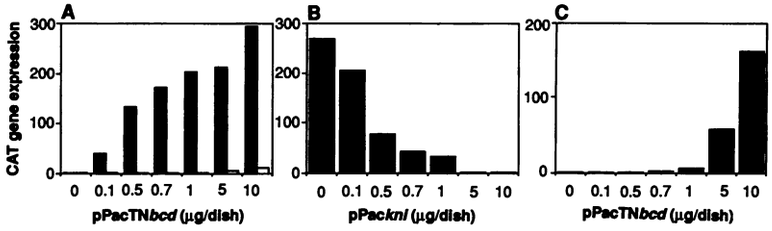

Fig. 3. The 16-bp element–mediated transcriptional activation by BCD and KNI in tissue culture cells. (**A**) *Drosophila* Schneider cells were co-transfected with various amounts of the *bcd* effector plasmid DNA pPacTN*bcd* (abscissa), and a constant amount (1 μg/dish) of CAT reporter gene construct DNA that lacked the 16-bp element (pPAdh*CAT*) or that contained seven copies of it (p*Kr*16AdhCAT) (*16, 17*). In experiments with p*Kr*16AdhCAT reporter plasmid (solid bars), CAT gene expression increased with the amount of *bcd* effector plasmid added. The control experiments with the pPAdhCAT reporter plasmid (open bars) indicate that no significant activation of gene expression occurred in the absence of the 16-bp element. Each bar represents the mean value from three independent experiments. (**B**) Co-transfection experiments with p*Kr*16AdhCAT reporter plasmid and the two effector plasmids pPacTN*bcd* and pPac*kni* (*16, 17*). Plasmid p*Kr*16AdhCAT (1 μg) and *bcd* effector plasmid (5 μg) were co-transfected with increasing amounts of *kni* effector plasmid, pPac*kni* (abscissa). Each bar represents the mean value from three independent experiments. The BCD-dependent CAT gene expression decreased with the amount of *kni* effector plasmid added. (**C**) Co-transfection experiments with constant amounts of p*Kr*16AdhCAT reporter DNA (1 μg), pPac*kni* effector DNA (0.5 μg), and increasing amounts of pPacTN*bcd* effector plasmid. Each bar represents the mean value from three independent experiments. The amount of BCD required to stimulate *Kr* 16–CAT gene expression in the presence of KNI is higher than that required in the absence of KNI (A).

receptors. There is no evidence that either one acts as receptor. This is supported by our results, which show that ectopically expressed TLL or KNI (Fig. 4, F through H) are equally functional in other embryonic regions. A ligand, if one exists, would then have to be uniformly distributed. Neither KNI nor TLL(181-517) binding sites resemble the palindromic or tandem repeat sequences recognized by vertebrate hormone receptors, which act principally as activators, not repressors, of transcription (*1, 14*).

Gene activation or repression by competition of opposing transcriptional regulators for the same binding sites (*22*), as established here for the 16-bp element, may provide a molecular basis for gap gene products to contribute to the spatial control of *Kr* gene expression (*24, 25*). The concentration of a gap protein at a particular point in the gradient of that protein across the embryo (*24–26*) may effectively block gene activation by the amount of BCD found at that location (*27*), thus restricting the initial gene activation in response to the gradient of BCD morphogen (*24, 27*).

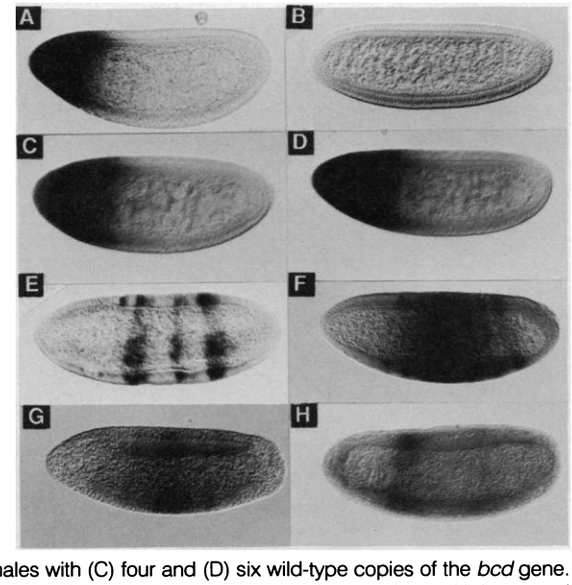

Fig. 4. Expression of *lacZ* directed by the 16-bp element and by the *Kr* 730 element in wild-type and experimentally manipulated embryos. In (A) through (D), antibody to β-galactosidase was used to stain whole mount embryos (*19*) carrying six copies of the 16-bp element in the *Kr* 16–*lacZ* reporter gene construct (*17*) in homozygous conditions. In (E) through (H), antibody to β-galactosidase and antibody to *even-skipped* protein were used together to stain whole mount embryos carrying both a *lacZ* reporter gene and an hsp70 effector gene construct (*20*). (**A**) Embryo derived from wild-type females (two copies of the *bcd* wild-type gene). (**B**) No *lacZ* expression was observed in embryos derived from bcd^{E1} mutant mothers (*20, 21*), that is, in the absence of *bcd* activity. (**C** and **D**) Expression of *lacZ* in embryos derived from females with (C) four and (D) six wild-type copies of the *bcd* gene. The expression domain extended more posteriorly in embryos derived from mothers with increased *bcd* activity. (**E**) Expression of *lacZ* and *even-skipped* in heat-shocked embryos containing one copy each of the *Kr* 16–*lacZ* and the hsp70*kni* genes (*20*). Heat shock leads to ectopic expression of *kni* activity throughout the embryo, resulting in a severe disruption of the *even-skipped* pattern and a lack of gene expression mediated by the 16-bp element. In a control experiment, heat shock of embryos carrying the *Kr* 16–*lacZ* gene [as in (A) through (D)], but no hsp70*kni* gene, did not lead to repression of *lacZ* expression, indicating that the lack of the 16-bp element–mediated gene expression is due to ectopic *kni* activity. (**F**) *Kr* 730–mediated gene expression in early blastoderm embryos (*11*). Staining of the *even-skipped* protein (*19*) served as an internal standard for the staining intensity. (**G**) *Kr* 730–mediated gene expression in transgenic embryos containing the hsp70*kni* gene (*20*). After heat shock (*24*), the amount of *Kr* 730–mediated gene expression was reduced in comparison to the amount of *even-skipped* protein staining. The altered stripe pattern is indicative of ectopic *kni* activity. (**H**) *Kr* 730–mediated gene expression in transgenic embryos containing the hsp70*tll* gene (*9*); after heat shock (*9*), gene expression was absent. The distribution of *even-skipped* protein was abnormal, indicative of ectopic *tll* activity. Methods, constructs, and mutants are described in (*17–20*).

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13. The DNA-binding region of TLL (nucleotides 181 through 517) (*4*) was expressed by use of the bacterial expression vector pET 3c system [F. W. Studier and B. A. Moffat, *J. Mol. Biol.* **189**, 113 (1986)]. Full-length KNI [M. J. Pankratz *et al.*, *Cell* **61**, 309 (1990)] and BCD (*11*) were produced as described. Transformation, induction of gene expression, preparation of bacterial extracts, footprinting, and Maxam-Gilbert reactions were performed as described (*11*). To show all of the overlapping binding sites for BCD and TLL(181-517), and for BCD and KNI in one footprinting experiment (Fig. 1A), we used portions of the *Kr*

730 element (base pairs 101 through 383 fused to 619 through 730; Fig. 2A) (*11*). Binding site 6 for TLL(181-517) was obtained with a different subfragment of the *Kr* 730 element and is not shown in Fig. 1A. TLL(181-517) binding sites were confirmed by use of a TLL(181-517) expression plasmid that produced the full-length TLL protein (data not shown).

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15. The consensus sequences for KNI and TLL(181-517) were established from footprints of the regulatory regions of three KNI and TLL target genes. Fourteen binding sites were used to generate the KNI binding consensus (AA/TCTAA/GATC), and fifteen binding sites were used to generate the TLL(181-517) consensus (AAATTAA). M. Hoch, C. Hartmann, H. Jäckle, unpublished results.
16. *Drosophila* Schneider line S2/M3 cells were grown [P. P. Di Nocera and I. B. David, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 7095 (1983)], plated, and transfected as described (*28*). For transfection, a total of 20 μg of DNA containing various amounts of effector plasmid DNA, 1 μg of reporter plasmid DNA, 3 μg of pPac*lacZ* reference plasmid DNA [see (*17*) and Fig 3], and Bluescript carrier DNA (Stratagene) were used. Transfected cells were grown for 60 hours, washed once with phosphate-buffered saline (PBS), and lysed by repeated freeze-thaw cycles. Cell debris was removed by centrifugation. The supernatant was assayed for β-galactosidase activity to account for variations of transfection efficiency. CAT assays were performed as described [C. M. Gorman, L. F. Moffat, B. H. Howard, *Mol. Cell. Biol.* **2**, 1044 (1982)]. The production and nuclear localization of KNI and BCD in transfected cells were controlled by antibody stainings (data not shown).
17. For construction of the *Kr* 16–*lacZ* reporter gene, a double-stranded oligonucleotide of the sequence 5′-ACTGAACTAAATCCGG-3′, derived from the *Kr* 730 element (Figs. 1 and 2B), was synthesized. After self-ligation, a six-copy fragment (five sites in tandem orientation, the last one in opposite orientation) was cloned into the KrZ P-element vector (*11*). The reporter plasmid p*Kr*16AdhCAT was constructed by insertion of seven copies of the 16-bp element (five in tandem orientation and two in opposite orientation; see above) into the unique Xba I site of the pPAdh86 vector [described in (*28*)]. The pPac*kni* effector plasmid was constructed by blunt ligation of a 2.5-kb Nru I fragment of the *kni* coding region into the Bam HI site of the pPac vector (*28*). The pPacTN*bcd* effector plasmid is a pPac derivative (*27*). The pPac*lacZ* reference plasmid contains the *lacZ* coding region followed by the SV40 polyadenylation signal of the pCaSpeR AUGβ-gal vector [C. S. Thummel, A. M. Boulet, H. D. Lipshitz, *Gene* **74**, 445 (1988)].
18. The fusion gene construct *Kr* 16–*lacZ* was integrated into the *Drosophila* genome by P-element–mediated germline transformation [G. M. Rubin and A. C. Spradling, *Science* **218**, 348 (1982)]. DNA constructs were injected into *w* sn^{w} homozygous mutant embryos. Transformant lines were established, and their embryonic progeny was assayed for *lacZ* expression [Y. Hiromi and W. J. Gehring, *Cell* **50**, 963 (1987)] with antibody staining (*19*). For each experiment, at least three independent transformant lines were analyzed.
19. Antibody stainings of whole mount embryos were carried out as described [P. Macdonald and G. Struhl, *Nature* **324**, 537 (1986)] with the Vectastain ABC Elite-horseradish peroxidase system (Vector Laboratories). For staining conditions and staining control experiments see (*11*). For the staining pattern of *even-skipped* see M. Frasch and M. Levine, *Genes Dev.* 1, 981 (1987).
20. Females containing extra copies of the *bcd* gene were derived from the fly strain *bcd*$^{+5/+8}$ described (*21*). Maternally mutant embryos were scored as described (*11*); the mutant strain *bcd*^{E1} has been described (*12*). The hsp70*kni* flies contain the *kni* coding region fused to an inducible hsp70 promoter; hsp70*tll* flies contain the *tll* coding region fused to an inducible hsp70 promoter. Embryos carrying the *kni* hsp70 P-element or the corresponding *tll* hsp70 P-element were heat-shocked as described (*4, 24*). Heat-shocked embryos were identified by staining with an anti-eve antibody (*19*).
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Molecular Cloning of the Interleukin-1β Converting Enzyme

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Interleukin-1β (IL-1β) mediates a wide range of immune and inflammatory responses. The active cytokine is generated by proteolytic cleavage of an inactive precursor. A complementary DNA encoding a protease that carries out this cleavage has been cloned. Recombinant expression in COS-7 cells enabled the cells to process precursor IL-1β to the mature form. Sequence analysis indicated that the enzyme itself may undergo proteolytic processing. The gene encoding the protease was mapped to chromosomal band 11q23, a site frequently involved in rearrangement in human cancers.

The cytokine interleukin-1 (IL-1) has been implicated in inflammation, septic shock, and other physiological situations, including wound healing and the growth of certain leukemias (*1*). There are two distantly related forms of IL-1 (*2*), IL-1α and IL-1β; the latter is the predominant species released by monocytes. Most efforts to develop an IL-1 antagonist have focused on inhibition of binding to the IL-1 receptor, which mediates biological responses to both forms of the cytokine (*3*). In some circumstances, it may be desirable to discriminate between the two forms of IL-1; an alternative approach specific to IL-1β involves the inhibition of the protease required for its biosynthesis. This protease (the IL-1β converting enzyme or convertase) cleaves the inactive 31-kD precursor of IL-1β between Asp^{116} and Ala^{117}, releasing the 153 COOH-terminal amino acids that constitute the mature cytokine (*4–6*). The critical function of this protease is indicated by the recent finding that cowpox virus encodes a highly specific inhibitor of the enzyme; this inhibitor is necessary for the virus to suppress the host inflammatory response (*7*). To pursue the study of this protease, we have cloned a cDNA that encodes a proteolytically active form of this converting enzyme.

The IL-1β converting enzyme was purified from the human acute monocytic leukemia cell line THP-1, and 20 amino acids from the NH_2-terminus were determined (Fig. 1) (*8*). The convertase cDNA was isolated in three stages. First, single-stranded cDNA prepared from THP-1 cells was amplified by polymerase chain reaction (PCR) with degenerate oligonucleotide primers based on the NH_2-terminal sequence (*9*). This action resulted in a cDNA

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