Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 858–862, February 1993
Developmental Biology

Characterization of downstream elements in a Raf-1 pathway

(tyrosine kinase receptor/nuclear receptor/tailless/pattern formation/gene evolution)

G.-J. LIAW*, E. STEINGRIMSSON*, F. PIGNONI*, A. J. COUREY†‡, AND J. A. LENGYEL*‡§

Departments of *Biology and †Chemistry and Biochemistry, and ‡Molecular Biology Institute, University of California at Los Angeles, Los Angeles, CA 90024

Communicated by David Eisenberg, October 16, 1992 (received for review August 24, 1992)

ABSTRACT **At the poles of the *Drosophila* embryo, cell fate is established by a pathway that begins with the activation of a membrane-associated tyrosine kinase (the torso gene product); this then leads to activation of a serine/threonine kinase (*Drosophila* Raf-1). Activated Raf-1 then leads, by an undefined mechanism, to the transcriptional activation of the tailless (*tll*) gene; the *tll* gene product, itself a transcription factor, subsequently regulates the expression of an array of target genes. To further define this pathway, we have utilized sequence comparison between *Drosophila melanogaster* and *Drosophila virilis* to identify conserved elements in the *tll* promoter region. As assessed by DNase I footprinting and promoter dissection experiments, two of these elements are potential regulatory targets of Raf-1-activated transcription factors. Sequence comparison also reveals that the unique residues in the DNA-binding domain of the tll protein, the next component in the pathway, are conserved. One of these residues, the alanine after the last cysteine in the first zinc finger, may be responsible for part of the difference between the tll protein DNA binding site and the closely related half-site of the retinoid/estrogen receptors. Consistent with the rapid turnover of the tll protein, it contains a PEST sequence (rich in proline, glutamate and aspartate, serine, and threonine) that is also conserved.**

Cell signaling pathways initiated by activated membrane-bound protein-tyrosine kinases play essential roles in cell growth control, determination, and differentiation (reviewed in ref. 1). One of the secondary signal transducer molecules believed to respond to these receptors is the protein-serine/threonine kinase Raf-1 (reviewed in ref. 2). That c-*raf-1* is a protooncogene (2) and that transfected v-Raf protein can transactivate early response promoters, including that of c-*fos* (3), suggest that Raf-1 plays an essential role in signal transduction. The mechanism of Raf-1 activation and the sequence of steps downstream of this activation that lead to new transcriptional activity are not well understood (2).

Our clearest understanding of a pathway involving Raf-1 is based on a genetic and molecular analysis of the terminal pattern-formation pathway in the early *Drosophila* embryo (reviewed in ref. 4). The maternally encoded components of the terminal system are required for formation of the embryonic head and the tail. An as yet unknown but spatially localized ligand is presumed to convert the torso (*tor*) gene product, a uniformly distributed membrane-bound tyrosine kinase receptor, to an active form at the poles of the embryo. This then leads to activation of the *Drosophila* homolog of the mammalian Raf-1 kinase. Raf-1 kinase activity is necessary for zygotic transcriptional activation, in symmetrical polar caps, of the tailless (*tll*) gene (5). A member of the nuclear receptor family of transcription factors (6), the tll protein then activates and represses zygotic genes involved in establishing cell fate in the termini (7).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Essential for an understanding of both the *Drosophila* terminal system and, more generally, mammalian cell signaling pathways is the identification of the transcription factor(s) activated by Raf-1. This will require characterization of elements in the *tll* promoter that, in response to activated terminal system components, control expression in the polar caps. Sequence comparisons between two distantly related *Drosophila* species, *Drosophila melanogaster* (*Dmel*) and *Drosophila virilis* (*Dvir*), have proven valuable in identifying gene regulatory elements (8). Comparison of the *tll* promoter sequence of these two species, described here, reveals a number of short, highly conserved elements. The biological significance of these elements was assayed by footprinting with early embryonic nuclear proteins and by testing the ability of promoter fusion constructs bearing various of these elements to drive correct terminal expression. Using this approach, we have identified putative terminal system response elements that can form the basis of a biochemical search for the Raf-1-activated transcription factor(s).

The *Dmel–Dvir* sequence comparison also provides information about the next step in the pathway, the function of the *tll* gene product as a DNA-binding transcription factor. The tll protein contains unique amino acid residues in the canonical nuclear receptor DNA-binding Zn^{2+} fingers and in the putative ligand-binding domain; conservation of these residues argues that they are essential to the function of the protein. We discuss these unique, conserved features, as they relate to the tll consensus binding site and the rapid turnover of the tll protein.¶

MATERIALS AND METHODS

*Hin*dIII fragments from phage C1 (6), which contains the *tll* gene of *Dmel*, were subcloned into pBluescript SK (Stratagene). Nested deletions were generated by using exonuclease III and S1 nuclease (9). Both strands were sequenced by the chain termination method (10). Some sequence was obtained by the Sequencing Facility at the University of California at Los Angeles. Sequences were compiled with the DB system (11).

A *Dvir* genomic library, constructed by partial Sau3A1 digestion and ligation into bacteriophage λ EMBL3, was generously provided by J. Tamkun (University of California, Santa Cruz). Five overlapping positive clones were recovered by using ^{32}P-labeled DNA of the *Dmel tll* cDNA N4 (6) as probe at a reduced stringency (42°C in 0.3 M NaCl/0.03 M sodium citrate, pH 7/20% formamide); all had in common a 4.9-kb *Xba* I–*Eco*RI genomic fragment that hybridized with the N4 cDNA on Southern blots. This *Xba* I–*Eco*RI fragment was divided into two regions (overlapping by 267 bp) and subcloned into pBluescript SK (Stratagene). Generation of

Abbreviations: *Dmel, Drosophila melanogaster; Dvir, Drosophila virilis.*

§To whom reprint requests should be addressed.

¶The sequences reported in this paper have been deposited in the GenBank data base [accession nos. L04954 (*Dmel*) and L04955 (*Dvir*)].

nested deletions and sequencing were then carried out as described above. The *Dmel* and *Dvir* sequences were compared by using the University of Wisconsin Genetics Computer Group software package.

RNA in whole embryos was detected by *in situ* hybridization as described (6).

Fragments of the D_3 region of the *tll* promoter (shown in Fig. 3*A*) were generated by digestion with appropriate restriction enzymes and oligomerized either as described (12) or by ligating *Eco*RI–*Pst* I fragments in the presence of a single-stranded DNA (5′-AATTTGCA-3′) and both restriction enzymes at room temperature. Oligomers containing four tandemly repeated copies of different portions of the D_3 region were inserted into a *P*-element vector, PwHZ16, and then injected into embryos to generate germ-line transformants, as described (13).

For DNase I protection experiments (14), a DNA fragment extending from nt −371 to +465 of the *Dmel tll* gene was labeled with [α-^{32}P]dCTP and Klenow fragment of DNA polymerase (9) and then incubated with nuclear extract of 0- to 4-hr embryos (15) before addition of DNase I.

RESULTS AND DISCUSSION

***tll* genomic DNA from *Dmel* and *Dvir*.** Because *Dmel* and *Dvir* diverged from one another >40 million years ago (16), comparison of the *tll* genes from the two species should identify, as the most conserved regions, functionally significant domains within the gene. This approach has been used successfully to guide the identification of gene regulatory elements; most conserved sequences identified in this way and then tested by mutagenesis have been found to play required roles in the control of gene expression (8).

Genomic *tll* DNA sequence was determined from previously isolated *Dmel* genomic DNA (6) and from *Dvir* genomic DNA isolated as described in *Materials and Methods* (Fig. 1). The deduced tll amino acid sequences from *Dvir* and *Dmel* are very similar (see below). In addition, *in situ* hybridization of *Dvir* genomic DNA to whole *Dvir* embryos shows a pattern of mRNA expression indistinguishable from that seen for the *Dmel tll* gene (data not shown). Taken together, these results indicate that the genomic DNA recovered from *Dvir* constitutes the *tll* gene of this species.

Conserved Sequence Elements in a *tor*-Responsive Portion of the *tll* Promoter. Previous dissection of the *Dmel tll* promoter indicated that immediately proximal to the transcription initiation site is a 365-bp element, referred to as D_3, that mediates activation by the terminal system (13). Comparison of the *Dmel* and *Dvir* genomic DNA in this D_3 region reveals five short nucleotide stretches of 15–30 bp (boxes I–V, Fig. 1) that are conserved in both sequence (87–96% identical) and position. In addition to these five elements, both D_3 regions contain multiple repeats of the sequence GAGAGA (doubly underlined in Fig. 1) within the D_3 region. This sequence is the binding site for the GAGA factor, a protein believed to control the expression of a number of pattern-formation genes in the early *Drosophila* embryo (17). The precise locations of the GAGA binding sites are not conserved.

DNase I Protection of Conserved Elements in the D_3 Region. Transcription factors present in the early *Drosophila* embryo are likely to bind *in vitro* to sequence elements in the *tll* promoter that play a role in activation of the gene. Five sites in the *Dmel* D_3 region are protected from DNase I digestion by nuclear extracts of early embryos (Fig. 2). Two of these footprints (C and D) map to the conserved elements II and III (Figs. 1 and 3*A*). The remaining three footprints, A, B, and E, are over the GAGA factor-binding sites.

Key Role of Region $D_{3.2}$ Shown by Germ-Line Transformation Studies. To examine the functional role of different sequence elements identified in the *Dmel* D_3 region, we prepared constructs carrying oligomerized fragments of this region inserted adjacent to a β-galactosidase reporter gene. The D_3 region was subdivided into three portions, $D_{3.1}$, $D_{3.2}$, and $D_{3.3}$; constructs carrying various combinations of these, and the corresponding *in vivo* expression patterns, are summarized in Fig. 3*A*. As shown previously (13), the intact region D_3 (construct G1) drives expression in the terminal region similar to that of the endogenous *tll* gene (Fig. 3*B*). $D_{3.2}$ alone (construct G5) drives a very low level of expression (detected only by *in situ* hybridization) at the posterior pole (Fig. 3*E*), while $D_{3.1}$ (construct G4) and $D_{3.3}$ (construct G6) give no detectable posterior expression on their own. We conclude that the three conserved elements within the $D_{3.2}$ region are likely to be direct targets for the terminal signal transduction pathway.

While $D_{3.1}$ and $D_{3.3}$ do not by themselves drive terminal expression, they both are capable of interacting with the terminal-specific element in $D_{3.2}$ in a positive manner. Specifically, both construct G2 (which contains $D_{3.1}$ and $D_{3.2}$) and construct G3 (which contains $D_{3.2}$ and $D_{3.3}$) drive much stronger terminal expression than does the construct bearing only $D_{3.2}$ (Fig. 3 *C* and *D*; see legend for details). Thus, $D_{3.1}$ and $D_{3.3}$ appear to serve partially redundant functions. We speculate that this redundant function could be provided by the GAGA binding sites found in both subregions. It should be noted that binding sites for the GAGA factor are found in a variety of promoter elements, and this factor is available uniformly throughout the early embryo (W. Soeller, personal communication).

The results of this analysis are consistent with the interpretation that elements II and III (which are conserved between *Dmel* and *Dvir* and also bind early embryonic nuclear proteins) mediate localized terminal system activation and that multiple nearby GAGA sequences act to significantly enhance the level of this expression.

Conservation and Significance of Unique Features of the tll Transcription Factor. The sequence comparison between the coding regions of the *Dvir* and *Dmel tll* genes contributes to an understanding of this next level of the pathway by revealing that unique motifs in the tll protein are conserved. As a member of the nuclear receptor superfamily, the tll protein contains a Zn^{2+} finger DNA-binding domain, C, and a ligand-binding and dimerization domain, E. The two other domains, A/B and D, are thought to be involved in transcriptional activation and nuclear localization, respectively (20, 23). The 95% conservation throughout the entire tll protein is unusually high for comparisons between the two species (reviewed in ref. 24; Fig. 1). This is most likely a function of the high conservation of nuclear receptors generally and the fact that the two most conserved domains, C and E, constitute most of the mass of the relatively "streamlined" tll protein.

Residues Involved in Specificity of DNA Binding. The C domains of the *Dmel* and *Dvir* tll proteins are 100% identical. Thus, the unique features of the tll DNA-binding domain are conserved and presumably play a role in determining the sequence specificity of DNA binding. One unique feature of the tll C domain is the presence of two additional amino acids in the D box (6, 21), usually a five-amino acid sequence in the second Zn^{2+} finger (see Fig. 1) that is required for discrimination between different half-site spacings in the DNA target (19). The exceptional D box of the tll protein may be related to the fact that the reported tll binding site does not contain a repeated half-site.

Unique residues in another portion of the C domain can be related to the unique tll half-site for DNA binding. Three amino acid residues (in bold below) in the P box of the DNA-binding domain, located in the first Zn^{2+} finger (see Fig. 1), determine sequence specificity of DNA binding (19). The retinoid, estrogen, and COUP receptors have a P-box

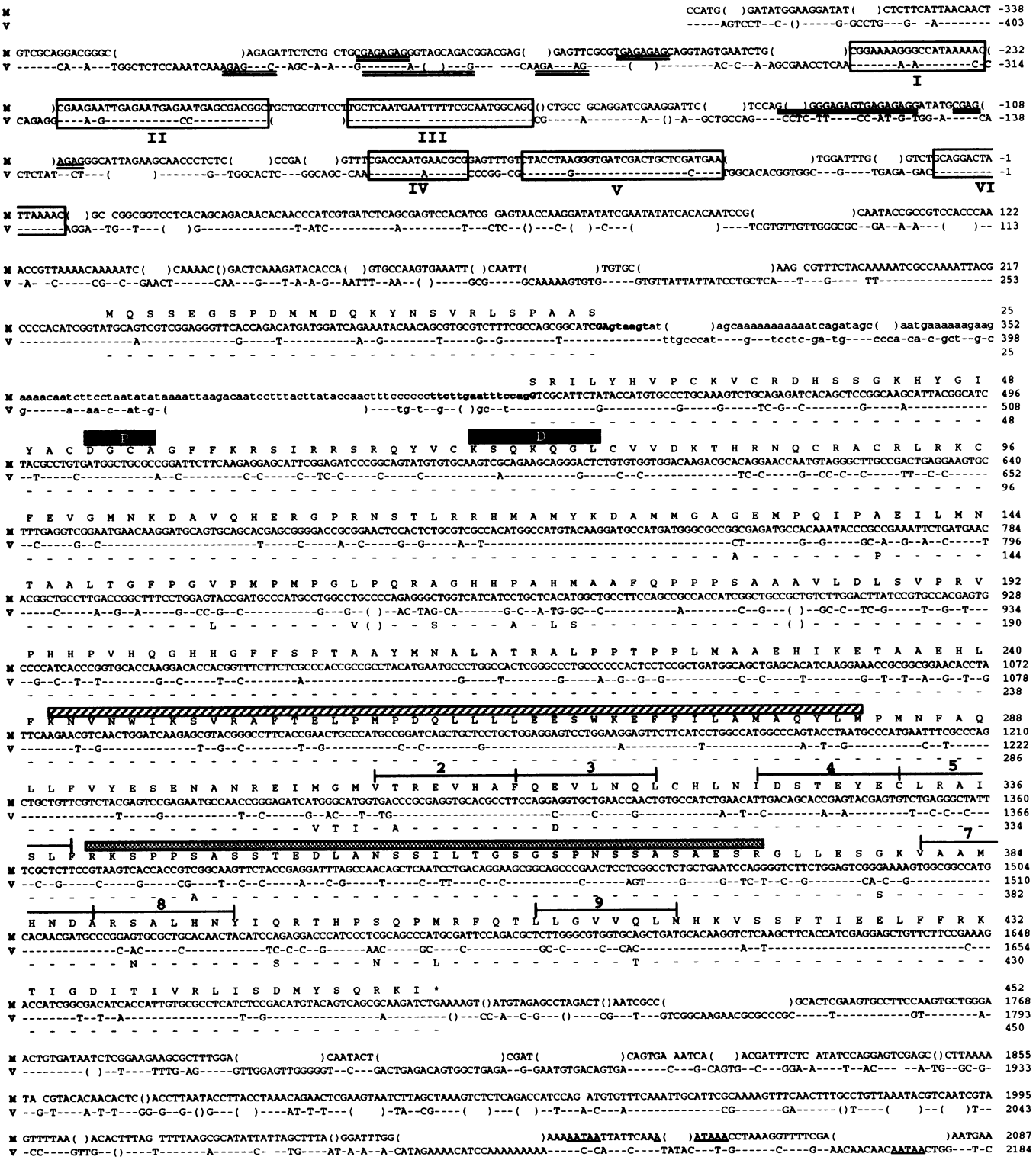

FIG. 1. Comparison of *tll* genomic sequence in *Dmel* and *Dvir*. The aligned genomic DNA sequence is in the central two lines (M = *Dmel*, V = *Dvir*). At both the nucleotide and the amino acid level, a dash in the *Dvir* sequence indicates no difference between the sequences; gaps are indicated by empty parentheses. In the noncoding upstream region, stretches of highly conserved nucleotide sequence are boxed (I–VI). Sequences homologous to the binding site of the GAGA factor (17) are doubly underlined. In the transcribed region, a 16-nt sequence that is identical between the two species (box VI) contains the transcription initiation site in both cases (13). The *Dmel* genomic *tll* sequence is consistent with that of the cDNA and also reveals the presence of a single small intron of 121 nt. The intron sequence (lowercase) lies between nt 256 and 257 of the cDNA (6). At the 5′ and 3′ ends of the intron are sequences (boldface) that show a high degree of similarity to characterized splice donor and acceptor sites (18), and that are well conserved between the two species. Putative poly(A) addition sites are underlined; the first site in the *Dmel* sequence is contained within the N4 cDNA sequence (6). The length of the predicted spliced mRNA, based on the positions of the transcription initiation site (13), the splice donor and acceptor sites, and the poly(A) addition site, is consistent with the 2.0-kb *tll* mRNA size determined from RNA gel blots (6). The amino acid sequence (single-letter code) is shown above the encoding nucleotide sequence for *Dmel* and below for *Dvir*. Various motifs are marked by bars above the amino acid sequence: P and D boxes (19), solid bars; transcription inhibition (Ti) domain (20, 21), hatched bar (amino acids 242–282); PEST sequence (ref. 22; PEST-FIND score = 10.98), stippled bar (amino acids 340–373). Each unit that aligns with one of the nine postulated heptad repeats (20, 21) is indicated by an overline labeled with the number

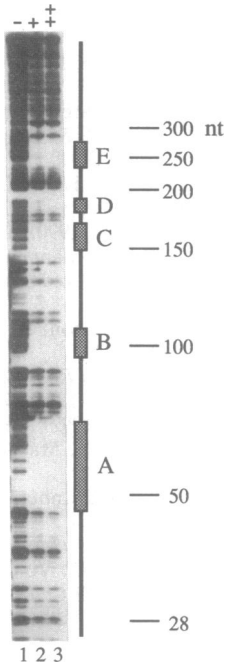

FIG. 2. DNase I footprints in the D_3 region. A *Dmel* DNA fragment extending from −371 to +465 was labeled as described in *Materials and Methods*. DNase I footprinting assays were performed with nuclear extract from 0- to 4-hr *Dmel* embryos. The probe was incubated with no extract (lane 1), 5 μl of extract (lane 2), or 10 μl of extract (lane 3). Boxes A–E indicate the regions protected from DNase I digestion by the nuclear extract. Numbers at right represent sequence coordinates determined from chemical sequencing markers (not shown).

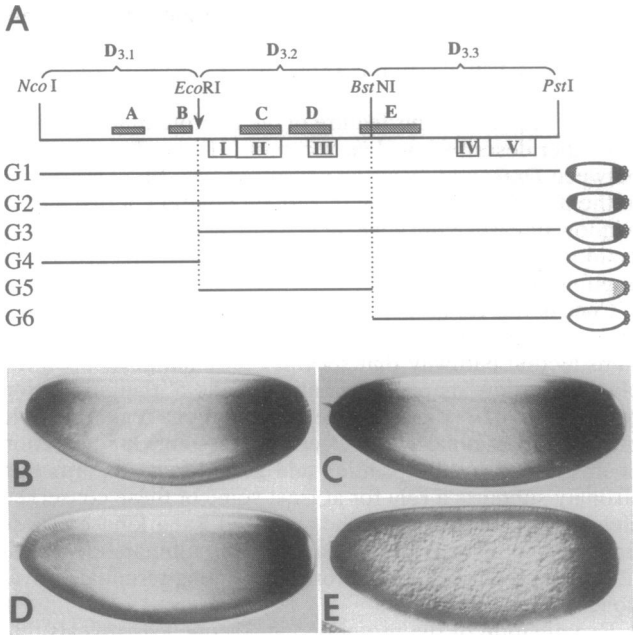

FIG. 3. Terminal expression driven by different portions of the D_3 region. (*A*) By digestion with restriction enzymes at the sites indicated (*Pst* I, −6; *Bst*NI, −134; *Nco* I, −371), the indicated subfragments of the D_3 region were generated; these fragments were oligomerized, inserted into a *P*-element vector, and transformed into the germ line as described in *Materials and Methods*. The *Eco*RI site at position −255 was generated by *in vitro* mutagenesis (9). The β-galactosidase expression pattern driven by each construct is shown in cartoons at right. The open boxes in the D_3 region (I–V) indicate the sequence stretches in this region that are highly conserved between *Dvir* and *Dmel* (Fig. 1). The shaded boxes (A–E) indicate sites that are protected from DNase I digestion by early embryonic nuclear extract (Fig. 2). Footprints A and B contain the GAGA sequences CGAGAGAG and TGAGAGAG (17) respectively. Footprint E contains three GAGA sequences: GGGAGAGAG, TGAGAGAG, and CGAGAGAG. (*B–E*) Embryos carrying the constructs G1 (*B*), G2 (*C*), and G3 (*D*) were fixed and stained with 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside at the cellular blastoderm stage. Note that the anterior cap of expression is stained more weakly in the embryos carrying constructs G1 and G3, whereas in the embryo carrying construct G2, both caps are stained equally. This indicates that within region $D_{3.3}$ is an element that mediates repression throughout the anterior cap by bcd (5, 13). While no β-galactosidase activity was detected in embryos carrying constructs G4, G5, and G6 (data not shown), a low level of β-galactosidase mRNA was detected by *in situ* hybridization in the posterior of embryos carrying the G5 construct (*E*).

sequence of Cys-**Glu-Gly**-Cys-Lys-**(Gly/Ala/Ser)** and bind to the half-site AGGTCA (reviewed in ref. 20). The tll protein has a related but unique P-box sequence, Cys-**Asp-Gly**-Cys-Ala-**Gly** (6, 21) and binds to the sequence AAAAGTCAA (25), within which is found the related half-site AAGTCA. The basis for the guanine/adenine difference in the second position of the two half-sites may be the alanine at position five of the tll P box; all other known P boxes contain a lysine at this position (21). Although mutagenesis experiments argue that it is required for transactivation rather than for DNA binding (26), in the crystal structure of the glucocorticoid receptor, this lysine contacts the guanine residue at the second position in the half-site (27).

Motifs Involved in Dimerization. The E domain of nuclear receptors was originally defined as the region required for ligand binding (23) and was later shown to contain domains involved in dimerization, and in release from transcriptional inhibition by binding of ligand (20, 28). It is not known whether there is a ligand for the tll protein, or whether the tll protein forms dimers.

That the tll DNA binding site does not contain direct or inverted half-site repeats suggests that dimerization may not be an essential feature of tll protein binding to DNA. Further evidence supporting this notion is that the tll consensus binding site is very similar to that of NGFIB, a member of the retinoid/estrogen/COUP group of receptors that binds to its response element (AAAAGGTCA) as a monomer (29).

While dimerization of the tll protein may not be required for DNA binding, a possible dimerization motif found in other nuclear receptors is nonetheless conserved in the tll protein. The nuclear receptor E domain contains a series of nine heptad repeats that are believed to mediate dimer formation by a coiled-coil interaction between amphipathic α-helices (20). Within the tll E domain is a sequence of heptad repeats that aligns with repeats of other nuclear receptors (21); of these, repeats 2–5 and 7–9 appear to fit the criteria for forming α-helix. Of the four amino acid substitutions between *Dmel* and *Dvir* in the heptad repeat region, none is in the first, fifth, or eighth residues of each repeat that are postulated to form the hydrophobic surface involved in dimerization (20). The conservation of these repeats suggests that dimerization (or perhaps oligomerization with some other factor) may be important for some aspect of tll protein function.

A Conserved PEST Sequence and Rapid Turnover of the tll Protein. Possession of a PEST sequence (a sequence with a high content of proline, glutamate, aspartate, serine, and threonine, with positively charged amino acids at each end) is believed to target a protein for rapid turnover (22). Many proteins that turn over rapidly, including transcription factors

of the repeat; our positioning of repeats 2 and 3 is slightly more amino-terminal than proposed previously (21). The C, D, and E domains discussed in the text begin with amino acids 34, 101, and 152, respectively.

involved in *Drosophila* early embryonic pattern formation, contain PEST sequences (22). At the position where heptad repeat 6 is usually found (21), the tll protein contains a 34-amino acid sequence (Fig. 1) that has the characteristics of a PEST sequence. This feature of the tll protein, not found in any other described nuclear receptor (21), is 97% conserved between *Dmel* and *Dvir*. The presence of a PEST sequence in the tll protein is consistent with its function during a brief window of early embryonic development (6, 30) and with the rapid loss of tll protein from the gastrulating embryo (E.S., unpublished data).

Concluding Remarks. Our comparison of the *Dmel* and *Dvir tll* genes has shed light on two steps in the signal transduction pathway that specifies terminal portions of the blastoderm fate map. Examination of the 5′ flanking region revealed several conserved elements within a region capable of directing terminal-specific gene expression. Our dissection of this region by footprinting and germ-line transformation strongly supports a role for at least some of these conserved elements in responding to the activated Raf-1 kinase. These findings should provide the basis for the ultimate identification of the transcription factor(s) mediating terminal activation.

Examination of the protein-coding sequences reveals that several unique features of the tll nuclear receptor are evolutionarily conserved. Thus these features are likely to play a role in the regulation by tll of genes downstream in the pathway. The destabilizing, cassette-like PEST sequence allows the expression of the tll protein to be tightly circumscribed, limiting its regulatory role in establishing the body plan to a brief period prior to gastrulation. The unique features in the P and D boxes provide a possible explanation for the sequence preference (and thus the target-gene specificity) of the tll transcription factor, a possibility that can be tested by further analysis of tll protein–DNA interactions.

We thank M. Rechsteiner for performing a PEST-FIND search on the tll protein sequence, and U. Banerjee, B. Blumberg, S. Crews, and K. Umesono for helpful comments on the manuscript. This work was supported by an American Cancer Society grant to J.A.L. and A.J.C. and a National Institutes of Health grant to J.A.L.

1. Pawson, T. & Bernstein, A. (1990) *Trends Genet.* **6,** 350–356.
2. Li, P., Wood, K., Mamon, H., Haser, W. & Roberts, T. (1991) *Cell* **64,** 479–482.
3. Jamal, S. & Ziff, E. (1990) *Nature (London)* **344,** 463–466.
4. St. Johnston, D. & Nüsslein-Volhard, C. (1992) *Cell* **68,** 201–219.
5. Pignoni, F., Steingrimsson, E. & Lengyel, J. A. (1992) *Development* **115,** 239–251.
6. Pignoni, F., Baldarelli, R. M., Steingrimsson, E., Diaz, R. J., Patapoutian, A., Merriam, J. R. & Lengyel, J. A. (1990) *Cell* **62,** 151–163.
7. Steingrimsson, E., Pignoni, F., Liaw, G.-J. & Lengyel, J. A. (1991) *Science* **254,** 418–421.
8. Fortini, M. E. & Rubin, G. M. (1990) *Genes Dev.* **4,** 444–463.
9. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), 2nd Ed.
10. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
11. Staden, R. (1984) *Nucleic Acids Res.* **12,** 505–519.
12. Hartley, J. L. & Gregori, T. J. (1981) *Gene* **13,** 347–353.
13. Liaw, G.-J. & Lengyel, J. A. (1992) *Mech. Dev.*, in press.
14. Kadonaga, J. M., Carner, K. R., Masiarz, F. R. & Tjian, R. (1987) *Cell* **51,** 1079–1090.
15. Soeller, W. C., Poole, S. J. & Kornberg, T. (1986) *Genes Dev.* **2,** 68–81.
16. Throckmorton, L. H. (1975) in *Handbook of Genetics*, ed. King, R. C. (Plenum, New York), Vol. 3, pp. 421–469.
17. Biggin, M. D. & Tjian, R. (1988) *Cell* **53,** 699–711.
18. Mount, S. M. & Steitz, J. A. (1984) *Modern Cell Biol.* **3,** 249–297.
19. Umesono, K. & Evans, R. M. (1989) *Cell* **57,** 1139–1146.
20. Forman, B. M. & Samuels, H. H. (1990) *Mol. Endocrinol.* **4,** 1293–1301.
21. Laudet, V., Hänni, C., Coll, J., Catzeflis, F. & Stehelin, D. (1992) *EMBO J.* **11,** 1003–1013.
22. Rechsteiner, M. (1988) *Adv. Enzyme Regul.* **27,** 135–151.
23. Krust, A., Green, S., Argos, P., Kumar, V., Walter, P., Bornet, J.-M. & Chambon, P. (1986) *EMBO J.* **5,** 891–897.
24. Neufeld, T. P., Carthew, R. W. & Rubin, G. M. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 10203–10207.
25. Pankratz, M. J., Busch, M., Hoch, M., Seifert, E. & Jäckle, H. (1992) *Science* **255,** 986–989.
26. Oro, A. E., Hollenberg, S. M. & Evans, R. M. (1988) *Cell* **55,** 1109–1114.
27. Luisi, B. F., Xu, W. X., Otwinowski, Z., Freedman, L. P., Yamamoto, K. R. & Sigler, P. B. (1991) *Nature (London)* **352,** 497–505.
28. Baniahmad, A., Köhne, A. C. & Renkawitz, R. (1992) *EMBO J.* **11,** 1015–1023.
29. Wilson, T. E., Paulsen, R. E., Padgett, K. A. & Milbrandt, J. (1992) *Science* **256,** 107–110.
30. Mahoney, P. A. & Lengyel, J. A. (1987) *Dev. Biol.* **122,** 464–470.