DNA Template and Activator-Coactivator Requirements for Transcriptional Synergism by *Drosophila* Bicoid

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The template and coactivator requirements for synergistic transcription directed by a single activator, Bicoid (BCD), bound to multiple sites have been determined. Mutagenesis studies in combination with protein binding experiments and reconstituted transcription reactions identified two independent activation domains of BCD that target different coactivator subunits ($TAF_{II}110$ and $TAF_{II}60$) of the basal transcription factor IID (TFIID). The presence of both coactivators is required for BCD to recruit the TATA binding protein (TBP)–TAF complex to the promoter and direct synergistic activation of transcription. Thus, contact between multiple activation domains of BCD and different targets within the TFIID complex can mediate transcriptional synergism.

A generally accepted model for transcriptional activation postulates that activator proteins bind selectively to enhancer elements of target genes, thereby positioning one or more activation domains for contact with specific targets in the basal transcription machinery (*1*, *2*). Most eukaryotic transcriptional regulatory regions contain multiple activator DNA binding sites required to achieve enhanced levels of transcription (*3*). In some cases a single activator bound to multiple DNA sites can activate transcription synergistically. An important clue to how this synergy may work mechanistically came with the observation that most eukaryotic transcription factors contain multiple activation domains (*4*, *5*). Recent studies show that different activation domains (such as glutamine-rich, acidic, and isoleucine-rich) contact distinct subunits of TFIID that comprise TBP and at least eight associated factors, called TAFs (*6*, *7*).

In the accompanying article, we determined how two distinct *Drosophila* activators, Bicoid (BCD) and Hunchback (HB), each bearing a single but distinct activation domain, act in concert with different coactivators of the TFIID complex to direct synergistic activation of *hunchback* (*hb*) transcription in vitro (*8*). In vivo studies suggest that BCD alone can also activate *hb* transcription (*5*). The transcription levels directed by BCD depend on the number of DNA binding sites, suggesting that there is a potential synergistic interaction between multiple bound BCD molecules. Here, we investigated how a single *Drosophila* activator, BCD, bearing multiple distinct activation domains (*5*), mediates synergistic transcription when bound to two or more sites on the DNA template.

We tested whether wild-type Bicoid protein activated transcription synergistically with templates bearing one, two, or three BCD binding sites by using a reconstituted *Drosophila* transcription system comprising recombinant basal factors TFIIA, B, E, F, purified TFIIH, and RNA polymerase II in combination with either recombinant TATA binding protein (TBP), endogenous TFIID, or in vitro assembled TBP-TAF_{II} complexes (*9*). The various truncated and fusion versions of activators used in this study were overproduced in Sf9 cells infected with recombinant baculovirus expressing epitope-tagged proteins (*10*). The templates used to program transcription consisted of plasmids containing the *hunchback* promoter region fused to enhancer fragments containing one, two, or three activator binding sites (*11*). Reconstituted transcription reactions supplemented with TBP failed to support activation by BCD (Fig. 1A) (*12*). Transcription reactions supplemented with TFIID and programmed with the template containing a single BCD binding site supported a modest level of activation (threefold) even at saturating concentrations of BCD protein (Fig. 1A). In contrast, a template bearing two BCD binding sites supported approximately a 10-fold level of activation. Thus, doubling the number of BCD binding sites from one to two resulted in a greater than additive effect on the level of transcription. The addition of a third BCD binding site to the template increased activation only by an additional 25%. Transcription reactions programmed with control template without any BCD binding sites failed to support detectable levels of activation (*13*). Our results indicate that high levels of synergistic transcription by BCD can be achieved with templates containing two or more BCD binding sites in the presence of TFIID. These results establish that BCD activation is TAF-dependent and most likely requires multiple activator-TAF interactions.

In vitro protein-protein interaction assays were performed to identify the TAFs that bind selectively to the activation domain (or domains) of BCD (*14*). An affinity resin saturated with epitope-tagged BCD retained both $TAF_{II}110$ and $TAF_{II}60$ but not $TAF_{II}250$, $TAF_{II}80$, or TBP (Fig. 1B). We tested the other *Drosophila* TAFs, as well as several basal factors, but none bound BCD selectively (*13*). Wild-type BCD, which likely contains more than one activation domain, can bind selectively to at

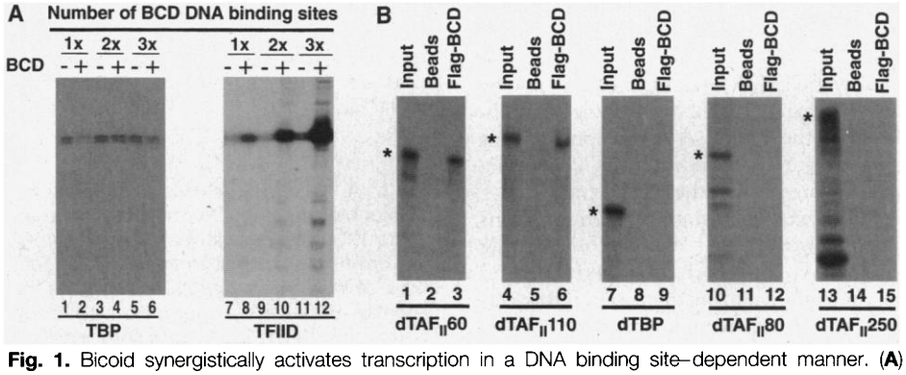

Fig. 1. Bicoid synergistically activates transcription in a DNA binding site–dependent manner. (**A**) Transcription reactions with a reconstituted *Drosophila* transcription system supplemented with either 1 ng of recombinant TBP (lanes 1 to 6) or ~25 ng of endogenous TFIID (lanes 7 to 12) with the reporter plasmids phbCAT-91 (lanes 1, 2, 7, and 8), phbCAT-231X (lanes 3, 4, 9, and 10), or phbCAT-298 (lanes 5, 6, 11, and 12) containing one, two, or three high-affinity BCD DNA binding sites, respectively (*11*). Transcription products were detected by primer extension from reactions containing no BCD (lanes 1, 3, 5, 7, 9, and 11) or 15 ng of BCD (lanes 2, 4, 6, 8, 10, and 12). (**B**) Bicoid interacts with $TAF_{II}60$ and $TAF_{II}110$. Autoradiograms representing protein-protein interaction assays in which Flag antibody resin saturated with Flag epitope–tagged BCD (lanes 3, 6, 9, 12, and 15) or resin devoid of BCD (lanes 2, 5, 8, 11, and 14) was incubated with the ^{35}S-methionine–labeled reticulocyte-expressed proteins indicated at the bottom of each panel. Protein complexes were separated by SDS-PAGE and bound proteins were detected by autoradiography. The input lane represents 25% of the starting material used in each reaction. Asterisks indicate the position of full-length proteins.

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least two distinct subunits of the TFIID complex, $TAF_{II}110$ and $TAF_{II}60$ (*6*, *15*).

In vivo studies suggested that BCD contains at least two tandem activation domains, including a glutamine-alanine–rich region (QA) and a COOH-terminal acidic region (*5*). To delineate the regions of BCD required for specific activator-TAF interactions, we generated fusion proteins comprising the glutathione-S-transferase (GST) coding region linked to different portions of BCD (Fig. 2, A and B) (*16*). The resulting GST-BCD fusion products were attached to glutathione beads and incubated with ^{35}S-labeled $TAF_{II}60$ or $TAF_{II}110$ (*17*). Both TAFs were retained on beads loaded with a fusion protein containing the Q and A domains (BCD-QA) but lacking the COOH-terminal acidic domain (Fig. 2, C and D). A deletion mutant that lacks the A region of the QA domain abolished the binding of $TAF_{II}60$ but still bound $TAF_{II}110$. Fusion proteins containing only the BCD DNA binding domain or the acidic domain failed to interact with either $TAF_{II}110$ or $TAF_{II}60$. GST alone also failed to interact with any TAFs. Thus, the glutamine domain of BCD (Q) binds to $TAF_{II}110$, whereas the alanine-rich domain (A) interacts with $TAF_{II}60$.

To determine the activator requirements for synergistic transcription, we tested BCD derivatives containing the dual activation domain, BCD-QA, or truncated versions containing either one (BCD-Q) or the other (BCD-A) activation domain (Fig. 3A) (*10*) in transcription reactions reconstituted with various in vitro assembled TBP-TAF_{II} complexes (*18*). Transcription reactions supplemented with the triple complex TBP, $TAF_{II}250$, and $TAF_{II}60$ supported activation by BCD-QA and BCD-A but not BCD-Q (Fig. 3B). In contrast, the TBP-$TAF_{II}250$-$TAF_{II}110$ triple complex mediated activation by BCD-QA and BCD-Q but not by BCD-A (Fig. 3C). These experiments establish that the Q and A activation domains can operate independently by contacting distinct targets with the TFIID complex.

Next, we tested transcription reactions supplemented with a complex containing TBP, $TAF_{II}250$, $TAF_{II}110$, and $TAF_{II}60$ for their ability to mediate synergistic activation. This quadruple complex supported simple activation by BCD-Q or BCD-A (Fig. 3D). However, BCD-QA, bound to two sites on the template, mediated synergistic activation in the presence of the quadruple complex. Neither of the two triple complexes lacking $TAF_{II}60$ or $TAF_{II}110$ supported synergistic activation by BCD-QA (Fig. 3, B and C). A template containing only one BCD DNA binding site also failed to mediate synergistic activation even in the presence of BCD-QA and the quadruple TBP-TAF_{II} complex (*13*). These results suggest that at least two different activation domains interacting with distinct coactivators in the TFIID complex are required for synergistic activation.

To further substantiate our hypothesis that synergistic activation requires direct interaction between multiple activation domains and distinct targets in the TFIID complex, we generated hybrid activators that consist of the BCD activation domains (Q or A) fused to the heterologous DNA binding domain of the *Drosophila* protein HB to form HB-Q and HB-A (Fig. 4A) (*10*). For these studies, we used a reporter plasmid containing one HB binding site and one BCD binding site fused to the *hb* promoter at position −55 (*19*). None of the activators alone or in combination supported synergistic transcription in the presence of the triple TBP-TAF_{II} complexes containing either $TAF_{II}110$ or $TAF_{II}60$ (*13*). However, a quadruple complex containing both $TAF_{II}60$ and $TAF_{II}110$ mediated strong synergistic activation when both BCD- and HB-derived fusion protein

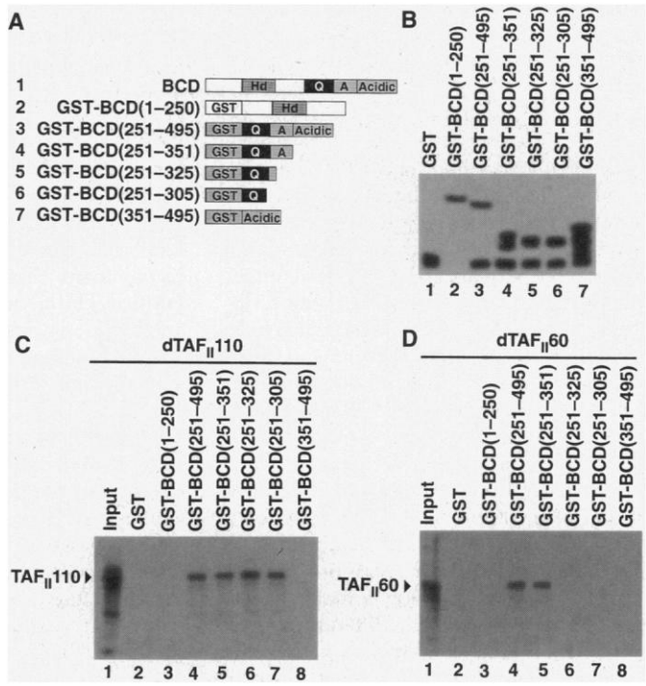

Fig. 2. $TAF_{II}60$ and $TAF_{II}110$ bind to different regions of the BCD activation domain. (**A**) Schematic representation of fusion genes encoding various GST-BCD fusion proteins used in the interaction assays performed in (C) and (D). Row 1, schematic drawing of full-length BCD; Hd, position of the homeo DNA binding domain. Q, A, and acidic indicate the glutamine-rich, alanine-rich, and acidic activation domain, respectively. Lanes 2 to 7 illustrate the architecture of fusion genes that comprise the GST coding region fused to different domains of BCD. (**B**) Coomassie-stained SDS-polyacrylamide gel showing GST (lane 1) and GST-BCD fusion proteins (lanes 2 to 7). Proteins were expressed in *E. coli*, coupled to glutathione-resin, separated by SDS-PAGE, and detected by Coomassie blue staining. (**C**) Autoradiogram of protein-protein interaction assays with GST (lane 2) or GST-BCD fusion proteins (lanes 3 to 8) coupled to glutathione resin together with ^{35}S-labeled in vitro expressed $TAF_{II}110$. Protein complexes were separated by SDS-PAGE and bound TAFs were detected by autoradiography. (**D**) Same as in (C) except that labeled $TAF_{II}60$ was used. Input lane (lane 1) represents 25% of the starting material used in the reactions. The position of full-length $TAF_{II}60$ and $TAF_{II}110$ is indicated.

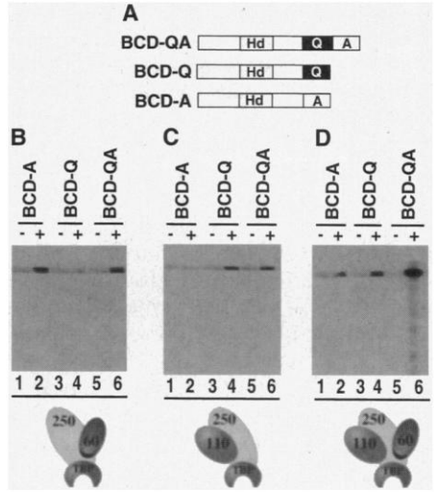

Fig. 3. (**A**) Schematic representation of Bicoid activator-proteins. BCD-QA comprises amino acids 1 to 351 containing both the glutamine-rich (Q) and alanine-rich (A) activation domain. BCD-Q encodes amino acids 1 to 305 and contains the Q-domain. BCD-A encodes amino acids 1 to 250 fused to amino acids 325 to 351 containing the A domain. Hd indicates the position of the homeo DNA binding domain. (B to D) In vitro transcription experiments with the reconstituted *Drosophila* transcription system supplemented with 4 nM of either the triple complex TBP-$TAF_{II}250$-$TAF_{II}60$ (**B**) or TBP-$TAF_{II}250$-$TAF_{II}110$ (**C**) or the quadruple complex TBP-$TAF_{II}250$-$TAF_{II}60$-$TAF_{II}110$ (**D**), using the reporter plasmid phbCAT-231X containing two BCD DNA sites (B to D). Primer extension was used to measure the products of reactions containing no activator (B to D, lanes 1, 3, and 5) or 5 ng of BCD-A (B to D, lane 2), 5 ng of BCD-Q (B to D, lane 4), or 5 ng of BCD-QA (B to D, lane 6).

containing different activation domains were bound to the template simultaneously (Fig. 4A). No synergism was observed when one transactivator was used in combination with a truncated partner lacking activation domains (*13*). These results strongly support the notion that two different activation domains, each contacting a different target in the TFIID complex, are required for synergistic transcription. Furthermore, each activation domain can transmit its signal to the basal machinery when present in the context of heterologous DNA binding domains.

The requirement for these specific and multivalent protein-protein interactions suggests that a key step in transcriptional synergism may be the recruitment of TFIID to the template. To address this question, we performed deoxyribonuclease I (DNase I) footprint protection assays with a DNA template containing two BCD binding sites (A2 and A3; Fig. 4B) and a single HB binding site derived from the *hb* enhancer (−231 to +115) (*20*). We used the activators at concentrations that protected ~90% of the DNA binding sites present in the reactions. Binding reactions containing both HB-Q and BCD-A (Fig. 4B) resulted in efficient recruitment of the quadruple TBP-TAF_{II} complex to the template, and occupancy of the TATA box region could be observed even at low concentrations of TFIID. By contrast, binding reactions with two activators bearing the same activation domain failed to recruit the binding of the TBP-TAF complex except at the highest concentration (Fig. 4B). Thus, the simultaneous interaction of different activation domains with distinct targets of the TFIID complex is necessary and sufficient to enhance the recruitment of TFIID to the promoter.

How multiple enhancer-bound activators function in concert to trigger high levels of transcription lies at the nexus of regulatory mechanisms governing gene expression in eukaryotes. Recent studies of activators and their interaction with components of the basal transcriptional apparatus have established that individual TAFs in the TFIID complex can serve as receivers of activation signals transmitted by individual activators tethered to the DNA template (*8*). However, it remained unclear how two or more activators can integrate transcriptional signals to achieve the enhanced levels of synergistic activation observed in vitro and in vivo. We describe one potential role of multiple activation domains within a single regulatory protein in mediating transcriptional synergism when bound to two or more DNA sites.

The *Drosophila* maternal regulator, BCD, contains at least two distinct activation domains (Q and A), each responsible for targeting a different coactivator subunit within the TFIID complex. Thus, when multiple binding sites on template DNA are occupied by BCD, one molecule of BCD can use its Q domain to contact $TAF_{II}110$ while a second molecule can rely on the A domain to bind $TAF_{II}60$. In this way, a single activator bound to multiple sites can simultaneously interact with different targets within TFIID and direct a wide range of activities depending on the number of DNA recognition sites, affinity of protein-DNA binding, concentration of activators, and specificity of activator-coactivator interactions. This situation is similar to that of BCD and HB binding to the *hb* promoter and mediating synergistic activation by virtue of multiple activator-coactivator interactions (*8*). An analogous situation has been reported for synergistic transcription directed by λ C_I and CRP when each contacts a different subunit of *Escherichia coli* RNA polymerase (*21*). Thus, multiple contacts between activators and basal transcription components may represent a general mechanism for achieving high levels of transcription by synergism.

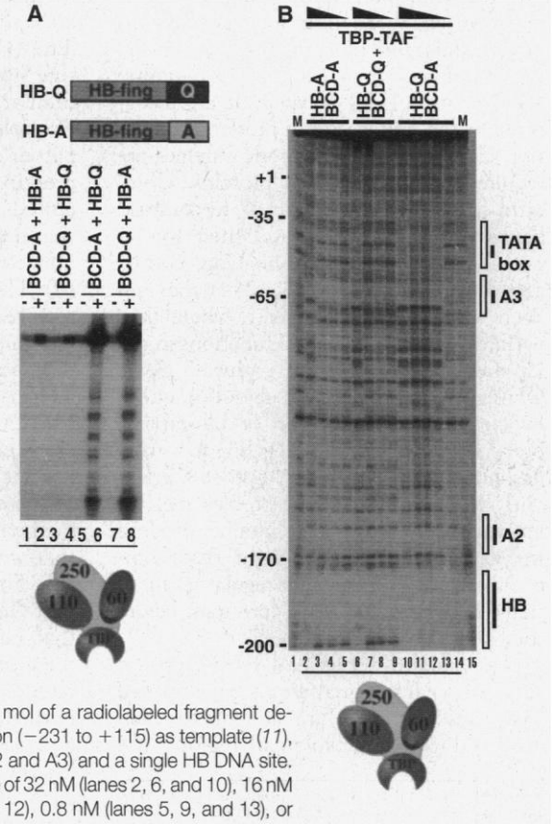

Fig. 4. Different activation domains synergistically activate transcription in concert. (**A**) Schematic representation (upper panel) of HB-BCD hybrid proteins comprising the HB zinc finger DNA binding domain (HB-fing) fused to the glutamine-rich (Q) or the alanine-rich (A) activation domain of BCD. In vitro transcription reactions (lower panel) with the recombinant *Drosophila* transcription system using the reporter plasmid pBlue*hb*-HBCAT, containing an enhancer element comprising a single BCD and a single HB DNA binding site. Primer extension was used to measure the products from reactions done in the absence or presence of different combinations of BCD and HB derivatives. Transcription reactions contained 4 nM of the quadruple complex TBP-TAF_{II}250-TAF_{II}60-TAF_{II}110 (lanes 1 to 8) and either no activator (lanes 1, 3, 5, and 7) or 5 ng of each of the activators indicated (lanes 2, 4, 6, and 8). (**B**) Enhanced binding of TBP-TAF_{II} complexes to the *hb* promoter in the presence of two different BCD activation domains. Autoradiogram of DNase I footprinting experiments with 0.2 mol of a radiolabeled fragment derived from the *hb* enhancer-promoter region (−231 to +115) as template (*11*), containing two BCD DNA binding sites (A2 and A3) and a single HB DNA site. Digestions were carried out in the presence of 32 nM (lanes 2, 6, and 10), 16 nM (lanes 3, 7, and 11), 4 nM (lanes 4, 8, and 12), 0.8 nM (lanes 5, 9, and 13), or 0.08 nM (lane 14) of the quadruple TBP-TAF_{II}250-TAF_{II}60-TAF_{II}110 complex and contained either 5 ng of BCD-A and 5 ng of HB-A (lanes 2 to 5) or 5 ng of BCD-Q and 5 ng of HB-Q (lanes 6 to 9) or 5 ng of BCD-A and 5 ng of HB-Q (lanes 10 to 14). Digests done with no protein are indicated by M (lanes 1 and 15). Rectangles, protected regions; and bars, consensus DNA binding sites.

REFERENCES AND NOTES

1. P. J. Mitchell and R. Tjian, *Science* **245**, 371 (1989); L. Zawel and D. Reinberg, *Curr. Opin. Cell Biol.* **4**, (1992); S. Buratowski, *Cell* **77**, 1 (1994); R. Tjian and T. Maniatis, *ibid.*, p. 5.
2. A. D. Frankel and P. S. Kim, *Cell* **65**, 717 (1991).
3. S. Small, A. Blair, M. Levine, *EMBO J.* **11**, 4047 (1992); G. Struhl, P. Johnston, P. A. Lawrence, *Cell* **69**, 1259 (1992); D. Herschlag and F. B. Johnson, *Genes Dev.* **7**, 173 (1993); J. Jiang and M. Levine, *Cell* **72**, 741 (1993); M. Simpson-Brose, J. Treisman, C. Desplan, *ibid.*, **78**, 855 (1994).
4. J. Ma and M. Ptashne, *Cell* **48**, 847 (1987); A. J. Courey and R. Tjian, *ibid.*, **55**, 887 (1988); M. Ptashne, *Nature* **335**, 697 (1988).
5. W. Driever, J. Ma, M. Ptashne, C. Nüsslein-Volhard, *Nature* **342**, 149 (1989); W. Driever and C. Nüsslein-Volhard, *ibid.* **337**, 138 (1989); G. Struhl, K. Struhl, P. M. Macdonald, *Cell* **57**, 1259 (1989).
6. T. Hoey *et al.*, *Cell* **72**, 247 (1993).
7. J. A. Goodrich, T. Hoey, C. J. Thut, A. Admon, R. Tjian, *ibid.* **75**, 519 (1993); J.-L. Chen, L. Attardi, C. P. Verrijzer, K. Yokomori, R. Tjian, *ibid.* **79**, 93 (1994); J. A. Goodrich and R. Tjian, *Curr. Opin. Cell Biol.* **6**, 403 (1994); X. Jacq *et al. Cell* **79**, 107 (1994); C. J. Thut, J.-L. Chen, R. Klemm, R. Tjian, *Science* **267**, 100 (1995).
8. F. Sauer, S. Hansen, R. Tjian, *Science* **270**, 1783 (1995).
9. The reconstituted *Drosophila* transcription system has been described [S. Hansen and R. Tjian, *Cell* **82**, 565 (1995)]. Assembly of partial TFIID complex was done as described (*8*).
10. The expression plasmid encoding Flag epitope–tagged BCD is described in (*8*). Baculovirus expression plasmids encoding truncated or hybrid activators were generated by fusing complementary DNA (cDNA) fragments to the COOH-terminal end of the Flag coding region in pSLFlag [S. Lichtsteiner and R. Tjian, *EMBO J.* **14**, 3937 (1995)]. To create BCD-QA, we inserted a 290–base pair (bp) Sal I–Ava I fragment (Ava I blunt end) into the Sal I–Sma I restriction sites of pSLFlag. For BCD-Q, a 170-bp Sal I–Hin PI (Hin PI

blunt ended) was inserted into the Sal I–Sma I restriction sites of pSLFlagBCD-Hd. For BCD-A, an oligonucleotide encoding BCD amino acids 321 to 351 was synthesized flanked by Sal I and Eco RI restriction sites and inserted into the corresponding restriction sites of pSLFlagBCD-Hd (*13*). For generating baculovirus expression plasmids encoding HB-A or HB-Q, fusion proteins composed of the HB zinc finger region (amino acids 185 to 407) and the BCD alanine-rich activation domain (A) or the BCD glutamine-rich activation domain (Q) oligonucleotides flanked by Eco RI restriction sites, encoding the A-domain or the Q-domain, were inserted into the corresponding restriction site of pSLFlagHB-finger (*13*). Proteins were expressed and purified as described (*8*).

11. The reporter plasmids phbCAT-91 and phbCAT-298 have been described (*5*). phbCAT-231X was generated as follows: A 269-bp Hin PI–Bam HI *hb* enhancer-promoter fragment (−154 to +115) derived from the plasmid phbCAT-231 (*5*) was fused to a synthetic oligonucleotide, encompassing the *hb* enhancer from position (−231 to −154), in which two low-affinity BCD DNA binding sites were eliminated by base-pair exchanges at position −220 and −210. The generated enhancer (−231 to +115) substituted the enhancer of phbCAT-231 to generate phbCAT-231X.
12. In vitro transcription with a reconstituted *Drosophila* transcription system was done as described (*8*). Briefly, 25 ng of template were preincubated with activators for 10 min at 4°C and then basal factors were added and the mixture was incubated for 15 min at 20°C. Transcription was started by adding nucleoside triphosphates to a final concentration of 1 mM. Products were detected by primer extension.
13. F. Sauer and R. Tjian, unpublished results.
14. Protein-protein interaction assays were done as described (*6–8*). Briefly, Flag epitope–tagged BCD was immobilized on Flag M2 antibody resin, then incubated with ^{35}S-methionine in vitro expressed TAFs or basal factors. Protein complexes were analyzed by SDS–polyacrylamide gel electrophoresis (SDS-PAGE) and visualized by autoradiography.
15. R. O. J. Weinzierl, S. Ruppert, B. D. Dynlacht, N. Tanese, R. Tjian, *EMBO J.* **12**, 5303 (1993).
16. Plasmids encoding GST-BCD fusion proteins were generated by fusing different parts of the BCD cDNA derived from pARBCD [T. Hoey and M. Levine, *Nature* **332**, 858 (1988)] to the GST coding region of the vector pGEX-2TKN (S. Ruppert and R. Tjian, unpublished results). The BCD cDNA fragments were cloned into restriction sites of pGEX-2TKN as follows: (i) GST-BCD (1 to 250), a 750-bp Nde I–Sal I fragment in Nde I–Sal I; (ii) GST-BCD (251 to 495), a blunt-ended 1-kb Sal I–Eco RV fragment into the Sal I–Sma I restriction sites to create pGEX-BCDSE; (iii) GST-BCD (251 to 351), a 300-bp Nde I–Acc I (Acc I blunt ended) fragment from pGEX-BCDSE into the Nde I–Sma I restriction sites; (iv) GST-BCD (251 to 325), a 270-bp Nde I–Rsa I fragment from pGEX-BCDSE into the Nde I–Sma I restriction sites; (v) GST-BCD (251 to 305), a 170-bp Nde I–Hin PI (Hin PI blunt ended) fragment from pGEX-BCDSE into the Nde I–Sma I restriction sites; and (vi) GST-BCD (351 to 495), a 430-bp Acc I–Eco RV fragment into the Bam HI (blunt ended)–Sma I restriction sites.
17. Protein-protein interaction assays were done essentially as described (*6, 7*) with the following modifications: The *Escherichia coli* DH 5α strain was used and protein expression was induced in cultures of these bacteria at an absorbance at 600 nm of 0.2 with 10 μM isopropyl-β-D-thiogalactopyranoside for 2 hours.
18. In vitro transcriptions with recombinant partial TBP-TAF complexes were done as described (*8, 12*) except that the partial TBP-TAF complexes were preincubated with the activator and template at 4°C for 5 min before the remaining basal factors were added.
19. In vitro transcriptions were done as described (*8, 9, 12*) except that 25 ng of the reporter plasmid pBlue*hb*-HBCAT, containing a single HB and BCD DNA binding site, were used (*8*).
20. DNase I footprinting was done as described (*8*) [T. Hoey, B. D. Dynlacht, M. G. Peterson, B. F. Pugh, R. Tjian, *Cell* **61**, 1179 (1990)]. As template we used the 346-bp *hb* enhancer-promoter fragment (−231 to +115) of phbCAT-231X (*11*). Template and activator (or activators) were preincubated at 20°C for 10 min, the partial complexes were added, and the mixture was incubated for an additional 10 min at 20°C. Digestion was initiated by adding 10 mM $MgCl_2$-$CaCl_2$ solution (50 μl) containing DNase I (5 μg/ml; Boehringer) and proceeded for 20 s.
21. J. K. Joung, L. U. Le, A. Hochschild, *Proc. Natl. Acad. Sci U.S.A.* **90**, 3083 (1993); J. K. Joung, D. M. Koepp, A. Hochschild, *Science* **265**, 1863 (1994).
22. We thank W. Driever for the phbCAT reporter plasmids; C. Nüsslein-Volhard and T. Hoey for various BCD and HB expression plasmids; K. Goodrich for oligonucleotide synthesis.; J.-L. Chen for providing baculovirus expression plasmids; C. Thut for the bacterial expression plasmid pET19bTAF$_{II}$60; all members of the Tjian lab for helpful discussions, especially H. Beckmann, J. A. Goodrich, and E. Wang; and D. Rio, M. Rothe, T. Hoey, H. Beckmann, C. Thut, J. A. Goodrich, S. Albright, and E. Wang for comments on the manuscript. Supported by a European Molecular Biology Organization long-term fellowship (F.S.), a fellowship from the University of Kopenhagen, Denmark (S.K.H.), and in part by an NIH grant (R.T.).

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Control of Cell Fate by a Deubiquitinating Enzyme Encoded by the *fat facets* Gene

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Ubiquitin is a highly conserved polypeptide found in all eukaryotes. The major function of ubiquitin is to target proteins for complete or partial degradation by a multisubunit protein complex called the proteasome. Here, the *Drosophila fat facets* gene, which is required for the appropriate determination of particular cells in the fly eye, was shown to encode a ubiquitin-specific protease (Ubp), an enzyme that cleaves ubiquitin from ubiquitin-protein conjugates. The Fat facets protein (FAF) acts as a regulatory Ubp that prevents degradation of its substrate by the proteasome. Flies bearing *fat facets* gene mutations were used to show that a Ubp is cell type– and substrate-specific and a regulator of cell fate decisions in a multicellular organism.

Ubiquitin-mediated protein degradation is an important mechanism for regulating diverse cellular functions in all eukaryotes (*1, 2*). Proteins conjugated to the 76–amino acid ubiquitin (Ub) polypeptide are recognized by the proteasome, a protein degradation complex. Many different enzymes are required to attach Ub to proteins. Ubiquitin-specific proteases (Ubps), in contrast, deubiquitinate proteins, and their role is not as well understood. The Ubps constitute a large protein family (*2–4*) that has been studied mainly in yeast, where they perform a variety of general functions in the Ub-mediated degradation pathway. Some Ubps generate monomeric ubiquitin, either by cleaving polymeric Ub or Ub-protein precursors (*3–5*) or by recycling Ub from partially degraded proteins, a process apparently required to clear the proteasome (*6*). Partly because of the large number of Ubps in yeast, it has been thought that Ubps could also perform specific regulatory functions by deubiquitinating proteins before they reach the proteasome (*2, 4, 7*).

The isolation of several yeast Ubps on the basis of functional assays has revealed that these enzymes are similar to each other primarily in two small regions, the so-called Cys and His domains, centered on a single cysteine (Cys) and two histidine (His) residues thought to be the active site of the protease (*4, 6*) (Fig. 1A). Similar Cys and His domains identify the *Drosophila* Fat facets (FAF) protein as a potential Ubp (*6*) (Fig. 1A). The FAF protein (2691 amino acids long) is required in a cell communication pathway that negatively regulates neural cell determination in the developing compound eye (*8*). The *fat facets* (*faf*) gene is specifically required for eye development, as *faf* null mutants are viable and have abnormal eye morphology (*8*). The most noticeable defect in *faf* mutant eyes is the inclusion of more than the normal complement of eight photoreceptors in each unit eye, or facet (*8*) (Fig. 2, A to H). The appearance of these ectopic photoreceptors is caused by the misdetermination of particular cells that would not normally become neurons (*8*). The only other aberration in *faf* mutant flies is that mutant females lay eggs that never reach cellularization, an early stage of embryogenesis (*8*). Thus, FAF is required in only two tissues of the fly for normal development, the ovary and the eye; it is necessary during eye development for the appropriate cell fate decisions of particular cells. We conducted a series of experiments to determine whether FAF indeed functions as a Ubp.

We first tested whether FAF had Ubp activity in a bacterial assay (*4, 5*) (Table 1).

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