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# $TAF_{II}$ mutations disrupt Dorsal activation in the *Drosophila* embryo

JUMIN ZHOU*, JÖRK ZWICKER†, PAUL SZYMANSKI‡, MICHAEL LEVINE*, AND ROBERT TJIAN†§

*Molecular and Cell Biology Department and †Howard Hughes Medical Institute, Molecular and Cell Biology Department, University of California, Berkeley, 401 Barker Hall, Berkeley, CA 94720-3204; and ‡Amplicon, Incorporated, New York, NY 11733

*Contributed by Robert Tjian, September 23, 1998*

**ABSTRACT** **In this study, we present evidence that the Dorsal activator interacts with limiting amounts of the TFIID complex in the *Drosophila* embryo. *In vitro* transcription reactions and protein binding assays implicate the $TAF_{II}110$ and $TAF_{II}60$ subunits of the TFIID complex in contributing to Dorsal-mediated activation. Mutations in $TAF_{II}110$ and $TAF_{II}60$ result in altered patterns of *snail* and *twist* transcription in embryos derived from *dl*/+ females. These results suggest that $TAF_{II}$s contribute to the activation of transcription *in vivo* and support the hypothesis that subunits of TFIID may serve as targets of enhancer binding proteins.**

Transcriptional activation via the interplay of enhancer binding proteins with the core machinery has been studied most extensively by *in vitro* biochemical strategies. A large body of evidence suggests that different activators can contact distinct components of the core machinery, including different subunits of the TFIID complex (1, 2). It has been found that certain classes of *Drosophila* and human activators require the presence of the $TAF_{II}$ subunits of TFIID to mediate activation *in vitro*. Moreover, various classes of activation domains have been shown to bind directly to specific $TAF_{II}$ subunits, and these interactions are important for mediating transcriptional activation *in vitro* (3–5). However, the *in vivo* role of $TAF_{II}$s in metazoan transcription has not been established as firmly.

Early studies of a temperature-sensitive mutant in $TAF_{II}250$ provided evidence for the function of $TAF_{II}$s in the transcription of cell cycle-regulated genes in mammalian tissue culture cells (6). Previously, an attempt was made to determine the effects of $TAF_{II}$ mutations on the expression of *hunchback* directed by the activator Bicoid in *Drosophila* embryos (7). However, the embryo staining results of this study recently were found to be incorrect (8) so that the *in vivo* role of $TAF_{II}$s in metazoan development remains obscure.

Here, we have examined the maternal Dorsal gradient to assess the role of $TAF_{II}$s in the *Drosophila* embryo. A nuclear gradient of Dorsal initiates the differentiation of the embryonic mesoderm, neurogenic ectoderm, and dorsal ectoderm (9–11). Dorsal is a member of the Rel-family of transcriptional enhancer factors and is regulated by a highly conserved signaling pathway that includes the Toll receptor and cactus inhibitor (12–14). Dorsal establishes distinct thresholds of gene expression and tissue differentiation through the regulation of different target genes in a concentration-dependent fashion (15). For example, high levels of Dorsal activate two regulatory genes, *twist* and *snail*, which initiate the differentiation of the mesoderm in ventral regions of precellular embryos (16–22). Low levels of the Dorsal gradient are insufficient to activate *twist* or *snail* but are able to trigger the expression of *rhomboid* and *short gastrulation*, which define the limits of the presumptive neurogenic ectoderm in lateral regions (23–25). These different thresholds of gene activity depend on the binding affinities of Dorsal operator sites within the target promoters and synergistic interactions between Dorsal and other adjacently bound activators (26). Dorsal not only functions as a sequence-specific transcriptional activator but also mediates repression by recruiting corepressor proteins to closely linked sites within the *zen* and *dpp* promoter regions (27, 28). There are several favorable considerations regarding the analysis of Dorsal–TFIID interactions. For example, *dorsal* gene activity is limiting in the early embryo, and the expression patterns of Dorsal target genes are relatively stable during cellularization and gastrulation. We have, therefore, chosen the Dorsal-*snail* system to reevaluate the potential role of $TAF_{II}$s in mediating transcriptional activation *in vivo*.

## METHODS

**Plasmids.** Expression plasmid encoding FLAG-tagged Dorsal protein was generated by using a *Nde*I/*Spe*I (blunt) fragment derived from pKS-Dl678 (10) inserted into pVL1392-FLAG (29) (PharMingen). The reporter plasmid was derived from E1BCAT by insertion of an oligo containing three copies of Dl binding sites of the rho NEE (−1,753 to −1,780) (24).

***In Vitro* Transcription.** *In vitro* transcription and primer extension analysis were performed as described (30) except that 100 ng of template DNA was used in 25 μl total volume. All transcription reactions contained 50 ng of dTFIIA, 25 ng of dTFIIB, 3 ng of hTFIIE56, 15 ng of hTFIIE34, and 1 μl of PolII/IIF/IIH-containing S300 fraction supplemented with 1 ng of TBP or 20 ng of peptide-eluted TFIID immunopurified from the Heparin 0.4 M fraction of *Drosophila* nuclear extracts. Reactions were preincubated for 10 min at 20°C, and transcription was initiated by adding rNTPs to 0.6 mM. *In vitro* transcription was allowed to proceed for an additional 30 min at 20°C. The mutant TAFs were preincubated with activator and template for 5 min before the addition of the other components of the transcription reaction.

**Protein Binding Assays.** Protein binding assays were performed by incubating purified FLAG-Dorsal protein (1 μg) immobilized on FLAG antibody resin (Eastman Kodak) in 0.1 M NaCl HEMG-NCDMP [25 mM Hepes, pH 7.6/12.5 mM $MgCl_2$/1 mM EDTA/10% glycerol/0.1% NP40/0.1% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate/1 mM DTT/1 mM sodium metabisulfite/0.2 mM 4-(2-aminoethyl)-benzene sulfonyl fluoride] with [$^{35}$S]methionine-labeled wild-type or mutant TAFs produced with the TNT-coupled reticulocyte lysate *in vitro* transcription/translation system (Promega) for 2 hours at 4°C. The resin then was washed extensively with 0.4 M NaCl HEMG-NCDMP resuspended in SDS loading buffer and was analyzed by SDS/PAGE.

**Protein Expression and Purification.** FLAG-Dorsal, FLAG-$TAF_{II}110\Delta C$, and FLAG-$TAF_{II}60YY$ (7) were expressed in Sf9 cells. Cell extracts were prepared 48 hours after infection by sonicating in 0.8 M NaCl HEG-NCDMP. After a high-speed spin, the extracts were diluted to 0.3 M NaCl and

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§To whom reprint requests should be addressed. e-mail: jmlim@uclink4.berkeley.edu.

were respun before they were incubated with FLAG-M2 antibody resin (Eastman Kodak) for 2 hours at 4°C. The resin was washed extensively with 1.0 M HEG NCDMP (1% NP40, 1% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate), and then the proteins either were used directly for protein binding assays or were eluted with 2 mg/ml FLAG peptide in 0.4 M HEG NCDMP. Dorsal was purified further by DNA affinity chromatography. The recombinant and partially purified basal factors were prepared as described (31).

***Drosophila* Strains.** $yw^{67}$ flies were used in this study as "wild-type" flies. A null allele of *Dorsal*, $dl^{15}$ (11, 32) in the form of $dl^{15}b/CyO$, was used. Two mutant alleles for $TAF_{II}110$, *S-466* and *XS-793* (encoding $TAF_{II}110\Delta C$ and $TAF_{II}110\Delta B$, respectively), and one mutant allele for $TAF_{II}60$, *XS-922* (7), were maintained over synthetic balancer chromosome T2B (abbreviation for TM3, *Sb e ry P[ry sev-RaslV12]*, (33). Deficiencies for $TAF_{II}110$ {*Df(3L)st-f13,Ki[1]roe[1]P[P]/[TM6B]*, 2993}, $TAF_{II}60$ {*Df(3L)KT02/TM6B]*, 3617}, and *TBP* {*DF[2R]PU-D17,NW[D]PIN[YT]/SM1]*, 2605} were obtained from the Bloomington Stock Center (Bloomington, IN). *M(2)53C/SM5* flies were from the Rubin laboratory (Univ. of California, Berkeley). Transgenic strain carrying the 1.6 *sna lacZ* transgene was as described (19).

***Drosophila* Crosses.** Virgin females doubly heterozygous for *dl* and each of the $TAF_{II}$ mutants or deficiencies were obtained by crossing $dl^{15}b/CyO$ males to virgin *TAF/T2B* or *TAF* deficiencies/TM6B females. *dl*, *TAF* double heterozygous females were crossed to either wild-type males to assess maternal effects of *TAFs* or *TAF/P[Kr-lacZ]* males to measure maternal and zygotic effects. *TAF/P[Kr-lacZ]* flies were generated from crossing *TAF/T2B* females to males homozygous for *P[Kr-lacZ]* on the third chromosome (from Gary Struhl, Columbia Univ., New York). *dl*, *Df(2)TBP* trans-heterozygous females were generated by crossing *dl/CyO* males to *Df(2)TBP/SM1* females. In control experiments, *dl* heterozygous females are generated from mating wild-type males with $dl^{15}b/CyO$ females.

***In Situ* Hybridization of *Drosophila* Embryos.** Embryos were collected and aged at 22–24°C. Embryos 2–4 hours old were hybridized with digoxigenin-UTP-labeled antisense RNA probes as described (16, 34).

## RESULTS

Strong gene dosage interactions were observed between *dl* and genes that encode bHLH activators (21, 35). For example, embryos derived from *dl/+* females that were also heterozygous for mutations in *twist*, *daughterless*, or *scute* exhibited severe patterning defects, including a reduction in the mesoderm (35). The sensitization achieved by removing one maternal dose of *dl* offered the opportunity to investigate gene dosage interactions between Dorsal and core components of the transcription complex. The initial experiments involved the use of chromosomal deletions that remove different basal factors, including TBP, $TAF_{II}110$, and $TAF_{II}60$. The expression patterns of the Dorsal target genes *snail* and *twist* were analyzed in embryos obtained from trans-heterozygous or double heterozygous females that contained one dose of $dl^{+}$ gene activity and a deficiency in a given basal factor. Embryos were collected and aged at room temperature (22–24°C), were hybridized with digoxigenin-labeled antisense RNA probes, and subsequently were stained with antidigoxigenin antibodies.

**The Early Embryo Contains Limiting Amounts of the TFIID Initiator Complex.** Embryos derived from *dl/+* heterozygotes exhibited an essentially normal *snail* expression pattern, which included a band ≈20 cells in width in the ventral and ventrolateral regions that encompass the presumptive mesoderm (Fig. 1*A*). Embryos derived from *dl/+*; *Df(3)TAF*$_{II}$*110/+* double heterozygotes exhibited a subtle but reproducible

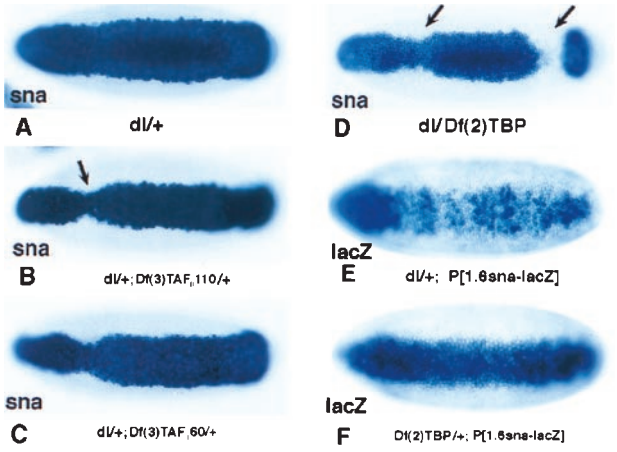


FIG. 1. *Drosophila* embryos deficient in $TAF_{II}$s or *TBP* are impaired for *dl*-dependent transcription of *sna*. Whole mount *in situ* hybridization of stage 5 *Drosophila* embryos with digoxigenin-UTP-labeled anti-sense RNA probe to *sna* (*A–D*) or *lacZ* gene (*E* and *F*) are shown. All embryos are oriented with anterior to the left and ventral surface facing the reader. Female flies with different genotypes were crossed to wild-type males (*A–D*) or wild-type males carrying a 1.6-kilobase *sna-lacZ* transgene (*E* and *F*) (19). (*A*) *sna* expression pattern in embryos from *dl/+* mothers. (*B*) *sna* expression in *dl/+; Df(3)TAF110/+* maternal background. There is a slight narrowing in the pattern, particularly in the cephalic furrow region. (*C*) *sna* expression in *dl/+; Df(3)*$TAF_{II}$*60/+* maternal background. A similar but milder effect is seen. (*D*) In *dl/Df(2)TBP* maternal background, the majority of the embryos are severely affected. (*E* and *F*) The 1.6-kilobase *snail-lacZ* transgene directs an abnormal expression pattern in embryos derived from dl/+ females (*E*); however, it directs a normal expression in embryos Df(2)TBP/+ from females (*F*).

change in the *snail* expression pattern (Fig. 1*B*). There was a slight narrowing in the pattern particularly in the vicinity of the cephalic furrow located near the head/trunk junction (Fig. 1*B*, arrow). Embryos derived from *dl/+*; *Df(3)*$TAF_{II}$*60/+* double heterozygotes exhibited a similar narrowing of the *snail* expression pattern (Fig. 1*C*), although a smaller proportion of the embryos exhibit this defect as compared with the *dl/+*; *Df(3)*$TAF_{II}$*110/+* double heterozygotes (see Table 1). The most severe defects were observed in embryos derived from *dl/DF(2)TBP* trans-heterozygotes (Fig. 1*D*). There was a dramatic narrowing in the *snail* expression pattern, and gaps appeared near the cephalic furrow and posterior transverse furrow (Fig. 1*D*, arrows).

The preceding results suggest that components of the TFIID complex, TBP, $TAF_{II}110$, and $TAF_{II}60$, are present at limiting concentrations in the early embryo. However, it is conceivable that the altered *snail* staining patterns result from a reduction

Table 1. Phenotypes of *sna* expression in different genetic background

| Maternal genotypes | Normal, % | Type I, % | Type II, % | *n* |
|---|---|---|---|---|
| *dl/+* | 92.4 | 7.6 | 0.0 | 471 |
| *dl/+; Df(3)*$TAF_{II}$*110/+* | 42.4 | 43.5 | 14.1 | 177 |
| *dl/+; Df(3)*$TAF_{II}$*60/+* | 59.0 | 37.8 | 3.2 | 188 |
| *dl/Df(2)TBP* | 18.6 | 29.4 | 52.0 | 19 |
| *dl/M(2)53C* | 83.2 | 16.8 | 0.0 | 125 |

"Normal" is defined as *sna* expression patterns indistinguishable from those of embryos derived from wild-type females. "Type I" embryos displayed a variable narrowing, especially near the cephalic furrow region. "Type II" embryos showed a more severe disruption of the *snail* staining pattern. *snail* expression domains were usually 10–15 cells wide around 50% egg length and 5 cells or less around the cephalic region. Embryos from crosses indicated in Fig. 1 (A–D) abd embryos derived from the mating of male *M(2)53C/+* X female *M(2)53C/dl1* were scored.

in the maternal transcription of *dl* during oogenesis. That is, the combined reduction in *dorsal* and TBP gene dose might lead to a severe reduction in the Dorsal nuclear gradient in early embryos. To test this possibility, we analyzed the expression of a sensitized *snail-lacZ* transgene in embryos derived from *Df(2)TBP*/+ females. The transgene contains the first 1.6 kilobases of 5′ flanking sequence from the *snail* gene and normally is expressed in the ventral-most 14–16 cells in response to high levels of the Dorsal gradient. As shown (19), this transgene directs an erratic and abnormal staining pattern in embryos derived from *dl*/+ heterozygotes (Fig. 1*E*). These results demonstrate that the *snail-lacZ* transgene is sensitive to just a 50% reduction in the levels of Dorsal. Nonetheless, a normal staining pattern is observed in embryos derived from *Df(2)TBP*/+ heterozygotes (Fig. 1*F*). This result indicates that a reduction in maternal TBP gene dose does not significantly alter the transcription of the *dorsal* gene during oogenesis. Instead, it would appear that the altered patterns of *snail* expression seen in the preceding gene dosage assays can be attributed to interactions between Dorsal and the TFIID complex on the *snail* target promoter (and/or the *twist* promoter; see below). However, a major limitation of these studies is that relatively large chromosomal deletions were used, so it is uncertain whether the altered *snail* expression patterns result from reduced levels of TBP, $TAF_{II}110$, or $TAF_{II}60$ as opposed to other genes, which are uncovered by the deletions. Both *in vitro* experiments and gene dosage assays with point mutations were used to investigate this issue.

***In Vitro* Transcription Activation by Dorsal Requires $TAF_{II}$s.** The observed alterations in the pattern of *snail* expression in embryos with deficiencies in TBP, $TAF_{II}110$, or $TAF_{II}60$ suggested that activation by Dorsal may depend on multiple subunits of the TFIID complex. To test directly the $TAF_{II}$ requirements for activation by Dorsal, we have reconstituted *in vitro* transcription reactions programmed with a DNA template bearing multiple Dorsal binding sites fused to the E1B core promoter. In the presence of purified *Drosophila* RNA polymerase II and all of the basal factors except for TFIID, no transcription was detectable (Fig. 2*A*). When purified recombinant *Drosophila* TBP was added to these reactions, accurate initiation of basal transcription was observed, but no Dorsal-dependent activation of transcription was seen. By contrast, in reactions reconstituted with affinity-purified TFIID complex, a robust Dorsal-dependent enhancement of transcription was obtained. Thus, *in vitro* activation of transcription by Dorsal appears to be $TAF_{II}$ dependent.

To determine which of the $TAF_{II}$ subunits of TFIID might serve as targets for interaction with Dorsal, we have carried out a series of activator:$TAF_{II}$ binding assays. Affinity resin containing either control beads or a FLAG–Dorsal fusion protein was used to bind individual *in vitro*-labeled $TAF_{II}$ subunits. Dorsal bound most efficiently to $TAF_{II}110$ and moderately well to $TAF_{II}60$ (Fig. 2*B*). We also observed weak binding of $TAF_{II}40$ to FLAG-Dorsal whereas no significant interaction was detected for $TAF_{II}$s 80, 30$\alpha$, or 30$\beta$ (Fig. 2*B*). These results suggest that at least $TAF_{II}110$ and possibly $TAF_{II}60$ and $TAF_{II}40$ can provide specific interfaces for interaction between Dorsal and subunits of TFIID.

**Mutant $TAF_{II}$s Squelch Dorsal-Dependent Activation *in Vitro*.** Mutant alleles for $TAF_{II}110$ and $TAF_{II}60$ ($TAF_{II}110\Delta C$, $TAF_{II}110\Delta B$, and $TAF_{II}60YY$) were isolated in a dominant modifier screen sensitized to transcription levels (7, 33). The two point mutations in $TAF_{II}110$ give rise to C-terminally truncated products whereas the $TAF_{II}60$ mutant bears an insertion of two tyrosine residues (Fig. 3*A*). Biochemical analysis of these mutant $TAF_{II}$ proteins revealed that they fail to bind the core subunit ($TAF_{II}250$) of TFIID and most likely do not incorporate into stable TFIID complexes (7). Consequently, these mutant proteins are expected to behave as transdominant negative inhibitors that can squelch activation.

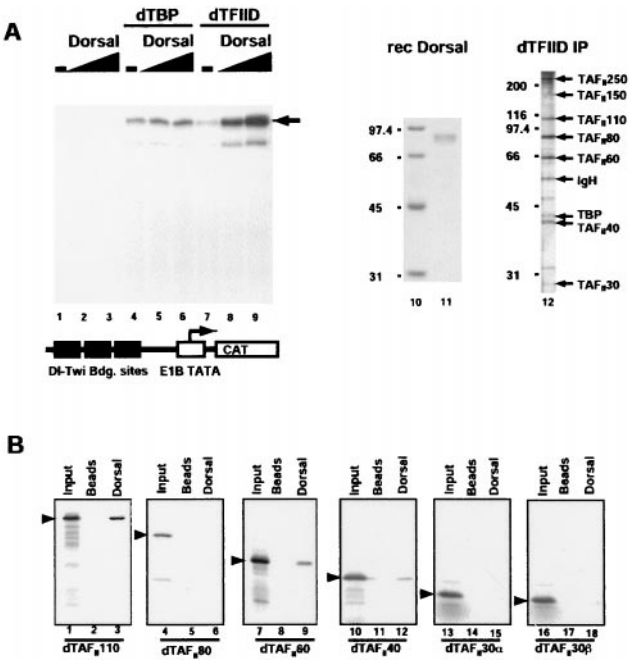


FIG. 2. Effect of $TAF_{II}$s on transcriptional activation by Dorsal *in vitro*. (*A*) Ability of TBP versus TFIID to mediate activation by Dorsal in a purified *in vitro* transcription system. (*Left*) *In vitro* transcription reactions containing the basal factors and RNA polymerase II but lacking TBP or IID (lanes 1–3) were programmed with 3 × DlTwiE1BCAT as template in the absence (lanes 1, 4, and 7) or presence of Dorsal (10 ng, lanes 2, 5, and 8 or 40 ng, lanes 3, 6, and 9) supplemented with TBP (lanes 4–6) or TFIID (lanes 7–9) and were assayed by primer extension. (*Center*) Coomassie blue-stained SDS polyacrylamide gel of purified Dorsal (lane 11). (*Right*) Silver-stained SDS polyacrylamide gel of purified TFIID (lane 12). The molecular sizes of protein standards (in kilodaltons) are indicated on the left. The identity of the TAF subunits is indicated on the right of lane 12. (*B*) Dorsal interacts with $dTAF_{II}110$ and $dTAF_{II}60$. Anti-FLAG antibody resin (lanes 2, 5, 8, 11, 14, and 17) or beads loaded with Dorsal (lanes 3, 6, 9, 12, 15, and 18) were incubated with the [$^{35}$S]methionine-labeled, *in vitro*-translated TAFs indicated at the bottom of each panel. Protein complexes were analyzed by SDS/PAGE. Bound TAFs were identified by autoradiography. Lanes 1, 4, 7, 10, 13, and 16 represent 10% of the input material for each binding reaction.

Because $TAF_{II}110$ and $TAF_{II}60$ interact with Dorsal, it should be possible to test, both *in vitro* and *in vivo*, whether these mutant $TAF_{II}$s play a role in mediating activation by Dorsal. First, we determined whether the mutant $TAF_{II}$ proteins retained the ability to bind the activator. Direct protein binding experiments revealed that FLAG–Dorsal interacts efficiently with all three of these mutant $TAF_{II}$ proteins (Fig. 3*B*). Next, we tested the ability of $TAF_{II}110\Delta C$ and $TAF_{II}60YY$ to inhibit activation of transcription by Dorsal. Fully reconstituted transcription reactions supplemented with purified Dorsal protein and affinity-purified TFIID can be significantly inhibited by the addition of purified $TAF_{II}110\Delta C$ or $TAF_{II}60YY$ protein (Fig. 3*C*). As expected, addition of increasing amounts of mutant $TAF_{II}$ protein reproducibly showed no effect on basal transcription but inhibited Dorsal activation (Fig. 3*C*, compare lanes 2, 4, and 6 to 7, 9, and 11). These results are consistent with the notion that the mutant $TAF_{II}$s are unable to incorporate stably into the TFIID complex but retain one or more coactivator domains that can squelch activation but do not inhibit basal transcription. These data suggest that the *in vitro* activation of transcription by Dorsal depends on $TAF_{II}110$ and $TAF_{II}60$.

**$TAF_{II}110$ and $TAF_{II}60$ Are Required for *snail* and *twist* Transcription *in Vivo*.** We investigated gene dosage interaction between *dorsal* and the mutant *TAF*$_{II}$s in the early embryo. The

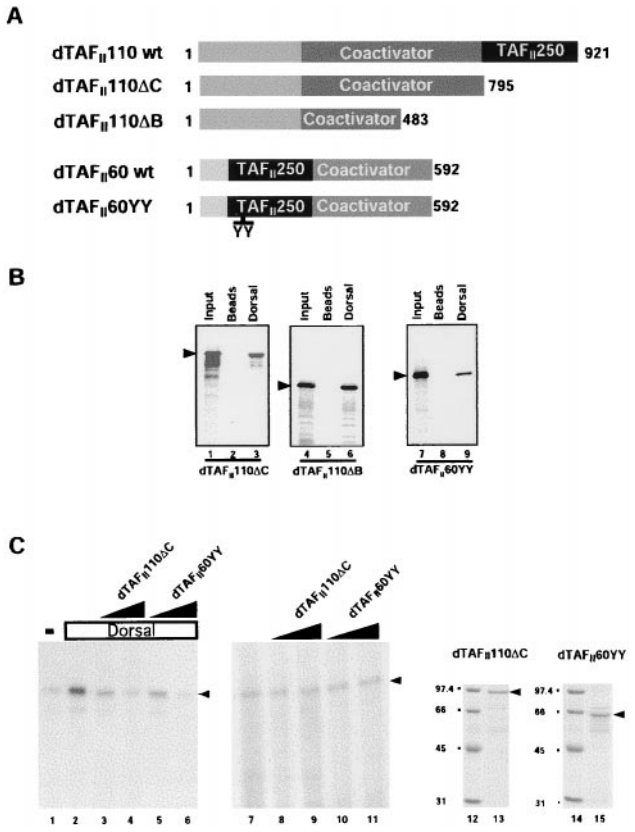


FIG. 3. (*A*) Schematic diagram of the domain structure of wild-type and mutant dTAF$_{II}$110 and dTAF$_{II}$60. The black boxed area represents the dTAF$_{II}$250 interaction domain of each TAF. The dark gray area (marked coactivator) delineates the domains that are thought to interact with activator proteins. (*B*) TAF$_{II}$110ΔC, TAF$_{II}$110ΔB, and TAF$_{II}$60YY retain the ability to bind selectively to Dorsal. Anti-FLAG antibody resin (lanes 2, 5, and 8) or beads loaded with Dorsal (lanes 3, 6, and 9) were incubated with [$^{35}$S]methionine-labeled, *in vitro*-translated mutant TAFs indicated at the bottom of each panel. Protein complexes were separated by SDS/PAGE. Bound mutant TAFs were identified by autoradiography. Lanes 1, 4, and 7 represent 10% of the input material for each binding reaction. (*C*) Mutant dTAF$_{II}$110 and dTAF$_{II}$60 squelch activation by Dorsal but not basal transcription. Reactions containing the basal factors, RNA polII, 3 × DlTwiE1BCAT DNA template, and 0 ng of Dorsal (lane 1 and lanes 7–11) or 40 ng of Dorsal (lanes 2–6) were assayed in the presence of increasing amounts (30 ng or 100 ng) of either TAF$_{II}$110ΔC (lanes 3 and 8 and lanes 4 and 9, respectively) or TAF$_{II}$60YY (lanes 5 and 10 and lanes 6 and 11, respectively). Coomassie blue-stained SDS/PAGE gels of mutant TAF$_{II}$110ΔC (lane 13) and TAF$_{II}$60YY (lane 15) as well as molecular weight markers (lanes 12 and 14) are shown.

initial series of experiments involved the mating of *dl*/+; *TAF*$_{II}$*110*/+ or *dl*/+; *TAF*$_{II}$*60*/+ double heterozygous females with wild-type males to assess potential interactions between Dorsal and maternally deposited TAF$_{II}$s (Fig. 4 *A–D*). The mutations in *TAF*$_{II}$*110* (Δ*C* and, less frequently, Δ*B*) exhibited similar genetic interactions with Dorsal (Fig. 4 *B* and *C*, respectively, and Table 2). There was a narrowing of the overall *snail* expression pattern; this narrowing was most severe in the cephalic region (Fig. 4 *B* and *C*, arrows; compare with Fig. 4*A*). A similar, but somewhat less frequent, change in the *snail* pattern was observed in embryos derived from *dl*/+; *TAF*$_{II}$*60YY*/+ double heterozygotes (Fig. 4*D* and Table 2). In all cases, the alterations of *snail* expression patterns were more severe than those observed with the chromosomal deficiencies (Fig. 1 *B* and *C*). This observation is consistent with the finding that TAF$_{II}$110 and TAF$_{II}$60 mutants encode dominant negative proteins (see above and *Discussion*).

It is conceivable that the most severely affected embryos are *TAF*$_{II}$/+ heterozygotes. In this regard, we note that *in situ* hybridization assays suggest that the *TAF*$_{II}$*110* and *TAF*$_{II}$*60* genes are transcribed quite early, during nuclear cleavage cycle 12/13 (data not shown). To investigate this issue of zygotic interactions, additional experiments were done with embryos derived from the mating of *dl*/*TAF*$_{II}$ double heterozygous females and *TAF*$_{II}$/*P[Kr-lacZ]* males. One-fourth of the resulting embryos corresponded to *TAF*$_{II}$/*TAF*$_{II}$ homozygotes. The males used in these matings carried a *Kr-lacZ* transgene so that one-half of the embryos lacking lacZ staining corresponded to TAF$_{II}$ homozygotes.

Although the putative *TAF*$_{II}$ homozygous embryos from *dl*$^{+}$ females displayed normal *snail* staining, the putative *TAF*$_{II}$ homozygous embryos exhibited severe changes in the *snail* expression pattern (Fig. 4 *F–H* and Table 2). In early embryos (at the midpoint of cellularization), there was a particularly marked narrowing of the *snail* expression pattern in anterior regions (Fig. 4 *F* and *G*, arrows). We also observed a significant number of embryos (4–8%) displaying even more severe phenotypes. For example, 8% of the TAF$_{II}$110ΔC mutants displayed either almost no *snail* staining in the anterior third of the embryo at early stage 5, or there was a pronounced gap near the cephalic furrow at mid- or late stage 5 (data not shown). These alterations in the *snail* pattern were more severe than those observed in embryos derived from double heterozygotes mated with wild-type males (Fig. 4 *B* and *C*). As seen for the chromosomal deletions, the *TAF*$_{II}$*110* mutants, especially *TAF*$_{II}$*110*Δ*C*, exhibited a somewhat more severe disruption in the *snail* pattern as compared with the *TAF*$_{II}$*60* mutant (compare Fig. 4*H* with Fig. 4 *F* and *G*; Tables 1 and 2).

Defects in the *snail* expression pattern were more pronounced in anterior regions of mutant embryos, particularly in the region spanning the presumptive cephalic furrow. These defects were similar to those observed in embryos containing reduced levels of *Dorsal* and *twist* (19, 35). It is conceivable that one or more repressors are required for the establishment of the cephalic furrow, thereby sensitizing the *twist* and *snail* expression patterns in this specific region of the embryo. We suggest that the residual *twist* and *snail* expression patterns observed in dl;TAF$_{II}$ mutant embryos resulted from the perdurance of normal TFIID initiator complexes present in the germline. It has not been possible to obtain germline clones for *TAF*$_{II}$ mutants, so that the *TAF*/+ heterozygous females used in this study always contained a wild-type copy of the gene.

The specificity of *dl*/*TAF*$_{II}$ interactions was tested by analyzing a minute mutation, *M53(C)*, which results in a general reduction in the rates of translation and embryogenesis (45). Embryos derived from the mating of *M53(C)*/*dl* females and *M53(C)*/+ males exhibited relatively minor effects on *snail* expression, as compared with the disruptions observed in embryos derived from dl;TAF$_{II}$ females (see Table 2).

The preceding analysis raises the possibility that Dorsal-TAF$_{II}$ interactions are important for the transcriptional activation of the *snail* promoter. However, it is also possible that such interactions occur on the *twist* promoter, which can be thought of as an immediate early target of the Dorsal gradient. Previous studies have shown that the Dorsal gradient activates *twist* and that the two proteins then work synergistically to activate *snail* (16–19). Thus, the disruptions we observed in the *snail* expression pattern could result, indirectly, from reduced levels of Twist. To test this possibility, embryos derived from the mating of dl;TAF$_{II}$ females and *TAF*$_{II}$/*P[Kr-lacZ]* males were hybridized with a *twist* antisense RNA probe (Fig. 4 *I–L*).

The *twist* staining pattern observed in embryos derived from *dl*/+ heterozygotes was essentially normal and encompassed the ventral-most 20–22 cells (Fig. 4*I*). The *twist* and *snail* patterns were similar, except that *snail* exhibited sharper borders of expression. Mutant embryos that were presumably homozygous for *TAF*$_{II}$*110* exhibited a narrowing in the *twist* pattern, particularly in anterior regions (Fig. 4 *J* and *K*, arrows). A similar, but somewhat less severe, alteration also was

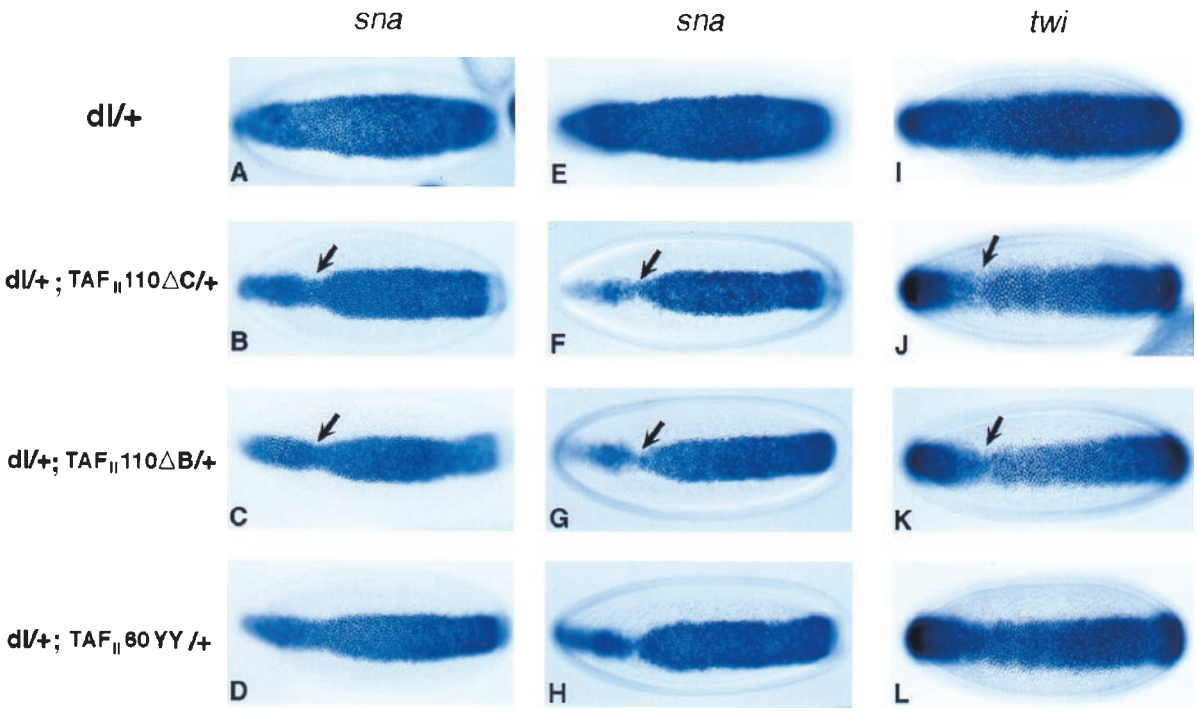


FIG. 4. TAF$_{II}$110 and TAF$_{II}$60 are required for Dorsal-dependent transcriptional activation of *twist* and *snail* in the early embryos. *In situ* hybridizations of mid-stage five embryos by using *snail* (*A–H*) or *twist* (*I–L*) antisense RNA probe are shown. Embryos from *dl/+* (*A*, *E*, and *I*), *dl/+; TAF$_{II}$110ΔC/+* (*B*, *F*, and *J*), *dl/+; TAF$_{II}$110ΔB/+* (*C*, *G*, and *K*), or *dl/+; TAF$_{II}$60YY/+* (*D*, *H*, and *L*) females that are mated with either wild-type males (*A*, *B*, *C*, *D*, *E*, and *I*), *TAF$_{II}$110ΔC/P[Kr-lacZ]* males (*F* and *J*), *TAF$_{II}$110ΔB/P[Kr-lacZ]* males (*G* and *K*), or *TAF$_{II}$60YY/P[Kr-lacZ]* males (*H* and *L*) are shown.

observed in *TAF$_{II}$60* mutants (Fig. 4*H*). In general, the changes in the *snail* patterns were more severe than those observed for *twist* (e.g., compare Fig. 4*F* with Fig. 4*J*). The simplest interpretation of these results is that Dorsal-TAF$_{II}$ interactions are important for the activation of both *twist* and *snail*. Reduced levels of Twist, together with a breakdown in Dorsal-TAF$_{II}$ interactions, resulted in a relatively severe disruption in the *snail* pattern.

## DISCUSSION

We have presented evidence that TFIID complexes are limiting in the early embryo and that two specific TAF$_{II}$s (TAF$_{II}$110 and TAF$_{II}$60) are required for Dorsal-mediated activation. One can imagine several nonexclusive models for Dorsal–TAF interactions in the *Drosophila* embryo. First, Dorsal may activate transcription through direct protein–protein interactions with TAF$_{II}$110 and/or TAF$_{II}$60. Second, mutations in TAF$_{II}$110 and TAF$_{II}$60 may alter TFIID structure or function and indirectly disrupt activation of the *twist* and *snail* target genes in sensitized *dl/+* embryos. Perhaps, mutant TAF proteins impair the recognition of the *twist* and *snail* promoters by TFIID; alternatively, the defective TFIID might affect activator interactions with other components of the transcription machinery. Finally, it is conceivable that *dl/+* embryos are sensitized to the point that any perturbation in the core transcription complex may disrupt *snail* and *twist* expression. At present, it is difficult to discriminate between these potential mechanisms.

Studies of yeast TAF$_{II}$ mutants suggested that disrupting the TFIID complex does not lead to detectable transcriptional defects (36, 37). However, more recent analysis of yeast mutant TAF$_{II}$s confirm earlier studies of mammalian TAF$_{II}$s (6) that transcription of several genes involved in cell cycle control, in fact, depend on yeast TAF$_{II}$145 (38, 39). The yeast studies have been interpreted to indicate that TAF$_{II}$145 plays a role in promoter recognition rather than mediating activation. It was, however, unclear whether mutations in TAF$_{II}$145, the homolog of *Drosophila*, and human TAF$_{II}$250 affected activation, promoter selectivity, or both because neither the identity of the activator nor the location of the critical cis-controlling elements responsive to TAF$_{II}$145 have been identified. By con-

Table 2. Phenotypes of *sna* expression in different genetic background

| | Kr-lacZ embryos, +/+ or *TAF*/+ | | | | Non-Kr-lacZ embryos, *TAF*/+ or *TAF*/*TAF* | | | |
|---|---|---|---|---|---|---|---|---|
| Maternal genotypes | Normal, % | Type I, % | Type II, % | *n* | Normal, % | Type I, % | Type II, % | *n* |
| wild-type | | | | | 96.2 | 3.8 | 0.0 | 400 |
| *TAF$_{II}$110ΔC/+* | | | | | 94.7 | 5.3 | 0.0 | 337 |
| *TAF$_{II}$110ΔB/+* | | | | | 94.4 | 5.6 | 0.0 | 285 |
| *TAF$_{II}$60YY/+* | | | | | 96.3 | 3.7 | 0.0 | 351 |
| *dl/+; TAF$_{II}$110ΔC/+* | 23.2 | 57.1 | 19.7 | 452 | 8.1 | 55.1 | 36.8 | 494 |
| *dl/+; TAF$_{II}$110ΔB/+* | 22.4 | 63.0 | 14.6 | 343 | 6.9 | 64.8 | 28.3 | 361 |
| *dl/+; TAF$_{II}$60YY/+* | 21.7 | 65.0 | 13.3 | 309 | 11.8 | 58.1 | 30.1 | 356 |

Normal, Type I, and Type II are as in Table 1. Wild-type females or female flies with designated genotype were mated with wild-type males or male flies with the corresponding *TAF$_{II}$* mutations over the *Kr-lacZ*-marked chromosome. Embryos with *Kr-lacZ* expression were either wild-type or contained one mutant copy of the indicated *TAF* gene; embryos without *Kr-lacZ* expression contained either one copy of mutant *TAF*s or were homozygous for the *TAF* mutation.

trast, studies of ts mutants of human $TAF_{II}250$ established that the core subunit of TFIID can serve as a target for activation and also can help direct promoter recognition at the *cyclin A* gene (40).

The promoter selectivity function of $TAF_{II}250$ is consistent with previous biochemical studies demonstrating the ability of certain TFIID subunits to contact core promoter DNA sequences directly (4, 41–43). *In vitro* transcription assays also indicated that $TAF_{II}250$, $TAF_{II}150$, and $TAF_{II}60$ may contribute to the binding of TFIID to specific core promoter sequences. However, these DNA binding functions of $TAF_{II}$s appear to be independent of activators, and, thus far, only three of nine $TAF_{II}$s have been found to bind DNA in a sequence specific manner. At present, there is no evidence to indicate that *Drosophila* $TAF_{II}110$ interacts with DNA.

It is possible that *dorsal*/*TAF*$_{II}$ dosage-sensitive interactions reflect a failure of TFIID complexes lacking $TAF_{II}110$ and $TAF_{II}60$ to perform core promoter recognition at the *snail* and *twist* gene sequences. However, Dorsal has been shown to be promiscuous in its ability to activate distinct core promoters, suggesting that promoter selectivity may not be the basis for the observed Dorsal/$TAF_{II}$ interactions (15). Indeed the *twist* and *snail* promoters appear to represent two distinct classes of core promoters (17, 19, 44) that are fully responsive to Dorsal, and both are affected by mutations in $TAF_{II}110$ and $TAF_{II}60$. Thus, it seems unlikely that the $TAF_{II}$-mediated activation of *snail* and *twist* by Dorsal depends solely on promoter selectivity.

Although the *in vivo* basis for Dorsal-$TAF_{II}$ interactions is uncertain, there are several arguments for direct protein–protein interactions. First, the Dorsal activator can bind selectively to at least two $TAF_{II}$s *in vitro*, and Dorsal is able to interact directly with purified TFIID in a protein binding assay in the absence of promoter DNA (data not included). Moreover, activation by Dorsal in reconstituted transcription reactions is $TAF_{II}$-dependent and is squelched by transdominant negative versions of these two $TAF_{II}$s. The mutant TAFs retain their ability to bind Dorsal but fail to incorporate into a stable TFIID (Fig. 3*B* and ref. 7) and therefore interfere with transcription when present in the embryo with wild-type $TAF_{II}$s. Consistent with this possibility is the finding that the mutant $TAF_{II}$s produce stronger effects than their corresponding deficiencies in gene dosage assays (Tables 1 and 2).

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