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# Insensitivity of the Preset *hsp26* Chromatin Structure to a TATA Box Mutation in *Drosophila**

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**The role of the TATA element in establishing the chromatin structure and inducible transcription of the *Drosophila melanogaster hsp26* gene has been analyzed. An *hsp26/lacZ* fusion gene with a mutant promoter, in which the TATA box sequence TATAAA was changed to CCCAAA, was introduced into *Drosophila* by P-element transformation. The mutation had little effect on formation of the preset chromatin structure observed prior to induction. However, the mutation dramatically reduced transcription levels following heat shock. Northern analysis indicated that weak, inducible expression of the mutant promoter occurred within the same period of heat shock as for the normal promoter, suggesting that TFIID was associated with the mutant promoter prior to heat shock. Biochemical analysis showed that the mutant promoter still bound TFIID *in vitro*, but with 3–5-fold less affinity than the normal promoter. DNase I footprinting revealed that the conformation of the TFIID·DNA complex differed significantly from that of the normal promoter. These results indicate that alterations in the conformation or the stability of the TFIID·DNA complex drastically reduce the level of induction, but do not dramatically affect chromatin structure formation. Formation of the requisite chromatin structure is either independent of, or highly tolerant of, changes in the TFIID·DNA complex.**

In eukaryotic cells, almost all of the DNA is packaged with histones into ordered arrays of nucleosomes. Biochemical and genetic analyses have established that the assembly of DNA into nucleosomes not only packages DNA effectively, but also provides an important means of transcriptional control. In many cases, nucleosomes appear to repress transcription by limiting the access of the transcriptional machinery and regulatory factors to the DNA template (1–5).

The heat shock genes of *Drosophila* are a good model system for understanding the relationship between chromatin structure and gene activation. These genes are rapidly induced by a variety of stress treatments. The promoters of these genes appear to be preset for rapid induction in a chromatin structure that is quite distinct from bulk chromatin, with certain regions showing a marked increase in sensitivity to DNase I. A variety of assays have revealed that RNA polymerase II is associated with the promoter region of heat shock genes prior to induction (6–9). The polymerase has initiated transcription but is paused in elongation in a region 20–40 base pairs downstream of the transcription start site. Genomic footprinting has revealed that the TATA box and the $(CT)_n \cdot (GA)_n$ repeats are constitutively bound by proteins, presumably TFIID (the TATA-binding protein complex) and the GAGA factor,[1] respectively (9–11). Mutagenesis of the heat shock gene promoters has shown that the $(CT)_n \cdot (GA)_n$ repeats and sequences downstream of the transcription start site contribute both to formation of the normal chromatin structure and stable association of polymerase with the heat shock genes in *Drosophila* prior to heat shock induction (12–14). Recently, the $(CT)_n \cdot (GA)_n$ repeats in the hsp70 promoter have been shown to mediate ATP-dependent disruption of nucleosomes upon addition of GAGA factor (15).

The interaction of TFIID and RNA polymerase II with the *hsp26* promoter implies a potential role of the TATA box in formation of an appropriate chromatin structure prior to induction. Biochemical analyses provide further support. If nucleosomes are reconstituted onto an *hsp70* promoter, subsequent addition of nuclear extracts from heat-shocked *Drosophila* embryos does not result in transcription. If yeast TATA-binding protein (TBP)[2] is bound prior to the reconstitution, addition of the nuclear extract leads to transcription. Hence, TBP establishes the transcriptional potential of the promoter in this model reaction (16). While these reconstitution experiments provide support for a simple model in which TBP alone might drive formation of the necessary chromatin structure, the situation is more complicated *in vivo*. Experiments with modified transgenes have shown that the TATA box and downstream sequences alone are insufficient to generate the DH sites *in vivo* (14). In addition, TBP is only one subunit of TFIID in higher eukaryotes. The remaining subunits are called TAFs (17–19). TAFs are required to reconstitute activator-dependent transcription (20–22). TAFs also appear to recognize specific sequence elements located downstream of the TATA element in the heat shock and histone promoters of *Drosophila* (23–25).

To better understand the function of the TATA box and TFIID in chromatin assembly and transcription of the heat shock genes, we have analyzed the consequences of mutating the TATA box in the *Drosophila hsp26* heat shock gene promoter. We have examined the effects of this mutation on formation of the DNase I-hypersensitive sites at the promoter and on heat shock induction of the gene *in vivo*. We have also analyzed the effects of the mutation on the binding of purified TFIID *in vitro*. Our results indicate that the wild type TATA box of the *Drosophila hsp26* gene promoter is essential for correct binding of

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[1] J. A. Weber and D. S. Gilmour, submitted for publication.

[2] The abbreviations used are: TBP, TATA-binding protein; DH site, DNase I-hypersensitive site; TAF, TBP-associated factor; kb, kilobase pair(s); HSE, heat shock element.

TFIID and for inducible expression, but not for formation of the DNase I-hypersensitive sites.

## EXPERIMENTAL PROCEDURES

*P-element Plasmids and Transformation*—The construction of pCarX has been described (26). pCarXmTATA was constructed as follows. The *Xba*I/*Sac*I fragment (−51 to +489) of *Drosophila hsp26* was cloned in M13mp18. The single-stranded form of the resulting clone was used as a template for oligonucleotide-directed mutagenesis (27), to mutate the TATA box sequence TATAAA at −30 to CCCAAA. The oligonucleotide used was 5′-TCTAGAAAAGCTCCAGCGGGCCCAAAAGCAGCGTCG-CTTGACGAACAG-3′ (underlined sequence indicates the mutant TATA box). A clone containing this mutation, M13mp18(X/S)mTATA, was identified by sequencing. The *Xba*I/*Sac*I fragment containing the TATA mutation was recovered from the replicative form of M13mp18(X/S)mTATA and used to replace the *Xba*I/*Sac*I fragment of pCarX, resulting in pCarXmTATA. *hsp26*/*lacZ* constructs were introduced into the *Drosophila* germline by P-element-mediated transformation (28, 29) using $ry^{506}$ as the host stock. Transformants were identified using the eye color marker; those containing independent single insertions of the P-element transgene were identified by Southern blot analysis. Multiple independent transformants were obtained. The integrity of the transgenes was confirmed by genomic restriction mapping using the 1.1-kb *lacZ* sequence (Fig. 1) as a probe (data not shown).

*Expression of hsp26*/*lacZ Transgenes*—The expression of the *hsp26*/*lacZ* transgenes was assessed by determining levels of β-galactosidase activity using CPRG assays (30) and a Northern analysis. For CPRG assays, individual males of each line were crossed to the host stock $ry^{506}$; adult $ry^{+}$ female progeny, which were heterozygous for the P-element insertion, were heat-shocked at 37 °C for 90 min. The level of β-galactosidase activity for each transgenic line was determined as previously described (12).

For analysis of induced mRNA synthesis, 5 adult flies were incubated in a tube at 37 °C (heat shock conditions) for the times indicated, and total RNA was isolated (31). This total RNA was size-fractionated on a 1.2% (w/v) agarose gel with formaldehyde using standard methods (32). Methods for transfer of the RNA onto a Nytran membrane, hybridization, and washing of the blot were the same as used for the indirect end-labeling analysis described (12, 14). The probes used were a mixture of the 1.1-kb *lacZ* fragment (Fig. 1) and a plasmid DNA containing the *rp49* gene (33), labeled with [$\alpha$-$^{32}$P]dCTP by the random hexamer method (34).

*Chromatin Structure Analysis*—For chromatin structure analyses, two independent transformed lines, which showed average β-galactosidase activity on induction as determined by CPRG assays, were selected. Methods used for the isolation of nuclei from larvae, DNase I or *Xba*I treatment of nuclei, DNA purification, and Southern blot analyses using indirect end-labeling are described elsewhere (14, 26). The probes used for detecting the DH sites and for quantitating the accessibility of *Xba*I sites within the proximal and distal DH sites are shown in Fig. 1.

To quantitate the accessibility of the *Xba*I site within the proximal DH site, nuclei were treated with an excess of *Xba*I. (Titration with increasing amounts of *Xba*I showed that maximum cleavage was obtained using 200 units of *Xba*I with 20 μg of genomic DNA in nuclei.) The genomic DNA was then purified, cleaved to completion with *Eco*RV, fractionated by electrophoresis through a 1% (w/v) agarose gel, and transferred to a nylon membrane; the membrane was probed with the 1.1-kb *lacZ* fragment (Fig. 1). The intensities of the bands on the autoradiographs were measured using a scanning densitometer (Molecular Dynamics). The percent accessibility of the proximal *Xba*I site was determined as the intensity of the band representing cleavage of the proximal *Xba*I site compared to the total intensity of bands resulting from cleavage at the proximal *Xba*I site, the distal *Xba*I site, and neither site by *Xba*I (the latter being the parental *Eco*RV band) in each sample.

To quantitate the accessibility of the *Xba*I site within the distal DH site, the DNA from nuclei treated with *Xba*I was purified and restricted to completion with *Sma*I and *Hpa*I. The DNA was size-fractionated on a 1% (w/v) agarose gel, transferred to a nylon membrane, and the membrane probed with a 0.6-kb DNA fragment from the 3′ region of the xanthine dehydrogenase gene (*xdh*); *xdh* is located upstream of the *hsp26* sequences in the constructs used here (Fig. 1). The percent accessibility of the distal *Xba*I site was determined using the methods described above for the proximal *Xba*I site. To simplify the comparison of relative values for the transgene CarXmTATA have been normalized to that of CarX. The variation in measurements of the *Xba*I accessibility by this method is ±5%.

*DNA Binding Assays with Immunopurified TFIID*—The binding assays with the collection of labeled DNA fragments and the DNase I footprinting analysis were performed as previously described (24, 25). The *Drosophila* TFIID was immunopurified from an 0.5 M KCl phosphocellulose fraction with monoclonal antibody 14C2-F4, which was generously provided by Robert Weinzierl and Robert Tjian. Increasing amounts of TFIID (2.5, 5, 10, and 20 μl of bed volume) were allowed to bind to the collection of promoter fragments in a total 50-μl volume of binding buffer (24). Binding reactions contained 1 μg of *Escherichia coli Hae*III-cut DNA and 120,000 Cerenkov counts of labeled promoters. The bound fragments were recovered and analyzed on an 8% denaturing polyacrylamide gel. Increasing amounts of protein bound increasing amounts of total DNA (data not shown).

The following mixture of DNA fragments was used in one binding assay. pCarXmTATA and pCarX span the region from −51 to +137. They were labeled with polynucleotide kinase at the *Ssp*I site at +137 and then cut at −51 with *Xba*I. The labeled strand of the pCarXmTATA was made 4 nucleotides longer than the normal counterpart by filling in the 5′ overhang of the *Xba*I site with DNA polymerase and deoxynucleotide triphosphates. The ΔTATA fragment is a derivative of the hsp70 promoter that has had the TATA box deleted (35). This fragment binds poorly to TFIID and provides a measure of the lower limit for specific binding. It was cut and labeled at an *Nru*I site that corresponds to −50 in the normal promoter. The other end was cut at a *Hpa*II site located downstream of +89 in the cloning vector, pUC13. The +43 and +61 fragments are derived from 3′ deletion constructs of the hsp70 promoter (24). These constructs bind TFIID with an affinity that is comparable to larger promoter constructs. They were prepared by cutting and labeling the appropriate clones at an *Nru*I site located at −50 and then cutting the DNA with *Bst*NI, which is downstream of the 3′ deletion end point.

The fragments from the normal and TATA mutant hsp26 promoters that were used for DNase I footprinting were prepared from pCarX and pCarXmTATA, respectively. Each was cut with *Ssp*I at +137 and labeled with polynucleotide kinase. The DNA was then cleaved with *Apa*LI at −118, and the appropriate fragment was gel-purified.

## RESULTS AND DISCUSSION

We have investigated the role of the TATA box in the *hsp26* heat shock gene promoter using an *in vivo* test system that employs P-element-mediated transformation. The TATA box sequence TATAAA at −30 was changed to CCCAAA by oligonucleotide-directed mutagenesis. The mutant construct and its wild type counterpart in fragments containing sequences from −1917 to +632 (except for the loss of sequences from −371 to −352) were fused in-frame to the *E. coli lacZ* gene, resulting in constructs pCarX and pCarXmTATA (see Fig. 1). The *hsp26*/*lacZ* fusion genes were then introduced into the *Drosophila melanogaster* genome by P-element-mediated transformation (28). Ten stable independent transformants were recovered for each construct; all of them contained single inserts. The effect of the mutation on the promoter was evaluated by measuring the levels of β-galactosidase activity that were induced by a 90-min heat shock at 37 °C. The TATA mutation severely inhibited promoter activity; the heat shock-induced level of β-galactosidase activity was, on average, 3% that of the wild type control (Fig. 2). However, the mutant gene was heat shock-inducible compared to the non-heat shock controls (see also the Northern analysis below), implying that some level of TBP must still interact with the promoter either before or during heat shock induction.

While mutation of the TATA box clearly has an effect on transcription *per se*, it is also of interest to determine whether or not this change affects establishment of the normal preset chromatin structure. Consequently, we analyzed the effect of the TATA mutation on the DNase I hypersensitivity of the promoter. Nuclei were isolated from third instar larvae of transformed lines and each preparation was treated with a range of DNase I concentrations. As shown in Fig. 3*A*, two DH sites were detected in the promoter region of both the wild type and mutant promoters. The patterns of cleavage in both cases were quite similar to that detected for the endogenous *hsp26* gene (11, 36). The proximal DH site extended from the *Xba*I site

at −51 to the *Eco*RI site at +7, covering the TATA box region. The similarities of these patterns indicated that the TATA box was not required to form the DNase I-hypersensitive sites.

Although the overall pattern of DNase I hypersensitivity was similar for the two promoters, it appeared that the level of sensitivity to DNase I in the mutant transgene was decreased compared to that of the wild type control CarX. We have previously established that the accessibility of a DH site can be quantitatively assessed by measuring the accessibility of a restriction site located within the DH site (12, 14, 26); there is an *Xba*I site located in the proximal and the distal DH sites of the *hsp26* promoter region (see Fig. 1). The accessibility of the proximal *Xba*I site (−51) and of the distal *Xba*I site (−351) in transgene CarXmTATA was determined to be 64 and 81% that of the wild type control transgene CarX, respectively (Fig. 3, *B* and *C*). Previously, we have shown that when the preset chromatin structure at *hsp26* is severely altered due to mutations at the $(CT)_n \cdot (GA)_n$ repeats, the accessibility of the proximal and the distal *Xba*I sites is reduced to 9 and 18% that of the wild type control, respectively (12). The results here indicate that the TATA box is not essential for the formation of DH sites at this promoter. On the other hand, mutation of the TATA box alters the accessibility of both of the DH sites, implying that proteins that bind (or are dependent on) the TATA box, such as TFIID, are contributing to DH site formation, particularly to the formation of the proximal site.

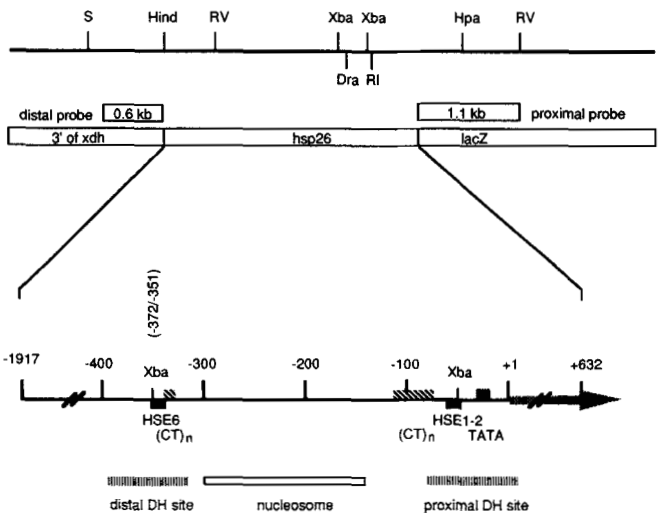


FIG. 1. **Map of the *hsp26/lacZ* construct and the probes used for chromatin structure analyses.** The structure of CarX is shown. *hsp26* sequences from −1917 to +632 (with the exception of sequences from −371 to −352, which are deleted) are fused in-frame to the *E. coli lacZ* gene. Restriction sites shown on the *top line* are those sites giving marker fragments or those used for mapping the chromatin structure in indirect end-labeling experiments. Probes used in chromatin structure analyses are indicated. The partial restriction map of *hsp26* sequences (−1917 to +632) is enlarged below with the $(CT)_n$ regions (*striped boxes*), the TATA box (*stippled box*), and two required HSEs (*HSE1–2* and *HSE6*, *filled boxes*) diagramed. Chromatin structural features of the *hsp26* gene are marked below. *Dra*, *Dra*I; *RI*, *Eco*RI; *RV*, *Eco*RV; *Hind*, *Hind*III; *Hpa*, *Hpa*I; *S*, *Sma*I; *Xba*, *Xba*I.

Given that the TATA mutation severely affected transcription from the promoter, we decided to examine the kinetics of induction for the wild type and mutant promoters. We reasoned that if TFIID were absent from the mutant promoter, it might exhibit a delayed response upon induction. The wild type promoter, already having a poised polymerase, would immediately begin synthesis of a transcript upon heat shock. As shown in Fig. 4, the mutant promoter responded to heat shock as rapidly as did the wild type template. In both cases, transcripts were detected within 5 min, although the detected level in CarXmTATA is slightly lower. If we allow 1 min for the temperature to rise in the chamber containing the flies and 3 min for elongation of the 3.6-kb fusion gene (8, 37), the appearance of transcript in 5 min suggests that the TFIID did interact with the promoter before induction in both the normal and mutant cases. The alternative, that TFIID is rapidly recruited to the promoter, is less likely because the binding of TFIID is a slow process (38). Since the *hsp26* promoter is preset before induction, the congruity of the kinetics argues that the RNA polymerase II is also transcriptionally engaged and paused at the mutant promoter. While the time of induction required for transcriptional activation is similar in both transgenes, it is notable that the amount of transcript that accumulates over time is much less for the mutant transgene.

Given that mRNA from both CarX and CarXmTATA was detected 5 min after heat shock, we thought that TFIID might interact with the promoter prior to activation, even though the TATA box had been mutated. Recent work has shown that sequences downstream of the TATA element are recognized by TFIID, and these interactions play a major role in the assembly of TFIID on the template (23–25). Thus, we tested directly whether or not TFIID still bound to the TATA mutant promoter. Antibody against the TBP subunit was used to purify TFIID from a protein fraction derived from a *Drosophila* nuclear ex-

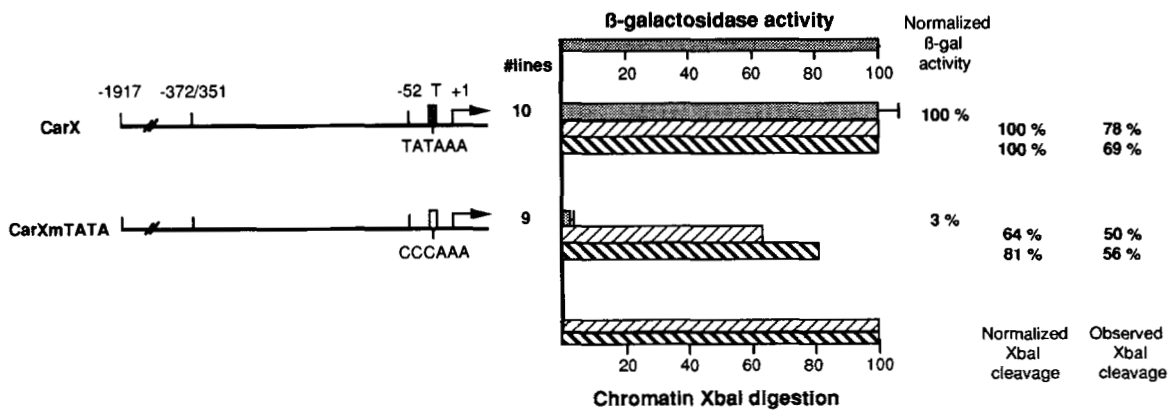


FIG. 2. **Expression and chromatin structure of the transgenes.** Transgenes are diagramed on the *left* side of the figure. A *filled box* labeled *T* indicates the wild type TATA box in CarX; an *open box* indicates sequence alterations in the TATA box in CarXmTATA. The number of independent transformed lines used to determine heat shock-inducible β-galactosidase activity is shown. The percentage values represent average levels of heat induced activity and accessibility of the *Xba*I sites for each transgene, shown normalized to the values obtained for CarX. Within the bar graph, the *top bar* (▨) for each transgene shows relative levels of heat shock-induced β-galactosidase activity; the *middle bar* (▨) shows relative values of accessibility of the proximal DH site (from results shown in Fig. 3*B*); the *bottom bar* (▨) shows relative values of accessibility of the distal DH site (from results shown in Fig. 3*C*). The actual percentage of *Xba*I cleavage in chromatin is also shown.

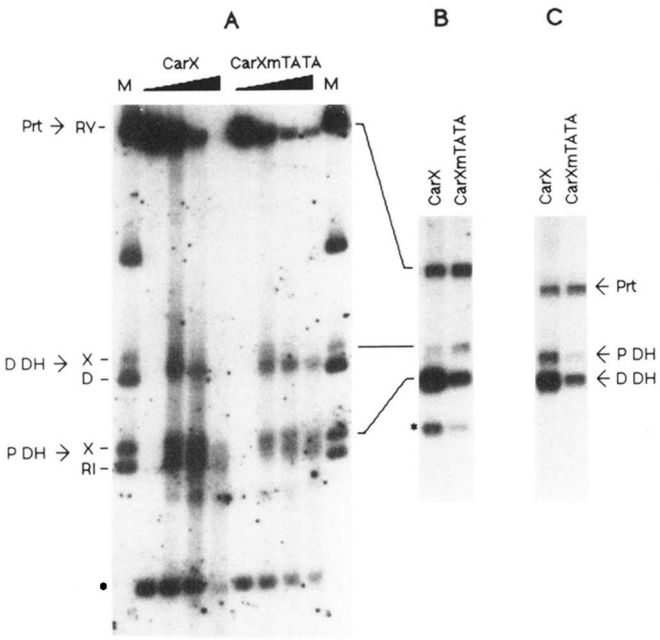


FIG. 3. **Chromatin structure analysis of the transgenes.** *Panel A*, DNase I analysis of the transgenes. Nuclei were treated with increasing amounts of DNase I, as indicated by the *wedge bars* above lanes. The genomic DNA was subsequently purified and cleaved with *Eco*RV. After size fractionation by electrophoresis through a 1% agarose gel and transfer to nylon membrane, the DNA was probed with the 1.1-kb *lacZ* fragment. *Panel B*, quantitation of the accessibility of the *Xba*I site within the proximal DH site. Nuclei were treated with *Xba*I, the genomic DNA was subsequently purified and restricted to completion with *Eco*RV. After size fractionation by electrophoresis through a 1% agarose gel and transfer to nylon membrane, the DNA was probed with the 1.1-kb *lacZ* fragment. *Panel C*, quantitation of the accessibility of the *Xba*I site within the distal DH site. Nuclei were treated with *Xba*I. The genomic DNA was purified and restricted to completion with *Sma*I and *Hpa*I. After size fractionation by electrophoresis through a 1% agarose gel and transfer to a nylon membrane, the DNA was probed with a 0.6-kb DNA fragment from the 3′ region of the xanthine dehydrogenase gene (*xdh*) (see Fig. 1). The *asterisk* indicates a fragment that hybridizes to the probe but is unrelated to the transgene.

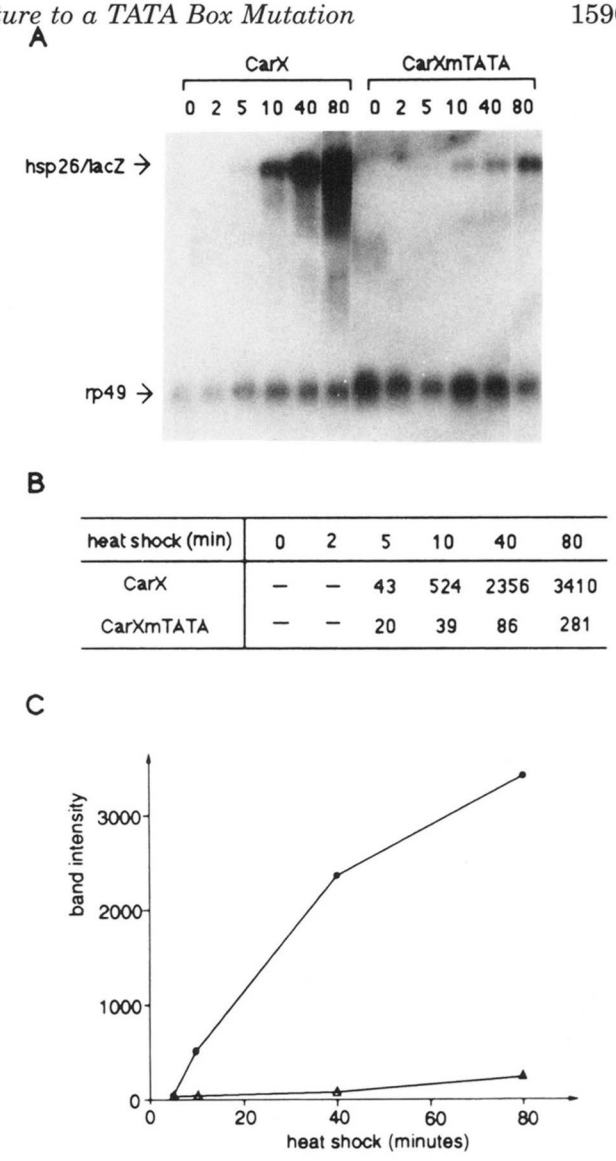


FIG. 4. **Northern blot analysis.** *Panel A*, total RNA from heat-shocked adults was fractionated on a 1.2% (w/v) agarose gel, transferred to a nylon membrane, and hybridized with a mixture of probes including the 1.1-kb *lacZ* fragment (see Fig. 1) and a plasmid containing the *Drosophila rp49* gene. The *number above* each *lane* indicates the time period (minutes) of heat shock. Transcripts are indicated with labeled *arrows*. *Panel B*, values of the *hsp26/lacZ* transcript levels (*panel A*), adjusted for equal RNA loading using rp49 values, as measured by a scanning densitometer. The *numbers* are the arbitrary opticle density unit of the scanning densitometer; – indicates undetectable values. *Panel C*, a graph of the band intensity from the Northern blot analysis in *panel A*, using the arbitrary numbers in *panel B*. *Open circles* represent measurements of mRNA at various time points from CarX; *open triangles* represent measurements of mRNA at various time points from CarXmTATA.

tract. The immunopurified TFIID was left immobilized on the protein G-Sepharose. Previously, it had been found that the immobilized TFIID retained its capacity to bind specifically to DNA fragments containing the *hsp70*, *hsp26*, or histone H3 promoters (24, 25). We presented different amounts of the immobilized TFIID with a constant mixture of end-labeled DNA fragments corresponding to the wild type and mutant *hsp26* promoters. As shown in Fig. 5, TFIID binds 3–5-fold less of the *hsp26* TATA mutant template (pCarXmTATA) than the wild type template (pCarX). The binding affinity of TFIID to the mutant template, however, was clearly higher than the binding affinity exhibited for an *hsp70* mutant promoter that had its TATA element deleted (Fig. 5). The wild type hsp26 promoter has an affinity for TFIID that was comparable to two wild type constructs of hsp70 (+61 and +43).

The binding of the *hsp26* TATA mutant appeared to be specific because it consistently bound better than the *hsp70* TATA deletion over a range of protein levels. The interaction between the immobilized TFIID and the individual promoter sequences was further examined by DNase I footprint analysis. For this analysis, the unbound DNA was washed away so that only the DNA retained on the bead by the TFIID was subjected to the DNase I. When the wild type promoter template was bound to TFIID, a DNase I footprint spanning the region from –44 to +35 was evident (Fig. 6). This was similar to the footprints produced on the wild type *hsp70* and histone H3 promoter templates (24, 25). Immobilized TFIID also produced a DNase I footprint on the CCCAAA-containing mutant promoter, but one that was strikingly different from that observed for the wild type promoter. The sharp boundaries at the –44 and +35 regions were entirely absent, and the hypersensitive sites that appear within the normal footprint were missing. Instead, regions of protection lacking sharp boundaries were evident over the CCCAAA sequence, the start site, and around +25 (Fig. 6).

The above analysis shows that TFIID can still associate with the *hsp26* promoter even though the TATA box has been mutated. This result combined with the rapid response upon induction leads us to propose that TFIID still participates in setting up the promoter for rapid induction. This hypothesis is further supported by analysis of other mutations in the *hsp26*

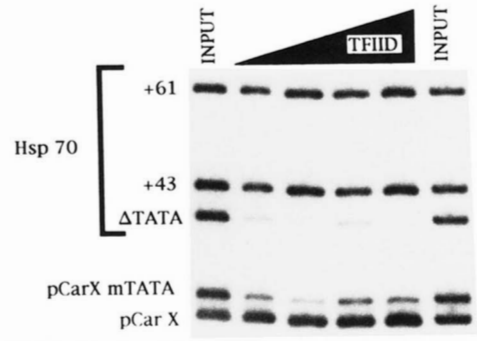


FIG. 5. **TFIID binds with specificity to the *hsp26* mutant promoter.** Binding of immunopurified TFIID with a collection of *hsp26* and *hsp70* promoter fragments. The +61, +43, and ΔTATA *hsp70* fragments are derivatives of the hsp70 promoter. The pCarXmTATA and pCarX are hsp26 derivatives. Binding reactions were set up with increasing amounts of immobilized TFIID associated with 2.5, 5, 10, and 20 μl of settled beads. The lane labeled *Input* indicates the end-labeled fragments before TFIID binding; the lane labeled *Bound* indicates the TFIID-bound fragments. Equal radioactive counts are loaded on the lanes, although increasing amounts of DNA were recovered with increasing amounts of TFIID.

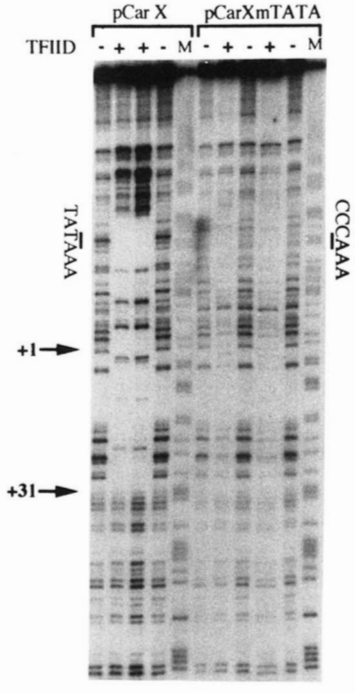


FIG. 6. **DNase I footprinting reveals an altered TFIID·DNA complex on the *hsp26* mutant promoter.** DNase I footprinting of the complexes formed between immobilized TFIID and either the wild type (pCarX) or mutant (pCarXmTATA) *hsp26* promoter fragment. Lanes labeled with a + or a – indicate the presence or absence of TFIID in the reactions; duplicate samples are included. *Arrows* labeled with numbers indicate nucleotide positions relative to the transcriptional start site.

and *hsp70* promoters. In the case of the *hsp26* promoter, the region between –135 and +7 is not sufficient for the formation of DH sites. This region contains a GAGA element (–135 to –85), TATA box, and the transcription start site. Addition of the region from –351 to –136, which contains a GAGA element, or of sequences from +8 to +632, which would provide the downstream contacts of TFIID (23, 25), restored the capacity for formation of the DH sites (14). In the case of the *hsp70* promoter, deletion of sequences downstream of the TATA element leads to reduced levels of paused polymerase when the sequences upstream of the TATA element extend to –89 (13). Insertion of additional copies of the GAGA element from the region between –38 and –89 caused a significant increase in the level of paused polymerase (13). All of these results point to the possibility that multiple interactions involving the GAGA factor and TFIID act in concert to insure the transcriptional potential of the heat shock gene promoters.

The dramatic effect of the mutation on the level of transcription following heat shock could then be inferred to be due to alterations in the TFIID·DNA interactions *per se*. Differences in the DNase I footprint formed by immunopurified TFIID suggest that the conformation of the complex is quite different on the wild type and mutant promoters. The cocrystal structure of the TBP and the TATA element indicates that the DNA is dramatically distorted causing the DNA to be sharply angled by 100° (39, 40). Mutation of the TATA box in the *Drosophila hsp26* gene may result in a failure of TFIID to induce the correct conformational changes in the DNA complex. This might disrupt protein-protein contacts at some stage in the transcription process. In addition, the stability of the TFIID·DNA complex might be reduced. The CCCAAA mutation could weaken the association of the TFIID complex so that fewer rounds of initiation occur before the TFIID dissociates and a new molecule must be recruited. It is interesting that transcripts are detected within 5 min for the mutant promoter (Fig. 4). We have recently suggested that the downstream contacts of TFIID could function primarily to set up the promoter (25, 41). The elongation of RNA polymerase II from the transcription start might disrupt many of the downstream contacts of TFIID leading to a situation in which the retention of TFIID for multiple rounds of initiation becomes largely dependent on upstream contacts of TFIID such as those involving the TBP subunit.

The effect of an altered TATA box on expression has been studied for a number of genes *in vitro* and *in vivo*. Studies including analysis of the chromatin structure of altered genes include those of the *hsp82* gene (42), the *PHO5* gene (43) and the *SUC2* gene (44) from yeast. The effects of TATA mutation on transcription and chromatin structure of the above yeast genes are consistent with what we have observed in studying the *hsp26* gene in *Drosophila*. That is, a dramatic reduction in transcription is observed, with little effect, if any, on chromatin structure formation. Whether or not the yeast TFIID can still bind to the mutant promoter in each of the above cases has not been determined.

Taken together, our analysis of TFIID is consistent with biochemical analysis by others (16, 45, 46) showing that TFIID is involved in establishing chromatin structure of gene promoters and the transcriptional potential. Further, our study indicates that the TATA element is not the only sequence in the promoter recognized by TFIID; contacts made downstream may allow TFIID to interact with the mutant TATA box. Mutation of the TATA box may alter the conformation of TFIID, which apparently does not dramatically interfere with its function in chromatin structure formation, but severely reduces the level of induced transcription.

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