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Drosophila P-element transposase is a transcriptional repressor *in vitro*

(fruit fly/transposable elements/transcription factor TFIID/RNA polymerase II)

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ABSTRACT **Mobility of *P* transposable elements in *Drosophila melanogaster* depends on the 87-kDa transposase protein encoded by the *P* element. Transposase recognizes a 10-base-pair DNA sequence that overlaps an A+T-rich region essential for transcription from the *P*-element promoter. We report here that transposase represses transcription from the *P*-element promoter *in vitro*. This transcriptional repression is blocked by prior formation of an RNA polymerase II transcription complex on the template DNA. Binding of transposase on the *P*-element promoter is blocked by prior binding of either the *Drosophila* RNA polymerase II complex or the yeast transcription factor TFIID. These data suggest that transposase represses transcription by preventing assembly of an RNA polymerase II complex at the *P*-element promoter.**

Transposable elements have been studied in many species and have provided numerous examples of regulated gene expression (1). The frequency or pattern of transposition is often limited by mechanisms that act to minimize the genetic damage caused by excessive DNA rearrangements (2). *P*-element transposition in *Drosophila*, for example, is negatively regulated in both a genetic and a tissue-specific manner (3–5). In germ-line tissues, full-length *P* elements express a trans-acting 87-kDa transposase protein, which is the one element-encoded protein required for forward transposition as well as precise and imprecise excision events (3–5). *P*-element transposase is a sequence-specific DNA-binding protein that recognizes internal sites present near both *P*-element termini (6).

The transposase binding site near the *P*-element 5′ end overlaps an A+T-rich region found ≈25 nucleotides (nt) upstream of the transcriptional initiation site (6). This region, known as the "TATA box," is present in the majority of RNA polymerase II promoters and serves as the binding site for an RNA polymerase II-associated transcription factor known as TFIID (7, 8). Biochemical studies of mammalian transcription have shown that TFIID functions at an early step during the assembly of transcription initiation complexes at RNA polymerase II promoters (9, 10). Diverse eukaryotic organisms such as yeast (11–14) and *Drosophila* (15, 16) have TFIID proteins that under some conditions are functionally interchangeable with their mammalian counterpart. These proteins have similar DNA-binding specificities, and all recognize A+T-rich DNA sequences (8, 17).

The overlap of the transposase binding site and the TATA box suggested that transposase might alter transcription from the *P*-element promoter. We therefore added purified transposase to an *in vitro* transcription system capable of accurately initiating *P*-element mRNA synthesis. Our results indicated that transposase represses transcription from the wild-type *P*-element promoter under conditions in which transposase is bound to its specific site within the promoter. Transcriptional repression occurs via the mutual exclusion of transposase binding and RNA polymerase II complex formation at the *P*-element promoter. Site-specific DNA binding of transposase and yeast TFIID are also mutually exclusive. We propose that *P*-element transposase acts as a transcriptional repressor by interfering with the TFIID–TATA box interaction, thereby blocking the assembly of an RNA polymerase II transcription complex at the *P*-element promoter.

MATERIALS AND METHODS

Proteins. The transposase used in these experiments was the TdT 0.3 M KCl chromatographic fraction, prepared essentially as described (6). Yeast TFIID was overproduced in *Escherichia coli* carrying plasmids pT7-IID (a gift of S. Buratowski and P. A. Sharp, Massachusetts Institute of Technology) and pGP1-2 essentially as described (18) and was purified to near homogeneity in HGKED buffer (6) by flowing through DEAE-Sepharose at 0.1 M KCl and then being eluted from S-Sepharose with a linear gradient of 0.1–1.0 M KCl. The purified *Drosophila* RNA polymerase II transcription system (a gift from the laboratory of James Kadonaga, University of California, San Diego) consisted of the RNA polymerase II, TFIIB, TFIID, and TFIIE+F fractions, as described (16).

***In Vitro* Transcription.** For run-off transcription, the plasmids pN/P2 [an 854-base-pair (bp) *Nae* I–*Pvu* II fragment of pπ25-1 (ref. 19), carrying the wild-type *P*-element promoter, inserted into *Sma* I-cleaved pUC8], pA2ΔPst (ref. 20; carrying the actin 5C promoter), and pHSX-LS-47-58 (ref. 6; carrying the mutant *P*-element promoter) were cleaved with *Dra* I (pN/P2) or *Pst* I restriction endonuclease, purified, and used as DNA templates. Transposase or buffer alone was added to the wild-type and control DNA templates (45 or 90 fmol of each template, in equimolar amounts) in a volume of 4 μl, and binding was allowed to reach equilibrium on ice for 20 min. *Drosophila* Kc cell nuclear extract (20) was added (8.5 μl, ≈0.7 mg of protein) with a transcription solution containing [α-^{32}P]GTP (20), to a 25-μl final volume. RNA was purified (20) and was analyzed by denaturing gel electrophoresis.

To assay transcription using the fractionated *Drosophila* RNA polymerase II system (16), 60 fmol (100 ng) of supercoiled pHSX-LS114-139 DNA (6) was used as template in a final volume of 30 μl. RNA was analyzed by S1 nuclease protection (21), using a single-stranded probe derived from a 225-bp *Xba* I–*Xho* I fragment of pHSX-LS-10-1 (6), 5′-end-labeled at the *Xba* I site.

DNase I Protection Analysis. RNA polymerase II and its cofactors were bound to DNA in HGKED buffer (6) at 50 mM KCl in the presence of 6.25 mM $MgCl_2$ and 0.05% Nonidet P-40. TFIID was bound to DNA as described (11) or in the same buffer used for RNA polymerase II transcription. The

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Abbreviation: nt, nucleotide(s).

DNase I protection analysis was performed as described (6, 22), using either a 225-bp *Xho* I–*Eco*RI *P*-element DNA fragment from pN/P175TpBXho (23), labeled at the 5′ end of the *Xho* I site, or a 0.9-kbp *Bam*HI–*Pvu* II *Drosophila* actin 5C gene fragment from pHSS-A2 (6), labeled at the 5′ end of the *Bam*HI site.

RESULTS

P-Element Transposase Represses P-Element Transcription. To test the effect of purified transposase on transcription initiation from the *P*-element promoter *in vitro*, transposase was prebound to various promoter-containing linear DNA templates (Fig. 1) before addition of *Drosophila* RNA polymerase II-containing nuclear extracts. When increasing amounts of transposase were bound to the DNA templates, transcription from the wild-type *P*-element promoter was specifically repressed compared with either of two internal control templates: the *Drosophila* actin 5C gene (Fig. 1, lanes 1–6) or a linker-scanning mutant of the *P*-element promoter termed LS 47-58 (lanes 7–12). Neither control promoter has a specific binding site for transposase (6). Densitometry showed that the extent of repression of the wild-type *P*-element promoter relative to either control promoter was similar, with a 4- to 5-fold reduction of transcription at a molar ratio of 15 transposase monomers to each template molecule (P.D.K., unpublished data). Site-specific binding of transposase to its recognition site is observed at similar molar ratios of transposase to DNA (2–20:1; ref. 6). In the reactions shown with the highest amount of transposase (20:1 molar ratio), transcription from the control templates also was reduced (Fig. 1, lanes 6 and 12), presumably as a consequence of the nonspecific DNA-binding activity of transposase observed at this protein/DNA ratio (6).

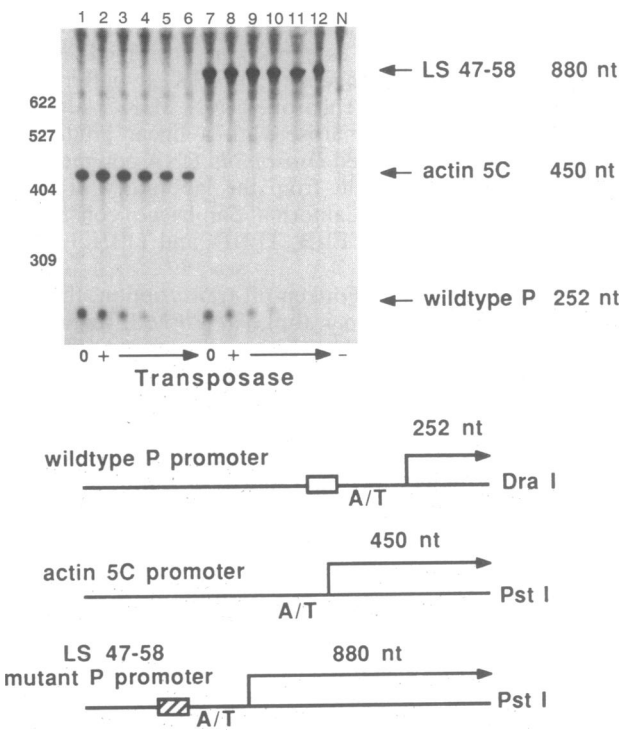

FIG. 1. Run-off *in vitro* transcription in the presence of *P*-element transposase. The wild-type *P*-element promoter DNA was transcribed in the presence of either *Drosophila* actin 5C promoter DNA (lanes 1–6) or the transposase binding-site mutant LS 47-58 DNA (lanes 7–12). Transposase was prebound to the template DNAs in a 2.5-fold (lanes 2 and 8), 5-fold (lanes 3 and 9), 10-fold (lanes 4 and 10), 15-fold (lanes 5 and 11), or 20-fold (lanes 6 and 12) molar excess over the wild-type *P*-element promoter DNA. Lane N shows labeling of nucleic acids in the absence of added DNA template. Positions of molecular size (nt) markers are indicated at left. The expected sizes of run-off transcripts are diagrammed below the autoradiogram. The boxes diagrammed on the *P*-element promoter templates represent the wild-type (open box) and mutant (striped box) transposase binding sites (6).

Repression of Transcription by Transposase and RNA Polymerase II Complex Formation Are Mutually Exclusive. *Drosophila* RNA polymerase II in conjunction with its general factors (TFIIB, -IID, -IIE, and -IIF) forms a stable, multiprotein complex on promoter DNA *in vitro* when nucleoside triphosphates are not present; this complex does not persist after the initiation of transcription (16, 24). We tested the effect of DNA binding by transposase on transcription-complex assembly, and vice versa, by assaying transcription in order-of-addition experiments. Prebinding of transposase to the wild-type *P*-element promoter inhibited transcription, even after the transposase-bound template was incubated in an RNA polymerase II-containing nuclear extract to allow formation of the transcription complex (Fig. 2*A*, lanes 1–3). However, if the nuclear extract was incubated with the DNA templates before addition of transposase, the repression was not observed at any level of transposase tested (Fig. 2*A*, lanes 4–6).

Next, S1 nuclease protection analysis was used to examine *P*-element transcripts synthesized *in vitro* by the fractionated RNA polymerase II transcription system. As in the experiments using nuclear extract (Fig. 2*A*), transposase repressed *P*-element transcription, and this repression could be blocked by prior formation of a stable RNA polymerase II complex (Fig. 2*B*). To observe a single round of transcriptional initiation, reinitiation was blocked by the addition of 0.2% sarkosyl after addition of nucleoside triphosphates (ref. 24; Fig. 2*B*, lanes 2–4). Other experiments showed that the degree of template specificity of transposase repression was diminished in the fractionated *Drosophila* transcription system compared with that observed in the nuclear extract (P.D.K., unpublished data). This may be due to the removal of distinct factors from the nuclear extract during fractionation that improve the specificity of the transposase–DNA interaction, or it may simply result from removal of nonspecific DNA-binding proteins during fractionation, allowing transposase to recognize more nonspecific sites on the template DNA. In any case, these order-of-addition experiments (Fig. 2) suggest that the transcription-repressing activity of transposase is mechanistically based on steric occlusion of RNA polymerase II complex formation at the *P*-element promoter.

DNA Binding by Transposase Is Mutually Exclusive with Binding by Yeast Transcription Factor TFIID or the *Drosophila* RNA Polymerase II Complex. Because transposase blocked assembly of an RNA polymerase II complex at the *P*-element promoter, we hypothesized that the RNA polymerase II complex, or more specifically, the TATA-binding transcription factor, TFIID, might block DNA binding by transposase. Yeast TFIID protein, which binds a number of TATA elements *in vitro* (17), protected the TATA-box regions of both the *P*-element and *Drosophila* actin 5C promoters from DNase I digestion (Fig. 3*A*). The specific TFIID binding site on *P*-element DNA observed at lower protein concentrations (nt 44–70, Fig. 3*A*, lanes 2–3) overlaps the previously defined region bound by transposase (nt 48–68; ref. 6; see Fig. 3*B*). At high molar ratios of TFIID to DNA, more nonspecific binding to A+T-rich regions is observed (ref. 17; Fig. 3*A*, lanes 4 and 5).

The location of the yeast TFIID and *P*-element-transposase binding sites on the *P*-element promoter suggested the possibility that TFIID and transposase directly compete for binding to overlapping DNA recognition sequences. To test this idea, we prebound one protein to *P*-element promoter DNA, then added the second protein.

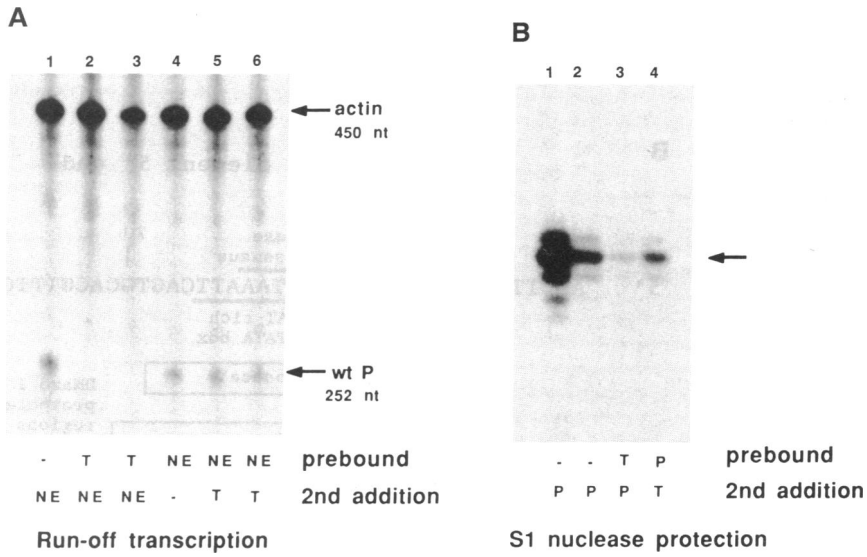

FIG. 2. Effect of transposase on transcription by preformed RNA polymerase II complexes. (*A*) Run-off transcription assay with either transposase or the nuclear extract preincubated with the DNA templates. Transposase was either omitted (lanes 1 and 4) or added at a 7.5-fold (lanes 2 and 5) or 15-fold (lanes 3 and 6) molar excess over the wild-type *P*-element template DNA. For lanes 1–3, transposase or chromatography buffer was preincubated with the template DNAs at room temperature for 20 min before transcription was initiated (24). For lanes 4–6, the template DNAs were incubated with the nuclear extract under transcription conditions in the absence of nucleoside triphosphates (NTPs) for 20 min at room temperature to allow complete RNA polymerase II complex formation (24). Transposase or buffer alone was then added along with NTPs, and transcription was allowed to proceed at 20°C for 30 min. Addition of transposase (T) and nuclear extract (NE) is indicated. (*B*) S1 nuclease protection analysis of transcription by the fractionated RNA polymerase II system. The protected fragment indicated by the arrow represents initiation of transcription at the *P*-element promoter. The protected fragment is 17 nt shorter than expected (51 rather than 68 nt; P.D.K., unpublished data); we believe that this results from instability of the mRNA/probe hybrid caused by the long A+T-rich stretches in this region. Four microliters of each protein fraction (≈0.17 μg of RNA polymerase II, 0.2 μg of TFIIB fraction, 8 μg of TFIID fraction, and 1.6 μg of TFIIE+F fraction; ref. 16) was used for each reaction. Lanes 1 and 2: RNA polymerase II complexes were formed on the template DNA at room temperature for 30 min (16), followed by addition of NTPs. Thirty seconds after the addition of NTPs, 2 μl of water (lane 1) or *N*-lauroylsarcosine (sarkosyl, to 0.2% final concentration; ref. 24) (lane 2) was added. Lane 3: the DNA template was preincubated with transposase (≈1.2 pmol, ≈0.1 μg, a 20-fold molar excess over template DNA) at room temperature for 30 min. RNA polymerase II and its cofactors were then added, and the mixture was incubated 30 min at room temperature before addition of NTPs. Sarkosyl was added to 0.2% final concentration 30 sec after addition of NTPs. Lane 4: same as lane 3, except that the order of addition of transposase and RNA polymerase II was reversed. Addition of transposase (T) and RNA polymerase II (P) is indicated.

This experiment was feasible because transposase and yeast TFIID yielded easily distinguishable DNase I protection patterns on the *P*-element promoter DNA (ref. 6 and Fig. 3*A*). This is especially true for the amount of TFIID used in this experiment, which gave a pattern of enhanced and protected bands that differed greatly from the unbound control DNA (Fig. 3*C*, compare lane 1 with lanes 2–7). The patterns of protection observed with either TFIID or transposase prebound to the DNA did not change over time in the presence of the second, subsequently added, protein; thus, DNA-bound transposase or TFIID mutually excluded the binding of the other protein (Fig. 3*C*). We also performed a similar experiment using the fractionated *Drosophila* RNA polymerase II transcription system and *P*-element transposase. Although the RNA polymerase II and transcription factor fractions provided only weak protection of the TATA-box region from DNase I digestion (Fig. 3*D*, lane 2), the pattern of enhanced DNase I cleavages adjacent to the TATA box, and the extent of protection within the TATA-box region in the presence of prebound RNA polymerase II alone (Fig. 3*D*, lane 2) differ from that seen with prebound transposase (lane 8). The RNA polymerase II and transcription factor fractions blocked quantitative binding of transposase to the *P*-element promoter (Fig. 3*D*, lanes 3–7). The strong protection of nt 48–68 from DNase I attack observed in the presence of transposase alone (ref. 6; Fig. 3*D*, lane 8) was never observed when the RNA polymerase II complex was formed prior to the addition of transposase (Fig. 3*D*, lanes 3–7). Further, the DNase I protection observed in the presence of prebound transposase did not change significantly over time following addition of the RNA polymerase II and transcription factor fractions (Fig. 3*D*, lanes 8–13). These results indicate that transposase and the RNA polymerase II complex bind and remain bound to the *P*-element promoter in a mutually exclusive manner, probably due to competition between TFIID and transposase for overlapping DNA sequences.

DISCUSSION

Because the transposase binding site on the 5′ end of *P*-element DNA overlaps the TATA box of the *P*-element promoter (6), we studied the effect of transposase on *P*-element transcription *in vitro*. Transcription from the wild-type *P*-element promoter was specifically repressed when transposase was prebound to template DNAs at molar ratios that allow site-specific DNA binding (Fig. 1). However, prior formation of an RNA polymerase II complex, derived either from nuclear extract or from fractionated material, blocked the ability of subsequently added transposase to repress *P*-element transcription (Fig. 2). These data suggested that DNA binding by transposase and the RNA polymerase II complex are mutually exclusive. This hypothesis was supported by DNA-binding experiments which indicated that transposase prevented either yeast transcription factor TFIID or the *Drosophila* RNA polymerase II complex from binding to the *P*-element promoter (Fig. 3 C and D). We therefore propose that transposase bound to its site at the 5′ end of *P*-element DNA prevents formation of the RNA polymerase II complex at the *P*-element promoter by preventing the interaction of TFIID with the TATA box.

These data suggest that transcription from the *P*-element promoter leads to the accumulation of transposase protein in germ-line cells, until a level of transposase is reached that

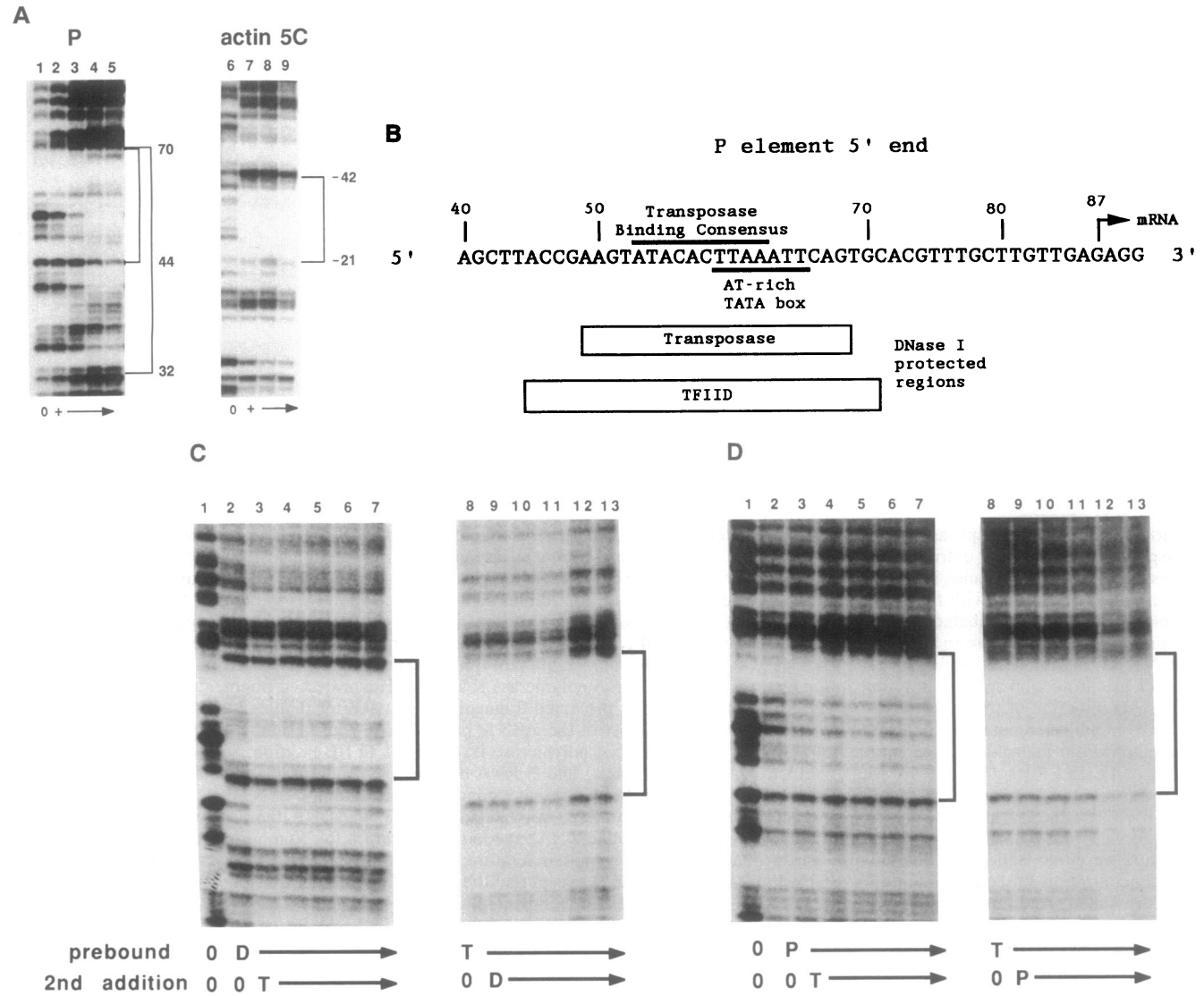

FIG. 3. DNase I protection analysis of proteins interacting with the *P*-element TATA box. (*A*) *Escherichia coli*-overproduced yeast TFIID binding to the *Drosophila P*-element (lanes 1–5) and actin 5C (lanes 6–10) promoters. The *P*-element DNA probe was incubated with buffer alone (lane 1) or with 10 ng (lane 2), 25 ng (lane 3), 100 ng (lane 4), or 200 ng (lane 5) of purified TFIID. The protected regions (*P*-element nt 44–70, at lower TFIID concentrations, nt 32–70 at higher concentrations; see ref. 25 for nucleotide numbers) are bracketed. The actin 5C probe was incubated with buffer alone (lane 6) or with 20 ng (lane 7), 80 ng (lane 8), or 200 ng (lane 9) of TFIID. The A+T-rich TATA box extends from bp −21 to −31 relative to the mRNA start site (7). (*B*) Binding sites for transposase and yeast TFIID on the *P*-element promoter. *P*-element nucleotide numbers (25) are shown above the line representing the DNA. (*C*) Mutual exclusion of transposase and TFIID for *P*-element promoter DNA binding. The DNA was preincubated with either buffer alone (lane 1), 50 ng of TFIID (lanes 2–7) or 5 ng of transposase (lanes 8–13) at room temperature for 25 min. Subsequently, either buffer (lanes 1, 2, and 8), 5 ng of transposase (lanes 3–7), or 50 ng of TFIID (lanes 9–13) was added to the preformed protein–DNA complexes. DNase I cleavage was then initiated at 1 min (lanes 3 and 9), 2.5 min (lanes 4 and 10), 4 min (lanes 5 and 11), 10 min (lanes 6 and 12), or 25 min (lanes 1, 2, 7, 8, and 13) after the addition of the second protein. T, addition of transposase; D, addition of TFIID. Brackets represent the regions bound by TFIID (lanes 2–7) or transposase (lanes 8–13), as diagrammed in *B*. (*D*) Mutual exclusion of transposase and *Drosophila* RNA polymerase II for binding to *P*-element promoter DNA. The probe DNA was preincubated with either buffer alone (lane 1), 0.25 μl of each of the RNA polymerase II, TFIIB, TFIID, and TFIIE+F fractions (ref. 16; lanes 2–7), or 5 ng of transposase (lanes 8–13) at room temperature for 30 min. At that point, buffer (lanes 1, 2, and 8), 5 ng of transposase (lanes 3–7), or 1 μl of the mixture of the four RNA polymerase II factor fractions (lanes 9–13) was added to the preformed protein–DNA complexes. DNase I cleavage was then performed as in *C*. T, addition of transposase; P, addition of the four RNA polymerase II fractions.

represses *P*-element transcription (Fig. 4). This initial accumulation may occur following a hybrid dysgenic cross in which a *P*-element-containing sperm fertilizes an oocyte that lacks *P* elements. This transcriptional repression is autoregulatory and would prevent accumulation of high levels of transposase. The level of transposase synthesized prior to repression of *P*-element transcription might be sufficient to allow a low frequency of transposition yet simultaneously prevent elevated levels of transposase synthesis that would result in high rates of transposition-induced mutation, DNA rearrangements, and cell lethality (26). This autoregulatory mechanism may thus operate *in vivo* to provide a sensitive indicator for the intracellular levels of transposase.

A mechanism of transcriptional repression involving steric occlusion of TFIID binding has been proposed for the *Drosophila* engrailed homeodomain protein (27). The engrailed protein, in addition to recognizing its cognate consensus binding sequence, can also interact with the TATA boxes of several promoters to block the TFIID interaction and can act as a transcriptional repressor *in vitro* (27).

Negative regulation of gene expression at the level of transcriptional initiation may be common to other eukaryotic

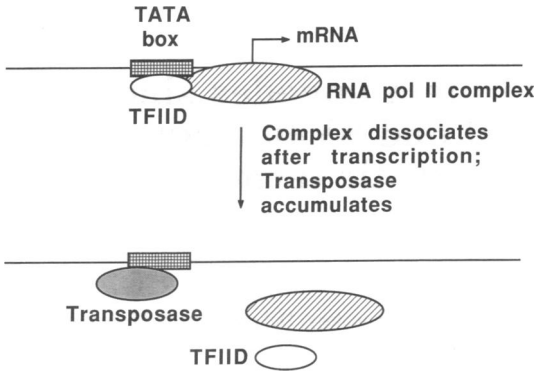

FIG. 4. Molecular interactions at the *P*-element promoter. The transcription start site is indicated by the bent arrow. The upper line shows the RNA polymerase II complex (striped oval) on the *P*-element promoter, with TFIID (white oval) binding the TATA box (checked). Transcription from the *P*-element promoter would result in the accumulation of transposase protein. Transposase (gray oval) would then bind its recognition site and prevent the interaction of TFIID and the RNA polymerase II complex with the *P*-element promoter, resulting in repression of transcription.

transposable elements. The *tnpA* gene product encoded by the *En-1* transposable element of *Zea mays* recognizes internal DNA sequences near the terminal inverted repeats of the transposon, including a DNA sequence that overlaps the TATA box of the *tnpA* promoter (28). TnpA is capable of suppressing expression of host genes containing *En-1* insertions (29).

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1. Berg, D. E. & Howe, M. M., eds. (1989) *Mobile DNA* (Am. Soc. Microbiol., Washington).
2. Kleckner, N. (1990) *Annu. Rev. Cell Biol.* **6**, 297–327.
3. Engels, W. R. (1989) in *Mobile DNA*, eds. Berg, D. E. & Howe, M. M. (Am. Soc. Microbiol., Washington), pp. 437–484.
4. Rio, D. C. (1990) *Annu. Rev. Genet.* **24**, 543–578.
5. Craig, N. L. (1990) *Cell* **62**, 399–402.
6. Kaufman, P. D., Doll, R. F. & Rio, D. C. (1989) *Cell* **59**, 359–371.
7. Parker, C. S. & Topol, J. (1984) *Cell* **36**, 357–369.
8. Nakajima, N., Horikoshi, M. & Roeder, R. G. (1988) *Mol. Cell. Biol.* **8**, 4028–4040.
9. Buratowski, S., Hahn, S., Guarente, L. & Sharp, P. A. (1989) *Cell* **56**, 549–561.
10. Van Dyke, M. W., Roeder, R. G. & Sawadogo, M. (1988) *Science* **241**, 1335–1338.
11. Buratowski, S., Hahn, S., Sharp, P. A. & Guarente, L. (1988) *Nature (London)* **334**, 37–42.
12. Cavallini, B., Huet, J., Plassat, J.-L., Sentenac, A., Egly, J.-M. & Chambon, P. (1988) *Nature (London)* **334**, 77–80.
13. Horikoshi, M., Wang, C. K., Fujii, H., Cromlish, J. A., Weil, P. A. & Roeder, R. G. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 4843–4847.
14. Schmidt, M. C., Kao, C. C., Pei, R. & Berk, A. J. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 7785–7789.
15. Hoey, T., Dynlacht, B. D., Peterson, M. G., Pugh, B. F. & Tjian, R. (1990) *Cell* **61**, 1179–1186.
16. Wampler, S., Tyree, C. & Kadonaga, J. T. (1990) *J. Biol. Chem.* **265**, 21223–21231.
17. Hahn, S., Buratowski, S., Sharp, P. A. & Guarante, L. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 5718–5722.
18. Tabor, S. & Richardson, C. C. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 1074–1078.
19. O'Hare, K. & Rubin, G. M. (1983) *Cell* **34**, 25–35.
20. Price, D. H., Sluder, A. E. & Greenleaf, A. L. (1987) *J. Biol. Chem.* **262**, 3244–3255.
21. Biggin, M. D. & Tjian, R. (1988) *Cell* **53**, 699–711.
22. Jones, K. A., Yamamoto, K. R. & Tjian, R. (1985) *Cell* **42**, 559–572.
23. Rio, D. C. & Rubin, G. M. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 8929–8933.
24. Kadonaga, J. T. (1990) *J. Biol. Chem.* **265**, 2624–2631.
25. Rubin, G. M. & Spradling, A. C. (1983) *Nucleic Acids Res.* **11**, 6341–6351.
26. Engels, W. R., Benz, W. K., Preston, C. R., Graham, P. L., Phillis, R. W. & Robertson, H. M. (1987) *Genetics* **117**, 745–757.
27. Ohkuma, Y., Horikoshi, M., Roeder, R. G. & Desplan, C. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 2289–2293.
28. Gierl, A., Lutticke, S. & Saedler, H. (1988) *EMBO J.* **7**, 4045–4053.
29. Grant, S. R., Gierl, A. & Saedler, H. (1990) *EMBO J.* **9**, 2029–2035.