Cooperative and Competitive Protein Interactions at the Hsp70 Promoter*

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Paul B. Mason, Jr. and John T. Lis‡
From the Department of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York 14853

**Drosophila** heat shock factor (HSF) binds to specific sequence elements of heat shock genes and can activate their transcription 200-fold. Though HSF has an acidic activation domain, the mechanistic details of heat shock gene activation remain undefined. Here we report that HSF interacts directly with the general transcription factor TBP (TATA-box binding protein), and these two factors bind cooperatively to heat shock promoters. A third factor that binds heat shock promoters, GAGA factor, also interacts with HSF and further stabilizes HSF binding to heat shock elements (HSEs). The interaction of HSF and TBP is explored in some detail here and is shown to be mediated by residues in both the amino- and carboxyl-terminal portions of HSF. This HSF/TBP interaction can be specifically disrupted by competition with the potent acidic transcriptional activator VP16. We further show that the acidic domain of the largest subunit of Drosophila RNA polymerase II (Pol II) associates with TBP in vitro and is specifically displaced from TBP upon addition of HSF. The region of TBP that mediates both HSF and Pol II acidic domain binding maps to the conserved carboxyl-terminal repeats and depends on at least one of the TBP residues known to be contacted by VP16 and to be critical for transcription activation. We discuss these findings in the context of a model in which HSF triggers hsp70 transcription by freeing the hsp70 promoter-paused Pol II from the constraints on elongation caused by the affinity of Pol II for general transcription factors.

Heat shock triggers formation of heat shock factor (HSF) protein trimers (1, 2) that bind tightly to heat shock elements upstream of the hsp70 promoter. HSF binding is concomitant with a rapid and vigorous (200-fold) increase in the rate of transcription. The uninduced heat shock promoter is primed for rapid activation. This promoter is contained in a chromatin structure that is open and easily accessible and contains one paused Pol II per promoter (3). A rate-limiting step in transcription appears to be the escape of this promoter-paused Pol II into productive elongation. Even after heat shock, when Pol II fires from the hsp70 promoter every 6 s, transient Pol II pausing can still be detected on hsp70 (4, 5) such that Pol II progression through this specific region at the 5′ end of the transcription unit remains rate-limiting.

The relative generality of transcriptional control at the level of paused polymerase (6) indicates that many upstream activators can act to stimulate escape of the paused polymerase into a productive elongation mode. How might this happen? We favor a model in which the paused polymerase is restrained via its strong association with the promoter and general transcription factors. In this scenario, polymerase recruitment is vigorous while escape (beyond the pause) is limiting. The activator could act to increase the rate of Pol II escape by modifying the polymerase complex to produce an elongationally competent form or perhaps more simply by competing with Pol II for one or more binding sites on the general transcription apparatus.

We have demonstrated previously that Pol II can bind TBP in vitro and can be displaced from TBP by competition with specific transcriptional activator proteins (VP16 and CTF1) (7, 8).

TBP is a good candidate for a heat shock factor target given its constitutive presence on hsp70 (4) and given the close proximity of the TATA element to the proximal HSF binding sites. Also, the potent acidic activator VP16 has been shown to associate with TBP (9), and it is known that acidic activators like GAL4 can activate an hsp70 promoter in transgenic fly lines (10). Finally, the carboxyl-terminal domain (CTD) heptad repeats and the acidic domain (the so-called “H” domain) of RNA polymerase have been shown to interact genetically with each other (8, 11), and both have been shown to bind to TBP in vitro (8, 12). In our hands, the H-domain/TBP interaction is stronger than the CTD/TBP interaction.

TBP-binding is only one of a variety of activities displayed by transcriptional activators. VP16 has been implicated in the recruitment of TFIIH (13). Since TFIIH has both DNA helicase activity and CTD-specific kinase activity, this suggests a role for activators in promoter melting and/or CTD phosphorylation. Such a modification of the polymerase complex might also play a role in the progression of the paused polymerase into productive elongation. VP16 has also been shown to associate with TFIIB in vitro (14). The multiple interactions of activators with basal factors are consistent with multiple layers of activator-mediated regulation and the synergistic effect of activators (15). A fraction of TBP is complexed in vivo, as TFID, with at least eight TBP-associated factors (TAFs), which also have been implicated as promoter-specific activator targets (reviewed in Ref. 16). The TBP core of TFID also serves as the foundation for assembly of the basal transcription apparatus (17). TBP is, therefore, capable of many interactions, some of which must occur simultaneously. This may be possible if these interactions are specific for small portions of the TBP surface (as has been shown for several basal factor-TBP interactions, see Ref. 17), allowing TBP to support additional activator contacts. Additionally, any of these protein-protein interactions may be quite dynamic, such that multiple factors could bind to the same site on the TBP surface.

Here we present first tests of a simple “competition” hypoth-
HSF Interacts with TBP

Plasmid Constructions—All DNA manipulations were carried out using standard procedures (18). Plasmid construction information will be made available from the authors upon request.

Protein Production—All recombinant proteins were produced in BL21 cells at an optical density of 0.5 by induction using 1 mM isopropyl-1-thio-
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HSF Interacts with TBP

concentration of GST-dHSF required for recovery of 50% of the HSF in the presence of HSF (compare lanes 3 and 5). Therefore, some portion of the role of GAGA factor in heat shock promoter function may be a consequence of direct interaction with HSF and stabilization of HSF binding.

In contrast, GAGA factor showed less binding to GST-TBP beads than to GST-HSF beads, and no cooperative DNA-binding was detected between GAGA factor and TBP. As shown in Fig. 4B, the GAGA factor footprint partially overlaps that of the TBP-TFIIB complex, and GAGA factor appears to compete with TBP for binding to DNA. The two bands at the 3’ end of the TATA region, indicated by an arrow in Fig. 4B, serve as an indicator of TBP binding in the presence of GAGA factor. GAGA factor appears to destabilize the TBP-TATA complex, presumably via steric competition (compare lanes 2–4). Thus, in the case where a GAGA site overlaps the TATA box region, the binding of the two factors to DNA appears to be competitive.

Localization of Domains Critical for HSF/TBP Binding—HSF, like a variety of transcriptional activators that interact with TBP, has an acidic activation domain. To assess whether TBP-binding is mediated exclusively by the acidic activation domain (27) located at the carboxyl terminus of HSF, we tested the ability of truncated (GST-fused) HSF proteins to bind TBP. As shown in Fig. 5A, both the amino- and carboxyl-terminal regions of HSF were sufficient to bind to TBP in this assay. This suggests that there are at least two distinct HSF surfaces that can mediate the HSF/TBP interaction.

The carboxyl-terminal repeats of TBP are highly conserved
across species, and are critical for cell viability (28, 29). To determine if the conserved region of TBP is necessary for HSF-binding, we used two truncated Drosophila TBP constructs, each fused to GST. As shown in Fig. 5B, a TBP derivative containing the carboxyl-terminal conserved repeats (and lacking nonconserved amino-terminal sequences) bound HSF efficiently in a standard bead-binding assay. Conversely, a TBP derivative lacking an intact conserved domain failed to associate strongly with HSF.

**HSF Competes with VP16 for TBP-binding**—VP16 is a well-characterized potent acidic activator that binds to TBP in vitro (30). The carboxyl-terminal sequences of HSF display similarities to sequences within the first activation domain of VP16, and we reasoned that HSF may associate with TBP in a fashion similar to that of VP16. Fig. 6A shows that 10–20% of input TBP remains bound to GST-VP16 beads after washing. If HSF is included in the binding mixture, however, a reduced fraction of the input material remains bound to the VP16 beads after washing. These results imply that HSF and VP16 can compete for binding to a common surface of TBP.

To more precisely probe the relationship of the specificities of HSF and VP16 binding to TBP, we measured HSF binding to both wild-type yeast TBP and the TBP point mutant L114K, which has been shown to be deficient in both in vitro VP16 binding and response to acidic transcriptional activators (31). This point mutation resides in the first of two highly conserved direct repeats of yeast TBP. As shown in Fig. 6B, L114K mu-
HSF Interacts with TBP

**Fig. 5.** Protein domains required for HSF/TBP binding. A, His6-TBP was equilibrated with beads containing GST or GST-HSF truncated derivatives as indicated. After washing, samples were electrophoresed and visualized by Western blotting using an antibody against TBP. B, His6-HSF was equilibrated with beads containing GST or the indicated GST-TBP truncated derivatives. After washing, retained fractions were subjected to SDS-PAGE followed by visualization using an antibody against dHSF.

**Fig. 6.** HSF competes with VP16 for binding to TBP. A, His6-TBP was equilibrated with equimolar amounts of MBP or various concentrations of MBP-HSF (equal protein concentration was maintained by supplementation with MBP) followed by addition of beads containing GST or GST-VP16. After further incubation, beads were washed, and bound fractions were analyzed by SDS-PAGE and visualized by immunoblotting using an antibody against TBP. B, His6-yTBP or His-yTBP/L114K were incubated with beads containing GST or GST/HSF. After washing, bound fractions were electrophoresed and visualized using an antibody against yTBP.

To examine the ability of the H-domain of Drosophila PolII to bind to TBP or TFIIIB, we incubated these factors with GST or GST-H-domain beads. As shown in Fig. 7A, TBP bound effectively to the H-domain, but TFIIIB showed weaker binding. To test the ability of HSF to disrupt the TBP-H-domain complex, we exposed TBP to beads containing GST or GST-H-domain in the absence or presence of HSF. As shown in Fig. 7B, in the presence of HSF, less TBP is retained by the H-domain beads, indicating that the TBP/polymerase interaction can be compromised by HSF.

If HSF and RNA polymerase compete for a specific binding site on TBP, it seems likely that a TBP mutation that reduces HSF binding would also reduce polymerase binding. To test this, we passed yTBP and the yeast TBP point mutant L114K over beads containing GST-H-domain and GST only. Fig. 7C shows that, like HSF, polymerase H-domain-TBP-binding is reduced by a point mutation in this hydrophobic TBP residue, which has been shown to be critical for response to acidic transcriptional activators in vitro (31) and in vivo (32).

**DISCUSSION**

We have shown here that the heat shock gene-specific activator, HSF, binds efficiently to the general transcription factor TBP in vitro. In these experiments, comparable fractions of input TBP were recovered by HSF affinity chromatography using either Drosophila nuclear extracts or purified recombinant TBP. A second general factor TFIIIB, which also has been reported to bind acidic activators (20), shows only weak affinity for HSF. The HSF/TBP interaction appears to influence the association of these factors with their DNA targets, in that we observe that purified HSF and TBP bind cooperatively to heat shock promoters in vitro. Likewise, GAGA factor, another component of the hsp70 and hsp26 promoters, also aids the binding of HSF to the hsp70 promoter in vitro. Both TBP and GAGA factor occupy these heat shock promoters prior to induction by heat shock and are thus positioned to facilitate HSF recruitment. These interactions, coupled with the open chromatin configuration of heat shock promoters (33), may help to explain the fact that HSF binding to HSEs in vivo is dependent on the presence of intact TFIIID and GAGA binding sites (34). In addition, these interactions of HSF with TBP and GAGA factor may stabilize promoter associations of these factors during multiple rounds of activated transcription when proposed contacts of these factors with RNA polymerase II and other components of the basal machinery are likely to be disrupted during each cycle of transcription.

The binding of HSF to TBP is mediated by residues in both the DNA-binding/trimerization domain and in the acidic carboxyl-terminal domain of HSF. This binding is targeted to the conserved carboxyl-terminal repeats of TBP. The binding of HSF to TBP is similar in both avidity and character to the

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tant TBP exhibited reduced binding to GST-HSF beads, relative to wild-type TBP. These experiments further indicate that the HSF/TBP interaction is mediated by the conserved region of TBP, and that a TBP residue that is critical for transcription activation and VP16 binding is also critical for HSF binding.

**HSF Competes with Pol II for TBP-Binding**—The carboxyl terminus of the largest subunit of Drosophila RNA polymerase II has sequence similarity to acidic transcription activators (8). The pattern of hydrophobic and acidic residues in this region resembles the activation domains of VP16 and GAL4. We have previously described the ability of the homologous region (the H-domain) of yeast RNA polymerase to bind to yeast TBP (8). This domain of the yeast polymerase functions as a potent transcriptional activator, similar in strength to the activation domain of VP16, when fused to a heterologous DNA-binding domain (8). This activating property of a domain of Pol II led us to hypothesize a mechanism of transcriptional activation that is centered on the competition between the activator and RNA polymerase for binding to one or more sites on the basal transcription apparatus (8).
binding of the acidic transcription activator VP16 to TBP. Both interactions are affected by a specific mutation in TBP (L114K). Moreover, VP16 and HSF compete for binding to TBP.

We have also shown that an acidic domain (H) of Drosophila RNA polymerase II binds to TBP in vitro in a manner similar to the polymerase/TBP interaction previously reported in yeast (8). This interaction is disrupted upon addition of HSF, suggesting that polymerase and HSF can compete for the same site on TBP. This site on TBP also maps to the conserved TBP carboxyl-terminal repeats and is specifically reduced by the L114K mutation. We suggest that some of the same polymerase-general factor contacts, including interactions in- volved in Pol II recruitment, How does the next Pol II molecule may then occupy a site that is important for the next round of Pol II contact by binding to the core promoter complex, HSF for multiple rounds of transcription. If HSF displaces a critical contacts of this strong promoter.

While this competition model is attractive in its simplicity, it does not exclude other mechanisms that might act alternatively or additionally to increase the rate of escape of Pol II from the pause site into productive elongation. For example, HSF may facilitate recruitment of other general factors, which could modify the promoter-paused Pol II and thereby affect its escape to productive elongation. Furthermore, we have examined here only one of what may be several common contacts of HSF and Pol II with general factors.

Eukaryotic transcription has many steps that can be fine tuned to the needs of the thousands of differentially regulated promoters. Many distinct regulatory steps have been documented, including TFIIID-recruitment (36–38), Pol II recruitment (39), promoter melting (40), and elongational control after promoter escape (41, 42). In each case, the slow step in transcription must be the target of regulatory factors that either enhance or inhibit one of many specific molecular interactions required for establishing a productive transcription complex.

The fact that regulatory factors and RNA polymerase interact with multiple general transcription factors provides the potential for modulation at any of multiple distinct steps.

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