A Highly Conserved Domain of RNA Polymerase II Shares a Functional Element with Acidic Activation Domains of Upstream Transcription Factors

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Received 24 June 1994/Returned for modification 25 July 1994/Accepted 17 August 1994

We report here that the largest subunit of yeast RNA polymerase II contains an acidic domain that is similar to acidic activators of transcription. This domain includes the highly conserved homology box H. A hybrid protein containing this acidic domain fused to the DNA-binding domain of GAL4 is a potent activator of transcription in the yeast *Saccharomyces cerevisiae*. Interestingly, mutations that reduce the upstream activating activity of this acidic domain also abolish the normal function of RNA polymerase II. Such functional defects can be rescued by the acidic activation domains of VP16 and GAL4 when inserted into the mutant derivatives of RNA polymerase II. We further show that this acidic domain of RNA polymerase II interacts directly with two general transcription factors, the TATA-binding protein and TFIIB, and that the acidic activation domain of VP16 can compete specifically with the acidic domain of the RNA polymerase for these interactions. We discuss the implications of this finding for the mechanisms of transcriptional activation in eucaryotes.

How upstream activator proteins stimulate transcription by RNA polymerase II remains an interesting and challenging question. Transcription by RNA polymerase II is a multistep process (reviewed in references 54 and 71), and any one of the steps leading to productive transcription could be rate limiting and a target for activator proteins. Early in the series of steps, RNA polymerase II and a collection of general transcription factors must first assemble onto a promoter to form a transcription preinitiation complex (10, 33). The assembly of a transcription-competent complex begins with the binding of the general transcription factor TFIID to the promoter, followed by the binding of TFIIB, RNA polymerase II, and other general transcription factors (10, 33; reviewed in references 9, 54, and 71). A number of activator proteins have been shown to interact directly with general transcription factors (see, for example, references 26, 38, 39, and 59; reviewed in reference 63), which could potentially recruit one or more of the general transcription factors to the promoter (14, 38, 67). Therefore, activator proteins may act at these early steps to stimulate transcription by facilitating the assembly of a transcription complex.

The assembly of a fully competent transcription complex on a promoter is, however, not always sufficient for productive transcription. In the case of many genes in higher eucaryotes, such as *Drosophila hsp70* and human *c-myc*, the rate-limiting step in the activation of transcription can occur subsequent to the initiation of transcription (19, 34, 45, 49, 52, 60). Thus, some activator proteins may influence a step(s) following complex assembly or the initiation of transcription.

Each cycle of transcription probably involves the assembly of a transcription complex and subsequent disassembly of at least part of the complex to allow RNA polymerase II to leave the

promoter and to proceed into productive transcription. Thus, understanding not only how RNA polymerase II enters a promoter complex but also how it can leave the promoter complex may be key to the understanding of transcriptional regulation. Recent studies have shown that the carboxy-terminal domain (CTD) of the RNA polymerase II largest subunit (16, 70) is involved in the recruitment of the RNA polymerase to the promoter through its direct interaction with the general transcription factor TFIID (15, 32, 61, 66). The CTD is a unique, highly conserved structural component of RNA polymerase II which consists of tandem repeats of a heptapeptide with the consensus YSPTSPS (reviewed in references 16 and 70). RNA polymerase II molecules are present in two electrophoretically distinct size classes (30, 51, 56); RNA polymerase IIO has a hyperphosphorylated CTD, and RNA polymerase IIA has a hypophosphorylated CTD. It has been shown that RNA polymerase IIA is preferentially associated with the preinitiation complex (13, 40, 47, 66), where it is converted to RNA polymerase IIO by a promoter-associated kinase concomitant with or immediately following the initiation of transcription (5, 36, 47, 48). It has been reported that mRNA synthesis in mammalian cells is catalyzed by the phosphorylated form of RNA polymerase II (12). More recently, RNA polymerase II paused early in elongation on a number of *Drosophila* genes has been shown to be largely hypophosphorylated, and passage of polymerase into an elongationally competent mode coincides with phosphorylation (44). It has been proposed that phosphorylation of the CTD may be a way to disrupt the contacts between the CTD of RNA polymerase II and the promoter complex, thereby allowing the polymerase to leave the promoter and proceed into elongation (18, 36).

Recently, we and others have shown that the CTD of yeast, *Drosophila*, and mouse RNA polymerase II can function upstream as an activator of transcription when fused to the DNA-binding domain of GAL4 (29, 57, 69). In addition, we have demonstrated that CTD-like heptapeptide repeats are an essential functional component of the proline-rich activation domain of CTF/NF1 and are important for the interaction of

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the proline-rich domain with the TATA-box-binding protein (TBP) (69). Thus, RNA polymerase II and an upstream activator protein share a structural element that can interact directly with a common target(s) in the general transcription factor-promoter complex. These results have led us to propose a tether-and-competition model for the transcriptional activation by upstream activator proteins (69). In this model, a common element is used both by RNA polymerase II for its recruitment to the general transcription factor-promoter complex and by upstream activators of transcription to displace the RNA polymerase from the general transcription factor-promoter complex so that it can proceed into productive elongation. Thus, by sharing an element with RNA polymerase II, an activator has the potential to act at multiple steps to stimulate transcription, both during and following the assembly of a transcription complex.

In this study, we have identified and characterized an acidic domain in the largest subunit of the yeast RNA polymerase II that possesses both sequence and functional similarities to the acidic activation domains of upstream activator proteins (24, 25, 41, 64). This acidic domain of the RNA polymerase is a potent activator of transcription when fused to the DNA-binding domain of GAL4 and interacts directly with two general transcription factors, TBP and TFIIB.

MATERIALS AND METHODS

Media and strains. Media were prepared according to standard methods (6). The medium used for galactose induction is synthetic medium with 2% galactose, 2% ethanol, and 2% glycerol. The *Saccharomyces cerevisiae* strain GGY1:171 (20), used for assays of GAL4 derivatives, was obtained from Mark Ptashne's laboratory. This strain lacks the entire GAL4 coding sequence. Strain JAY47 (4), used for assays of the RNA polymerase II largest subunit, and its mutant derivatives contains a *GAL10-RPO21* fusion in place of the *RPO21* locus. The expression of this *GAL10-RPO21* gene can be repressed by growing yeast cells on glucose-containing media; thus, the growth of the yeast cells depends on a second copy of the *RPO21* gene whose expression is not under glucose control.

Site-directed mutagenesis and plasmid constructions. All DNA manipulations were carried out by using standard methods (53). The GAL4 derivatives were constructed in the yeast vector pHGX1, a single-copy plasmid that contains sequences encoding GAL4 residues 1 to 147 under the control of the yeast heat shock factor promoter (69).

The GAL4-RNA polymerase II acidic domain fusion (see Fig. 2, line d, pGRPIIAD) was constructed as follows. The sequence AGATCA at positions 4483 to 4488 of *RPO21* (this and subsequent numbering follows that of Allison et al. [2]) was changed to an *EcoRI* site GAATTC, and CCCGGA at positions 4909 to 4914 was changed to a *SmaI* site CCCGGG, by site-directed mutagenesis. This *EcoRI-SmaI* fragment encoding the entire acidic domain and an *NruI-XhoI* fragment containing the transcription termination site of *RPO21* from pGYR8 (69) were cloned into *EcoRI*- and *SalI*-digested pHGX1. The sequence following the *NruI* site is CGATG ATATAG which provides an in frame stop codon (underlined).

Deletion derivatives of this *RPO21* acidic domain were generated by using the naturally occurring restriction sites *BglII* AGATCT at positions 4758 to 4763, *ClaI* ATCAGT at positions 4645 to 4650, and *KpnI* GGTACC at positions 4621 to 4626 and mutationally created restriction sites *HincII* (by changing GTAAAA at positions 4663 to 4668 to GTATAC) and *ApaI* (by changing GTGCTT at positions 4550 to 4555 to

GGGCCC). A stop codon was generated at the 3' deletion endpoint of each derivative by fusion either to the *NruI-XhoI* fragment from pGYR8 (see above) or to a *HincII-XhoI* fragment containing the final 13 codons, including the stop codon and transcription termination site of *RPO21*, from pYCDsh (69). pGRPIIAD.AS contains sequences between the *ApaI* and *SmaI* sites, pGRPIIAD.RB contains sequences between the *EcoRI* and *BglII* sites, pGRPIIAD.AB contains sequences between the *ApaI* and *BglII* sites, pGRPIIAD.I contains sequences between the *EcoRI* and *HincII* sites, pGRPIIAD.KS contains sequences between the *KpnI* and *SmaI* sites, pGRPIIAD.CS contains sequences between the *ClaI* and *SmaI* sites, pGRPIIAD.II contains sequences between the *HincII* and *SmaI* sites, and pGRPIIAD.BS contains sequences between the *BglII* and *SmaI* sites.

pGVP16 was constructed by cloning sequences encoding the carboxy-terminal 79 amino acids and sequences containing the transcription termination site of the yeast heat shock factor into pHGX1. pGAL4 contains sequences encoding GAL4 under the heat shock factor promoter control in pHGX1. The phenylalanine codon TTT at positions 4633 to 4635 was changed to a proline, alanine, or tyrosine codon, and the valine codon GTC at positions 4594 to 4596 was changed to a phenylalanine codon, by site-directed mutagenesis.

All RNA polymerase II derivatives were constructed in plasmid JAY102, a single-copy plasmid that contains a copy of the wild-type *RPO21* gene (4). Deletion of acidic subdomain II of the largest subunit of RNA polymerase II was made by removing sequences between the *HincII* (position 4666) and *SmaI* (position 4911) sites. Alterations of the CTD of the yeast RNA polymerase II were made in plasmid pYCDx (69). Site-directed mutagenesis was performed to create an *NruI* site in the 8th, 11th, and 13th repeats of the CTD in pYCDx to generate pYCDR8nr, pYCD11nr, and pYCD13nr, respectively, by changing C to G at positions 5078 and 5141 and TCCT to GCGA at positions 5181 to 5184 of *RPO21*; a *BamHI* site was created in the 24th repeat by changing ATC to GGA at positions 5409 to 5411 to generate pYCD24b; a *SalI* (*HincII*) site was created at the 3' side of the final repeat of the CTD by changing CAA to GTC at positions 5452 to 5454 to generate pYCDsh. These restriction sites were used to generate CTD variants with 8 to 52 heptapeptide repeats.

A maltose-binding protein (MBP)-VP16 fusion was made by subcloning an *EcoRI-XhoI* fragment from pGV16 containing the VP16 sequences into *EcoRI*- and *SalI*-digested pMAL-c2 (New England Biolabs, Inc.). An MBP-RPIIAD fusion was made by subcloning an *EcoRI-PstI* fragment from pGRPIIAD containing the entire acidic domain of the yeast RNA polymerase II largest subunit into *EcoRI*- and *PstI*-digested pMAL-c2. To create MBP-RPIIAD.I, an *EcoRI-PstI* fragment from pGRPIIAD.I was subcloned into *EcoRI*- and *PstI*-digested pMAL-c2.

Yeast transformation and β -galactosidase assays. Plasmid constructs were introduced into yeast cells as described previously (23). Transformed yeast cells were grown in selective medium to an A_{600} of 1.0 to 1.5. Cells were centrifuged in a 1.5-ml Eppendorf tube in a microcentrifuge for 2 min. The cell pellets were resuspended in β -galactosidase assay buffer (50 mM K_2PO_4 [pH 7.4], 1 mM $MgCl_2$) and were permeabilized with chloroform and sodium dodecyl sulfate as previously described (21). β -Galactosidase activity was determined by using the substrate chlorophenol red- β -D-galactopyranoside (Boehringer Mannheim) as described elsewhere (58).

Protein blot analysis of yeast whole-cell extracts. Yeast whole-cell extracts were prepared as described previously (55). The extracts were analyzed by protein blotting with antibodies

against the CTD of yeast RNA polymerase II or the DNA-binding domain of GAL4, using the Amersham ECL (enhanced chemiluminescence) detection system.

Protein affinity chromatography. Sequences that contain the activation domain of VP16 or the acidic domain of the yeast RNA polymerase II were fused to *Escherichia coli* MBP. The proteins were then expressed in *E. coli* and purified by affinity chromatography. The purified proteins were covalently coupled to Affi-Gel 10 to about 4 mg/ml of resin. The resin was packed into 0.5-ml minicolumns, which were washed extensively with affinity chromatography buffer (ACB) (20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid [HEPES; pH 7.5], 1.0 mM EDTA, 5 mM MgCl₂, 0.2 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride, 10% glycerol) containing 100 mM KCl.

E. coli extracts containing yeast TBP, yeast TFIIB, and human TFIIB were prepared as follows. Plasmids containing yeast TBP, yeast TFIIB, and human TFIIB under T7 promoter control were transformed into *E. coli* BL21(DE3), which harbors a T7 RNA polymerase under *lac* promoter control. These proteins were induced with 0.2 mM isopropylthiogalactopyranoside (IPTG) for 2 to 3 h at 37°C. Cells were harvested and resuspended in 20 mM HEPES (pH 7.5)–1.0 mM EDTA–5.0 mM MgCl₂–1.0 mM dithiothreitol, 1.0 mM phenylmethylsulfonyl fluoride, 100 mM KCl. The cell suspensions were frozen at –20°C overnight and then thawed and broken by sonication. Following centrifugation at 10,000 × *g* for 30 min, the supernatant was dialyzed extensively in ACB–100 mM KCl. Before use, the extracts were adjusted to a final concentration of about 5 mg of total protein per ml.

For protein affinity chromatography, 1.5-ml aliquots of *E. coli* extracts (see above) were loaded onto each minicolumn (see above) over a period of 30 min. The columns were then washed with 10 to 20 column volumes of ACB–100 mM KCl. The bound protein(s) was eluted with 1.5 ml of ACB–800 mM KCl and analyzed by polyacrylamide gel electrophoresis and silver staining.

For competition assay, total *E. coli* extracts containing TBP or TFIIB were loaded onto an MBP-VP16 or MBP-RPIIAD column and washed extensively with ACB–100 mM KCl (see above). Bound proteins were then eluted with ACB–50 mM KCl–2 mg of MBP-VP16 per ml or, as a control, with MBP. Yeast TBP or human TFIIB was detected by using the Amersham ECL detection system with anti-yeast TBP or anti-human TFIIB antibodies.

RESULTS

The largest subunit of yeast RNA polymerase II contains a domain with sequence similarities to acidic transcriptional activation domains. To identify and characterize elements common to RNA polymerase II and upstream activators of transcription, we examined sequences of the yeast RNA polymerase II subunits for similarities with known transcriptional activation domains. We found that a region of the largest subunit of yeast RNA polymerase II, consisting of the conserved domain H and residues between H and the CTD (Fig. 1A), has a high content of acidic residues, which is characteristic of the acidic activators of transcription (24, 25, 41, 64). This region of about 140 amino acids contains a total of 27 acidic amino acids with a net negative charge of –22. Figure 1B shows the sequence of a portion of this acidic domain and for comparison sequences from the corresponding domain of *Drosophila* RNA polymerase II (27) and from a number of acidic activators of transcription. Amino acid similarities between this acidic domain of the RNA polymerase and the

FIG. 1. The largest subunit of RNA polymerase II contains an acidic domain with sequence similarities to acidic activators of transcription. (A) Structural organization of the largest subunit of yeast RNA polymerase II (70). Black boxes indicate the approximate positions and sizes of the conserved regions, which have been termed homology boxes A to H, and the hatched box represents the CTD, which consists of repeats of a conserved heptapeptide, YSPTSPS. The acidic domain identified consists of two subdomains, I and II; subdomain I corresponds to the highly conserved homology box H, and subdomain II corresponds to the adjacent sequences between box H and the CTD. (B) Sequence comparisons of a portion of this acidic domain of yeast and *Drosophila* (DROS.) RNA polymerase II with acidic activators of transcription. Aromatic and bulky hydrophobic residues in the RNA polymerase that match those in the acidic activation domains are in open boxes. The arrowhead indicates the phenylalanine residue at amino acid position 1441 of the yeast RNA polymerase II largest subunit, which is aligned with F-442 of the acidic domain of VP16. Amino acids in the acidic activation domains that are similar or identical to those in the polymerase sequences are in shaded boxes.

acidic activators of transcription are evident, in particular around the phenylalanine residue. This phenylalanine residue is absolutely conserved at this position of RNA polymerase II (1, 8, 27), which suggests its functional importance. Also noteworthy is the pattern of hydrophobic and aromatic residues, which is similar between the acidic activators of transcription (17, 50) and the RNA polymerase II largest subunit. Triezenberg and colleagues have shown that such sequence features are important for the transcriptional activation function of the acidic domain of VP16 (17, 50). In addition, the entire acidic domain of yeast RNA polymerase II shows a pattern of distribution of acidic residues interspersed with hydrophobic and aromatic residues, which appears to be a general feature of acidic activation domains of transcription (reviewed in reference 63). For simplicity of discussion, we have termed the region corresponding to the conserved homology box H acidic subdomain I and the adjacent region acidic subdomain II (Fig. 1A).

The acidic domain of RNA polymerase II can act upstream as a potent activator of transcription when fused to the DNA-binding domain of GAL4. To determine the structural and functional relationships between the acidic domain of the RNA polymerase II largest subunit and the acidic transcriptional activation domains, we tested the ability of the acidic domain of the RNA polymerase to stimulate transcription when tethered to a site upstream of the TATA box. To this end, we constructed and expressed hybrid proteins that contain

		ACTIVATOR	S-GAL (UNITS)
a	pGAL4 (1-147)	GAL4 (1-147) (NONE)	<1
b	pGAL4	GAL4 (1-147) (GAL4)	13980
c	pGVP16	GAL4 (1-147) (VP16)	7974
		I II	
d	pGRPIIAD	GAL4 (1-147) [REDACTED]	14799
e	pGRPIIAD.AS	GAL4 (1-147) [REDACTED]	6928
f	pGRPIIAD.RB	GAL4 (1-147) [REDACTED]	3770
g	pGRPIIAD.AB	GAL4 (1-147) [REDACTED]	1229
h	pGRPIIAD.I	GAL4 (1-147) [REDACTED]	3207
i	pGRPIIAD.KS	GAL4 (1-147) [REDACTED]	3263
j	pGRPIIAD.CS	GAL4 (1-147) [REDACTED]	3236
k	pGRPIIAD.II	GAL4 (1-147) [REDACTED]	1747
l	pGRPIIAD.BS	GAL4 (1-147) [REDACTED]	664

FIG. 2. The acidic domain of the yeast RNA polymerase II largest subunit functions upstream as a potent activator of transcription. The black box under I corresponds to the conserved homology box H, and the shaded box under II corresponds to the region between box H and the CTD (see Fig. 1A). The entire acidic domain or parts of it were fused to the GAL4 DNA-binding domain, and the hybrid proteins were assayed in *S. cerevisiae* GGY1:171 (20) for the ability to stimulate transcription from a *GAL1-lacZ* reporter gene. The values shown represent the average units of β -galactosidase activity from three to five independent assays. The variation in relative β -galactosidase activities was within 10%.

the acidic domain of the yeast RNA polymerase II largest subunit fused to the DNA-binding domain of GAL4 and tested the ability of the hybrid proteins to stimulate transcription from a *GAL1-lacZ* reporter gene in yeast cells. As shown in Fig. 2, this acidic domain of the RNA polymerase is a potent activator of transcription. The level of transcriptional activation by this acidic domain is as high as that of the wild-type GAL4 and about twofold higher than that of the acidic domain of VP16 (Fig. 2; compare lines *b* and *c* with line *d*). Deletion analysis showed that segments of this acidic domain of the yeast RNA polymerase II also stimulate transcription (lines *e* to *l*). This scattered pattern of localization of transcriptional activity is typical of many acidic activation domains of transcription (24, 25, 41, 64).

To examine further the structural and functional properties of the acidic domain of the yeast RNA polymerase II largest subunit and its relationship to acidic activators of transcription, we introduced point mutations into acidic subdomain I at amino acid position 1441, which is analogous to F-442 of the acidic activator of VP16 (Fig. 1B). Triezenberg and colleagues showed that point mutations at this position of VP16 can have dramatic effects on its transcriptional activity (17, 50). As shown in Fig. 3, substitution of the phenylalanine with a proline or alanine residue within subdomain I reduced the transcriptional activity by more than twofold (compare line *b* with *c* and lines *d*), whereas substitution with a structurally similar tyrosine residue had only a minor effect (compare line *b* with line *e*). These results further indicate that the sequence similarity of acidic subdomain I of the RNA polymerase II largest subunit with the acidic activator of VP16 is functionally significant in the context of an upstream activator of transcription. The less dramatic effect of substitution at F-1441 of subdomain I than at F-442 of VP16 may reflect the fact that

FIG. 3. Point mutations can either decrease or increase the transcriptional activity of the acidic domain of yeast RNA polymerase II. Point mutations were introduced at either of the two positions in subdomain I as indicated; a phenylalanine was changed to a proline, alanine, or tyrosine residue at amino acid position 1441 (*c*, *d*, and *e*), and a valine was changed to a phenylalanine at amino acid position 1428 (*f*). This V-1428→F substitution is identical to a previously reported mutation that was identified as a suppressor of a partial CTD truncation (43). These mutant derivatives of subdomain I were fused to the DNA-binding domain of GAL4 and assayed as in Fig. 2. An asterisk indicates a point mutation. β -Galactosidase (β -GAL.) levels were determined as in Fig. 2.

this domain of the RNA polymerase contains a total of three phenylalanine residues, whereas the VP16 subdomain contains only one phenylalanine which is also the only aromatic residue. The importance of phenylalanine or aromatic residues for the transcriptional activity of an activation domain is further demonstrated in Fig. 3, line f, which shows that substitution of a phenylalanine for the valine at amino acid position 1428 significantly enhanced the transcriptional activity of subdomain I.

Structural elements that are required for function in the context of an upstream transcriptional activator are also required for normal RNA polymerase II function. The fact that the acidic domain of the yeast RNA polymerase II largest subunit can act upstream as a potent activator of transcription does not necessarily mean that the structural elements required for function in the context of an upstream activator of transcription are also functionally significant for the RNA polymerase. To test this, we constructed yeast RNA polymerase II derivatives that contain deletion and point mutations in the acidic domain of the largest subunit and assayed the mutant derivatives for their ability to support growth of yeast cells. As shown in line *c* of Fig. 4, a mutant derivative with a deletion of acidic subdomain II cannot support normal cell growth. Yeast cells carrying this mutant RNA polymerase as the sole source of RNA polymerase II grow more slowly than cells carrying the wild-type RNA polymerase and are temperature sensitive (compare line *a* with line *c*). The mutant growth phenotype is even more severe when the number of heptapeptide repeats in the CTD was reduced from the 26 found in the wild type to 17 (compare line *b* with line *d*).

To show that the inability of the mutant RNA polymerase II to support normal cell growth is not a result of a change in spacing between the CTD and other domains (Fig. 1A), we constructed RNA polymerase II derivatives that contain, in place of acidic subdomain II, sequences of similar sizes but without transcriptional activity (as tested with fusions to the GAL4 DNA-binding domain) (Fig. 4, lines *e* and *f*). Substitu-

FIG. 4. The acidic domain is required for the normal function of yeast RNA polymerase II. Only the acidic domain (both subdomains I and II) and the CTD are shown. *a* is the wild-type largest subunit, which contains both subdomains I and II and all 26 heptapeptide repeats in the CTD. The number of heptapeptide repeats in each derivative is indicated in parentheses. NO ACTIVATION refers to sequences that showed no stimulation of transcription when fused to the DNA-binding domain of GAL4. RS1 (*e*) encodes 80 residues between homology box H and the CTD of *Drosophila* RNA polymerase II, and RS2 (*f*) encodes 79 residues from a nonconserved region of the second largest subunit of yeast RNA polymerase II. VP16 (*g*) refers to the acidic activation domain (amino acids 413 to 488) of VP16, and VP16d (*h*), amino acids 454 to 488 (the distal subdomain) of VP16 (64). GAL4I (*i* and *k*) is the sequence encoding amino acids 147 to 211 of GAL4 (35), which contains a weak transcriptional activity (41). Derivatives *j* to *l* contain substitution of a proline for the conserved phenylalanine residue in subdomain I (refer to Fig. 3). All of the derivatives were introduced into *S. cerevisiae* JAY47 (see Materials and Methods). Transformed cells were tested for growth at 30 and 37°C on solid medium with glucose as the carbon source. Protein blot analysis using an antibody against the yeast RNA polymerase II largest subunit showed that the wild-type largest subunit and its mutant derivatives were produced in very similar amounts in transformed yeast cells (data not shown).

tions with these sequences to restore the spacing did not restore the function of the RNA polymerase (lines *e* and *f*). We then inserted sequences of the acidic activation domains of VP16 (lines *g* and *h*) and GAL4 (line *i*). Insertions of the acidic activation domains of VP16 and GAL4 rescued the growth defects (lines *g* to *i*). These results indicate that acidic subdomain II is important for the normal function of yeast RNA polymerase II and that in the context of RNA polymerase II, the acidic activation domains of VP16 and GAL4 are functionally similar to the acidic domain of the RNA polymerase.

To test whether the transcriptional activity of acidic subdomain I is also required for the normal function of yeast RNA polymerase II, we constructed an RNA polymerase II derivative that contains an F-to-P mutation (as in Fig. 3, line *c*) in addition to a deletion of acidic subdomain II (line *j*). This

FIG. 5. Functional relationship between the CTD and the acidic domain of yeast RNA polymerase II. Both wild-type and variant acidic domains (*a* to *d*; see Fig. 3 and 4 for a description of the acidic domain variants) were tested for their requirements for heptapeptide repeats in the CTD. For the majority of the derivatives, this was done with truncations in the wild-type CTD (26 heptapeptide repeats) at intervals of 2 to 4 heptapeptide repeats (the positions of truncations are indicated by circles). Derivatives containing longer CTDs (35, 42, and 52 heptapeptide repeats) were also constructed in the context of the deletion of subdomain II (*b*). All of the derivatives were introduced into *S. cerevisiae* JAY47, and transformed cells were tested for growth at 30 and 37°C on solid medium with glucose as the carbon source, as described in the legend to Fig. 4. Open circles indicate no growth; black circles indicate wild-type or nearly wild-type growth; shaded circles indicate intermediate growth rates (many of these derivatives also showed temperature sensitivity); the most lightly shaded circles indicate extremely slow growth (tiny colonies were seen only after prolonged incubation). In many cases, we noted a gradual reduction in growth rate as the CTD was progressively truncated.

F-to-P mutation, which reduced the transcriptional activity of subdomain I (Fig. 3; compare lines *b* and *c*), enhances the mutant growth phenotype conferred by the deletion of acidic subdomain II (Fig. 4; compare lines *c* and *j*). The enhancement of the mutant growth phenotype conferred by the F-to-P point mutation is also clearly seen when subdomain II is replaced with a weak activation domain, acidic domain I of GAL4 (Fig. 4; compare lines *i* and *k*). In contrast, the growth defects were fully rescued by insertion of the strong acidic activation domain of VP16 in place of subdomain II (Fig. 4, line *l*). These results lend further support to the idea that the overall transcriptional activity of the acidic domain is required for normal function of RNA polymerase II.

A functional relationship between the acidic domain and the CTD of the RNA polymerase II largest subunit. As shown in lines *b* and *d* of Fig. 4, a deletion of acidic subdomain II rendered RNA polymerase II more sensitive to truncations of the CTD, suggesting a functional relationship between the acidic domain and the CTD. This relationship was further tested by examining RNA polymerase II derivatives with either weaker or stronger transcriptional activities in the acidic domain (as defined in the context of hybrid GAL4 activators) for their requirements for the CTD. To this end, we constructed RNA polymerase II derivatives with mutations in the acidic domain in combination with various numbers of heptapeptide repeats in the CTD. We then tested these derivatives for the ability to support cell growth at different temperatures. The results are summarized in Fig. 5. Yeast RNA polymerase II with the intact acidic domain requires only 11 of the 26 or 27 CTD heptapeptide repeats to support cell growth at 30°C and 13 or more heptapeptide repeats to support normal growth at all temperatures (Fig. 5, line *a*). This finding is consistent with previously published results (3, 42). However, an RNA polymerase II derivative lacking acidic subdomain II requires 35 or more heptapeptide repeats in the CTD to support normal growth and a minimum of 19 heptapeptide repeats to support

even very slow growth (Fig. 5, line *b*). These results demonstrate that RNA polymerase II with a weak activator domain requires a longer CTD (or more heptapeptide repeats) for normal function, while RNA polymerase II with a strong activator domain requires fewer heptapeptide repeats, since a deletion of subdomain II reduced the transcriptional activity of the acidic domain by almost fivefold (Fig. 2; compare lines *d* and *h*).

This functional relationship between the acidic domain and the CTD is also clearly demonstrated in Fig. 5, lines *c* and *d*. The two RNA polymerase II derivatives are identical except that one (Fig. 5, line *c*) contains an F-to-P mutation in subdomain I that reduced transcriptional activity of the subdomain (refer to Fig. 3, line *c*), while the other (Fig. 5, line *d*) contains a V-to-F mutation that increased transcriptional activity of the subdomain (refer to Fig. 3, line *f*). The RNA polymerase II derivative with the weaker activator domain requires more CTD repeats to support cell growth, whereas the one with the stronger activator domain requires fewer CTD repeats to support cell growth (Fig. 5; compare lines *c* and *d*). This V-to-F mutation at position 1428 is identical to a previously reported suppressor mutation in homology box H (termed acidic subdomain I in this study) of the yeast RNA polymerase II largest subunit (43). In this case, the V-to-F mutation was isolated as an intragenic suppressor of the cold-sensitive phenotype of an RNA polymerase II derivative with only 11 heptapeptide repeats in the CTD (43). These results further demonstrate that a strong activator domain can alleviate the requirement for the CTD heptapeptide repeats. It should be mentioned, however, that the CTD cannot be replaced entirely by acidic activation domains of upstream transcription factors, suggesting that the acidic domain and the CTD possess overlapping as well as distinct functions.

The results presented above have interesting implications for the functional relationship between the CTD and the acidic domain of RNA polymerase II from higher eucaryotes. The acidic domain of the yeast RNA polymerase II largest subunit includes the conserved homology box H and the adjacent region between homology box H and the CTD (Fig. 1A). However, we noticed that in the largest subunit of RNA polymerase II of higher eucaryotes, an excess of acidic amino acids is confined to homology box H only (not shown, but see references 1, 8, and 27). As we have demonstrated in this study (Fig. 4 and 5), acidic subdomain II is necessary for the normal function of yeast RNA polymerase II but can be functionally substituted either by the acidic activation domains of VP16 and GAL4 or by additional heptapeptide repeats in the CTD. These results suggest that the requirement for a longer CTD (40 or more heptapeptide repeats) in the largest subunit of higher-eucaryotic RNA polymerase II (7, 72) may be due, at least in part, to an acidic domain that is weaker than the one in yeast RNA polymerase II.

The acidic domain of RNA polymerase II interacts directly with TBP and TFIIB. The acidic activation domain of VP16 has been shown to interact with both TBP (28, 59) and TFIIB (39). Since the RNA polymerase II acidic domain shows sequence and functional similarities to the acidic activation domains of transcription, it may also interact directly with TBP and TFIIB. To test this, we constructed, expressed, and purified two hybrid proteins, MBP-RPIIAD and MBP-VP16, coupled these fusion proteins covalently to Affi-Gel 10 columns, and passed total *E. coli* extracts containing recombinant TBP or TFIIB through these columns. As shown in Fig. 6A, the yeast TBP is retained specifically on MBP-VP16 and MBP-RPIIAD columns (lanes 4 to 6) but not on control columns without protein or with MBP only (lanes 2 and 3). The

FIG. 6. The acidic domain of the RNA polymerase II largest subunit interacts directly with TBP and TFIIB. (A) Total *E. coli* extracts (lane 1) containing recombinant yeast TBP (yTBP) were loaded onto Affi-Gel 10 columns either without (lane 2) or with the indicated covalently coupled proteins (lanes 3 to 6). Eluted proteins from these columns were visualized by silver staining, and identities of yeast TBP, yeast TFIIB (yIIB), and human TFIIB (hIIB) were confirmed by protein blot analysis with anti-yeast TBP and anti-human TFIIB antibodies. (B) Total *E. coli* extracts containing both yeast TFIIB and human TFIIB were analyzed as in panel A. yIIBc is a degradation fragment from the yeast TFIIB which may correspond to the carboxy terminus of the protein.

interaction between the acidic domain of RNA polymerase II and TBP is strong and highly specific, since TBP can be purified from total *E. coli* extracts to near homogeneity by a single passage of total *E. coli* extracts through the column with the MBP-RPIIAD fusion protein (compare lanes 1 and 5 in Fig. 6A).

The acidic domain also interacts directly with the general transcription factor TFIIB. As shown in Fig. 6B, both the yeast TFIIB and human TFIIB are retained specifically on columns with the MBP-VP16 and MBP-RPIIAD fusion proteins (lanes 3 to 5) but are retained not at all or very little on the control column with MBP only (lane 2). These results demonstrate that the acidic domain of the yeast RNA polymerase II largest subunit interacts directly with both TBP and TFIIB.

As both subdomains I and II can act synergistically or independently as strong activators of transcription (Fig. 2, lines *h* and *k*), they both contribute to the interaction with TBP and TFIIB. As can be seen from Fig. 6, TBP and TFIIB are retained specifically on the MBP-RPIIAD.I column but more strongly on the MBP-RPIIAD column (compare lanes 5 and 6 for TBP in Fig. 6A and lanes 4 and 5 for TFIIB in Fig. 6B). It is also interesting that the acidic activation domain of VP16 shows a higher affinity for human TFIIB than for yeast TFIIB, while the acidic domain of yeast RNA polymerase II shows a higher affinity for yeast TFIIB than for human TFIIB (Fig. 6B; compare lanes 3 and 4). This species specificity might reflect functional significance, since in yeast cells the acidic domain of the yeast RNA polymerase II is a stronger activator than the acidic domain of VP16 (Fig. 2; compare lines *c* and *d*).

The acidic activation domain of VP16 competes with the acidic domain of RNA polymerase II for interaction with TBP and TFIIB. The result that both the acidic domain of the RNA polymerase and the acidic activation domain of VP16 interact with TBP and TFIIB suggests the possibility that they interact

FIG. 7. Competition assay for the specificity of interaction of the acidic domain of the RNA polymerase II largest subunit with TBP and TFIIB. (A) Experimental design. As shown, TBP was bound to a column through its direct interaction with an acidic domain (AD) covalently coupled to the column. A second protein (as indicated by the shaded circle following the arrow) was used to elute TBP from the column. (B) TBP (lanes 1 and 2) or TFIIB (lanes 3 and 4) bound to an MBP-VP16 column was much more efficiently eluted with the MBP-VP16 fusion protein (E1, lanes 2 and 4) than with MBP (E1, lanes 1 and 3). To show that the lower levels of elution of TBP and TFIIB with MBP were not due to less protein on the columns to begin with, the columns were subsequently eluted with elution buffer containing 800 mM KCl (E2). Lanes 1 and 2 (also 3 and 4) in the second elution (E2) show that very similar amounts of proteins were loaded on the columns. (C) An assay identical to that in panel B except that TBP (lanes 5 and 6) or TFIIB (lanes 7 and 8) was bound to a column containing the acidic domain of the RNA polymerase largest subunit and eluted with MBP (E1, lanes 5 and 7) or MBP-VP16 (E1, lanes 6 and 8). In both panel B and panel C, TBP and TFIIB were detected by protein blot analysis using anti-yeast TBP and anti-human TFIIB antibodies.

with the same surface or structural domain of the TBP or TFIIB protein. As a simple test for this, we performed a competition assay (see Materials and Methods). Figure 7A shows the experimental design for the competition assay. MBP-VP16 or MBP-RPIAD was covalently coupled to an Affi-Gel 10 column; TBP or TFIIB was then bound to the column through its direct interaction with the acidic domain of VP16 or of yeast RNA polymerase II. The bound TBP or TFIIB was then eluted with a second protein, MBP or MBP-VP16. Figure 7B demonstrates the specificity of this assay. Both TBP and TFIIB bound to an MBP-VP16 column can be eluted efficiently with the MBP-VP16 fusion protein (lanes 2 and 4) but much less efficiently with the MBP protein (lanes 1 and 3). We then tested the ability of the MBP-VP16 fusion protein to elute TBP or TFIIB from an MBP-RPIAD column. As shown in Fig. 7C, TBP or TFIIB bound to the MBP-RPIAD column can be eluted efficiently with the MBP-VP16 fusion protein (lanes 6 and 8) but much less efficiently with the MBP protein (lanes 5 and 7). These results provide evidence that the acidic activation domain of VP16 and the acidic domain of the yeast RNA polymerase II largest subunit interact with similar or identical structural domains or motifs of TBP and of TFIIB.

DISCUSSION

We have shown here that an acidic domain including the highly conserved homology box H and an adjacent region of

the largest subunit of RNA polymerase II possesses sequence and functional similarities to acidic activation domains of well-defined transcription factors. This acidic domain of RNA polymerase II, like the acidic activation domain of VP16, is a potent activator of transcription when fused to the DNA-binding domain of GAL4. As shown here, this acidic domain interacts directly and strongly with at least two of the general transcription factors, TBP and TFIIB.

To form a transcription-competent complex, RNA polymerase II must associate with the general factor-promoter complex through direct interactions of domains of the RNA polymerase and its associated factors (11) with general factors assembled on the promoter. It has been shown that only two general factors, TBP and TFIIB, are both necessary and sufficient for promoter-specific transcription by RNA polymerase II on supercoiled DNA templates (46). Previous studies have shown that the CTD of RNA polymerase II is one of the domains that interact directly with TBP (61, 66, 69). This interaction apparently is important for the recruitment of the RNA polymerase to the general factor-promoter complex (32, 61, 66). RNA polymerase II has also been shown to interact directly with the general transcription factor TFIIB (22, 65); however, the domain(s) of the RNA polymerase responsible for this latter interaction has not been determined. Our results demonstrate that an acidic domain of RNA polymerase II, including a highly conserved region, interacts directly and strongly with both TBP and TFIIB. Thus, this acidic domain, in concert with the CTD, could play a major role in the recruitment of the polymerase to the general factor-promoter complex.

The idea that the acidic domain and the CTD of RNA polymerase II both act to recruit the RNA polymerase to the general factor-promoter complex is further supported by results from our *in vivo* genetic and functional analyses. In this study, we have demonstrated complementation between the acidic domain and the CTD of the RNA polymerase (see also reference 43). The *in vitro* interaction of the acidic domain of RNA polymerase II with TBP and TFIIB is likely to have functional significance. Deletion of subdomain II not only reduced the affinity of the acidic domain for both TBP and TFIIB (Fig. 6) but also abolished the normal function of the RNA polymerase (e.g., Fig. 4, line c). In addition, the functional defect due to the deletion of subdomain II can be rescued by inserting into the mutant RNA polymerase the acidic activation domain of VP16, which can also interact directly with both TBP and TFIIB (28, 39, 59).

The acidic domain of the largest subunit of yeast RNA polymerase II includes homology box H (termed acidic subdomain I in this study), which is highly conserved through evolution (reviewed in references 16 and 70). Indeed, we have found that homology box H of the largest subunit of the *Drosophila* RNA polymerase II can functionally substitute for the corresponding domain of yeast RNA polymerase II (68a). Furthermore, homology box H of the *Drosophila* RNA polymerase II strongly stimulates transcription in yeast cells when fused to the DNA-binding domain of GAL4 (68a). These results lend further support to a conserved, fundamental function of homology box H of the largest subunit of RNA polymerase II.

The model presented in Fig. 8 (see also reference 69) proposes that acidic activators may have the potential to modulate transcriptional activity at several levels. To start transcription, general factors and RNA polymerase II must be assembled on a promoter to form a transcription-competent complex (10, 33). The assembly of this complex begins with the binding of TFIID to the basal promoter elements, and TFIIB

FIG. 8. A tether-and-competition model of transcriptional activation. For simplicity, only the upstream activator GAL4-acidic domain (AD), the general transcription factors TFIID (D) and TFIIB (B), and RNA polymerase II (RNAPII) with its acidic domain are shown. By sharing a common domain (AD) with RNA polymerase II, the upstream activator GAL4-AD could act both during and following the assembly of a transcription complex. Direct interaction of AD with TFIID and TFIIB may help recruit the general factors to the promoter (a) or block potential interactions of TBP with negative regulatory factors (not shown), thereby facilitating the subsequent assembly of a transcription complex (b). Following the assembly of the transcription complex, the potential competitive interaction of the upstream activator AD with TFIID and TFIIB may destabilize or displace the contacts of the AD of RNA polymerase II with TFIID and TFIIB, thereby accelerating the release of the RNA polymerase from the general promoter complex (c). For multiple rounds of transcription, the relative rate constants for association with and dissociation from TFIID and TFIIB of the upstream activator AD and of the AD of RNA polymerase II may be a factor in determining the frequency at which RNA polymerase II is recruited to and subsequently released from the general factor-promoter complex (b to d).

then binds the TFIID-promoter complex (10) or binds as part of a larger RNA polymerase II holoenzyme complex (31, 33). The recruitment and binding of TFIID and TFIIB are shown in the model to be promoted by acidic activators of transcription through their direct interactions with the general transcription factors (Fig. 8, line a). The association of RNA polymerase II with this D-B-promoter complex is likely to involve direct interactions of multiple RNA polymerase domains such as the CTD (32, 61, 66, 69) and the acidic domain (line b) with TFIID and TFIIB.

Following assembly, the transcription complex, or part of the transcription complex, must be disassembled to allow at least the RNA polymerase to leave the promoter to proceed into productive elongation. This means that whatever contacts the RNA polymerase makes to assemble into the general transcription complex must be disrupted or disconnected before the RNA polymerase can move out from the complex and proceed into transcription. An upstream activator that shares a domain with RNA polymerase II for interaction with the same target(s) in the general transcription machinery has the potential to displace the contacts of RNA polymerase II with the

general factor-promoter complex, thereby accelerating the release of the RNA polymerase from the general factor-promoter complex to proceed into transcription (Fig. 8, lines c and d). Such displacements are reconstituted *in vitro*, in which case both TBP and TFIIB bound to the acidic domain of RNA polymerase II on columns are eluted with the acidic activation domain of VP16 (Fig. 7).

The model in Fig. 8 provides a simple mechanistic explanation for the ability of an acidic activator such as that of VP16 to function at both preinitiation and postinitiation steps in the stimulation of transcription. Studies on acidic activators from several laboratories have shown that they act to promote the assembly of a transcription complex (for example, see references 14 and 67). The acidic activator of VP16 has also been shown to stimulate transcription when tethered to a promoter-proximal RNA sequence by fusion of the acidic domain to an RNA-binding domain (62). This latter result indicates that the acidic activator can also act at a step following the initiation of transcription (62).

The sharing of elements by RNA polymerase II and upstream activators of transcription is not limited to acidic activators and the polymerase. It has recently been shown that the proline-rich domain of the upstream activator CTF/NF1 contains a sequence identical to the heptapeptide repeat that constitutes the CTD of RNA polymerase II (29, 69). We have demonstrated that this CTD-like sequence is important for the interaction of the proline-rich domain with TBP and is essential for its transcriptional activation function (69). Thus, an element is also shared by RNA polymerase II and a proline-rich activator of transcription. These results support the generality of the tether-and-competition model proposed in Fig. 8 (see also reference 69).

We have focused our discussion on two potential steps at which an acidic activator may act. However, an acidic activator might have additional effects or act at other steps as well. For example, when bound to DNA, it might affect chromatin structure by interacting with histones or other chromosomal proteins, as has been proposed (37, 68). This might, in turn, affect the rate of transcription. In this regard, the acidic domain of the RNA polymerase II largest subunit may work in concert with upstream activators of transcription to augment their function. Thus, the model proposed in Fig. 8 provides a simple mechanistic basis for the future study of transcriptional activation by upstream regulatory proteins in eucaryotes.

ACKNOWLEDGMENTS

We thank J. Archambault, S. Triezenberg, and M. Ptashne for providing strains and plasmids; M. Ptashne for the antibody against the GAL4 DNA-binding domain; Burgess for antibody against the CTD of yeast RNA polymerase II; J. Greenblatt for antibodies against yeast TBP and human TFIIB and for recombinant plasmids expressing yeast TBP and TFIIB and human TFIIB; and members of the Friesen and Lis laboratories, E. Rasmussen, C. Giardina, T. O'Brien, D. Jansma, and I. Donaldson, for critical reading of the manuscript.

This work was supported by grants from the Medical Research Council (MRC) of Canada to J.D.F. and from NIH (United States) to J.T.L. H.X. was a recipient of a postdoctoral fellowship from the MRC of Canada.

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