A Gal4-σ54 Hybrid Protein That Functions as a Potent Activator of
RNA Polymerase II Transcription in Yeast*

Bo-Shiun Chen‡, Zu-Wen Sun‡§, and Michael Hampsey¶

From the Department of Biochemistry, Division of Nucleic Acids Enzymology, Robert Wood Johnson Medical School,
Piscataway, New Jersey 08854-5635

Promoter specificity of bacterial RNA polymerases (RNAPs) is conferred by sigma factors that bind the core RNAP to generate the α,β′σ holoenzyme. In addition to σ70, which is required for transcription of most bacterial genes, there are several alternative sigma factors that typically promote transcription of genes involved in physiologically related pathways. σ54 was initially identified as an alternative sigma factor, although subsequent studies revealed that σ54, like σ70, is involved in transcription of physiologically diverse genes (1, 2).

The bacterial σ54 protein associates with core RNA polymerase to form a holoenzyme complex that renders cognate promoters enhancer-dependent. Although unusual in bacteria, enhancer-dependent transcription is the paradigm in eukaryotes. Here we report that a fragment of Escherichia coli σ54 encompassing amino acid residues 29–177 functions as a potent transcriptional activator in yeast when fused to a Gal4 DNA binding domain. Activation by Gal4-σ54 is TATA-dependent and requires the SAGA coactivator complex, suggesting that Gal4-σ54 functions by a normal mechanism of transcriptional activation. Surprisingly, deletion of the AHC1 gene, which encodes a polypeptide unique to the ADA coactivator complex, stimulates Gal4-σ54 DNA binding activity and enhances the toxicity of Gal4-σ54. Accordingly, the SAGA and ADA complexes, both of which include Gen5 as their histone acetyltransferase subunit, exert opposite effects on transcriptional activation by Gal4-σ54. Gal4-σ54 activation and toxicity are also dependent upon specific σ54 residues that are required for activator-responsive promoter melting by σ54 in bacteria, implying that activation is a consequence of σ54-specific features rather than a structurally fortuitous polypeptide fragment. As such, Gal4-σ54 represents a novel tool with the potential to provide insight into the mechanism by which natural activators function in eukaryotic cells.

All forms of bacterial RNAP bind promoter DNA in at least two steps: formation of a closed promoter complex followed by isomerization to an open complex where the start strand becomes bound to promoter DNA only upon open complex formation (3, 4). However, this process is fundamentally different at σ54-dependent promoters, where the σ54 holoenzyme binds promoter DNA to form a fully stable closed complex (5). Isomerization from a closed to open complex requires ATP hydrolysis and occurs only upon contact of a remote activator protein with the σ54 holoenzyme (6). The uncoupling of promoter recognition from promoter melting enables σ54 to respond to activators bound at distal sites (7).

σ54 is structurally distinct from σ70 and is the only sigma factor that is not a member of the σ70 family (8). Unlike σ70, σ54 is a modular protein comprising at least three functional domains: the N-terminal region regulates DNA melting and transcriptional activation (7, 9–12), the central region binds core RNAP (13, 14), and the C-terminal region binds promoter DNA (11, 15). σ54 includes several structural characteristics found in eukaryotic transcription factors, including a glutamine-rich region near the N terminus, an acidic region that overlaps a hydrophobic heptad repeat near the central region, and a helix-turn-helix motif within the C-terminal promoter binding region. Despite overall structural disparity, σ54 and the σ70 family of proteins share a region of sequence similarity involved in RNAP binding, located at the end of a heptad repeat within the central region of σ54 (13).

The mechanism of transcription initiation by the σ54 holoenzyme is reminiscent of the mechanism of initiation by eukaryotic RNA polymerase II (RNAP II). Eukaryotic transcriptional activators bind cognate enhancer sequences and stimulate transcription by interacting, either directly or indirectly, with components of the general machinery. In addition to RNAP II, the general transcription factors include the TATA-binding protein (TBP), TFIIB, TFIIE, TFIIF, and TFIIF (16). These factors are either assembled de novo at the core promoter or are recruited in association with RNAP II as a complex, in either case forming a stable closed complex over the core promoter. Transcription initiation is dependent upon subsequent isomerization to an open promoter complex in an ATP-dependent manner (17).

Activation of RNAP II promoters involves at least two steps, including recruitment of TFIID (TBP plus TBP-associated factors or TAFs) and recruitment of the RNAP II holoenzyme (18). Strong evidence in support of “activation by recruitment” comes from tethering experiments where the DNA binding domain of a transcription factor is fused to a component of the core machinery. The resulting hybrid proteins stimulate transcription, independent of an activator (19–23). The reciprocal experiment, where an activation domain is fused to a compo-
YMH567 (strains YMH511 (MATD UASGAL1-TATAGAL1-lacZ) disruption of the indicated genes as described previously (28). Strain TRP1 CEN (pM1532) hybrid activators were constructed in a minicell chromatin. We reasoned that contact points such that the holoenzyme is recruited to the promoter by the TFIIB residues that interact with RNAP II/TFIIF, suggesting that contact points are required when fused to a DNA binding domain as described previously (28). Strain H-224 was created by one-step disruption of the AHC1 locus using host strain W303-1A (MATa leu2-3 can1-100 ura3-1 ade2-1 his3-11, 15 trp1-1) (29). With the exception of strain CBY14a, all strains harbor the reporter plasmid UASGAL1-TATAAGAL1-GAL1-Z (GAL1-Z) or UASGAL1-TGATAGAL1-lacZ as described previously (21).

Gal4-54 Constructs—Unless otherwise noted, all Gal4-54 plasmids are derivatives of the yeast Gal4 two-hybrid plasmid pPC97 (LEU2 CEN) (30). The Gal4-54-(29–194) (pM979) and Gal4-54-(29–182) (pM982) plasmids were constructed by ligating the SacI-BglII or SacI-BamHI DNA fragments of the E. coli rpoN gene, encoding amino acids 29–194 or 29–182 of α4, respectively, into pPC97 immediately downstream of the Gal4 DNA binding domain (Gal4-1–147). The Gal4-54-(29–177) (pM1544), Gal4-54-(29–168) (pM1545), Gal4-54-(29–138) (pM1546), Gal4-54-(84–194) (pM986), Gal4-54-(84–182) (pM985), Gal4-54-(84–138) (pM981), and Gal4-54-(138–177) (pM773) plasmids are pPC97 derivatives constructed by polymerase chain reaction amplification of rpoN DNA using synthetic primers that include flanking SacI and BglII recognition sequences with the indicated α4 end points. The Gal4-54-(29–182) (pM1533) and Gal4-54-(29–194) (pM1532) hybrid activators were constructed in a TRPI 1 cen vector by transferring the Kpn1-BamHI DNA fragment of pPC97 into plasmid pRSS14, creating plasmid pM867, followed by ligation of the SacI-BamHI DNA fragment encoding Gal4-54 (29–182), or the α4 fragment of rpoN (α4-84) (29–194), into the SacI-BglII sites of pRSS14. Two Gal4-VP16(412–490) hybrid constructs were made by ligation of the Xho1-BamHI DNA fragment encoding VP16 amino acid residues 412–490 into the SacI-BglII sites of either the LEU2 CEN vector (pM936) or the TRPI 1 cen vector (pM1536).

Site-directed Mutagenesis—The Gal4-54-(29–177) L31R, E32K, and L37R mutants were constructed by site-directed mutagenesis using the QuickChange Kit (Stratagene) and Gal4-54-(29–177) (pM1544) as template DNA. All mutations were verified by DNA sequencing.

β-Galactosidase Assays—Yeast strains harboring UASGAL1-lacZ reporter constructs were grown at 30 °C to an A600 of 0.6, harvested by centrifugation and resuspended in 500 μl of buffer containing (100 mM Tris-HCl, pH 8.0, 1% dithiothreitol, 2% glycerol, 2 mM phenylmethylsulfonyl fluoride). Cell extracts were prepared by vortexing with 0.5 mm glass beads six times in 15 s bursts. β-galactosidase assays were determined in duplicate for three independent transformants as described previously (31). Activities are reported as (1.7 ml × A420/0.0045 × cell extract [ml] × reaction time [min] × protein [mg/ml]) ± S.D.

RESULTS

Identification of a Gal4-54 Fusion Protein as a trans-Activator in Yeast—Artificial recruitment of TFII B to promoter DNA stimulates transcription (25). Activation is dependent upon TFII B residues that interact with RNP II/TFII F, suggesting that when tethered to promoter DNA, TFII B stimulates transcription by recruiting the RNP II holoenzyme complex; alternatively, TFII B is a component of the holoenzyme complex such that the holoenzyme is recruited to the promoter by the Gal4 DNA binding domain. We reasoned that contact points between TFIIB and other components of the preinitiation complex might be identified as amino acid replacements that affect the activation potential of TFIIB when fused to the Gal4 DNA binding domain.

TFII B derivatives were generated by polymerase chain reaction amplification of the SUA7 gene under error-prone conditions, followed by ligation of amplified DNA behind the Gal4 DNA binding domain. The resulting Gal4-IIB library was introduced into yeast strain CBY14a, which carries chromosomally integrated LYS2::UASGAL1-HIS3 and URA3::UASGAL1-lacZ reporters (26). A single transformant exhibiting 3-aminoantizole-resistance (3-AT^+), because of HIS3 overexpression, was identified. This strain also turned intensely blue on X-gal indicator medium, indicating elevated UASGAL1-lacZ expression.

Plasmid DNA was recovered from the 3-AT^+ strain and sequenced. Surprisingly, sequence analysis revealed that the DNA fragment fused to Gal4 did not correspond to SUA7. Instead, comparison of this sequence with the databases identified a fragment of the E. coli rpoN gene, which codes for α4. The insert corresponds to the rpoN open reading frame, encoding amino acids 29–194 of the 478 residue α4 protein (Fig. 1). This rpoN DNA fragment is flanked by SacI and BglII restriction sites, which are the same sites included in the SUA7 amplification primers for cloning purposes. Therefore, rpoN DNA appears to have been cloned from contaminating E. coli chromosomal DNA in our SUA7 plasmid preparation.

Comparison of the Activation Potential of Gal4-α4^24 with Gal4-VP16—The intensely blue color of the lacZ reporter strain suggested that Gal4-α4^24 is a potent transcriptional activator in yeast. Activation by Gal4-α4^24 was quantified by β-galactosidase assays and compared with that of the well-characterized Gal4-VP16 activator. Gal4-α4^24-(29–194) and Gal4-VP16(412–490) plasmids were introduced into strain YMH171, and β-galactosidase activities expressed from the UASGAL1-TATAGAL1-lacZ reporter were assayed. Consistent with the qualitative assay, 1700 units of β-galactosidase activity were detected in the strain expressing Gal4-α4^24-(29–194), corresponding to 2,400-fold stimulation over the Gal4 alone (Fig. 2). This compares with 2,800 units of activity and 4,000-fold activation by Gal4-VP16(412–490). A point mutation in the TATA element (UASGAL1-TATAGAL1-lacZ) diminished activation by Gal4-α4^24-(29–194) to ~5% of the activity from the UASGAL1-TATAGAL1-lacZ reporter (Fig. 2). This result is comparable to the effect of the TATA element on activation by Gal4-VP16(412–490) and demonstrates that activation is dependent upon an intact TATA box. Activation is specific to a subfragment of α4 since full-length Gal4-α4^24-(1–478) resulted in only 2.4 units or less than 10-fold stimulation (Fig. 3). These results define a subfragment of α4 as a potent transcriptional activator when fused to a DNA binding domain in yeast, displaying ~60% of the activity of Gal4-VP16(412–490). Furthermore, the requirement for the TATA core promoter element implies that Gal4-α4^24-(29–194)-mediated activation occurs by a normal RNP II activation mechanism.

Delimitation of the Gal4-α4^24 Activation Requirements—Gal4-α4^24-(29–194) includes the domain of α4 that interacts with core RNAP, as well as part of the N-terminal region I that affects RNAP isomerization and open promoter complex formation (7, 9–12). In an effort to define the mechanism of transcriptional activation by Gal4-α4^24-(29–194), we delimited the α4 sequences responsible for activation. In this series of assays the Gal4-α4^24-(29–194) construct yielded 1300 units of β-galactosidase, compared with 0.3 units for the Gal4 vector alone (Fig. 3). Deletion of α4 residues 29–83, creating Gal4-α4^24-(84–194), diminished activation 5-fold, resulting in 270 β-galactosidase units. Activation was further diminished by deleting
residues 29–137, such that Gal4-s54-(138–194) yielded only 68 units. These results demonstrate that the N-terminal region I of s54 is important for the activation function of Gal4-s54-(29–194).

The C-terminal requirement for activation by Gal4-s54-(29–194) was also characterized by deletion analysis. Notably, deletion of the C-terminal 17 residues, generating Gal4-s54-(29–177), enhanced activation to 2900 β-galactosidase units (Fig. 3), indicating that the C terminus of Gal4-s54-(29–194) partially masks its activation potential. Deletion of an additional 9 residues, creating Gal4-s54-(29–168), diminished activation relative to Gal4-s54-(29–177), yielding 2500 units. Activation was further diminished by deleting back to residue 138, such that Gal4-s54-(29–138) yielded 850 units. Gal4-s54-(138–177) yielded 510 β-galactosidase units, which compares to only 68 units for Gal4-s54-(138–194). This result is consistent with the negative effect of s54 residues 178–194 observed by comparing activation in response to Gal4-s54-(29–194) and Gal4-s54-(29–177). Western blot analysis using antibody to the Gal4 DNA binding domain confirmed the relative molecular masses of the Gal4-s54 hybrid proteins and that none of the deletion mutations affected Gal4-s54 synthesis or stability (data not shown).

Taken together, these results demonstrate that both the N-terminal and C-terminal regions of s54-(29–194) are required for the potent activation function of Gal4-s54 and that C-terminal residues 178–194 are deleterious for activation.

Suppression of Gal4-s54-(29–182) Toxicity by Mutations in Components of the SAGA Coactivator Complex—The Gal4-s54-(29–182) construct is toxic in yeast, a result reminiscent of the toxicity of Gal4-VP16 in yeast (32). Gal4-VP16 toxicity is thought to be a consequence of activator-mediated titration of transcription factors, or “squelching” (32). This effect was exploited by Guarente and co-workers (32, 33) to identify components of the SAGA coactivator complex, a multifunctional coactivator that includes the Gcn5 histone acetyltransferase, as well as ADA and SPT proteins. These components are organized into distinct subcomplexes that exert differential effects on transcription (34–38).

To identify the genetic requirements for activation by Gal4-s54, we asked if Gal4-s54-(29–182) toxicity is suppressed by ada2Δ, ada3Δ, or gcn5Δ deletions. Although the original Gal4-s54-(29–194) construct had a mildly adverse effect on cell growth, the Gal4-s54-(29–182) construct was extremely toxic, yielding colonies only upon prolonged incubation (Fig. 4). However, the toxic phenotype was fully suppressed by ada2Δ, ada3Δ, or gcn5Δ deletions. The slow-growth phenotype associated with Gal4-s54-(29–182) was restored in the ada2Δ, ada3Δ, or gcn5Δ mutants by plasmid-borne ADA2, ADA3, or GCN5, respectively, confirming that suppression is caused by the indicated gene disruptions (data not shown). These results estab-
associated with Gal4-2 Gal4-demonstrate that transcriptional activation by Gal4-effect, diminishing activity 400-fold to 4.4 units. These results alone (Gal4-gcn5, 130 and 140 units, respectively, corresponding to 12- to 13-fold reduction into strain YMH171 carrying the UASGAL1-TATAGAL1-lacZ reporter (TA/GIZ) and β-galactosidase activities were determined as described in Fig. 2.

To test this possibility and to further investigate the mechanism as a cofactor in toxicity.

The SAGA Complex Is Required for Activation by Gal4-—Suppression of Gal4-D(29–182) mediated toxicity by mutations in components of the SAGA complex suggested that activation by Gal4-D(29–194) is dependent upon SAGA. To test this possibility and to further investigate the mechanism of Gal4-D-mediated activation, we tested the effects of ada2Δ, ada3Δ, gcn5Δ or spt7Δ deletions on activation by Gal4-D(29–194).

For this set of experiments, strain YMH171 and the indicated isogenic derivatives harboring the lacZ reporter plasmid were assayed for β-galactosidase activities. Compared with 1700 units of activity in the normal strain, activity was reduced 32-fold, to 54 units, in the isogenic gcn5Δ deletion strain (Fig. 5). Activity was diminished in the ada2Δ and ada3Δ strains to 130 and 140 units, respectively, corresponding to 12- to 13-fold effects on activation. The spt7Δ deletion had the most severe effect, diminishing activity 400-fold to 4.4 units. These results demonstrate that transcriptional activation by Gal4-D is differentially dependent upon ADA, SPT, and Gcn5 components of the SAGA complex. This effect is consistent with previous reports that Spt7 is required for all SAGA-mediated functions, whereas other subunits, including the Gcn5 histone acetyltransferase, are required for a subset of SAGA functions (34, 37–39).

The ADA Complex Adversely Affects Activation by Gal4-D—The ADA histone acetyltransferase complex includes Gcn5 as its catalytic subunit but is structurally and functionally distinct from SAGA (29). The Ahc1 protein is a unique component of the ADA complex and is essential for complex integrity. To determine whether Gal4-D activation requires the ADA complex we assayed UASGAL1-TATAGAL1-lacZ expression in isogenic Ahc1 and ahc1Δ strains, and results were compared with the effect of ahc1Δ on Gal4-VP16-mediated activation. In strain W303–1A harboring the UASGAL1-lacZ reporter, the Gal4-D(29–194) activator yielded 500 units of β-galactosidase activity, compared with 1100 units for Gal4-VP16 (Fig. 6). However, in contrast to the effects of SAGA mutations (Fig. 5), the ahc1Δ deletion enhanced activation by Gal4-D(29–194), producing 970 units, or 2-fold stimulation of UASGAL1-lacZ expression (Fig. 6). Activation by Gal4-VP16 was similarly affected by ahc1Δ (Fig. 6).

If the SAGA and ADA complexes exert opposite effects on transcriptional activation, then the ahc1Δ deletion would not be expected to suppress Gal4-D(29–182) toxicity. Indeed, Gal4-D(29–182) is equally toxic in isogenic Ahc1 and ahc1Δ strains (Fig. 7). Furthermore, the slow-growth phenotype associated with Gal4-D(29–194) relative to the Gal4 vector alone is enhanced by the ahc1Δ deletion (Fig. 7). Thus, deletion of the Ahc1 component of the ADA complex facilitates activation by Gal4-D and Gal4-VP16 and enhances the growth defect associated with Gal4-D(29–194) expression, whereas deletion of the Ada2, Ada3, or Gcn5 components of the SAGA complex impair activation and suppress activator-associated growth defects (Figs. 4 and 5).
Gal4-σ^54-mediated Activation

Fig. 6. Deletion of the AHC1 component of the ADA histone acetyltransferase complex enhances activation by Gal4-σ^54-(29–194) and Gal4-VP16-(412–490). β-galactosidase activities associated with Gal4-σ^54-(29–194), Gal4-VP16-(412–490), or the Gal4 DNA binding domain vector are shown for strain W303-1A (WT) or its ahc1Δ derivative. Activities were determined as described in the legend to Fig. 2 using the UASGAL4::TATAA_GAL1-lacZ reporter TA/G1Z.

![Diagram of β-galactosidase units](image_url)

Fig. 7. Enhancement of toxicity associated with Gal4-σ^54 constructs by the ahc1Δ deletion. Gal4-σ^54-(29–182), Gal4-σ^54-(29–194) or the Gal4 DNA binding domain alone (Gal4−), was introduced into strain W303-1A or its ahc1Δ derivative. Transformants were plated on −Trp selection medium and incubated for 3 days at 30 °C. In contrast to the effect of deleting SAGA components (Fig. 4), deletion of the ADA-specific AHC1 gene does not suppress Gal4-σ^54-(29–182) toxicity and enhances Gal4-σ^54-(29–194) toxicity.

Gal4-σ^54 Function Is Dependent upon σ^54-specific Features—The requirement for physically disparate regions for maximal activation by Gal4-σ^54 suggests that activation is because of σ^54-specific features and not to a fortuitous consequence of a peptide fragment that is structurally appropriate for activation. To test this possibility more directly, we generated specific amino acid replacements in the N-terminal region of σ^54 shown previously to affect enhancer-dependent promoter melting in E. coli. Syed and Gralla (9) found that extensive, random substitutions within the N-terminal 40 residues of σ^54 were tolerated without loss of function. However, no replacements were uncovered at positions Leu-33, Glu-36, and Leu-37, suggesting that these positions are critical for transcription in vivo (9).

Subsequent site-directed mutagenesis demonstrated that L33R, E36K, and L37R replacements are nonfunctional in vivo: L33R and L37R are specifically defective for activator-dependent, open complex formation, whereas E36K appeared to affect reinitiation. Because these residues are included in the Gal4-σ^54-(29–177) activator, we constructed L33R, E36K, and L37R replacements and scored their effects on Gal4-σ^54-(29–177)-mediated toxicity. The L31R and E32K replacements, which had no effect on transcription in E. coli, were generated as controls. Remarkably, the L33R, E36K, and L37R derivatives relieved the toxicity conferred by Gal4-σ^54-(29–177), yielding a population of Leu+ transformants, whereas the L31R and E32K derivatives displayed the same toxicity as Gal4-σ^54-(29–177) (Fig. 8). Thus, the toxicity conferred by Gal4-σ^54-(29–177) is dependent upon specific amino acid residues that are also critical for promoter melting by σ^54 in bacteria. These results suggest that activation by Gal4-σ^54 in yeast reflects a normal function of σ^54 in bacteria.

There is a notable difference between relief of Gal4-σ^54 toxicity conferred by the cis-acting amino acid replacements and the trans-acting SAGA mutations. Whereas ada2Δ, ada3Δ and gcn5Δ deletions yielded transformants of uniform colony size (Fig. 4), the L33R, E36K, and L37R replacements yielded heterogeneous transformants (Fig. 8). A similar effect on toxicity is apparent for activation-defective derivatives of Gal4-VP16 (33). This effect precluded accurate quantification of the effects of the L33R, E36K, and L37R replacements on activation by Gal4-σ^54-(29–177). Although each of these replacements (but neither L31R nor E32K) diminished activation, the magnitude of the effect varied in proportion with relief of toxicity defined...
by transformant colony size (data not shown). Nonetheless, the replacements that affect promoter isomerization by σ34 in *E. coli* clearly correlate with effects on cell growth by Gal4-σ34 in yeast.

**DISCUSSION**

In this study we define a fragment of *E. coli* σ34 that functions as a potent transcriptional activator when fused to a Gal4 DNA binding domain in yeast. The initial Gal4-σ34-(29–194) construct displays ~60% of the activity of the Gal4-VP16-(412–490) activator, and the activities of these two hybrid activators were nearly indistinguishable when the C-terminal 17 residues of Gal4-σ34-(29–194) were deleted. Gal4-σ34-mediated activation is TATA-dependent and also requires the SAGA histone acetyltransferase coactivator complex, with the relative effects of *ada2Δ, ada3Δ, gen5Δ*, and *spl7Δ* deletions on Gal4-σ34-mediated activation comparable with their effects on Gal4-VP16-mediated activation. Thus, Gal4-σ34-(29–177) is a potent activator of RNAP II transcription that is dependent upon a common RNAP II core promoter element and a general coactivator that facilitates chromatin remodeling by catalyzing histone acetylation.

Yeast contains a second Gen5 histone acetyltransferase complex, designated ADA, which is distinct from SAGA (29). Deletion of the *AHC1* gene, which encodes a subunit unique to the ADA complex, affects Gal4-σ34 activity in a manner opposite to deletion of genes encoding SAGA components: *ahc1Δ* enhanced activation by Gal4-σ34 and not only failed to suppress Gal4-σ34-(29–182) toxicity, but rendered the Gal4-σ34-(29–194) construct toxic. Although *ahc1Δ* was reported previously to have no effect on Gal4-VP16-mediated activation (29), we found that *ahc1Δ* stimulates activation by Gal4-VP16 and Gal4-σ34, in both cases by about 2.2-fold. Although we do not understand the basis for this discrepancy, it is clear that the SAGA and ADA complexes can be functionally distinct with respect to their effects on transcriptional activation, despite having a common histone acetyltransferase subunit.

The structure of σ34 can be divided into at least three functional domains (Fig. 1). One is the C-terminal DNA binding domain (region III), encompassing residues 329–478, which enables σ34 to bind the promoter element centered at position −24 (11, 15). The second domain (region II) interacts with core RNAP and is located between residues 120 and 215 (13, 14). The most functionally complex domain encompasses the N-terminal 50 amino acids (region I) and plays a critical role in regulating the ATP-dependent isomerization of the σ34-DNA promoter complex (7, 9–12). Accordingly, the Gal4-σ34-(29–177) activator includes sequence elements that affect RNAP binding and promoter isomerization (Fig. 1), suggesting that either or both of these activities might be responsible for Gal4-σ34-mediated activation.

With one exception, progressive deletions from either end of the σ34 moiety of Gal4-σ34-(29–194) diminish activation function (Fig. 3). The exception is a 1.7-fold increase in activity associated with deletion of the C-terminal 17 amino acids. Sequence comparisons with the databases revealed that this region includes 8 of 10 residue identity to a conserved sequence within yeast Rpb5 (Fig. 1), a subunit common to RNAP I, II, and III, and homologous to a subunit of archaeal RNA polymerase (40, 41). The only region of structural similarity between σ34 and σ70 is also located in this region and similarity core RNAP (13). Limited sequence similarities between bacterial σ factors and the GTFs, including TBP, TFIIH, and both subunits of TFIIF, have been noted previously, consistent with the proposed distribution of σ factor activities among the GTFs in eukaryotic cells (42–45). The sequence similarity between σ34 and Rpb5 suggests that distribution of σ factor activities extends to a subunit of eukaryotic RNAPs. Whether there is functional significance to this sequence similarity and how it might account for the deleterious effect of the C-terminal 17 residues of Gal4-σ34-(29–194) remains to be investigated.

Does the ability of Gal4-σ34 to function as an activator in yeast reflect a normal activity of σ34 or does Gal4-σ34 simply include a polypeptide fragment that is structurally appropriate for activation? This is a valid concern because a small percentage of random *E. coli* DNA sequences are known to encode transcriptional activators when fused to a DNA binding domain (46). Two observations suggest that Gal4-σ34 activation reflects a normal function of σ34. First, activation by Gal4-σ34 is dependent upon a subfragment of σ34 that encompasses 150 amino acids: deletions from either the N- or C-terminal region of σ34 within Gal4-σ34-(29–177), spanning parts of two functional domains of σ34 (Fig. 1), dramatically reduced reporter gene activation (Fig. 3). This compares to random bacterial activator sequences that are generally short, in several cases less than 20 amino acids in length (46). Second, specific amino acid replacements that render σ34 defective in activator-responsive promoter melting in *E. coli* suppress the toxicity of Gal4-σ34-(29–177) in yeast (Fig. 8). Conversely, adjacent amino acid replacements that are without effect on σ34 have no effect on Gal4-σ34-(29–177). Thus, the activity of Gal4-σ34 in yeast appears to reflect, in part, a normal function of σ34 in bacteria.

The Gal4-σ34 activator might stimulate transcription by directly recruiting RNAP II to the core promoter. However, we were unable to detect a physical interaction between Gal4-σ34 and purified RNAP II by a co-immunoprecipitation assay (data not shown), even though RNAP II interacts with TFIIH and the Ssu72 protein in the same assay (47). Earlier studies also failed to detect a direct interaction between σ34 and bacterial RNAP in a co-immunoprecipitation assay that detected interaction of RNAP with σ70 and σ32 (48). Despite the presence of a domain in Gal4-σ34-(29–177) implicated in RNAP binding, we have no evidence that Gal4-σ34 stimulates transcription as a consequence of direct interaction between σ34 and RNAP II.

It is striking that the same amino acid replacements in σ34 that adversely affect promoter melting in *E. coli* relieve the toxicity of Gal4-σ34 in yeast, an effect that correlates with Gal4-σ34 activation potential. This result is consistent with the premise that the Gal4-σ34 activator is dependent upon a normal function of σ34 and suggests that Gal4-σ34 stimulates RNAP II transcription by enhancing or stabilizing open complex formation. Thus, Gal4-σ34 might not activate transcription by recruiting RNAP II or other components of the initiation complex, but instead by facilitating promoter melting. Furthermore, the SAGA requirement for activation by Gal4-σ34 suggests that SAGA might also play a role in promoter isomerization. One possibility is that Gal4-σ34 binds and stabilizes the fork junction that forms at the interface of double and single stranded DNA, as proposed recently to account for activator-induced promoter melting by σ34 in bacteria (49, 50). Further characterization of Gal4-σ34 has the potential to provide novel insights into the mechanism by which natural activators function in eukaryotic cells.

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