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The C Terminus of the α Subunit of RNA Polymerase Is Not Essential for Transcriptional Activation of σ^{54} Holoenzyme

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Several activators of σ^{70} holoenzyme whose binding sites lie upstream of the −35 region of promoters require the C-terminal region of the α subunit of RNA polymerase to activate transcription. (These are among class I activators, which require the C-terminal region of the α subunit for transcriptional activation.) Because transcription by σ^{54} holoenzyme universally depends upon activators whose binding sites lie well upstream (or downstream) of promoters, we determined whether the C-terminal region of the α subunit was also required for transcription from the σ^{54}-dependent promoter for the *glnA* operon. Nitrogen regulatory protein C-dependent activation from the *glnA* promoter remained good when RNA polymerases containing C-terminal truncations of the α subunit were employed. This was also the case for nitrogen fixation protein A-dependent activation if a nitrogen fixation protein A-binding site was appropriately placed upstream of the *glnA* promoter. These results lead to the working hypothesis (as yet untested) that activators of σ^{54} holoenzyme, which appear to make direct physical contact with the polymerase to catalyze a change in its conformation, activate the σ^{54} holoenzyme by contacting the σ subunit rather than the α subunit of the core enzyme.

The RNA polymerase core enzyme of eubacteria is composed of four subunits, $\alpha_2\beta\beta'$. Association of core with a σ factor determines its promoter specificity. Whereas the vast majority of promoters are recognized by core enzyme associated with σ^{70}, a few promoters are recognized by the alternative holoenzyme form containing σ^{54} (7, 19). σ^{54} is unique in showing no sequence similarity to other σ factors (24), all of which show similarity to one another (for a review, see reference 7). σ^{54} is also unique in its ability to bind to some promoters in the absence of core polymerase; in this way, it resembles the eukaryotic basal transcription factor TBP (TATA-binding protein) rather than other bacterial σ factors (1, 4, 37).

The α subunit of RNA polymerase from *Escherichia coli* is composed of 329 amino acid residues (for reviews, see references 15, 31, and 40). Whereas the amino-terminal region of the α subunit is required to nucleate the assembly of active core enzyme, the carboxy-terminal region is not; truncated forms of the α subunit lacking 73 or 94 amino acid residues from the carboxy terminus (called α-256 or α-235, respectively, to designate the remaining amino acids) allow the assembly of functional core enzyme (6, 11, 12). As indicated by studies of the effects of amino acid substitutions as well as truncations, the C-terminal region of the α subunit plays an essential role in mediating activation of transcription by many transcriptional activators (3, 5, 13, 14, 31, 36). Previous studies with truncated forms of the α subunit have distinguished between two classes of activators of σ^{70} holoenzyme (13, 15)—those that fail to activate mutant forms of polymerase bearing truncated α subunits (called class I activators), and those that retain the ability to activate the mutant polymerases. The latter include class II activators, which appear to contact the σ^{70} subunit (18, 21). Binding sites for several (but not all) class I activators lie upstream of the −35 region of promoters, whereas binding sites for known class II activators overlap the −35 region. Results with the truncated polymerases were particularly striking for the catabolite gene activator protein (CAP), which behaved as both a class I activator and a class II activator. CAP failed to activate the truncated polymerase from distant sites (*lacP1*, *uxuAB*), but was able to do so and behaved as a class II activator of gene expression from nearby sites (*galP1*).

We have studied the function of truncated α subunits at the *glnA* promoter of *Salmonella typhimurium*, which is σ^{54} dependent. This was of particular interest because transcription from all known σ^{54}-dependent promoters requires an activator bound to sites distant from the promoter, and activation appears to require physical contact between activator and polymerase (19). The mechanism of transcriptional activation at σ^{54}-dependent promoters has been studied extensively at the major promoter for the *glnA* gene, which encodes glutamine synthetase. Transcription from this promoter is activated by the NTRC protein (nitrogen regulatory protein C; also called NR_I), which binds to two sites more than 100 bp upstream of the transcriptional start site; these sites have been demonstrated to have the properties of a eukaryotic transcriptional enhancer (29). To activate transcription, NTRC bound to the enhancer contacts promoter-bound RNA polymerase by means of DNA loop formation (30, 33, 35, 38). NTRC catalyzes the isomerization of closed promoter complexes to open complexes in a reaction that depends upon ATP hydrolysis (28, 34). The NIFA protein (nitrogen fixation protein A), a homolog of NTRC, activates transcription from the *nifH* promoter. At *nifH*, DNA loop formation is assisted by the integration host factor (IHF), which introduces a sharp bend between the upstream binding site for NIFA and the promoter (10, 32, 33).

In this report, we show that both NTRC and NIFA can activate transcription from the *glnA* promoter by mutant forms of σ^{54} holoenzyme containing truncated α subunits. To study the activity of NIFA at the *glnA* promoter, we used a template carrying the *nifH* promoter-regulatory region in which the *nifH* promoter itself was precisely replaced with

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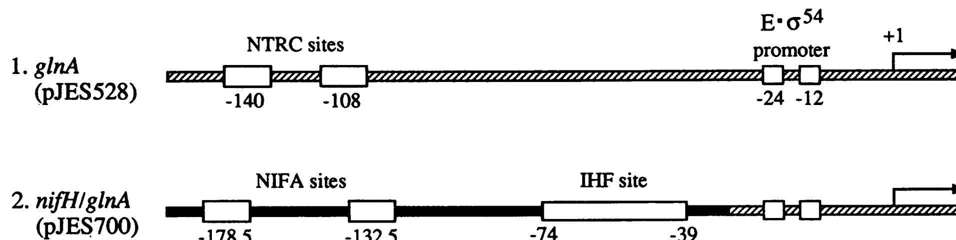

FIG. 1. Structure of the promoter-regulatory regions of templates used for transcription. Promoter elements, activator-binding sites, and a binding site for IHF are indicated. Conserved promoter sequences lie in the −12 and −24 regions with respect to the transcriptional start site at position +1 (27). Numbers under the activator-binding sites indicate their centers, whereas numbers under the IHF-binding site indicate the boundaries of protection from DNase I digestion (32). Arrows indicate the start of transcripts (27). The *nifH/glnA* template carries the upstream *nifH* regulatory region, which contains the NIFA- and IHF-binding sites, fused to the *glnA* promoter region (position −34 of *nifH* was joined to position −30 of *glnA*) (33). Numbers given for this template refer to the wild-type *nifH* configuration. The two NTRC-binding sites indicated at *glnA*, which are those most upstream from the promoter, constitute the *glnA* enhancer and are those important for activation of transcription. The NIFA-binding site at position −178.5 in the *nifH* promoter-regulatory region may function in activation of transcription from the divergent *nifJ* promoter rather than in activation from the *nifH* promoter.

the *glnA* promoter (designated *nifH/glnA*; Fig. 1). (Although NIFA can activate *glnA* transcription in vivo in the absence of a specific binding site nearby [26], activation is greater on a template that carries such a site.)

We tested activation from the *glnA* promoter in a purified transcription system as described previously (28, 33), using the (supercoiled) plasmid templates (Fig. 1) and proteins described below. Plasmid pJES528 carries the *glnA* promoter regulatory region (39). Plasmid pJES700 is a hybrid template (*nifH/glnA*) that carries the upstream *nifH* regulatory region (NIFA- and IHF-binding sites) fused to the *glnA* promoter (33). Core RNA polymerase was reconstituted from purified subunits as described previously and carried wild-type or C-terminally truncated α subunits (14). Because all α subunits were purified by gel filtration chromatography and all reconstituted core enzymes were purified by ion-exchange chromatography as described previously (14), core enzymes containing truncated α subunits were not detectably contaminated by core enzymes containing the wild-type α subunit. This was verified by demonstrating that core enzymes containing truncated α subunits could not be activated by CAP at the wild-type *lac* promoter (not shown). Native (as opposed to reconstituted) core enzyme was a gift from R. Burgess. σ^{54} (27), NTRC (9, 39), nitrogen regulatory protein B (NTRB) (16), and IHF (25) were purified as previously described. Purification of MBP-NIFA (a fusion of the soluble maltose-binding protein to the amino-terminal methionine of NIFA) is described elsewhere (20). We used MBP-NIFA because it has not yet been possible to obtain purified native NIFA that is active.

DNA template (5 nM) and proteins (100 nM σ^{54}, 60 nM core RNA polymerase, and 100 nM NTRC plus 50 nM NTRB [plasmid pJES528] or 2 μM MBP-NIFA plus 60 nM IHF [plasmid pJES700]) were mixed in transcription buffer (50 mM Tris-acetate, pH 8, 100 mM potassium acetate, 8 mM magnesium acetate, 27 mM ammonium acetate, 1 mM dithiothreitol, 3.5% polyethylene glycol) and incubated at 30°C for 10 min, and then a single nucleotide (1.6 mM dideoxy ATP) was added to allow formation of open complexes. After 10 min, heparin (0.1 mg/ml) was added, and 10 min later a single cycle of transcription was initiated by adding a mixture of nucleotides (10 mM ATP, 0.4 mM GTP, 0.4 mM UTP, 0.1 mM CTP, 5 μCi of [α-^{32}P]CTP [10 mCi/mmol]; Du Pont Corp.). After 10 min, nucleic acids were precipitated with ethanol and transcripts were analyzed on a 6% denaturing polyacrylamide gel. Radioactivity in transcripts was quantitated with a phosphorimager (Molecular Dynamics). The protein and DNA concentrations indicated are those immediately prior to initiation of transcription.

NTRC-mediated activation of transcription from the *glnA* promoter was almost as good when σ^{54} holoenzyme was formed with reconstituted core polymerase containing the full-length α subunit (Fig. 2A) as it was when formed with native core polymerase (data not shown; not tested at *nifH/glnA*). When σ^{54} holoenzyme was formed with core enzyme containing truncated α subunits, NTRC-mediated activation from the *glnA* promoter remained good (Fig. 2A); it was 46% (α-235) and 50% (α-256) of that obtained when holoenzyme was formed with reconstituted core enzyme containing the full-length α subunit. (As observed previously [14], truncation of the α subunit slightly impaired initiation of transcription even at promoters that are not activated—σ^{70}

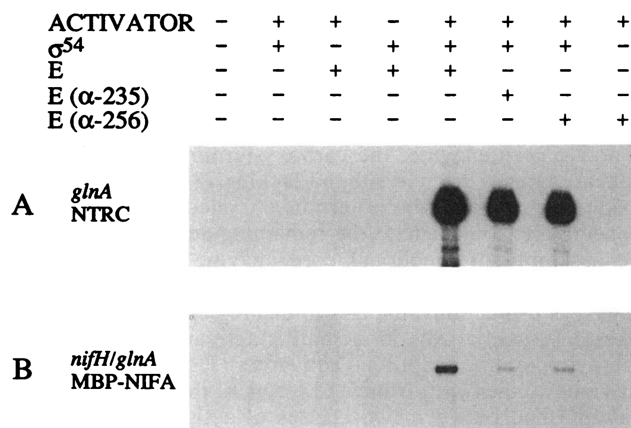

FIG. 2. NTRC- and NIFA-mediated activation of transcription by $E\sigma^{54}$ carrying full-length or truncated α subunits. Open complexes were detected by their ability to yield transcripts in a single-cycle transcription assay (see text). Templates (Fig. 1 and text) and activators were as indicated in the individual panels. + and −, presence and absence, respectively, of a component in the reaction mixture. E designates core polymerase with the full-length α subunit, whereas E (α-235) and E (α-256) designate core polymerase containing the truncated α subunits α-235 and α-256, respectively. In addition to the components indicated, IHF was present for the experiments shown in panel B.

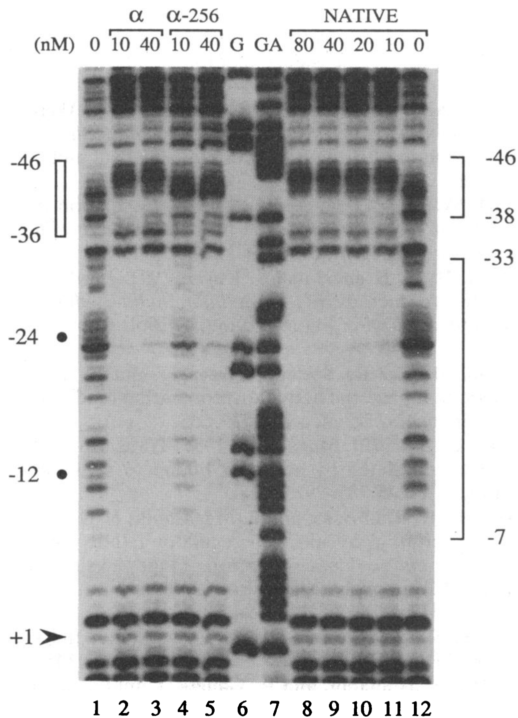

FIG. 3. Binding of $E\sigma^{54}$ containing a full-length or truncated α subunit to the *glnA* promoter in closed complexes. Binding was assessed by protection of the bottom (template) strand (prepared as described in reference 27) from DNase I digestion (33). α and α-256 designate $E\sigma^{54}$ formed from reconstituted core polymerase with the full-length α subunit or the truncated α subunit α-256, respectively. Native designates $E\sigma^{54}$ formed from native core polymerase. Concentrations above the lanes are those of core polymerase, which are assumed to equal those of σ^{54} holoenzyme because σ^{54} was present in fivefold excess over core enzyme (see text). Brackets to the right indicate regions in which $E\sigma^{54}$ yields protection from DNase I digestion, and the open bar to the left indicates the upstream region of the promoter in which there are differences in protection (enhancement) between α-256 and the α subunit (or native). The conserved doublets in the −24 and −12 regions of the promoter are indicated by dots to the left and are aligned with respect to the G and GA lanes, which are Maxam and Gilbert sequencing lanes (23).

holoenzyme formed with core enzyme containing a truncated α subunit was about 30% as active in initiation of transcription from *lacUV5* as holoenzyme formed with core enzyme containing a full-length α subunit [not shown].) NIFA-mediated activation of transcription from the *glnA* promoter (*nifH*/*glnA* template) was also good with $E\sigma^{54}$ containing truncated α subunits (Fig. 2B). Activation was 16% ($E\sigma^{54}$ [α-235]) and 23% ($E\sigma^{54}$ [α-256]) of that with $E\sigma^{54}$ containing the full-length α subunit.

We used DNase I footprinting (performed as described in reference 33) to compare the initial binding of $E\sigma^{54}$ and mutant $E\sigma^{54}$ containing the truncated α subunit α-256 to the *glnA* promoter in closed complexes (Fig. 3). Holoenzyme was formed with a fivefold excess of σ^{54} to core enzyme and was kept on ice for about 20 min before use. (The numbers given above the lanes in Fig. 3 are the concentrations of core enzyme, which we assume to be the concentrations of holoenzyme.) Whereas holoenzyme formed with native or reconstituted wild-type core enzyme protected the *glnA* promoter region from DNase I digestion at similar concentrations, equivalent protection by holoenzyme formed with mutant core enzyme required a two- to fourfold higher concentration. In addition, mutant holoenzyme yielded a slightly different pattern of protections and enhancements in the upstream region of the promoter. These results indicate that binding of the mutant holoenzyme to the *glnA* promoter in closed complexes is somewhat impaired but that the concentrations of holoenzyme used to assess transcription at *glnA* were saturating. The footprinting results are congruent with the view that α subunits may contact the upstream region of the promoter, as is indicated by footprinting and transcription experiments at the *rrnBP1* promoter (17, 31a, 36). Although binding of isolated σ^{54} to the *glnA* promoter has not been demonstrated, σ^{54} alone binds to the downstream region of other promoters (1).

In conclusion, the C-terminal region of the α subunit of core polymerase is not required for activation of transcription from the *glnA* promoter. Although NTRC and NIFA might contact another region of the α subunit (or another subunit of core enzyme) to bring about isomerization of closed to open complexes at *glnA*, an attractive hypothesis is that they contact σ^{54}. It has been noted that CAP fails to work with σ^{70} holoenzyme carrying C-terminal truncations of the α subunit at promoters at which it stimulates primarily binding of σ^{70} holoenzyme in closed complexes (e.g., *lacP1* or "CC" [symmetric (RP-binding site] + 20 *melR*) (2, 17, 22). CAP retains the ability to work with mutant holoenzyme forms at promoters at which it stimulates primarily isomerization of closed to open complexes (e.g., *galP1* or CC *melR*) (2, 8, 17). Although binding sites for activators of σ^{54} holoenzyme must be distant from the *glnA* promoter to be functional (unlike binding sites for CAP at *galP1* or CC *melR*, which overlap the −35 region), the activators stimulate isomerization of closed to open complexes at *glnA*; their ability to work with σ^{54} holoenzyme carrying truncated α subunits fits the above paradigm. Contact of these activators with σ^{54} holoenzyme, which is achieved by DNA looping, may be analogous to that of class II activators of σ^{70} holoenzyme which bind in the −35 region.

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