Recombinant Thermus aquaticus RNA Polymerase, a New Tool for Structure-Based Analysis of Transcription

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The three-dimensional structure of DNA-dependent RNA polymerase (RNAP) from thermophilic Thermus aquaticus has recently been determined at 3.3 Å resolution. Currently, very little is known about Thermus aquaticus transcription and no genetic system to study Thermus aquaticus RNAP genes is available. To overcome these limitations, we cloned and overexpressed Thermus aquaticus RNAP genes in Escherichia coli. Overproduced Thermus aquaticus RNAP subunits assembled into functional RNAP in vitro and in vivo when coexpressed in E. coli. We used the recombinant Thermus aquaticus enzyme to demonstrate that transcription initiation, transcription termination, and transcription cleavage assays developed for E. coli RNAP can be adapted to study Thermus aquaticus transcription. However, Thermus aquaticus RNAP differs from the prototypical E. coli enzyme in several important ways: it terminates transcription less efficiently, has exceptionally high rate of intrinsic transcript cleavage, and is highly resistant to rifampin. Our results, together with the high-resolution structural information, should now allow a rational analysis of transcription mechanism by mutation.

Most bacterial RNA polymerase (RNAP) core enzymes consist of four core subunits (β', β, and a dimer of identical α subunits). Binding of one of the several σ factors converts the core into the holoenzyme, which is able to initiate transcription from promoters. In vivo activity of several bacterial RNAPs can be recovered after separation of individual subunits in the presence of denaturing agents and subsequent mixing of the subunits and dialysis under controlled conditions (19). In vitro reconstitution of Escherichia coli RNAP from cloned and individually purified and expressed subunits provides a means of obtaining RNAP harboring lethal mutations in quantities sufficient for biochemical analyses (16). Structure-function studies of reconstituted recombinant E. coli RNAP mutants have provided crucial insights into the transcription mechanism and regulation and are primarily responsible for our increased understanding of this enzyme (see, for example, references 6, 8, and 17).

Recent structural analysis of RNAP from Thermus aquaticus provided the first tantalizing view of the bacterial RNAP core enzyme structure at 3.3 Å resolution (18). The availability of a relatively high-resolution structure qualitatively raises the importance of a rational structure-function analysis of RNAP. Despite obvious homology between RNAPs from Thermus aquaticus and E. coli, it would be highly desirable to perform mutational and structural studies using Thermus aquaticus RNAP rather than the better-studied E. coli counterpart. The important advantages of studying Thermus aquaticus RNAP include the ability to reduce assembly effects of mutations to a minimum and the ability to perform structural analysis of mutants. The disadvantages of the Thermus aquaticus system are the lack of functional assays, the general absence of data on gene transcription in this organism, and, more importantly, the inability to manipulate Thermus aquaticus RNAP subunit (rpo) genes. To overcome these limitations and to make full use of the available structural information, we cloned each of the Thermus aquaticus rpo genes in E. coli expression vectors and overproduced recombinant subunits. We show that recombinant Thermus aquaticus RNAP can be assembled in vitro and can be studied using assays developed for E. coli system.

Materials and Methods

Cloning and expression of Thermus aquaticus rpo genes. Fragments of genes coding for the core subunits of Thermus aquaticus RNAP were cloned previously (18). We used conventional cloning and PCR to assemble entire rpo genes in plasmid pT7Blue (Novagen), and we used site-directed mutagenesis to introduce an EcoRI site after the termination codon of every rpo gene and an NdeI site (CATATG) overlapping with the initiation ATG codon of every rpo gene (note that Thermus aquaticus rpo genes contain no NdeI or EcoRI sites) (18). Thermus aquaticus rpo genes were recovered by NdeI-EcoRI treatment of pT7Blue-based recombinant plasmids and were subcloned into appropriately treated pET21 and/or pET21 T7 RNAP expression vectors. The resultant plasmids, pET28TaA, pET28TaB, pET28TaC, and pET28TaZ, express Thermus aquaticus RNAP α, β, β', and σ, respectively, with N-terminal hexahistidine tags. Similar plasmids of the pET21 series express untagged Thermus aquaticus RNAP enzymes. E. coli BL21(DE3) cells transformed with the resulting plasmids overproduced individual Thermus aquaticus core RNAP subunits at high level on induction with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG), and the overexpressed proteins segregated into inclusion bodies.

A ~600-bp fragment of Thermus aquaticus rpoD was amplified from Thermus aquaticus genomic DNA using degenerate primers specific for highly conserved regions 2 and 4, and its sequence was determined. The entire rpoD gene sequence was then obtained by applying inverse PCR to SakI- and BglII-digested Thermus aquaticus DNA. The entire rpoD gene was then subcloned into pET system vectors. Expressed RpoD was found in inclusion bodies.

Plasmid pET28TaABCZ, coexpressing Thermus aquaticus rpoA, rpoB, rpoC, and rpoZ genes, was constructed from pET28TaC, which was treated with HindIII and Klenow enzyme followed by EcoRI and ligated to a Thermus aquaticus rpoB-containing fragment prepared from pET21TaB by treating with BglII and Klenow followed by EcoRI. The resultant plasmid, pET28TaBC, has two genes, rpoB and rpoC, in opposite orientations, each being preceded by the T7 RNAP promoter. pET28TaBC was treated with EcoRI and ligated with the EcoRI-treated PCR fragment of pET21TaA. Oligonucleotides used for PCR were designed to anneal upstream of the T7 RNAP promoter of pET21TaA and downstream of the termination codon of rpoA, and each contained an EcoRI site. The resultant
plasmid, pET28TaABC, has _T. aquaticus_ rpoA and an upstream T7 RNAP promoter inserted between the _rpoB_ and _rpoC_ genes, with rpoA having the same orientation as rpoC. Plasmid pET28TaABCZ was constructed by ligating the SgrAI-treated pET28TaABC with the SgrAI-treated PCR fragment of pET21bTaZ. Oligonucleotides used for PCR were designed to anneal upstream of the T7 RNAP promoter of pET21bTaZ and downstream of the termination codon of _rpoZ_, and each contained an SgrAI site. The resultant plasmid, pET28TaABCZ, has three untagged genes, _rpoZ_, _rpoC_, and _rpoA_, in the same orientation, and C-terminally hexahistidine-tagged RNAP in the opposite orientation.

**RNAP assembly and purification.** RNAP was reconstituted by a published procedure (2, 16). The molar ratio of α, β, and mutant β′ in the reconstitution reaction mixtures was 1:4:8. After reconstitution, RNAP preparations were either used directly or further purified by fast protein liquid chromatography and gel filtration on Superose-6 and Resource Q columns (Pharmacia-LKB Inc., Piscataway, N.J.) as described previously (2), concentrated by filtration through a 50% glycerol storage buffer at −20°C.

To purify the _T. aquaticus_ core from _E. coli_ cells coexpressing _T. aquaticus_ rpo genes, BL21(DE3) cells harboring pET28TaABCZ were induced with 1 mM IPTG for 3 h. Cells were collected, and the cell pellet was resuspended in 20 volumes of buffer containing 50 mM Tris-HCl, 100 mM KCl, 0.1% Tween 20, 1 mM phenylmethylsulfonyl fluoride, and 1 mM EDTA (pH 7.9). The cells were lysed by sonication, and the cleared lysate was incubated at 65°C for 60 min. After centrifugation, the proteins in the supernatant were precipitated with 40% saturation of ammonium sulfate, redissolved in IMAC starting buffer (10 mM Tris-HCl, pH 7.9, 40 mM KCl, 10 mM MgCl₂, 0.1 mM EDTA, 10 mM phenylmethylsulfonyl fluoride, and 10 mM β-mercaptoethanol), and loaded onto a Resource Q column equilibrated with the same buffer. The column was washed with buffer containing 20 mM imidazole, and RNAP was eluted with 100 mM imidazole in the buffer. The fraction was diluted fivefold with TGE buffer (50 mM Tris-HCl, 5% glycerol, 50 mM NaCl, 1 mM β-mercaptoethanol) and loaded onto a Resource Q column equilibrated with the same buffer. The column was developed with a linear gradient of NaCl concentrations in TGE buffer. Chromatographically pure RNAP eluted at ca. 400 mM NaCl and was concentrated and stored as above.

**Affinity labeling.** Reaction volumes of 10 μl contained 40 mM Tris-HCl (pH 7.9), 40 mM KCl, 10 mM MgCl₂, 10 μg of RNAP core, 0.5 to 1.0 mM initiating AMP derivatized with an aldehyde group (3), and 100 ng of poly(dA·dT). The reaction mixtures were supplemented with 10 mM BH₄ and incubated at 37°C for 10 min. [α-32P]UTP (3,000 Ci/mmol) was added to 0.3 mM (final concentration), and incubation was continued for 30 min at 37 or 65°C. Control experiments demonstrated that the resulting 32P labeling of RNAP depended on the addition of the tetralolate DNA (data not shown).

**KMrO₄ footprinting.** The 106-bp EcoRI DNA fragment containing the T7 A2 promoter (positions −84 to +32) was prepared as described previously (14). The fragment was 32P-end labeled by filling in EcoRI sticky ends with Klenow enzyme in the presence of [α-32P]dATP. The fragment was then treated with HncHII (which cuts at position +22) to obtain bottom-strand-labeled fragment. Promoter complexes were formed in 20-μl reaction mixtures containing 200 mM RNAP holoenzyme, 100 mM [32P]-end-labeled DNA fragment, 40 mM Tris-HCl (pH 7.9), 40 mM KCl and 10 mM MgCl₂. The reaction mixtures were preincubated for 15 min at 37°C (E. coli RNAP) or 65°C ( _T. aquaticus_ RNAP). Promoter complexes were then treated with 1 mM KMrO₄ for 15 s at 37°C. The reactions were terminated by the addition of β-mercaptoethanol to 300 mM followed by phenol extraction, ethanol precipitation, and 10% piperidine treatment. Reaction products were analyzed by denaturing polyacrylamide gel electrophoresis (PAGE) (6% polyacrylamide).

**In vitro transcription and transcript cleavage reactions.** Abortive initiation reaction mixtures (10 μl) contained 40 mM Tris-HCl (pH 7.9), 40 mM KCl, 10 mM MgCl₂, 50 mM RNAP core enzymes, and 100 nM recombinant _E. coli_ αβ or _T. aquaticus_ RpoD. The reaction mixtures were preincubated for 10 min at 45°C, and transcription was initiated by the addition of 100 mM Cpa and 0.5 mM [α-32P]UTP (3,000 Ci/mmol) and allowed to proceed for an additional 10 min at 35°C. The reactions were terminated by the addition of an equal volume of urea-containing loading buffer, and the products were analyzed by denaturing gel electrophoresis (7 M urea, 20% polyacrylamide) and autoradiography.

To determine transcription elongation rates, elongation complexes stabilized at position +20 (EC20) were prepared in 50-μl reaction mixtures containing 40 mM Tris-HCl (pH 7.9), 40 mM KCl, 10 mM MgCl₂, 20 mM DNA fragment containing the T7A1 promoter fused to the αR2 terminator (15), 50 nM RNAP, 0.05 mM Cpa, 0.05 mM ATP, 50 μM (each) ADP and GTP, and 2.5 μM [α-32P]CTP (3,000 Ci/mmol). The reaction mixtures were incubated for 15 min at 23°C ( _E. coli_ ) or 45°C ( _T. aquaticus_ ). EC20 were synchronously restarted by the addition of nucleoside triphosphates (NTPs). Reactions proceeded for 0 to 600 s at 45°C. The reactions were terminated by the addition of formamide-containing loading buffer. Products were analyzed by urea-PAGE (7 M urea, 6% polyacrylamide), followed by autoradiography and PhosphorImager analysis.

To monitor transcript cleavage, EC20 was prepared by incubating immobilized purified EC20 with 25 μM UTP for 5 min at room temperature ( _E. coli_ RNAP) or 42°C ( _T. aquaticus_ RNAP). Immobilized complexes were washed and left in ~20 μl in the presence or absence of recombinant _E. coli_ GreA, for 10 min at 37°C ( _E. coli_ RNAP complexes) or 65°C ( _T. aquaticus_ RNAP complexes). Reactions were terminated by the addition of an equal volume of formamide-containing loading buffer and analyzed by denaturing gel electrophoresis (7 M urea, 20% polyacrylamide) and autoradiography.

**Nucleotide sequence accession numbers.** The nucleotide sequences of the _rpoA_ gene (encoding the α subunit of RNAP), the _rpoB_ and _rpoC_ genes (encoding the β and β′ subunits of RNAP, respectively), and the _rpoZ_ gene (encoding the α subunit of RNAP) have been deposited in GenBank under accession nos. Y19922, Y19223, and AJ295839, respectively.

**RESULTS**

_T. aquaticus_ RNAP assembles in vitro. As a preliminary feasibility experiment, we wished to establish that _T. aquaticus_ RNAP assembles in vitro after denaturation. A highly pure _T. aquaticus_ RNAP core sample prepared as described by Zhang et al. (18) was incubated in a denaturing buffer containing 7 M guanidine and then dialyzed in a low-salt buffer under conditions favoring RNAP reconstitution. The resulting preparation was assayed for nonspecific transcription on synthetic template poly(dA·dT). Transcription activity was recovered with high yield (data not shown), suggesting that _T. aquaticus_ RNAP withstands the in vitro denaturation-renaturation cycle.

Cloning and overexpression of _T. aquaticus_ RNAP core subunit genes, and in vitro assembly of the recombinant _T. aquaticus_ RNAP core. The sequences of _T. aquaticus_ RNAP core subunit genes were determined previously (18). Each of the _T. aquaticus_ rpo genes was cloned in _E. coli_ pET expression vectors as described in Materials and Methods. Plasmids expressing _rpo_ genes with or without affinity hexahistidine tags were constructed. _E. coli_ cells harboring the expression plasmids overproduced high levels of recombinant _T. aquaticus_ RNAP core subunits (Fig. 1A). Most of the overexpressed subunits were found in inclusion bodies and could be recovered by standard procedure (2).

A separate plasmid pET28TaABCZ, simultaneously expressing all four _T. aquaticus_ rpo genes from T7 RNAP promoters, was also prepared. _E. coli_ BL21(DE3) cells transformed with pET28TaABCZ expressed _T. aquaticus_ RNAP subunits at low levels. The amount of overproduced _T. aquaticus_ β and β′ was only slightly larger than the amount of the endogenous _E. coli_ RNAP largest subunits, which form a characteristic double band on sodium dodecyl sulfate (SDS)-containing gels of whole-cell lysates (Fig. 1B, lanes 1 and 2). Coexpressed _T. aquaticus_ subunits formed a complex that remained soluble after high-temperature treatment of _E. coli_ extract (lane 3) and, when purified to homogeneity by IMAC and Resource Q chromatography, appeared indistinguishable from RNAP core enzyme purified from _T. aquaticus_ cells (lane 5).

Recombinant _T. aquaticus_ RNAP was also组装ed from individually expressed subunits in vitro, as judged by the appearance of characteristic chromatographic peaks in the course of purification that separates assembled enzyme from assembly intermediates and unassembled subunits (reference 2...
and data not shown). The catalytic proficiency of recombinant *T. aquaticus* RNAP was demonstrated by promoter-independent transcription of synthetic template poly(dA-dT) at the high temperature of 65°C (data not shown) and template-dependent affinity labeling with an initiating substrate derivative (Fig. 1C). In this reaction, recombinant, in vitro-reconstituted *T. aquaticus* RNAP was cross-linked to derivatized AMP (3). The reaction mixtures were then supplemented with poly(dA-dT) template and [α-32P]UTP. As controls, labeling reactions were also performed with RNAP purified from *T. aquaticus*, as well as with *E. coli* RNAP. As explained elsewhere, in *E. coli* the affinity-labeling protocol results in covalent attachment of the radioactive pApU dinucleotide tag to the β subunit Lys1065 (3, 8). Previous studies demonstrated that the cross-linkable AMP derivative modifies β-like subunits in RNAP from bacterial, archaeal, and eukaryal systems and that residues homologous to the universally conserved regions 2 and 4, responsible for promoter recognition. In striking contrast, *T. aquaticus* σ completely lacks the N-terminal conserved region 1. Instead, it contains a ~100-amino-acid segment without homology to any of the published sequences. *T. aquaticus* rpoD (σ70) gene is very similar to *E. coli* rpoD (σ70) gene from *T. aquaticus* (see Materials and Methods). Alignment of the deduced amino acid sequence revealed that the product of the *T. aquaticus* rpoD gene is very similar to the recently published sequence from *T. thermophilus* (11), as expected. *T. aquaticus* σ is also very similar to σ70 in conserved regions 2 and 4, responsible for promoter recognition. In striking contrast, *T. aquaticus* σ completely lacks the N-terminal conserved region 1. Instead, it contains a ~100-amino-acid segment without homology to any of the published sequences. *T. aquaticus* rpoD was cloned in the *E. coli* expression vector, and recombinant *T. aquaticus* σ was purified to homogeneity. The addition of *T. aquaticus* σ stimulated the synthesis of the abortive trinucleotide CpApU from the CpA initiator and UTP by the recombinant *T. aquaticus* core in the presence of a DNA fragment containing the T7 A1 promoter (Fig. 2A, lane 5). In contrast, the addition of *T. aquaticus* σ failed to stimulate transcription by the *E. coli* core enzyme (lane 3). The patterns of abortive products synthesized by *E. coli* and *T. aquaticus* holoenzymes on the T7 A1 promoter fragment in the presence of limiting sets of NTP substrates were identical, thus proving
that the observed transcription initiation was promoter specific (data not shown). In addition, KMnO₄ probing established that promoter complexes formed by the *T. aquaticus* holoenzyme on the well-studied T7 A2 promoter (14) are very similar or identical to the *E. coli* complexes (Fig. 2B).

**In vitro transcription by the *T. aquaticus* RNAP holoenzyme.** Having established conditions for promoter-specific transcription initiation by *T. aquaticus* RNAP, we wished to compare the transcription elongation, transcription termination, and transcript cleavage properties of *T. aquaticus* RNAP with those of the prototypic *E. coli* enzyme. Immobilized RNAP was used to obtain stalled elongation complexes containing 20-mer RNA on the T7 A1 promoter-containing DNA fragment (15). Purified elongation complexes were incubated in the presence of different concentrations of NTP, to monitor the transcription elongation rate and transcription termination on the rho-independent λ trR2 terminator located downstream. Alternatively, complexes containing 20-mer RNA were walked to position 21, purified, and incubated in the absence of NTP with or without *E. coli* GreA protein to monitor transcript cleavage. The most important conclusions from these experiments are summarized below. (i) *T. aquaticus* RNAP elongated RNA chains less efficiently than the *E. coli* enzyme did; in general a 10-fold-higher nucleotide concentration was required to achieve comparable elongation rates. Both enzymes exhibited transcription pausing, evident at shorter times (Fig. 3A, lanes 2 and 5); however, the pattern appeared to be distinct. (ii) *T. aquaticus* RNAP

**FIG. 2.** Transcription initiation by recombinant *T. aquaticus* RNAP. (A) The indicated RNAP core enzymes were combined with recombinant *E. coli* (Ec) σ⁷⁰ or *T. aquaticus* (Taq) RpoD in the presence of the T7 A1 promoter-containing DNA fragment, CpA primer, and [α-³²P]UTP substrate. The reaction mixtures were incubated at 45°C for 15 min (lanes 1 to 3 and 4 to 6, respectively), and the products were resolved by denaturing PAGE and revealed by autoradiography. (B) Transcription complexes were formed at the indicated temperatures on the T7 A2 promoter-containing DNA fragment radioactively end labeled on the bottom strand, using recombinant *E. coli* (Ec) or *T. aquaticus* (Taq) RNAP holoenzymes, and probed with KMnO₄. Reaction products were resolved on a 6% denaturing polyacrylamide gel and revealed by autoradiography. The permanganate-sensitive bands were assigned using G/A markers (not shown) (14).

**Fig. 3.** Transcript elongation, termination, and cleavage by recombinant *T. aquaticus* RNAP. (A) Elongation complexes stalled at position 20 were prepared using DNA fragment containing the T7 A1 promoter and λ trR2 terminator as a template (lanes 1 and 4). Transcription was resumed at 45°C by the addition of the indicated concentrations of NTPs; at the times indicated, aliquots were withdrawn and reactions were terminated by the addition of formamide containing stop buffer. Reaction products were analyzed by 6% urea PAGE followed by autoradiography. (B) Immobilized elongation complexes stalled at position 20 (lane 1) were walked to position 21 (lane 2). The complexes were desorbed from Ni²⁺-nitrilotriacetic acid agarose and supplemented with recombinant *E. coli* GreA (0.04 μg), reaction mixtures were incubated for 15 min at 37 and 65°C (for *E. coli* and *T. aquaticus* RNAP, correspondingly), and the products were resolved by denaturing PAGE in a 20% urea gel. In lane 5, RNA was extracted from the stalled *T. aquaticus* elongation complex with phenol and then incubated in the presence of *T. aquaticus* RNAP and *E. coli* GreA.
aquaticus RNAP recognized the tR2 terminator less efficiently (transcription termination efficiency of ~20%, compared to 86% with the E. coli enzyme). (iii) T. aquaticus RNAP actively cleaved 21-mer RNA in the stalled elongation complex in the absence of added cleavage factor. This activity was not further stimulated by E. coli GreA and was not due to RNase contamination, as shown by the control lane of Fig. 3B (lane 5) (in the control reaction, 21-mer RNA was prepared by phenol extraction of stalled complexes and pure RNA was then incubated with T. aquaticus RNAP before being loaded onto the gel).

Structural analysis of the T. aquaticus core indicates that sites in subunit β that correspond to sites of known rifampin resistance mutations in E. coli form a cluster in the ceiling of DNA binding channel of the enzyme (18). Previous reports indicated that T. aquaticus RNAP is highly resistant to rifampin (5). With the exception of T. aquaticus β Thr<sup>566</sup>, which corresponds to E. coli β Arg<sup>687</sup>, which defines Rif cluster III (6), all T. aquaticus amino acids that can be involved in rifampin binding are identical to the corresponding amino acids in the wild-type, rifampin-sensitive E. coli β subunit. To test whether Thr<sup>566</sup> is responsible for rifampin resistance, we substituted it for Arg, assembled the mutant enzyme in vitro, and determined its sensitivity to rifampin in a steady-state T7 A1 promoter transcription assay. The result, presented in Fig. 4, shows that in agreement with earlier data, T. aquaticus RNAP was highly resistant to rifampin and continued to synthesize full-sized transcripts even in the presence of 1,000 μg of rifampin per ml in the reaction mixture. In contrast, synthesis of the full-sized transcripts by the E. coli enzyme was completely inhibited in the presence of 10 μg of the drug per ml. T. aquaticus RNAP harboring the T566R substitution in subunit β was unaffected in its level of rifampin resistance. Therefore, the determinants of the resistance of T. aquaticus RNAP to rifampin must lie outside sites determined by genetic studies of resistance in E. coli and other sensitive bacteria.

**DISCUSSION**

The recent advances in our understanding of the mechanism and regulation of bacterial transcription are largely due to recombinant E. coli RNAP technology that has allowed the preparation of sufficient quantities of RNAP mutants harboring lethal mutations for biochemical analysis. Until recently, this powerful approach had a significant limitation, since mutations that affected RNAP assembly could not be studied. The recent determination of the high-resolution structure of the T. aquaticus RNAP core allows one to perform a precise, structure-based analysis of bacterial RNAP function.

As our results demonstrate, recombinant T. aquaticus RNAP can be prepared in large amounts, and the discriminative transcription assays developed for the E. coli enzyme can be adapted to study this thermophilic enzyme. However, the T. aquaticus enzyme differs from the E. coli enzyme in several significant ways. Promoter complexes formed by the T. aquaticus holoenzyme are indistinguishable from E. coli RNAP complexes, suggesting that the promoter specificities of T. aquaticus RpoD and E. coli σ<sup>70</sup> are the same. However, the two sigmas are not interchangeable. The functional specialization of sigmas is not due to the lack of formation of hybrid holoenzymes (data not shown). The unusual N-terminal extension present in T. aquaticus RpoD and/or the long (300-amino-acid) insertion in T. aquaticus β′, which is absent from E. coli counterpart and is located close to the conserved segment C involved in σ binding (1), may be responsible for the inability of hybrid holoenzymes to recognize promoters.

As with sigma, the E. coli transcript cleavage factor GreA did not alter the properties of T. aquaticus RNAP. In fact, T. aquaticus RNAP appears to be very active in factor-independent transcript cleavage even at low pH. High levels of intrinsic transcript cleavage were observed when T. aquaticus RNAP was purified from E. coli coexpressing T. aquaticus rpo genes or
when the enzyme was prepared by in vitro reconstitution from isolated subunits (data not shown). Thus, the cleavage activity probably reflects the true properties of *T. aquaticus* RNAP rather than contamination with the *E. coli* Gre factor(s). High levels of cleavage activity may be essential for an enzyme which transcribes DNA at high temperature, a condition known to stimulate transcription arrest in *E. coli* (9). Despite the relatively slow elongation, *T. aquaticus* RNAP terminates transcription less efficiently, lending further support to the idea that the relationship between the transcription elongation rate and transcription termination is more complex than was previously thought (10).

The unusually high resistance of *T. aquaticus* RNAP to rifampin is not due to *T. aquaticus* β-Thr^{655}, the only *T. aquaticus* β amino acid that is different from *E. coli* residues known to be involved in rifampin resistance. This result suggests that additional amino acids weaken rifampin binding to *T. aquaticus* RNAP. It is not clear why mutational changes in these amino acids were not detected during intensive screens for rifampin resistance in *E. coli* and other rifampin-sensitive bacteria (7, 13). One possible explanation is that such changes lead to a lethal phenotype. Nevertheless, this result suggests that details of rifampin interaction with *T. aquaticus* RNAP may be significantly different from those of the interaction in rifampin-sensitive enzymes.

To summarize, the availability of recombinant *T. aquaticus* RNAP and discriminative transcription assays for this enzyme should make it possible to test, by means of genetic engineering and biochemical analysis, many of the predictions of the structure-functional model of transcription put forward by Zhang et al. (18). The ability to purify mutant *T. aquaticus* RNAP directly from *E. coli* cells should also allow a structural analysis of RNAP mutants.

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