Dissection of Two Hallmarks of the Open Promoter Complex by Mutation in an RNA Polymerase Core Subunit*

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Deletion of 10 evolutionarily conserved amino acids from the β subunit of Escherichia coli RNA polymerase leads to a mutant enzyme that is unable to efficiently hold onto DNA. Open promoter complexes formed by the mutant enzyme are in rapid equilibrium with closed complexes and, unlike the wild-type complexes, are highly sensitive to the DNA competitor heparin (Martin, E., Sagitov, V., Burova, E., Nikiforov, V., and Goldfarb, A. (1992) J. Biol. Chem. 267, 20175–20180). Here we show that despite this instability, the mutant enzyme forms partially open complexes at temperatures as low as 0 °C when the wild-type complex is fully closed. Thus, the two hallmarks of the open promoter complex, the stability toward a challenge with DNA competitors and the sensitivity toward low temperature, can be uncoupled by mutation and may be independent in the wild-type complex. We use the high resolution structure of Thermus aquaticus RNA polymerase core to build a functional model of promoter complex formation that accounts for the observed defects of the E. coli RNA polymerase mutants.

The formation of the transcription-competent binary promoter complex by Escherichia coli RNA polymerase (RNAP)1 holoenzyme is a complex, multi-step process. Kinetic analysis using quantitative abortive initiation assays demonstrates that when RNAP first recognizes the promoter it forms the relatively unstable, “closed” complex (reviewed in Ref. 1). The closed complex is fully reversible: it easily dissociates back to free RNAP and DNA and is therefore highly sensitive to DNA competitors such as heparin, which bind free RNAP and prevent rebinding to promoter DNA. If RNAP commits itself to the template, the closed complex undergoes a series of poorly characterized conformational changes that lead to the formation of the transcription-competent “open” complex. Kinetic analysis at standard (37 °C) temperature indicates the existence of at least one kinetically significant, short-lived intermediate between the closed and the open complex (2). On most promoters the open complex formed at standard temperature is essentially irreversible and is fully resistant to DNA competitors.

Direct structural analysis of closed and intermediate complexes at standard temperature is complicated since they are short-lived. In contrast, the open complex, which is the dominant species at 37 °C on most promoters, can be easily studied. DNase I footprinting of open complexes at several promoters shows extensive DNA protection from −55 to +20 relative to the transcription start point at +1. On most promoters, a hypersensitive DNase I site exists in the otherwise well protected area at about position −25 (3). This hypersensitive site has been interpreted as the site of sharp bending in the DNA (3). Indeed, since the amount of DNA protected in the open complex exceeds the largest linear dimension of RNAP holoenzyme, DNA in the complex must be bent or curved (2, 4). Probing with single strand-specific reagents such as KMnO4 demonstrates that pyrimidines between −12 to +2 are accessible in the open complex but not in the closed complex. Thus, a fragment of promoter DNA in the open complex is either severely distorted or melted (the so-called transcription bubble).

On most promoters the open complex is temperature-sensitive and is rapidly and cooperatively inactivated when the reaction temperature is lowered to about 20 °C or below (5). Footprinting analysis reveals several distinct binary promoter complexes at lower temperatures (6–9). All these additional complexes are characterized by the absence of DNA reactivity to single strand-specific reagents. In addition, the region of DNA protected by RNAP from DNase I attack is reduced at the reaction temperature is lowered to about 20 °C or below (5). Footprinting analysis reveals several distinct binary promoter complexes at lower temperatures (6–9). All these additional complexes are characterized by the absence of DNA reactivity to single strand-specific reagents. In addition, the region of DNA protected by RNAP from DNase I attack is reduced at lower temperatures, mostly in the region downstream of transcription start point, and the degree of protection also varies. The dominant binary complex at 0 °C shows an alternating pattern of protection and deprotection between the positions −55 to −5, suggesting that RNAP interacts with only one side of the DNA molecule in this complex (7–9). The 0 °C binary complex is highly sensitive to DNA competitors, whereas some of the complexes obtained at higher, 10–15 °C, temperatures are relatively resistant.

The mechanistic significance of binary complexes present at lower temperatures is not known. The limited number of careful kinetic and thermodynamic studies on RNAP-promoter interactions on λ Pr and lac UV5 promoters suggests that binary complexes trapped at lower temperatures may correspond to transient kinetic intermediates of the open complex formation mechanism at 37 °C. Thus, the 0 °C complex may correspond to kinetically defined, heparin-sensitive closed complex, whereas other trapped complexes can correspond to various stages of isomerization from fully closed to fully open conformation. However, recent detailed analysis revealed that DNase I footprints of the 0 °C complex on the phage λ prmu-1 Δ265 promoter had both similarities to and differences from true closed complex formed at higher temperatures (10) and, thus, may be off the true promoter opening pathway.

Analysis of mutant RNAPs defective in promoter complex

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‡ The abbreviation used is: RNAP, E. coli RNA polymerase.

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formation provides an alternative and complementary way to trap reaction intermediates and potentially can lead to a better understanding of structure/function relationships in transcription initiation. As expected, mutational analysis indicates that the specificity subunit \( \sigma \) contributes to the initial promoter recognition, initiation of DNA melting, and possibly promoter complex stability (11–13). However, mutations in the core enzyme that affect promoter complex stability and structure have also been isolated. RNAP carrying an engineered 10-amino acid deletion in the evolutionarily conserved segment C of the \( \beta \) subunit, \( \beta RV \) (14), was unable to efficiently hold onto promoter DNA, resulting in heparin-sensitive open complexes. More recent studies resulted in isolation of additional mutations that destabilize RNAP-promoter complexes in vitro (15). One point mutation, 4348, occurred close to \( \Delta RV \), underscoring the importance of \( \beta \) segment C in open complex stability.

Another engineered mutation, \( \Delta (186–433) \), removed most of the \( \beta \)-dispensable region I, which is adjacent to segment C (Fig. 1, Ref. 16), and resulted in RNAP that formed open promoter complexes that were more resistant to low temperature inactivation than the wild-type open complexes. The mutant complexes remained sensitive to KMnO\(_4\) attack even at \(-20^\circ C\), whereas the wild-type complexes became unreactive below \(+15^\circ C\), as expected (16). Interestingly, the destabilizing \( \Delta RV \) mutation, and the stabilizing \( \Delta (186–433) \) are immediately adjacent to each other in the \( \beta \) subunit primary sequence. Here we reinvestigate promoter complex formation by RNAP carrying the \( \Delta RV \) mutation. We confirm that \( \Delta RV \) decreases open promoter complex resistance to heparin. Surprisingly, \( \Delta RV \) also increases open promoter complex ability to withstand low temperature and, thus, resembles \( \Delta (186–433) \). Thus, the two hallmarks of the open promoter complex, stability toward DNA competitors and sensitivity toward temperature downshift, can be uncoupled by mutation.

MATERIALS AND METHODS

Genetic Engineering.—The starting plasmid for genetic manipulation was pMKSe2 containing the wild-type \( \rho \) box gene under control of lac promoter (17). The \( \Delta RV \) mutation was constructed by treating pMKSe2 with EcoRV introduces two in-frame cuts in \( \rho \) box that are 30 base pairs apart (14) and religating the plasmid. To construct the \( \Delta \)Hes, two complementary oligonucleotides, 5'-p-AGGATCCAT- CACGACATCATCGAAGAGC (DN1) and 5'-p-GTGTCATGCTGTCGCTA- GTGATGCCTC-3' (DN2), were used. Both oligonucleotides contain BarnHI recognition site (underlined). DN1 and DN2 were annealed to each other and ligated to EcoRV-digested pMKSe2 plasmid. Ligase was then inactivated by heating, and reactions were treated with a high concentration of BarnHI (does not cut pMKSe2). BarnHI was inactivated by phenol extraction, and the plasmid was reagitated and transformed in E. coli cells. The DN1/DN2 linker when inserted in the right orientation should introduce eight amino acids, Arg-Ile-His\(_6\), in place of segment C. An RI DNA fragment containing 300 mM NaCl, RNAP was eluted with TGE containing 600 mM imidazole. The 200 mM imidazole fraction, which contained RNAP, was diluted 3-fold with TGE buffer (10 mM Tris-HCl, 5% glycerol, 0.1 mM EDTA) and applied on a 1-m column with HiTrap column (Amersham Pharmacia Biotech). After washing the column with TGE buffer containing 300 mM NaCl, RNAP was eluted with TGE containing 600 mM NaCl. The purified enzyme was concentrated by dialysis against storage buffer (20 mM Tris-HCl, pH 8.0, 300 mM NaCl, 5 mM \( \beta \)-mercaptoethanol, 50% glycerol) to \(-1\) mg/ml and stored at \(-20^\circ C\).

Footprinting Reactions.—The 106-base pair EcoRI DNA fragment containing the T7 A2 promoter (\(-84 to +32\)) was prepared as described (16). The fragment was 32P-end-labeled by filling-in EcoRI sticky ends with Klenow enzyme in the presence of \( [\delta P]dATP \). The fragment was then treated with Accl (cuts at position \(-70\)) to obtain top strand-labeled fragment or HincII (cuts at position \(+22\)) to obtain bottom strand-labeled fragment. Promoter complexes were formed in 20-m reactions containing 0.4 pmol of wt or mutant RNAP, 0.2 pmol of 32P-end-labeled DNA fragment, 40 mM Tris-HCl, pH 7.9, 40 mM KCl, and 10 mM MgCl\(_2\). Reactions were preincubated for 15 min at 37 \( ^\circ C \). DNase footprinting reaction was initiated by the addition of DNase I (2 \( \mu M \) DNase I, Worthington). The reaction proceeded for 10 s at 37 \( ^\circ C \) and was terminated by the addition of EDTA to 15 mM followed by phenol extraction and ethanol precipitation. For KMnO\(_4\) probing, promoter complexes were treated with KMnO\(_4\) (1 mM) for 15 s at 37 \( ^\circ C \). Reactions were terminated by the addition of \( \beta \)-mercaptoethanol to 300 mM followed by phenol extraction, ethanol precipitation, and 10% piperidine treatment. Where indicated, reaction mixtures were challenged by the addition of 100 \( \mu M \) heparin immediately before footprinting.

To footprint promoter complexes formed at low temperatures, RNAP and 32P-end-labeled T7 A2 promoter-containing fragments were combined on ice and incubated for 15 min on ice, and the footprinting reaction was performed. The same conditions were used for KMnO\(_4\) probing, but at low temperature, since control experiments demonstrated that KMnO\(_4\) modification was complete after 15 s. Products of footprinting reactions were analyzed by urea-polyacrylamide gel electrophoresis (7 M urea, 6% polyacrylamide) followed by autoradiography.

RESULTS

Genetic Context of the Conserved Segment C in the \( \beta \) Subunit.—Segment C is one of the nine co-linear segments of high sequence conservation found in all \( \beta \) subunit homologs. On the \( N \)-proximal side of segment C is dispensable region I (21), a long region of primary sequence (E. coli residues 149–433) that is present in proteobacteria but is partially absent from homologs from other eubacteria, archaea, and eukaryotes (Fig. 1). Sequence C-terminal to C is not particularly conserved and is followed by the Rf region (Ref. 17 and residues 505–574, Fig. 1).

Mutations at or close to segment C used in this study are shown on Fig. 1. \( \Delta RV \) removes amino acids 436–445 at the left side of segment C (14). The region affected by the deletion contains several highly conserved amino acids and has sequence similarity with barnase-type bacterial \( \beta \) nases. \( \Delta (186–433) \) is a long deletion of all dispensable region I and is immediately downstream of \( \Delta RV \) (21). 4348 mutations was chosen because their inability to confer prototrophy on \( \Delta\) Ara spT strains occurred close to \( \Delta RV \), changing arg\(_452\) to His (15). \( \Delta Hes \) is a new mutation. It was constructed by site-specific mutagenesis and introduces eight unnatural amino acids, Arg-Ile-His\(_6\), in place of segment C amino acids removed by \( \Delta RV \) (see "Materials and Methods").

The in vivo phenotype of \( \Delta Hes \) was tested in the E. coli...
AJ2005 tester strain (18). AJ2005 cells have an amber mutation in the chromosomal copy of \( rpoB \) that is suppressed by the \( supU \) suppressor carried by an F-factor. The F-factor also carries the \( lacZ \) gene, and AJ2005 cells are therefore \( lac^{-} \). When a plasmid bearing a functional \( rpoB \) allele is introduced into AJ2005, the F-factor can be lost, and the plasmid-borne \( rpoB \) gene becomes the only source of the \( lac \)-like subunits in the cell. As a result, such cells become \( lac^{-} \), and they can be easily distinguished from parental cells using indicator media. AJ2005 cells transformed with the \( rpoB \) expression plasmid pMKSe2 bearing \( rpoB^{+} \) or \( \Omega H_{6} \) segregated \( lac^{-} \) colonies (data not shown). In contrast, no \( lac^{-} \) colonies were observed when AJ2005 was transformed with pMKSe2 bearing \( rpoB \) (data not shown, Ref. 14). This result suggests that the insertion functionally compensates for defects caused by \( rpoB \) and that the total length of this part of segment C is more important for function than the sequence per se.

The Open Promoter Complex Formed by RNAP^{ARV} Is Shortened in the Downstream Region—To better understand the marked differences in promoter complex stability caused by the two adjacent mutations in the \( \beta \) subunit, \( \Delta RV \) and \( \Delta 186-433 \), promoter complexes formed by RNAP^{ARV}, RNAP^{ARV+433}, and RNAP^{WT} were studied by DNase I footprinting (Fig. 2). RNAP^{H6} was also included in this analysis.

The DNase I footprint of the RNAP^{WT} on the A2 promoter of bacteriophage T7 was typical for open complexes on \( \sigma^{70} \) promoters. Upstream of the transcription start site, protection started at around position -40, followed by a region of hypersensitivity at around position -25. The -10 promoter region was completely protected from DNase I attack, and this protection extended through the transcription start site to about position +20 (Fig. 2A, lane 5). As described previously (16), RNAP^{ARV+433} produced an identical footprint upstream of the transcription start site; however, position +1 was only partially protected, and there was no protection beyond position +5 (Fig. 2A, lane 4). The RNAP^{ARV} footprint was also shortened in the downstream direction and, thus, was qualitatively similar to RNAP^{ARV+433} footprint. Unlike RNAP^{ARV+433}, RNAP^{ARV} failed to protect the promoter DNA fully, suggesting that significant portion of the enzyme dissociated from the promoter during the 15-s treatment with DNase (note that in this experiment we used 10 times as much RNAP^{ARV} as other enzymes). This result agrees well with the previous findings obtained by filter binding and quantitative abortive initiation assays (14). Kinetic analysis indicated that even at very high RNA concentrations the steady-state level of mutant promoter complexes was 30% of that of the wild type (14). In contrast, RNAP^{H6} footprint was indistinguishable from the wild-type footprint; it was fully extended; and protection of DNA was complete (lane 3), in agreement with the results of the in vivo phenotypic analysis (above).

The DNA strand separation in open complexes formed by the wild-type and mutant RNAPs was investigated using KMnO_{4} probing. The wild-type and the \( \Omega H_{6} \) complex appeared the
we tested if RNAPD extend beyond the downstream region are lost, and DNA melting does not the T7 A2 promoter (0.2 pmol of a 106-base pair DNA fragment containing the transcription initiation start point. 0.2 pmol of a 106-base pair DNA fragment containing the T7 A2 promoter (~84 to +32) 32P-end-labeled on the bottom strand was combined with 0.4 pmol of wild type (WT) or indicated mutant RNAP in 20-μl reactions containing 40 mM Tris-HCl, pH 7.9, 40 mM KCl, and 10 mM MgCl2. 4 pmol of RNAPRV was used in reactions. Reactions were preincubated for 15 min at 37 °C and footprinted with DNase I (A) or probed with KMnO4 (B). Reaction products were resolved on a 6% sequencing gel and visualized by autoradiography.

same, with thymines at −15, −12, −11, −9, −7, −5, −4, and −3, as well as cytosine at −14 being modified by KMnO4 (Fig. 2B, lanes 3 and 5). As described elsewhere (16), the single-stranded region did not extend as far downstream in complexes formed by RNAP3186−433; only thymines at positions −15, −12, −11, and −9 and cytosine at −14 were modified by KMnO4 (Fig. 2B, lane 4). As expected, probing of RNAPRV complex revealed similar shortening of the transcription bubble (Fig. 2B, lane 2).

RNAPRV Promoter Complexes Remain Open at 0 °C—The results of the footprinting experiment suggest that in RNAPRV-T7 A2 promoter complexes, protein-DNA contacts in the downstream region are lost, and DNA melting does not extend beyond the −5 position relative to transcription start. RNAP3186−433 also forms such shortened complexes, which are heparin-resistant and remain sensitive to permanganate at temperatures as low as −20 °C (16). In contrast, the wild-type complexes close at around 15 °C on this promoter (16). Since at 37 °C RNAPRV and RNAP3186−433 promoter looked similar, we tested if RNAPRV complexes also remain sensitive to permanganate at lower temperatures. Fig. 3 demonstrates that this was indeed the case. In this experiment, RNAP and radioactively labeled promoter fragment were combined at either 37 or 0 °C and incubated for 15 min to allow the complex formation, and KMnO4 probing was performed. As can be seen, there was no modification of thymines in the wild-type complex at 0 °C (lane 3), indicating that the complex was closed, as expected. In contrast, in the ARV complex thymines in the upstream portion of the transcription bubble remained sensitive to permanganate attack even at low temperature (lane 5). Assuming 100% opening at 37 °C, more than 50% of the mutant complex was open at low temperature, similar to the value previously obtained for RNAP3186−433 (16).

β Segment C Point Mutation That Destabilizes Open Complex Does Not Result in Temperature Resistance—Recently, an rpoB 4348 point mutation that changes evolutionarily conserved segment C Arg454 to His and that renders RNAP-promoter complexes sensitive to heparin attack was described (15). To find out whether unstable open complexes are generally more resistant to low temperature, we purified the RNAP carrying the 4348 mutation, formed promoter complexes at 37 and 0 °C, and probed them with KMnO4. The Arg → His substitution caused by the 4348 mutation is only 10 amino acids away of the ARV deletion (Fig. 1), and we therefore expected that it destabilizes the complex through a similar mechanism. This expectation was not fulfilled. As can be seen, at 37 °C the mutant enzyme produced a fully open “wild-type” complex, which was highly sensitive to heparin attack (Fig. 4A, lane 6). When probing was performed at 0 °C, no sensitive thymines were observed, indicating that the mutant enzyme was unable to melt promoter DNA at these conditions (Fig. 4A, lane 7). Additional experiments established that RNAP3186 complexes were indistinguishable from the wild-type complexes, i.e. they were heparin-resistant at high temperature and closed at low temperature (data not shown). It was previously suggested that heparin may actively displace RNAP from promoter complex instead of simply acting as a trap for free RNAP. The experiment presented on Fig. 4 was repeated with poly[dA-dT] as a competitor, and the same result was obtained (data not shown).

Promoter Complexes Formed By RNAP From B. subtilis Are Shortened in the Downstream Region—in Gram-positive bacteria, the region on the N-terminal side of segment C contains insertions and deletions relative to the E. coli sequence (Fig. 1). It is conceivable that compared with the E. coli enzyme, insertions/deletions in these organisms change the position of segment C with respect to other RNAP functional sites. In agreement with this idea it was reported that RNAP from B. subtilis, which contains a long, 90-amino acid insertion relative to E. coli close to segment C (Fig. 1), as well several deletions closer to the N-terminal segment B, forms open promoter complexes that are highly sensitive to heparin (25). Moreover, DNase I
footprinting established that promoter complexes formed by *B. subtilis* enzyme were shortened in the downstream region as compared with *E. coli* RNAP complexes (25). We purified *B. subtilis* RNAP and investigated the T7 A2 promoter complexes formed by this enzyme using KMnO₄ footprinting. Purified *B. subtilis* RNAP recognized the T7 A2 promoter with low efficiency, and high amounts of the enzyme were necessary to observe promoter complex formation. In agreement with the published data, *B. subtilis* complexes were sensitive to heparin (Fig. 4B, lane 5). Importantly, the pattern of KMnO₄ sensitivity in the transcription bubble in 37 °C complexes formed by *B. subtilis* RNAP was shortened in the downstream direction as compared with the *E. coli* RNAP complexes and was highly similar or identical to RNAPΔRVA and RNAΔRV complexes (Fig. 4B, right). However, at 0 °C the KMnO₄ sensitivity in *B. subtilis* complex disappeared (Fig. 4B, lane 6). We are currently performing deletion mutagenesis in cloned *B. subtilis* rpoB gene to see if deletions in the *B. subtilis* β subunit β-dispensable region I can result in temperature resistance.

**DISCUSSION**

Open promoter complexes formed by *E. coli* RNAP at the physiological temperature of 37 °C are highly resistant to polyanion heparin, which acts as a DNA competitor. When the reaction temperature is lowered to about 15 °C, heparin-sensitive promoter complexes are formed (23). Thus, it appeared that heparin resistance of promoter complexes and the ability to partially melt promoter DNA are related to each other. One would expect then that mutations that render open complexes heparin-sensitive will also make them more sensitive to lower temperatures. Obviously, RNAP with a short deletion in the β-conserved segment C studied here, RNAΔRVA, does not meet this expectation; at 37 °C the mutant complexes are as sensitive to heparin as closed complexes (14), yet they remain partially open at 0 °C, when the wild-type complexes are fully closed. Thus, the two hallmarks of transcription-competent open promoter complex, resistance to inactivation by heparin and sensitivity to low temperature, can be uncoupled. Consequently, RNAP structural elements that underlie the localized melting of promoter DNA and ability to resist heparin challenge must also be distinct.

A seminal paper by Zhang *et al.* (26) reveals the structure of core RNAP from thermophilic bacterium *Thermus aquaticus* (Taq) at 3.3 Å resolution. The Taq molecule has a characteristic crab claw-like shape, with a deep, ~27-Å-wide channel separating the jaws of the claw. The catalytic Mg²⁺ ion is located deep in the middle of the channel that likely binds the template DNA. The area of the 27-Å channel that is to the left of catalytic Mg²⁺ in the representation of Fig. 5B is thought to contact the downstream, double-stranded DNA; the area to the right makes contacts with transcription bubble, DNA-RNA heteroduplex, and upstream DNA. The upper RNAP jaw is composed mostly of β and is bilobate; the downstream lobe is composed entirely of β-dispensable region I and portions of conserved segment C; the second, upstream lobe is composed of the remainder of conserved segment C and segment D. The region of Taq β that corresponds to *E. coli* positions 186–433 is shown in orange in Fig. 5; Taq amino acids corresponding to those removed by ΔRV are shown in red. As can be seen, Δ(186–433) entirely removes the downstream lobe, whereas ΔRV removes amino acids at the outside (downstream) edge of this lobe.

We propose that the bilobed structure of the top RNAP jaw holds the key to the understanding of biochemical phenotypes of the mutant enzymes studied here. We envision that both lobes make interactions with promoter DNA and that these interactions occur in a concerted, interdependent manner. The upstream lobe cooperates with the σ subunit (binds to the coiled-coil structure of β' located immediately below (Ref. 27, Fig. 5C)) and maintains the initial strand separation at the upstream edge of the transcription bubble. We propose that these upstream protein-DNA interactions are temperature-insensitive and that locking of the upstream jaw also engages, via an allosteric mechanism, the downstream lobe, which results in extended footprint and propagation of melting in the downstream direction. The allosteric mechanism that controls the movement of both lobes is organized such that only two conformations are stable; both lobes are unlocked (i.e. closed complex) or both lobes are locked (open complex). We propose that the locking of the downstream lobe is temperature-sensitive. The Δ(186–433) mutation removes the downstream lobe; ΔRV leaves it intact but destroys the allosteric mechanism. As a result, promoter complexes formed by mutant enzymes are held together by upstream lobe-promoter DNA interactions; they are shortened in the downstream direction, are partially melted, and are temperature-resistant.
In the wild-type E. coli RNAP complex or in the Δ(186–433) complex the upstream interactions are both temperature-resistant and heparin-resistant. However, mutations such as R454H (shown in cyan and heparin-resistant. However, mutations such as R454H (shown in green) and the α dimer in blue, β is in green, and ω is in yellow. The active center Mg$^{2+}$ is shown in red. A, view roughly parallel with the main axis of the RNAP channel. The active site Mg$^{2+}$ is seen through the secondary channel. B, view A rotated 90° clockwise about the vertical axis; C, view B rotated 90° toward the viewer about the horizontal axis, giving a view of the top of the β jaw. The segment of Taq β corresponding to E. coli amino acids removed by Δ(186–433) is shown in orange. The segment of β corresponding to E. coli amino acids removed by ΔRV is shown in red. Taq β Arg334, corresponding to E. coli Arg434, is shown in cyan and space-fill. Segment C amino acids located between the end point of ΔRV and Arg434 are shown in cyan. Taq amino acids Ser411, Ala412, and Thr443, corresponding to E. coli amino acids Ser531, Ala532, and Thr563, correspondingly, are shown in space-fill and magenta.

Our “two stroke” model of promoter opening is supported by data of Guo and Gralla (24), who studied the interaction of RNAP holoenzyme with artificial DNA templates containing nucleotide polymerase core mutation destabilizing open complex.
Saecker, R. M., and Record, M. T., Jr. (1998) J. Mol. Biol. 283, 741–756
24. Guo, Y., and Gralla, Y. D. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 11655–11660
25. Whipple, F. W., and Sonenshein, A. L. (1992) J. Mol. Biol. 223, 399–414
26. Zhang, G., Campbell, L., Minakhin, L., Richter, C., Severinov, K., and Darst, S. A. (1999) Cell 96, 811–824
27. Arthur, T. M., and Burgess, R. R. (1998) J. Biol. Chem. 273, 31381–31387
28. Zhou, Y. N., and Jin, D. J. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 2908–2913
29. Korzheva, N., Mustaev, A., Malhotra, A., Nikiforov, V., Goldfarb, A., and Darst, S. A. (2000) Science, in press
30. Mustaev, A., Kashlev, M., Lee, J. Y., Polyakov, A., Lebedev, A., Zalenskaya, K., Grachev, M., Goldfarb, A., and Nikiforov, V. (1991) J. Biol. Chem. 266, 23927–23931
31. Severinov, K., Mustaev, A., Severinova, E., Kozlov, M., Darst, S. A., and Goldfarb, A. (1995) J. Biol. Chem. 270, 29428–29432
32. Severinov, K., Mustaev, A., Severinova, E., Bass, I., Kashlev, M., Landick, R., Nikiforov, V., Goldfarb, A., and Darst, S. A. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 4591–4595
33. Severinov, K., Mustaev, A., Kukarin, A., Muzzin, O., Bass, I., Darst, S. A., and Goldfarb, A. (1996) J. Biol. Chem. 271, 27969–27974