Regulation of RNA polymerase during initiation, elongation, and termination of transcription is mediated in part by interactions with intrinsic regulatory signals encoded in the RNA and DNA that contact the enzyme. These interactions include contacts to an 8–9-bp RNA:DNA hybrid within the active-site cleft of the enzyme, contacts to the melted nontemplate DNA strand in the vicinity of the hybrid, contacts to exiting RNA upstream of the hybrid, and contacts to ~20 bp of duplex DNA downstream of the active site. Based on characterization of an amino acid substitution (G1161R) and a deletion (∆1149–1190) in the jaw domain of the bacterial RNA polymerase largest subunit (β'), we report here that contacts of the jaw domain to downstream DNA at the leading edge of the transcription complex contribute to regulation during all three phases of transcription. The results provide insight into the role of the jaw domain-downstream DNA contact in transcriptional initiation and pausing and suggest possible explanations for the previously reported isolation of the jaw mutants based on reduced ColEI plasmid replication.

Cellular, multisubunit RNA polymerases (RNAPs) participate in a complex cycle of conformational changes to initiate, elongate, and terminate RNA transcripts. Each step in this cycle is mediated by a network of protein-nucleic acid interactions composed of interconnected parts of RNAP that contact DNA and product RNA (1–9). During elongation, most nucleic acid contacts are made by two large subunits of structure and sequence in prokaryotic and eukaryotic RNAPs, called β' and β in bacteria or RPB1 and RPB2 in eukaryotes. During initiation, these contacts are supplemented by sequence-specific DNA contacts made by auxiliary initiation factors (σ in bacteria) that mediate promoter engagement.

In both initiation and elongation complexes, a key component in this protein-nucleic acid interaction network occurs between ~20 bp of duplex DNA downstream of the polymerization site and a channel in RNAP composed of a trough formed mostly by β(RPB1) and a cover formed by the lobe domain of β(RPB2). During promoter engagement, establishment of this contact is coupled to formation of the ~15 bp melted transcription bubble and insertion of the template DNA strand into the active site of RNAP (Refs. 1 and 10, and references therein). Upon promoter escape, when RNAP forms a transcription elongation complex (TEC), the downstream contact persists and participates in the response of RNAP to pause, arrest, and termination signals (11–16).

The downstream DNA interaction, which stretches from the position of duplex melting 1–3 nt in front of the catalytic center to ~20 bp further downstream, can be subdivided into active-site proximal and active-site distal sets of contacts (Fig. 1, B and C). The active-site proximal set of contacts is made at +5 to +8 by the lobe and domain called the clamp (formed mostly by β'), both of which can move relative to the central core of the enzyme (2, 8, 9). The active-site distal set of contacts is made at +10 to +20 by the clamp and by another feature termed the jaw. In eukaryotic RNAPII, the jaw is extensive and includes an external domain of RBP1 and part of the RPB5 subunit, neither of which are present in bacterial RNAP. In bacterial RNAP, the jaw contact involves only a C-terminal segment of β'. Both major groove and backbone contacts are involved in the downstream DNA interaction of RNAP (7, 9), but it seems likely they will be largely sequence-nonspecific to ensure facile translocation of the duplex during transcription.

Ederth et al. (17) recently reported that a deletion (∆1149–1190) or a single amino acid substitution (G1161R) in the portion of the Escherichia coli RNAP β' subunit that forms the bacterial jaw domain dramatically reduces the copy number of ColEI-type plasmids. They propose that these changes alter the relative activity of the promoters for the two RNA regulators of ColEI plasmid replication, RNAII and RNAI. Because RNAII primes plasmid replication and RNAI negatively regulates RNAII formation (18), a decrease in RNAII or an increase in RNAI would reduce plasmid copy number. Changes in contacts of RNAP to downstream DNA appear able to reduce promoter activity by reducing the longevity of open complexes formed at certain promoters (Refs. 19 and 20; see “Results”). Alternatively, it is possible that changes in transcriptional elongation and pausing, which are mediated in part by the downstream DNA contact, could alter the folding pathway of RNAI or RNAII (21). To investigate these ideas further and to test the role of the RNAP jaw-downstream DNA contact in RNAP function, we purified RNAPs containing these alterations and tested their effects on transcriptional elongation and pausing in vitro using assays that reveal separate steps in the transcription cycle.
Address correspondence to I. Artsimovitch, V. Svetlov, K. S. Murakami, and R. Landick, manuscript in preparation.

Fig. 1. Location of β′ alterations relative to RNAP structure and downstream DNA duplex. A, location of β′G1161IR and β′Δ(1149–1190). Conserved regions of β′ are shown in pink (A–H), with its structural domains labeled (8, 49). S1 indicates the position of a 187-aa sequence insertion in E. coli β′ that is not present in most bacterial species. The sequence of the E. coli β′ subunit in the vicinity of the alterations (Ec, top) is aligned with the corresponding β′ sequence from Thermus aquaticus (Ta, bottom; the source of the bacterial RNAP crystal structure; Ref. 49). Lines and italicized letters above the amino acid sequences indicate positions of insertions in various organisms (from left to right: a1, 32 aa in Aquasae aeolicus; a2, 21 aa in A. aeolicus; euk., 155 and 162 aa in yeast and human RNAPII, respectively (the eukaryotic jaw domain); c, 16 aa in Corynebacterium glutamicum; and m, 11 aa in Mycobacterium tuberculosis and Mycobacterium leprae. B, the crystal structure of T. aquaticus RNAP (49) with relevant features labeled and the surface affected by β′Δ(1149–1190) colored magenta. C, a model of the bacterial TEC with the clamp domain rotated to match the position observed in the yeast RNAPII crystal structure (9). The downstream DNA duplex is numbered based on TEC convention, with +1 being the first base downstream of the active site (the 3′ nt is assumed to be in the NTP-binding or i+1 subsite of the active site; Refs. 3 and 9). This is equivalent to the +3 position in an open complex, which by convention is indexed with +1 in the priming or i subsite of the active site. D, close-up of the jaw domain with β′ 1149–1190 shown as a magenta worm and the location of Gly-1161 shown in green. E and F, surfaces of wild-type and β′Δ(1149–1190) RNAPs color-coded by surface charge potential (scheme shown below). The view is the same as in D; the DNA is rendered semitransparent.
indicated in figure legends when transcription is initiated in the absence of UTP, with ApU at 150 μM, ATP and GTP at 2.5 μM, [α-32P]CTP at 1 μM. Halted complexes were formed for 15 min at 37 °C and stored on ice prior to use.

**Elongation Rate Assays**—Halted A29 complexes were formed on a linear DNA template containing a portion of the rpoB gene (Table I). Transcription was restarted by addition of NTPs to 40 μM each, and heparin to 50 μg/ml. Samples were combined at times shown in the figures and after a final 5-min incubation with 250 μM each NTP (chase) with an equal volume of stop buffer and analyzed as described below.

**Single Round Pause Assays**—Halted complexes were formed as described above in 50 μl of transcription buffer. Reaction was re-started by addition of nucleotides (ATP, CTP, and UTP to 150 μM; GTP to 10 μM) and heparin to 50 μg/ml. Samples were combined at times shown in the figures and after a final 5-min incubation with 250 μM each NTP (chase) with an equal volume of stop buffer and analyzed as described below.

**Termination Assay**—Halted [α-32P]CTP-labeled A20 elongation complexes were prepared as above in 20 μl of transcription buffer with 40 nM linear DNA template and 50 nM RNAP holoenzymes containing σ20. Elongation was started by addition of NTPs to 400 μM each, KCl to 100 mM, and heparin to 25 μg/ml. Reactions were incubated at 37 °C for 15 min and stopped by addition of an equal volume of the stop buffer.

**RNA Analysis**—Samples were heated for 2 min at 90 °C and separated by electrophoresis in denaturing 5–15% polyacrylamide gels (19:1 acrylamide:bisacrylamide) in 7 M urea, 44 mM Tris borate, pH 8.3, 1.25 mM Na2EDTA). Gels were dried, and the RNA products were visualized and quantified using PhosphorImager screens and ImageQuant Software (Amersham Biosciences). Pause half-life (time during which half of the complexes re-enter the elongation pathway) and pause efficiency (fraction of transcribing RNAP molecules that pause) were determined by nonlinear regression analysis as described (25). Termination efficiencies were calculated as described by Reynolds et al. (15).

## RESULTS

**Jaw Mutant RNAPs Decrease Open Complex Longevity in Vitro**—We began by testing a simple explanation for the finding that RNAI1 activity decreases in the jaw deletion mutant, whereas P11 activity increases (17), namely that the jaw mutants reduce the kinetic stability of open initiation complexes. Although the detailed mechanism of open complex formation involves at least three steps and can vary among promoters (10), it can be generalized as an initial, RNAP concentration-dependent binding step to form closed complexes, which is rapid and characterized by an equilibrium constant $K_B$, and subsequent RNAP-concentration-independent isomerization steps to form open complexes, which are characterized by composite rate constants $k_2$ and $k_{−2}$ (Refs. 26 and 27; Reaction 1).

$$K_B = \frac{[\text{RNAP}][\text{TEC}]}{[\text{RNAP}]_0}$$

When $k_{−2}$ is fast relative to $k_2$ (making open complexes kinetically unstable), the overall rate of initiation is sensitive to changes in $k_{−2}$, whereas when $k_{−2}$ is slow relative to $k_2$ (making kinetically stable open complexes), the overall rate of initiation is insensitive to changes in $k_{−2}$, and can even increase in vivo as a result of indirect effects (19, 28, 29). This is true for two reasons. First, short-lived open complexes exist in a dynamic competition between collapse back to the closed complex
versus NTP binding and productive initiation. An increase in \(k_{-2}\) will shift the competition away from productive initiation at these promoters while having little effect on initiation at promoters for which open complexes are long-lived relative to the time required for NTP binding and productive initiation. Second, the majority of cellular transcription is mediated by kinetically unstable open complexes formed at rRNA promoters. Thus, factors that decrease transcription from rRNA promoters (e.g. by increasing \(k_{-2}\) generally) also indirectly increase initiation at promoters that form long-lived open complexes by increasing the pool of RNAP available for promoter binding in cells (28, 29). Because downstream DNA contacts are known to contribute to open complex longevity (19, 20) and because \(P_{rRNAI}\) is known to form kinetically stable open complexes by increasing the pool of RNAP available for promoter binding (Fig. 2). However, the persistence of open complexes also affected the half-life of open complexes formed at this promoter (Fig. 2). Therefore, factors that decrease transcription from rRNA promoters (e.g. by increasing \(k_{-2}\) generally) also indirectly increase \(P_{rRNAI}\) activity and indirectly increasing \(P_{rRNAI}\) activity.

To test this possibility, we examined the kinetic stability of open complexes formed at \(P_{rRNAI}, P_{rRNAII}\), and three additional, well-studied promoters: \(rrnBP1, lacUV5,\) and \(AP_{R}\). To measure open complex lifetimes, we incubated pre-formed open complexes with heparin, which acts as a competitor to trap free RNAP (31), and then added radiolabeled NTPs to samples taken at increasing times to measure the fraction of complexes still able to form a productive RNA transcript (Ref. 24; see “Experimental Procedures”). The mutant RNAPs exhibited dramatically decreased half-lives for \(P_{rRNAI}, rrnBP1, lacUV5,\) and \(AP_{R}\), as reflected by rapid disappearance of the ability to make RNA transcripts after addition of heparin (by factors of \(10^2\)–\(40\); Fig. 2 and Table II; note that the half-lives for different promoters cannot be compared with each other directly because the conditions differ among assays). In contrast, the longevity of open complexes formed at \(P_{rRNAI}\) was too long to measure, and thus we were unable to determine whether the mutants also affected the half-life of open complexes formed at this promoter (Fig. 2). However, the persistence of open complexes at \(P_{rRNAI}\) provides a useful control showing that the mutant RNAPs were fully active and did not yield inherently defective open complexes; rather, the relatively uniform \(10^2\)–\(40\)-fold effect on \(k_{-2}\) of the jaw mutants likely fails to increase \(k_{-2}\) at \(P_{rRNAI}\) to a range that can be measured.

We conclude that altering the downstream DNA jaw of RNAP dramatically reduces open complex longevity at several promoters. In principle, this could result either from a decrease in the thermodynamic stability of open complexes or a decrease in the activation barrier between open and closed complexes. The latter effect, however, would also increase the rate of open complex formation (\(k_2\)) and counteract reduced initiation caused by increasing \(k_{-2}\). Because the jaw alterations were found to decrease transcription from \(P_{rRNAI}\) in vivo (17), the most reasonable interpretation of our results is that the jaw domain of bacterial RNAPs makes a contact to downstream DNA that stabilizes the open complex.

**Jaw Mutant RNAPs Exhibit an Increased Elongation Rate**—It also is possible that the jaw mutants affect elongation by RNAP and that changes in transcript elongation cause or contribute to lowered ColEI plasmid copy number. Downstream DNA contacts are known to influence pausing by RNAP (11, 13, 32, 33) and the pattern or extent of pausing could influence folding or interaction of RNAI and RNAII (21), which is the central event in the inhibition of replication primer formation by RNAI (18). To ask how jaw alterations affect the activities of the TEC, we first tested their effects on the overall rate of transcript elongation. We formed initially halted TECs (at position 29 by withholding UTP during initiation) on a template that specified a fragment of the E. coli rpoB gene downstream from the hairpin (see “Experimental Procedures”). Upon adjustment of all four NTP concentrations to 40 \(\mu\)M each, wild-type and mutant RNAPs resumed elongation and formed a run-off transcript in less than 1 min. However, \(\beta'(1141–1190)\) RNAP reached the template end approximately twice as fast as wild type (half the TECs completed the run-off transcript in \(10\) s, rather than \(40\) s for wild type). \(\beta'GR1161\) exhibited a much less dramatic increase in elongation rate. The increased elongation rate of \(\beta'(1141–1190)\) RNAP was reflected in a decrease in pausing at several prominent sites within the rpoB gene (Fig. 3A, arrows). We concluded that the downstream jaw contact of bacterial RNAP is partially responsible for recognition of transcriptional pause sites. Thus, reduced pausing and consequent effects on RNA folding also might influence the competition between RNAI-RNAII interaction and replication primer formation in ColEI plasmids.

**Jaw Mutant RNAPs Were Defective in Two Types of Transcriptional Pausing**—To investigate the role of the jaw domain in transcriptional pausing further, we tested examples of two classes of pause signals: the class I his leader pause signal, for which escape from the paused state is inhibited by RNA secondary structure called a pause hairpin, and the class II pheP ops pause signal, for which escape from the paused state is inhibited by backtracking of RNAP along the RNA and DNA chains (34). Both types of pausing occur in a two-step mechanism: isomerization to the paused state in competition with

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**FIG. 2.** The jaw mutant RNAPs decrease the half-lives of open complexes in vitro. Half-lives were measured as described under “Experimental Procedures.” A, electrophoretic separation of RNAs formed on a supercoiled plasmid template containing \(P_{rRNAI}\) and \(P_{rRNAII}\) (Table I) by preformed open complexes at various times (given below each lane) after addition of heparin to 10 \(\mu\)g/ml. B–E, plots of open complex remaining as a function of time for \(P_{rRNAI}, rrnBP1, lacUV5,\) and \(AP_{R}\) promoters, respectively.
sequence of the downstream DNA.  

We found that deletion of the β′ jaw decreased pausing at both classes of pause signals, whereas the G1161R substitution had less effect (Figs. 4 and 5). Interestingly, the deletion decreased both the efficiency and half-life of pausing at both classes of pause signals. However, the magnitude of these effects differed. Whereas the deletion decreased half-life and efficiency by factors of 3 and 4, respectively, at the class II ops signal, it decreased half-life by a factor of 6 at the class I his pause signal while decreasing efficiency by only a factor of 1.5. We conclude that the β′ jaw plays a positive role in recognition of both classes of pause signal and in stabilizing both types of paused conformation, most likely through an interaction with the downstream DNA duplex. This is consistent with current models of transcriptional pausing (see “Discussion”) and with the possibility that changes in RNAI-RNAII interaction mediated by transcriptional pausing could contribute to the plasmid copy number phenotype of the mutant RNAPs.

Pauing also is an initial step in transcriptional termination at ρ-independent terminators (37, 38). To ask whether the β′Δ(1149–1190) RNAP would manifest a defect in transcriptional termination as a result of its defect in pausing, we tested the effect of the deletion on readthrough of the T7 terminator, which has previously been found to require a downstream DNA interaction for efficient termination in vitro (12). We found that the deletion increased readthrough of the T7 terminator from 10 to 20%, relative to wild type, under transcription conditions similar to those of Figs. 4 and 5 (data not shown; see “Experimental Procedures”).

Jaw Mutant RNAPs Exhibit Lesser Effects on TEC Stability and Abortive Initiation—A possible explanation for some of the in vitro phenotypes we observed for the jaw deletion RNAP would be a change in the affinity of RNAP for the nucleic acid scaffold (although decreased termination would suggest an increased affinity, whereas decreased open complex longevity would suggest a decreased affinity). To test directly for simple changes in affinity for either the DNA or RNA, we examined the effect of the deletion on the thermal stability of TEC and on abortive initiation. If the jaw deletion RNAP lost affinity for the nucleic acid scaffold in a TEC, we would expect an increase in the rate of thermal inactivation of TECs. If the jaw deletion RNAP lost affinity for the RNA product, we would expect an increase in the rate of abortive initiation.

We tested the stability of TECs, by making halted A29 complexes on the his pause template (shown in Fig. 4) and challenging them with high salt (1 M KCl) and high temperature (45 °C). Both the jaw deletion RNAP and β′G1161R RNAP exhibited a slightly shorter TEC half-life compared with the wild type (reduced by a factor of 1.5 at most; Fig. 6). This TEC inactivation could result either from dissociation of the TEC or from backtracking into an arrested state (35, 39, 40). In either case, the result suggests only a minor thermal instability of the mutant RNAPs themselves or of their interactions with the

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3 M. Palangat, personal communication.
nucleic-acid scaffold, but not of the magnitude that could explain their effects on open complex longevity or transcriptional pausing.

We compared the ratio of abortive to productive RNA products during A29 complex formation in experiments such as those shown in Figs. 4 and 5. We found that neither RNAP exhibited an altered abortive/productive RNA ratio (data not shown), suggesting that the jaw does not influence the affinity of RNAP for the RNA product, consistent with its predicted interaction exclusively with the downstream DNA in transcription complexes.

We conclude that the jaw contributes slightly to the stability of TECs through interactions with DNA. However, the major effects that the jaw deletion manifests on open complex longevity and transcriptional pausing do not appear to be simple consequences of weakened affinity for the nucleic acid scaffold in transcription complexes. Rather, the contact of the jaw to the downstream DNA is likely to affect the rates of kinetic transitions in transcription complexes that are important for open complex longevity and pausing during transcript elongation.

**DISCUSSION**

Our chief conclusions are that the β′ jaw of bacterial RNAP plays a significant role in both open complex longevity and in transcriptional pausing. These findings are consistent with recent ideas about the role of the downstream DNA duplex in these processes, as well as molecular modeling that suggests the downstream DNA interacts with the β′ jaw in both open complexes and TECs.

**Interaction of the Downstream DNA Duplex with the β′ Jaw of RNAP**—The path of the downstream DNA duplex in both open initiation complexes and TECs formed by *E. coli* RNAP has been modeled based on cross-linking and fluorescence resonance energy transfer analyses of the position of the phosphate backbone relative to segments of the β and β′ subunits (41–43). These models offer a generally consistent picture in which duplex DNA enters RNAP between flexible regions of the lobe and clamp in a trough formed mostly by (see Introduction and Fig. 1 (B and C)). This positioning of the downstream DNA duplex also is consistent with the weak electron density observed for the downstream DNA duplex in the x-ray diffraction pattern of an RNAPII TEC crystal (9).

The jaw deletion removes a segment of β′ near the DNA

**Fig. 4. Pausing at the his pause site.** A, schematic of his pause template showing sequence in region of the his pause (34). B, electrophoretic separation of RNAs formed on the his pause template as a function of time (shown below each lane). Preformed [α-32P]CMP-labeled A29 complexes were incubated with 10 μM GTP, 150 μM ATP, CTP, and UTP on the his pause template. The chase lanes contain samples that were incubated for an additional 5 min with 250 μM each NTP after completion of the time course. Prominent transcripts are hisP (pause RNA transcript; 71 nt) and RO (run-off RNA transcript). The fractions of paused RNA were plotted against time; pause half-lives and pause efficiencies were determined as described under “Experimental Procedures” (25).

**Fig. 5. Pausing at the ops pause site.** A, schematic of ops pause template showing sequence in region of the opspause (34). Underlined sequence, ops pause signal. B, electrophoretic separation of RNAs formed on the ops pause template as a function of time (shown below each lane). Preformed [α-32P]CMP-labeled A29 complexes were allowed to elongate at 10 μM GTP, 150 μM ATP, CTP, and UTP. The chase lanes contain samples that were incubated for an additional 5 min with 250 μM each NTP after completion of the time course. Prominent transcripts are opsP (pause RNA transcript; 62 and 64 nt) and RO (run-off RNA transcript). The fractions of paused RNA were plotted against time; pause half-lives and pause efficiencies for the major pause site (64 nt) were determined as described under “Experimental Procedures” (25).
makes sequence-nonspecific, hydrophobic contacts to bases in the downstream duplex, as proposed for the more extensive jaw region of RNAPII (7).

Role of Downstream DNA Interaction in Open Complex Longevity—The conversion of bacterial RNAP from a closed to an open initiation complex is accompanied by extension of its DNA contacts from +1 to +20, a transition proposed to correspond to insertion of the downstream DNA duplex into the DNA entry channel and concomitant unwinding of the duplex in the vicinity of the active site (4, 10). Once the open complex has formed, the downstream duplex lies near the β’ jaw as described in the preceding section (41, 43). Either disruption of the jaw structure by the G1161R substitution or removal of the jaw by the (β’1149–1190) deletion appears to reduce the stability of the open complexes, so that the downstream duplex more readily falls out of the entry channel.

The importance of the jaw-DNA contact for transcriptional regulation is underscored by its apparent targeting by the T7 phage transcriptional inhibitor gp2. Substitutions in the jaw (E1158K and E1188K) prevent binding by gp2, as does β’Δ(1148–1198) (46). gp2 blocks open complex formation by E797 holoenzyme at −35-element-dependent promoters, but not at −35-element-independent promoters (so-called extended −10 promoters). This led Nechaev and Severinov (46) to propose that gp2 directly or indirectly blocks binding of sigma region 4.2 to the −35 promoter element. Our finding that the jaw region contributes to open complex longevity suggests an alternative interpretation for the effect of gp2 on −35-dependent promoters. Promoters dependent on the −35 sequence may be poised near a balance point of open complex stability because they lack the stabilizing interaction of sigma region 3.0 with the extended −10 sequence (5, 47). In the equilibrium between open complexes with DNA bound in the downstream channel versus closed complexes with gp2 bound in the channel, promoters lacking the extended −10 may lack the energy required to displace gp2 and form open complexes. Thus, gp2 binding to the jaw may simply tip the scales against a near even balance of open complex versus closed complex at −35-element-dependent promoters, but have little effect on extended −10 promoters where open complex formation is more strongly favored.

It is unclear whether the decrease in longevity of PRNAI open complexes alone is sufficient to account for the entire −4-fold shift in PRNAI/PRNAII activity caused by the jaw alterations in vivo (17). The PRNRAI k−2 observed for β’Δ(1141–1190) RNAP (−10−2 s−1) is still at least 300 times slower than the wild-type k−2 for rrnB P1 (Table II), at which a 4–30-fold increase in k−2 abrogates growth rate control of rrnB P1 initiation that is mediated by short-lived open complexes (48). It also is too slow to be on the same time scale of initiation, which would preclude direct effects of short-lived open complexes on RNAII synthesis. It is possible that other factors in vivo decrease the longevity of PRNRAI open complexes to this time scale or that principal effects of the jaw alterations on the more short-lived rrn open complexes affect RNAI/RNAII indirectly by increasing the concentration of free RNAP in the cell (19, 28). It is also possible that the loss of jaw-downstream DNA interaction affects steps in open complex formation other than just open complex longevity. Further work will be necessary to assign the cause of altered RNAI/RNAII transcription definitively; our primary finding here is that the jaw-DNA contact is an important contributor to open complex longevity.

Role of Downstream DNA Interaction in Transcriptional Pausing—We found that alterations to the β’ jaw suppressed both recognition of and escape from two different classes of pause signals by RNA polymerase (Figs. 5 and 6). Current
models for transcriptional pausing posit an initial isomerization to an unactivated state that competes with nucleotide addition at the pause site (34, 36). This unactivated (or initially paused) state can be stabilized by interactions that slow re- establishment of the reactive alignment of nucleotides in the active site, including interaction of an RNA secondary structure with the RNA exit channel of RNAP (class I pause), or backtracking of the unactivated TEC along the RNA and DNA chains (class II pause). Our current findings strongly suggest that interactions of downstream DNA with the jaw may both stabilize the paused RNAP against reformation of the active state (as reflected in the effect on pause half-life), and promote isomerization to the initially paused (unactivated) intermediate (as reflected by the effect on pause efficiency), thereby affecting both classes of transcriptional pausing.

How the downstream DNA exerts its effect on pausing (as well as on termination and arrest) remains an important question. The effect on pausing is known to depend on a characteristic of the downstream duplex other than its ease of melting and to extend to ~15 bp downstream of the pause site (12, 13). However, the precise sequence determinants of this effect and the positions within the ~15 bp downstream DNA duplex that are critical for it have not been elucidated. Our current results add three important pieces of information to this subject, which nevertheless will require additional study for a full understanding. First, at least part of the downstream DNA effect is mediated by contacts of the +5 to +15 region to the jaw domain. Second, the contribution of this downstream DNA contact to pausing may be partly sterical, rather than dependent on side-chain base-interactions, because removal of the jaw by βΔ(1149–1190) suppressed pausing more effectively than disruption of its structure by βG1161R (in contrast to their effects on open complex longevity). If the effect of downstream DNA on pausing depends on its structural distortion, for instance bending, as was suggested for its effects on termination and arrest (12, 14), then the absence of the jaw in the deletion may interfere with this effect more than does the disrupted jaw structure of the G1161R substitution. Third, the downstream contact (at least in the +5 to +15 region) appears to favor pausing rather than elongation because removal of the contact in the jaw deletion RNAP suppresses pausing, the opposite of what would be expected if pausing was accompanied by loss of a downstream contact that ordinarily favored nucleotide addition.

Conclusion—Taken together, our results define an important component of the network of RNAP-nucleic acid interactions that regulates transcription: the jaw-downstream DNA contact. Effects of this contact on both initiation and elongation may contribute to the reduction in plasmid copy number caused by βΔ(1149–1190) than βG1161R. The effects on initiation are especially interesting because altered transcription as a consequence of destabilizing open complexes is the proposed mechanism of the bacterial stringent response. In that case, ppGpp, by destabilizing open complexes, redistributes RNAP from promoters that form short-lived open complexes to promoters that form long-lived open complexes (19, 28, 29). Interestingly, however, neither βΔ(1149–1190) nor βG1161R suppress the multiple amino acid auxotrophy of so-called ppGpp strains,4 which is a hallmark of previously characterized mutant RNAPs that decrease open complex half-life (20). Future studies will address whether the sensitive plasmid copy number selection used to isolate βΔ(1149–1190) and βG1161R yielded mutants with weaker effects, or whether the role of the β jaw-downstream DNA interaction in open complex formation exhibits special features.

4 J. Ederth, unpublished results.