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The Downstream DNA Jaw of Bacterial RNA Polymerase Facilitates Both Transcriptional Initiation and Pausing*

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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 (Δ1149–1190) 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)[1] 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 similar 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 (Δ1149–1190) 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 transcription initiation and elongation *in vitro* using assays that reveal separate steps in the transcription cycle.

EXPERIMENTAL PROCEDURES

Reagents and Proteins—Oligonucleotides were obtained from Operon Technologies (Alameda, CA); NTPs from Amersham Biosciences; [α-^{32}P]CTP and [α-^{32}P]UTP from PerkinElmer Life Sciences; and other chemicals from Sigma. Wild-type and mutant RNAPs were purified by chitin-affinity chromatography and intein-mediated removal of the

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[1] The abbreviations used are: RNAP, RNA polymerase; TEC, transcription elongation complex; nt, nucleotide(s); aa, amino acid(s).

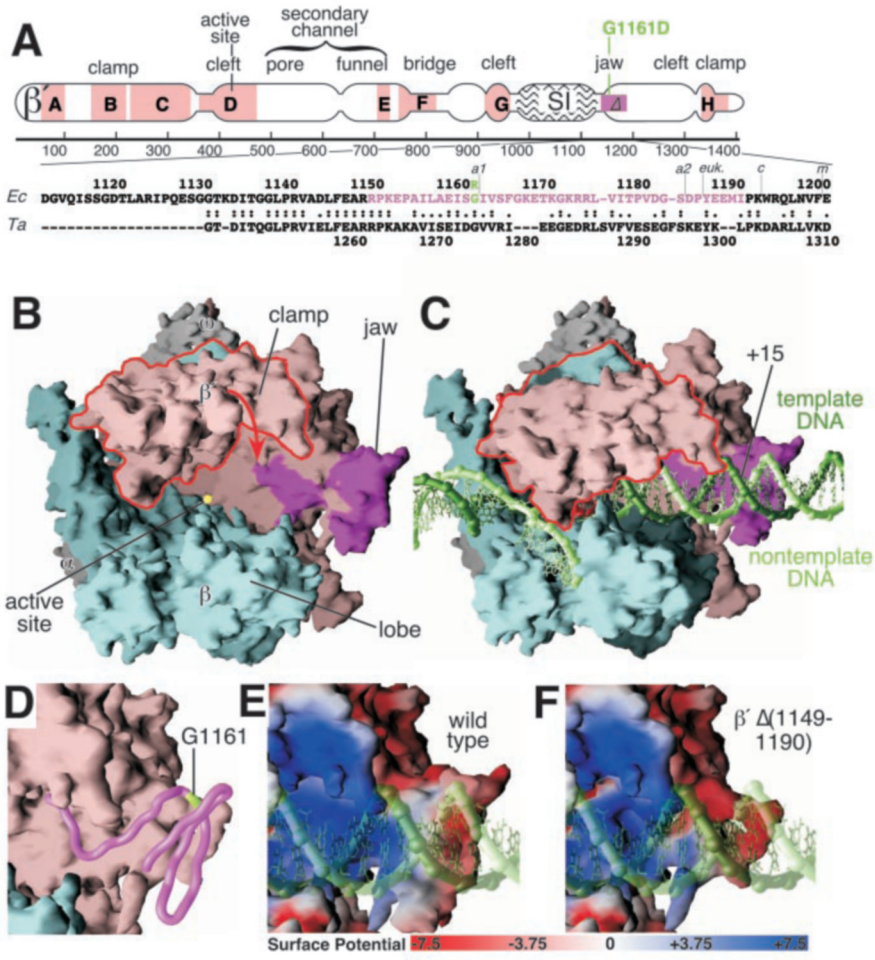

FIG. 1. **Location of β′ alterations relative to RNAP structure and downstream DNA duplex.** *A*, location of β′G1161R and β′Δ(1149–1190). Conserved regions of β′ are shown in *pink* (*A–H*), with its structural domains labeled (8, 49). *SI* indicates the position of a 187-aa sequence insertion in *E. 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 *Aquafex 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.

chitin-binding domain tag after overexpression from a T7 RNAP-based expression plasmid, as described elsewhere.[2] To obtain overexpression plasmids encoding β′G1161R and β′Δ(1149–1190), *rpoC* fragments containing these mutations were PCR-amplified from strains JE1134 or JE221 (17) using oligonucleotides 101E and 102E (Table I), digested with *Sal*I and *Bsp*EI, and then ligated into *Sal*I, *Bsp*EI-cut pRL663, which expresses C-terminally His_6-tagged β′ from a *lacI*-regulated *trc* promoter (22). The mutant *rpoC* genes were then transferred to the RNAP overexpression plasmid pIA423[2] on a *Bsm*I to *Xho*I fragment that is unique in both plasmids. Wild-type and mutant RNAP holoenzymes (core $\beta'\beta\alpha_2$ plus σ^{70}) were prepared by incubating a 5-fold molar excess of σ^{70} with core enzyme for 30 min at 30 °C. σ^{70} was purified as described previously (23).

DNA Templates—DNA templates were either PCR products amplified from plasmid DNA or supercoiled plasmid DNA, as described in the figure legends (plasmids, PCR primers, and templates are listed in Table I). Linear templates for *in vitro* transcription were generated by PCR amplification and purified using QIAspin PCR purification reagents (Qiagen, Valencia, CA). Supercoiled plasmids were isolated using the Qiagen midiprep reagents. DNA templates for open complex stability measurements (plasmids and primers for amplification of linear templates) were kindly provided by M. Barker and R. Gourse (University of Wisconsin, Madison).

Lifetimes of Open Complexes—Lifetimes of open complexes were measured on supercoiled plasmid DNA (*rrnB* P1 and RNAII) or linear DNA templates (*lac*UV5 and λP_R) using single-round transcription assays (24). RNAP and template DNA were incubated ~15 min at 30 °C in initiation buffer (40 mM Tris·HCl, pH 8.0, 10 mM $MgCl_2$, and 1 mM dithiothreitol) with different concentrations of NaCl (*rrnB* P1, 7.5 mM; RNAII, 35 mM; *lac*UV5, 100 mM; λP_R, 150 mM). Aliquots (10 μl) were removed to a tube containing 1.5 μl of NTPs to yield final concentrations of 200 μM ATP (1 mM ATP for the *rrnB* P1 promoter), 200 μM GTP, 200 μM CTP, and 10 μM [α-^{32}P]UTP (50 Ci/mmol) at different times after heparin addition (10 μg/ml final concentration). Transcription was stopped after 10 min by addition of an equal volume of formamide loading buffer, and RNA samples were analyzed as described below.

Halted Complex Formation—Elongation complexes were formed with 40 nM linear DNA template and 50 nM RNAP holoenzyme in 20–100 μl of transcription buffer (20 mM Tris·HCl, 20 mM NaCl, 3 mM $MgCl_2$, 14 mM 2-mercaptoethanol, 0.1 mM EDTA, pH 7.9). On the T7 A1 promoter templates, elongation complexes were halted at positions

[2] I. Artsimovitch, V. Svetlov, K. S. Murakami, and R. Landick, manuscript in preparation.

TABLE I
Plasmids, DNA primers, and transcription templates

Name	Description	Source or note
Plasmids		
pRLG770	General transcription vector	(50)
pRLG6555	pRLG770 containing *rrnB* P1 (−66+9)	(47)
pRLG3422	pRLG770 *lac* UV5 (−46+1)	R. Gourse
pRLG3265	pRLG770 RNAI (−61+1)	R. Gourse
pRLG934	pRLG770 RNAII (−150+50)	R. Gourse
pIA146	T7 A1 promoter-A29-*rpoB* transcription template plasmid; nt 223–3958 of *rpoB* inserted at *Spe*I site 22 nt after A29 in pCL102b (51)	This work
pIA171	T7 A1 promoter-A29-*his* pause transcription template plasmid; *Spe*I to *Age*I of pCL102b (51) replaced with *Spe*I to *Age*I pCL185 (33)	This work
pIA251	T7 A1 promoter-A29 *pheP ops* pause transcription template plasmid	(35)
pIA228	λP_R-A26-*his* pause-transcription template plasmid	This work
pIA263	λP_R-A26-T7 terminator transcription template plasmid; PCR fragment containing T7 terminator from pAR1707 (52) inserted between *Spe*I and *Bgl*II of pIA228	This work
Primers		
101E	5′-ATTCTCGCTCAAACAGGTCACTGC	*rpoC* upstream
102E	5′-AATGCTCTTTCCCTAAACTCCCCC	*rpoC* downstream
021	5′-GTCTGTCTCTGGGCGATCTG	*rpoB* internal
645	5′-CAGTTCCCTACTCTCGCATG	T7 A1 downstream
947	5′-GGAGAGACAACTTAAAGAG	T7 A1 upstream
1454	5′-GGCTTTCTCATGCGTTCATG	λP_R downstream
3071	5′-CGTTAAATCTATCACCGCAAGGG	λP_R upstream
3073G	5′-GTTTATGGCGGGCGTCCTG	p770 downstream
3158G	5′-CGAAAAGTGCCACCTGACG	p770 upstream
Transcription templates		
P_{RNAI}	pRLG934	
P_{RNAII}	pRLG934	
rrnB P1	pRLG6555	
*lac*UV5	PCR of pRLG3422 with 3158G and 3073G	
λP_R	PCR of pIA228 with 3071 and 1457	
rpoB′	PCR of pIA146 with 947 and 021	
his pause	PCR of pIA171 with 947 and 645	
ops pause	PCR of pIA251 with 947 and 645	
T7 terminator	PCR of pIA263 with 3071 and 1454	

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-^{32}P]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 5, 10, 15, 20, 30, 45, 60, 90, 120, 240, 480, and 600 s with an equal volume of stop buffer (10 M urea, 98 mM Tris borate, pH 8.3, 25 mM Na_2EDTA, 0.05% bromphenol blue) and analyzed as described below.

Single Round Pause Assays—Halted complexes were formed as described above in 50 μl of transcription buffer. Transcription was restarted 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.

Elongation Complex Stability Assays—A29 complexes were formed on a linear DNA template (PCR-amplified fragment of pIA171) for 15 min at 37 °C. At time 0, KCl was added to a final concentration of 1 M, and samples were shifted to 45 °C to facilitate dissociation of the TECs. At increasing times (see Fig. 6), aliquots of the TEC were removed to 37 °C and transcription was restarted by addition of NTPs to 250 μM and heparin to 50 μg/ml. Following a 5-min incubation, reactions were quenched by the addition of an equal volume of stop buffer and analyzed as described below.

Termination Assays—Halted [α-^{32}P]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 σ^{70}. 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 Na_2EDTA). 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 RNAII promoter (P_{RNAII}) activity decreases in the jaw deletion mutant, whereas P_{RNAI} 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).

$$\mathrm{RNAP} + \mathrm{P} \overset{K_B}{\rightleftharpoons} \mathrm{RP_c} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{RP_o} \xrightarrow{+\,NTP} TEC$$

REACTION 1

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_{RNAI} is known to form kinetically stable open complexes (24, 28–30), it is plausible that the RNAP jaw alterations could reduce plasmid copy number by directly reducing P_{RNAII} activity and indirectly increasing P_{RNAI} activity.

To test this possibility, we examined the kinetic stability of open complexes formed at P_{RNAI}, P_{RNAII}, and three additional, well studied promoters: *rrnB* P1, *lac* UV5, and λP_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_{RNAII}, *rrnB* P1, *lac* UV5, and λP_R, as reflected by rapid disappearance of the ability to make RNA transcripts after addition of heparin (by factors of 10–40; 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_{RNAI} 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_{RNAI} provides a useful control showing that the mutant RNAPs were fully active and did not yield inherently defective open complexes; rather, the relatively uniform 10–40-fold effect on k_{-2} of the jaw mutants likely fails to increase k_{-2} at P_{RNAI} 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_{RNAII} *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 ColE1 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

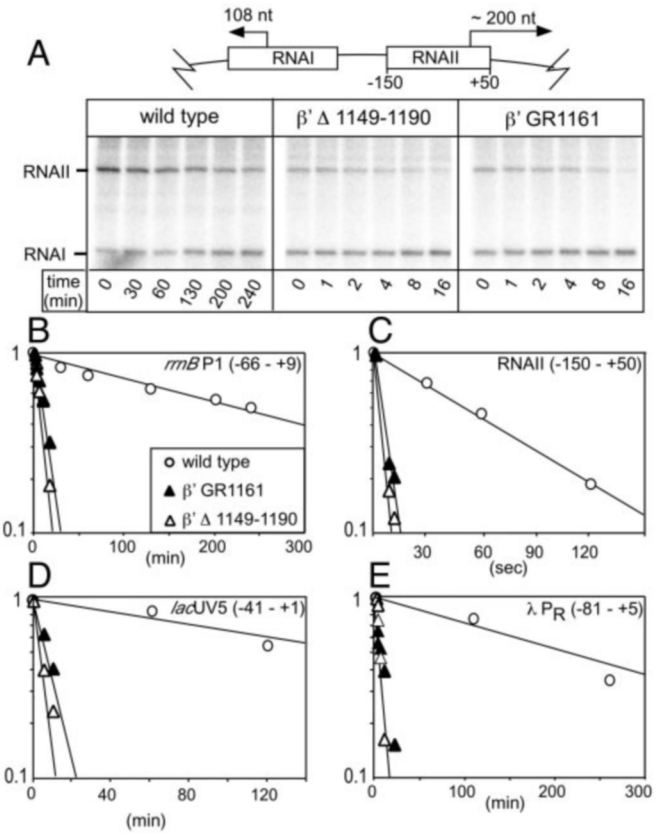

FIG. 2. **The jaw mutant RNAPs decrease the half-lives of open complexes *in vitro*.** Half-lives were measured as described under "Experimental Procedures." *A*, electrophoretic separation of RNAs formed on a supercoiled plasmid template containing P_{RNAI} and P_{RNAII} (Table I) by preformed open complexes at various times (given *below* each *lane*) after addition of heparin to 10 μg/ml. *B–E*, plots of open complex remaining as a function of time for P_{RNAII}, *rrnB* P1, *lac*UV5, and λP_R promoters, respectively.

(at position 29 by withholding UTP during initiation) on a template that specified a fragment of the *E. coli rpoB* gene downstream from the halt site (see "Experimental Procedures"). Upon adjustment of all four NTP concentrations to 40 μM each, wild-type and mutant RNAPs resumed elongation and formed a run-off transcript in less than 1 min. However, $\beta'\Delta$(1141–1190) RNAP reached the template end approximately twice as fast as wild type (half the TECs completed the run-off transcript in ~20 s, rather than ~40 s for wild type). β'GR1161 exhibited a much less dramatic increase in elongation rate. The increased elongation rate of β'(1141–1190) RNAP was reflected in a decrease in pausing at several prominent sites within the *rpoB* gene (Fig. 3*A*, *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

TABLE II
Half-lives of open complexes formed by wild-type and mutant RNAPs

Promoter	Conditions[a]	RP_o $t_{1/2}$		
		Wild type	β′Δ(1149–1190)	β′G1161R
			min	
rrnB P1 (+66−9)	7.5 mM, SC	0.8 ± 0.07	0.05 ± .01	0.07 ± 0.003
RNA II (+150−50)	35 mM, SC	260 ± 8	8 ± 2	10[b]
lacUV5 (−46+1)	100 mM, linear	130 ± 11	7 ± 2	4 ± 1
λP_R	150 mM, linear	270[b]	7[b]	7[b]

[a] Concentrations given are of NaCl in the initiation reactions. SC, supercoiled plasmid template; linear, linear DNA template.
[b] Single measurement.

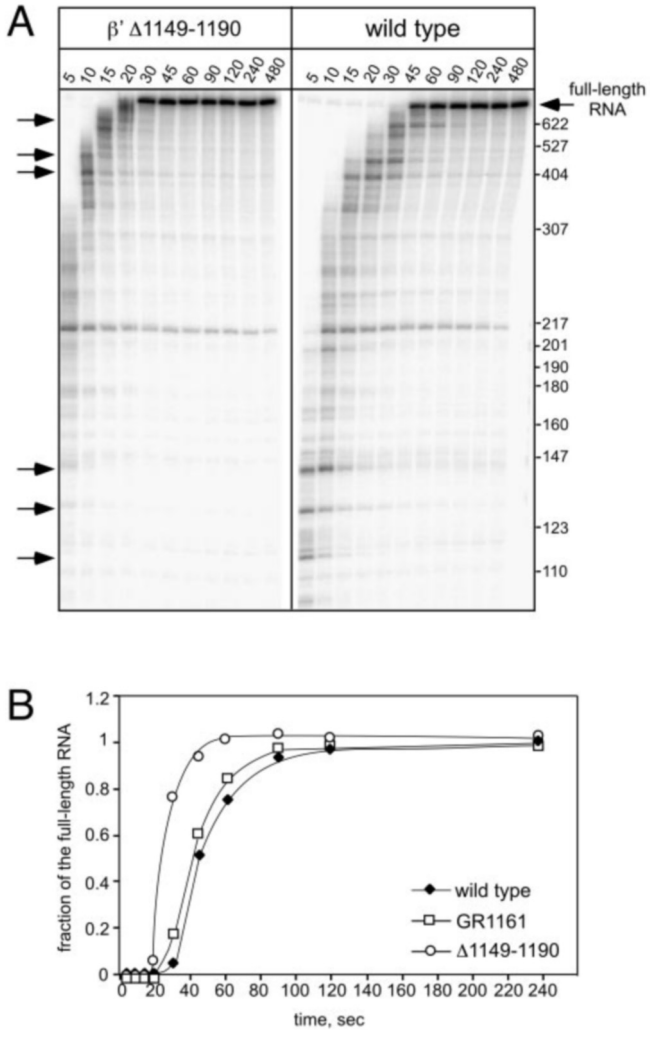

FIG. 3. **Elongation rates of the jaw mutant RNAPs.** *A*, electrophoretic separation of RNAs formed on the *rpoB* template as a function of time (shown *above* each *lane*; see "Experimental Procedures"). Prominent pause sites are indicated by *arrows*. *B*, fraction of the full-length RNA as a function of time. *WT*, wild type.

elongation past the site, followed by slow escape from the paused state. For the class I *his* pause, the downstream DNA sequence, as well as the RNA:DNA hybrid region and the bases in the active site, influence both steps (22); these three components plus the pause hairpin additively delay escape from the pause state by nucleotide addition (32, 33). The determinants of the class II *ops* pause have not been analyzed. However, by analogy to the class II human immunodeficiency virus type 1 pause signal that affects human RNAPII, they include the thermodynamic stability of the RNA:DNA hybrid in the active conformation of the TEC, the hybrid stability in the backtracked TEC that forms at the pause site (35, 36), and the sequence of the downstream DNA.[3]

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 signal. 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.

Pausing 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

[3] M. Palangat, personal communication.

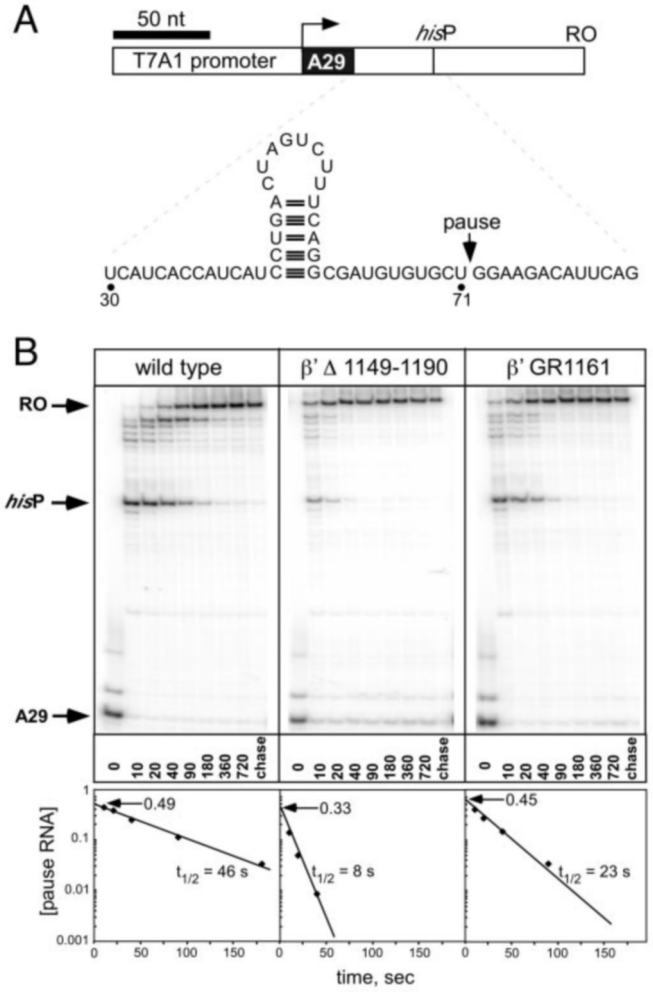

FIG. 4. **Pausing at the *his* pause site.** *A*, schematic of *his* pause template showing sequence in region of the *his* pause (34). *B*, electrophoretic separation of RNAs formed on the *his* pause template as a function of time (shown *below* each *lane*). Preformed [α-^{32}P]CMP-labeled A29 complexes 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).

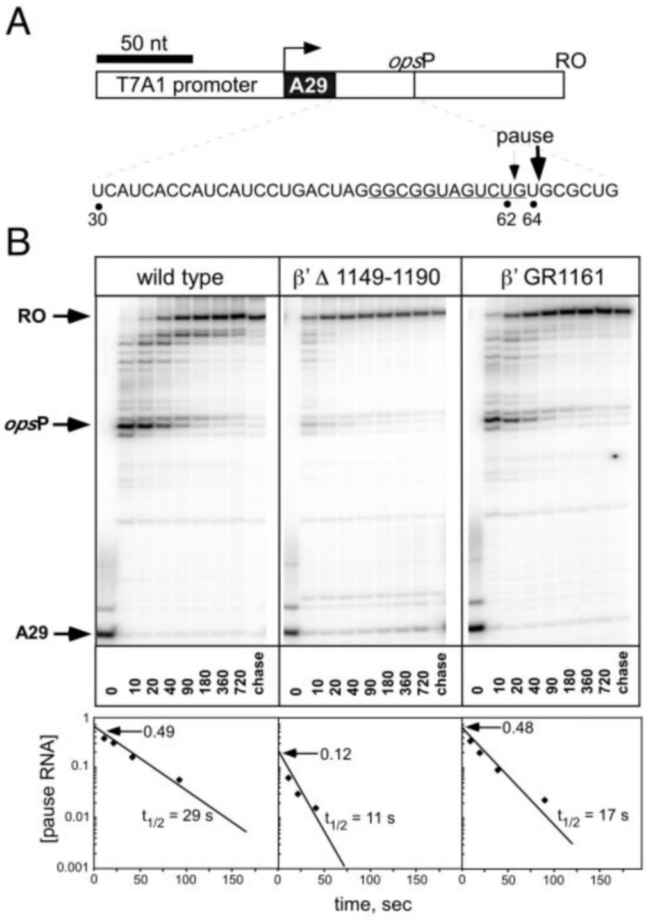

FIG. 5. **Pausing at the *ops* pause site.** *A*, schematic of *ops* pause template showing sequence in region of the *ops* pause (34). *Underlined* sequence, *ops* pause signal. *B*, electrophoretic separation of RNAs formed on the *ops* pause template as a function of time (shown *below* each *lane*). Preformed [α-^{32}P]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).

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 β′ 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 β′ 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 β and β′ termed the lobe and clamp in a trough formed mostly by β′ (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

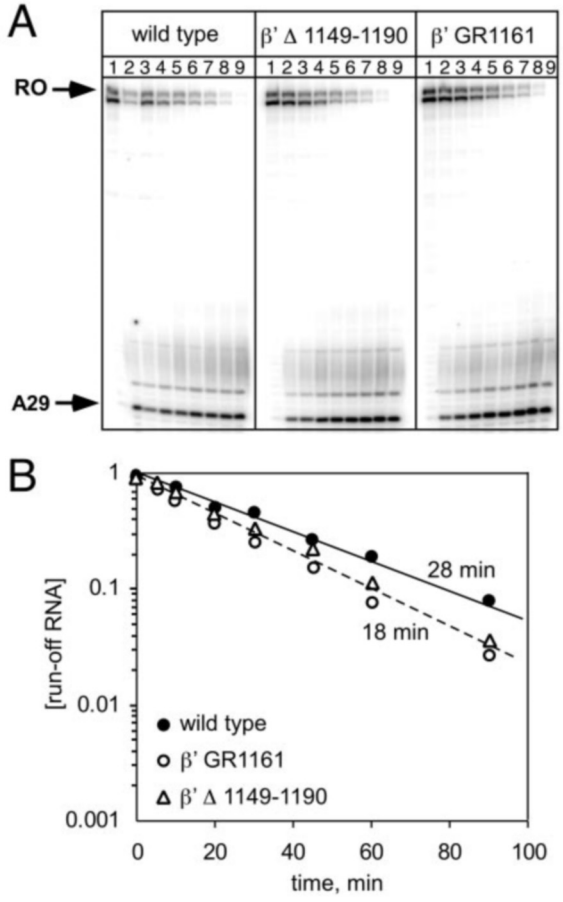

FIG. 6. **TEC stability of jaw mutant RNAPs.** Preformed A29 complexes were challenged with high salt (1 M KCl) and high temperature (45 °C) for increasing times and then incubated with NTPs (250 μM) to determine the fraction of active TECs remaining (see "Experimental Procedures"). *A*, electrophoretic separation of RNAs in wild-type, $\beta'\Delta$(1149–1190), and β'GR1161 TECs after stability challenge for 0, 5, 10, 20, 30, 45, 60, 90, and 120 min (*lanes 1–9* for each TEC, respectively). *RO*, run-off RNA formed when active TECs transcribed to the template end. *B*, amount of active TEC remaining as a function of time as determined from the run-off RNA in *A*. The 5-min sample for wild-type TECs was omitted because of a gel loading error. TEC half-lives are given on the plot and were indistinguishable for the $\beta'\Delta$(1149–1190) and β'GR1161 TECs.

duplex from ~10 to ~20 bp downstream of the active site, with the jaw itself being closest to a segment from +15 to +20 (Fig. 1, *C* and *D*). The sequence in this region folds into a three-stranded antiparallel β-sheet that cross-links to the nontemplate DNA strand near +15 (3), is variable in sequence among bacterial RNAPs (Fig. 1*A*), and lies near an insertion of 187 aa that is present only in some bacteria (44). Gly-1161, however, is invariant among bacterial RNAPs and occurs at a turn leading to the first β strand. Given this high conservation and the known structural role of Gly residues in protein loops (45), we postulate that the G1161R substitution disrupts the structure of the bacterial jaw. The region removed by the jaw deletion is nearly neutral (7 each acidic and basic residues in the *E. coli* β'(1149–1190); 9 acidic and 7 basic residues in the corresponding *T. aquaticus* segment depicted in Fig. 1 (*B–F*)). However, the basic residues mostly line the DNA entry channel whereas the acidic residues mostly face away from the nucleic acid path, yielding a net positive charge in part of the channel affected by the deletion (Fig. 1, *D* and *E*). The deletion may remove principally ionic contacts to the DNA phosphodiester backbone in the +5 to +15 region (Fig. 1*F*). However, we cannot exclude the possibility that this segment of the DNA entry channel also 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 $\beta'\Delta$(1148–1198) (46). gp2 blocks open complex formation by Eσ^{70} 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 P_{RNAII} open complexes alone is sufficient to account for the entire ~4-fold shift in P_{RNAI}/P_{RNAII} activity caused by the jaw alterations *in vivo* (17). The P_{RNAII} k_{-2} observed for $\beta'\Delta$(1141–1190) RNAP ($\sim 10^{-2}$ 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 P_{RNAII} 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 reestablishment 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 (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 steric, rather than dependent on side chain-base interactions, because removal of the jaw by $\beta'\Delta$(1149–1190) suppressed pausing more effectively than disruption of its structure by β'G1161R (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 $\beta'\Delta$(1149–1190) than β'G1161R. 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 $\beta'\Delta$(1149–1190) nor β'G1161R suppress the multiple amino acid auxotrophy of so-called ppGpp^0 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 $\beta'\Delta$(1149–1190) and β'G1161R 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.

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REFERENCES

1. Craig, M. L., Tsodikov, O. V., McQuade, K. L., Schlax, P. E., Jr., Capp, M. W., Saecker, R. M., and Record, M. T., Jr. (1998) *J. Mol. Biol.* **283,** 741–756
2. Darst, S. A., Opalka, N., Chacon, P., Polyakov, A., Richter, C., Zhang, G., and Wriggers, W. (2002) *Proc. Natl. Acad. Sci. U. S. A.* **99,** 4296–4301
3. Korzheva, N., Mustaev, A., Kozlov, M., Malhotra, A., Nikiforov, V., Goldfarb, A., and Darst, S. A. (2000) *Science* **289,** 619–625
4. Murakami, K. S., Masuda, S., Campbell, E. A., Muzzin, O., and Darst, S. (2002) *Science* **296,** 1285–1290
5. Murakami, K. S., Masuda, S., and Darst, S. (2002) *Science* **296,** 1280–1284
6. Landick, R. (2001) *Cell* **105,** 567–570
7. Cramer, P., Bushnell, D., Fu, J., Gnatt, A., Maier-Davis, B., Thompson, N., Burgess, R., Edwards, A., David, P., and Kornberg, R. (2000) *Science* **288,** 640–649
8. Cramer, P., Bushnell, D., and Kornberg, R. (2001) *Science* **292,** 1863–1876
9. Gnatt, A., Cramer, P., Fu, J., Bushnell, D., and Kornberg, R. D. (2001) *Science* **292,** 1876–1882
10. Saecker, R., Tsodikov, O., McQuade, K., Schlax, P., Jr., Capp, M., and Record, M., Jr. (2002) *J. Mol. Biol.* **319,** 649–671
11. LaFlamme, S. E., Kramer, F. R., and Mills, D. R. (1985) *Nucleic Acids Res.* **13,** 8425–8440
12. Telesnitsky, A., and Chamberlin, M. (1989) *Biochemistry* **28,** 5210–5218
13. Lee, D. N., Phung, L., Stewart, J., and Landick, R. (1990) *J. Biol. Chem.* **265,** 15145–15153
14. Kerppola, T. K., and Kane, C. M. (1990) *Biochemistry* **29,** 269–278
15. Reynolds, R., Bermúdez-Cruz, R. M., and Chamberlin, M. J. (1992) *J. Mol. Biol.* **224,** 31–51
16. Keene, R. G., Mueller, A., Landick, R., and London, L. (1999) *Nucleic Acids Res.* **27,** 3173–3182
17. Ederth, J., Isaksson, L., and Abdulkarim, F. (2002) *Mol. Genet. Genomics* **267,** 587–592
18. Tomizawa, J. (1990) *J. Mol. Biol.* **212,** 683–694
19. Barker, M. M., Gaal, T., and Gourse, R. L. (2001) *J. Mol. Biol.* **305,** 689–702
20. Bartlett, M. S., Gaal, T., Ross, W., and Gourse, R. L. (1998) *J. Mol. Biol.* **279,** 331–345
21. Pan, T., Artsimovitch, I., Fang, X. W., Landick, R., and Sosnick, T. R. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 9545–9550
22. Wang, D., Meier, T., Chan, C., Feng, G., Lee, D., and Landick, R. (1995) *Cell* **81,** 341–350
23. Gribskov, M., and Burgess, R. R. (1983) *Gene* (*Amst.*) **26,** 109–118
24. Leirmo, S., and Gourse, R. L. (1991) *J. Mol. Biol.* **220,** 555–568
25. Landick, R., Wang, D., and Chan, C. (1996) *Methods Enzymol.* **274,** 334–352
26. Chamberlin, M. J. (1974) *Annu. Rev. Biochem.* **43,** 721–775
27. McClure, W. R. (1985) *Annu. Rev. Biochem.* **54,** 171–204
28. Zhou, Y. N., and Jin, D. J. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95,** 2908–2913
29. Barker, M. M., Gaal, T., Josaitis, C. A., and Gourse, R. L. (2001) *J. Mol. Biol.* **305,** 673–688
30. Gaal, T., Bartlett, M., Ross, W., Turnbough, C., Jr., and Gourse, R. (1997) *Science* **278,** 2092–2097
31. Pfeffer, S. R., Stahl, S. J., and Chamberlin, M. J. (1977) *J. Biol. Chem.* **252,** 5403–5407
32. Chan, C. L., and Landick, R. (1993) *J. Mol. Biol.* **233,** 25–42
33. Chan, C., Wang, D., and Landick, R. (1997) *J. Mol. Biol.* **268,** 54–68
34. Artsimovitch, I., and Landick, R. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97,** 7090–7095
35. Nudler, E., Mustaev, A., Lukhtanov, E., and Goldfarb, A. (1997) *Cell* **89,** 33–41
36. Palangat, M., and Landick, R. (2001) *J. Mol. Biol.* **311,** 265–282
37. McDowell, J. C., Roberts, J. W., Jin, D. J., and Gross, C. (1994) *Science* **266,** 822–825
38. Gusarov, I., and Nudler, E. (1999) *Mol. Cell* **3,** 495–504
39. Komissarova, N., and Kashlev, M. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 1755–1760
40. Komissarova, N., and Kashlev, M. (1997) *J. Biol. Chem.* **272,** 15329–15338
41. Naryshkin, N., Revyakin, A., Kim, Y., Mekler, V., and Ebright, R. H. (2000) *Cell* **101,** 601–611
42. Korzheva, N., Mustaev, A., Nudler, E., Nikiforov, V., and Goldfarb, A. (1998) *Cold Spring Harbor Symp. Quant. Biol.* **63,** 337–345
43. Mekler, V., Kortkhonjia, E., Mukhopadhyay, J., Knight, J., Revyakin, A., Kapanidis, A. N., Niu, W., Ebright, Y. W., Levy, R., and Ebright, R. H. (2002) *Cell* **108,** 599–614
44. Zakharova, N., Bass, I., Arsenieva, E., Nikiforov, V., and Severinov, K. (1998) *J. Biol. Chem.* **273,** 24912–24920
45. Geetha, V., and Munson, P. J. (1997) *Protein Sci.* **6,** 2538–2547
46. Nechaev, S., and Severinov, K. (1999) *J. Mol. Biol.* **289,** 815–826
47. Barne, K. A., Bown, J. A., Busby, S. J., and Minchin, S. D. (1997) *EMBO J.* **16,** 4034–4040
48. Barker, M. M., and Gourse, R. L. (2001) *J. Bacteriol.* **183,** 6315–6323
49. Zhang, G., Campbell, E. A., Minakhin, L., Richter, C., Severinov, K., and Darst, S. A. (1999) *Cell* **98,** 811–824
50. Ross, W., Thompson, J. F., Newlands, J. T., and Gourse, R. L. (1990) *EMBO J.* **9,** 3733–3742
51. Chan, C., and Landick, R. (1989) *J. Biol. Chem.* **264,** 20796–20804
52. Studier, F. W., Rosenberg, A. H., Dunn, J. J., and Dubendorff, J. W. (1990) *Methods Enzymol.* **185,** 60–89 .0