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Dual regulation of open-complex formation and promoter clearance by Arc explains a novel repressor to activator switch

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ABSTRACT **In studies of variants of the P_{ant} promoter of bacteriophage P22, the Arc protein was found not only to slow the rate at which RNA polymerase forms open complexes but also to accelerate the rate at which the enzyme clears the promoter. These dual activities permit Arc, bound at a single operator subsite, to act as an activator or as a repressor of different promoter variants. For example, Arc activates a P_{ant} variant for which promoter clearance is rate limiting in the presence and absence of Arc but represses a closely related variant for which open-complex formation becomes rate limiting in the presence of Arc. The acceleration of promoter clearance by Arc requires occupancy of the operator subsite proximal to the −35 region and is diminished when Arc bears a mutation in Arg-23, a residue that makes a DNA-backbone contact in the operator complex.**

Transcription initiation by *Escherichia coli* RNA polymerase is a multistep process (1). RNA polymerase first forms a competitor-sensitive closed-promoter complex that then isomerizes to a competitor-resistant open-promoter complex. Polymerase bound in the open complex is competent to initiate transcription in the presence of NTPs, and after synthesis of approximately eight nucleotides, the enzyme clears the promoter and becomes committed to elongation (1). Any of these steps in initiation could potentially be regulated by transcription factors. In most cases, however, regulators bound to a single site affect only one step in either a positive or negative fashion.

During late lytic growth of bacteriophage P22, Arc represses transcription from the P_{ant} and P_{mnt} promoters in the *immunity I* operon (2, 3). This activity of Arc is mediated by binding to tandem subsites of the *arc* operator, positioned between the −10 and −35 promoter elements (3). Each operator subsite binds a single Arc dimer, and cooperative interactions between tandemly bound dimers contribute to stabilization of the protein-operator complex (4, 5). Here, we present evidence that Arc affects multiple steps in transcription initiation at P_{ant}. Using variants of the P_{ant} promoter, we show that Arc both slows open-complex formation and accelerates promoter clearance. These dual and opposing activities permit Arc to act as an activator or as a repressor, depending upon the intrinsic rates of different initiation steps for a given promoter and how Arc changes these rates. An Arc dimer bound at the operator subsite next to the −35 promoter element of P_{ant} is both necessary and sufficient for the acceleration of promoter clearance. This positive activity is also diminished by the RA23 mutation of Arc which removes a phosphate-backbone contact in the protein-operator complex (5, 6).

MATERIALS AND METHODS

Proteins. Arc and variants of Arc contained the st6 or st11 C-terminal extensions that minimize proteolysis *in vivo* and allow purification by nickel-affinity chromatography (7). Neither addition affects the activity of Arc (7). The Arc-SL35 variant was used for most studies. This mutant binds normally to single operator subsites but is defective in cooperative binding of Arc dimers to tandem subsites (8). *E. coli* σ^{70} RNA polymerase holoenzyme was purified as described (9).

Promoter Construction and Template Preparation. P_{ant} variants were cloned as *Eco*RI–*Bst*EII cassettes into the backbone of pSA660 (10), a derivative of pSA600 (7). Linear templates were generated by PCR using a pair of primers with the 5′ ends of the primers located at −87 and at +85 with respect to the start site of transcription. The −87 primer was end-labeled with ^{32}P using T4 polynucleotide kinase. To allow normalization of the templates in the reactions, the same stock of labeled primer was used in PCR reactions to synthesize different promoter fragments. The 173-bp PCR products were purified by gel electrophoresis.

Transcription, Footprinting, and Gel Mobility-Shift Assays. Unless noted, experiments were performed at 37°C in a buffer containing 30 mM Hepes·KOH (pH 7.5), 100 mM potassium glutamate, 10 mM $MgCl_2$, 1.5 mM $CaCl_2$, 0.1 mM Na_2EDTA, 100 μg/ml BSA, 1 mM dithiothreitol, 0.02% Nonidet P-40, and 0.2 nM template DNA. RNA polymerase and Arc-SL35 were used at concentrations of 7.5 nM and 75 nM, respectively, unless indicated. The final concentrations of NTPs in transcription assays were 167 μM ATP, GTP, and CTP, and 5 μM UTP. In run-off transcription assays, [α-^{32}P]UTP was used (1.4×10^4 cpm/pmol). RNase inhibitor (Promega; 1 unit per reaction) was included in the transcription and open-complex formation reactions. Transcription, footprinting, and gel mobility shift assays were quantified using a Molecular Dynamics PhosphorImager and IMAGEQUANT software.

For run-off transcription assays, promoter DNA was incubated with or without Arc-SL35 in the presence of NTPs. Transcription reactions were initiated by the addition of RNA polymerase and were quenched after 2 min with heparin, KCl, and Na_2EDTA at final concentrations of 100 μg/ml, 0.35 M, and 25 mM, respectively. The products of the transcription reactions were analyzed on 12% polyacrylamide, 8 M urea, and 1× TBE gels.

To assay open-complex formation, RNA polymerase (7.5 nM) was added to promoter DNA (0.2 nM), and the reaction was quenched after different times by the addition of heparin to 100 μg/ml and sucrose to 5%. Bound and free DNA fragments were resolved on 4% polyacrylamide, 0.5× TBE gels.

To assay promoter clearance by footprinting, RNA polymerase and promoter DNA were allowed to form open complexes, the reactions were diluted 50-fold into buffer containing 0.1 μg/ml heparin with or without Arc-SL35, and NTPs were added to initiate transcription. At different times, aliquots were removed and added to DNAse I (final concentration of 18 ng/ml) for 60 s, and cleavage was quenched by addition of an equal volume of 2.5 M ammonium acetate, 20 mM Na_2EDTA, and 10 μg/ml salmon sperm DNA. Digestion products were analyzed on 6% polyacrylamide, 8 M urea, and

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1× TBE gels, and loss of the RNA polymerase footprint was quantified.

To assay promoter clearance by run-off transcription assays, preformed open complexes were diluted 1.6-fold into heparin (4 μg/ml final concentration) with or without Arc-SL35 (75 nM final concentration). NTPs were then added to initiate transcription. Aliquots were removed at different times and quenched with heparin, KCl, and Na_2EDTA at final concentrations of 100 μg/ml, 0.35 M, and 25 mM, respectively. In the single time point assays shown in Fig. 3*B*, Fig. 4, and Fig. 5, the concentrations of the Arc variants were increased to 300 nM. For the experiments shown in Fig. 3*B* and Fig. 5, aliquots from the same open-complex pool were added to different Arc variants to test for effects on promoter clearance.

RESULTS

Promoter Variants. Two P_{ant} promoter variants were used for the majority of the studies described below. As diagrammed in Fig. 1*A*, the NC promoter contains a single subsite for binding of an Arc dimer proximal to the −35 region. The C promoter is identical except at position −8, where it contains a consensus base pair for σ^{70} promoters (1). In both the NC and C promoters, the operator subsite proximal to the −10 region of wild-type P_{ant} was inactivated by mutation. A divergent and overlapping promoter, P_{mnt}, was also inactivated by mutation. To avoid cooperative binding of additional Arc dimers at nonspecific sites adjacent to a dimer bound at the active subsite, a cooperativity-defective variant (Arc-SL35) was also used for most studies (8).

A Repressor to Activator Switch. In run-off transcription assays, Arc-SL35 represses the NC promoter and activates the C promoter (Fig. 1*B*). The extent of transcriptional repression or activation is modest but reproducible (Fig. 1*B*). The −8 position, at which the NC and C promoters differ, is more than 10 bp from the active operator subsite and would not be expected to influence Arc binding. In fact, the binding of Arc-SL35 to the NC and C templates is indistinguishable, as assayed by the protein concentrations required for half-maximal binding (not shown) or by the pattern of the DNAse I footprint (Fig. 1*C*). In addition, the DNAse I footprint of the RNA polymerase open complex is identical on the two promoters (Fig. 1*C*). Since no obvious differences in the DNA interactions of Arc-SL35 or RNA polymerase were observed on the NC and C promoter templates, we reasoned that the ability of Arc-SL35 to repress one promoter and activate the other might indicate that Arc is capable of differentially regulating distinct steps in transcription initiation.

Arc Slows Open-Complex Formation. Fig. 2 shows the results of open-complex formation assays in the presence and absence of Arc-SL35. Open-complex formation is about 10-fold faster on the C promoter than the NC promoter, but Arc-SL35 slows open-complex formation on both promoters (Table 1; Fig. 2). The decreased rate of open-complex formation on the NC promoter in the presence of Arc-SL35 is consistent with the repression of this promoter in transcription assays. The slowing of open-complex formation on the C promoter would also be expected to repress transcription but instead activation of this promoter is observed. Hence, under the experimental conditions used, formation of open complexes on the C promoter must not be rate limiting in the presence of Arc-SL35, and the protein must be capable of activating this promoter by influencing another step in transcription initiation.

Arc Accelerates Promoter Clearance. Arc-SL35 increases the rate of promoter clearance from both the NC and the C promoters by 2- to 3-fold in both footprinting and run-off transcription experiments (Fig. 3; Table 1). This stimulatory activity of Arc on promoter clearance has not been described previously. For these experiments, open complexes were

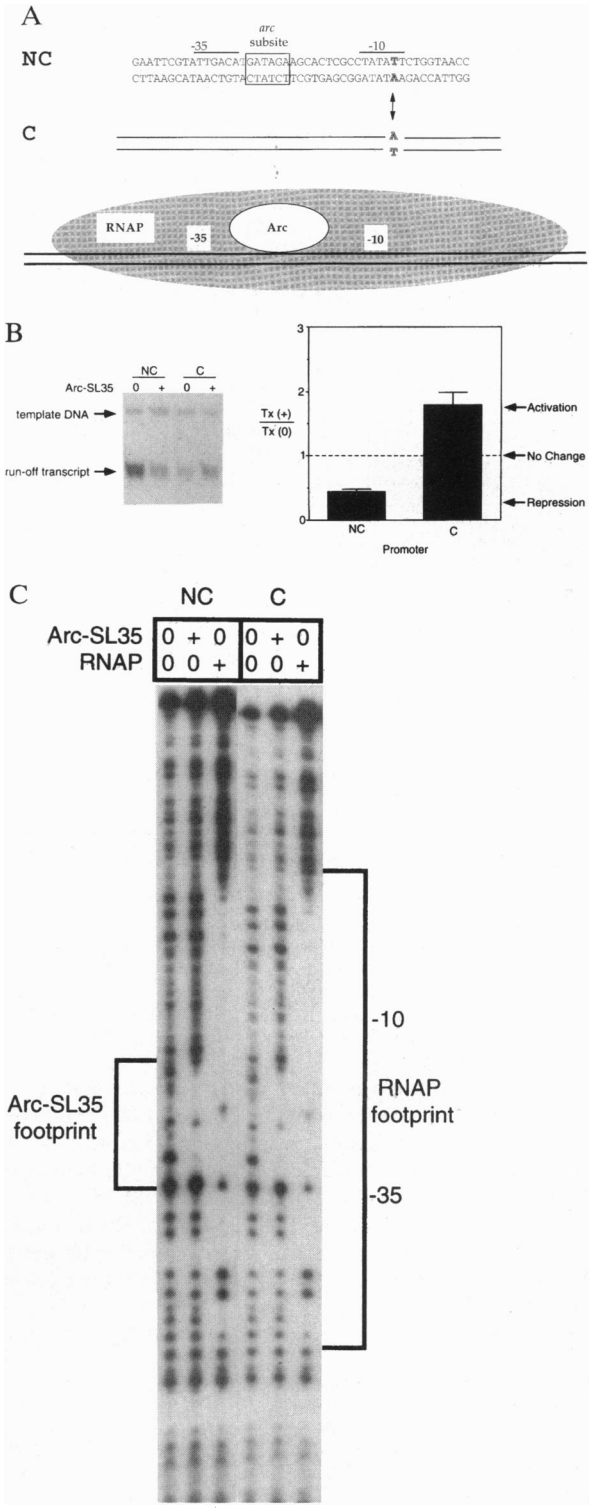

FIG. 1. (*A*) Sequence of the NC variant of the P_{ant} promoter (the C promoter is identical except at position −8). Base pairs contacted by an Arc dimer bound to the active subsite next to the −35 region are boxed (5). The diagram shown below indicates roughly how Arc bound to the active subsite and RNA polymerase bound in the open complex should be closely apposed. (*B*) Run-off transcription assays. Left, the radiolabeled DNA template and 85 base run-off transcript bands are marked. Right, a plot of the average (±SD, $n \geq 3$) of the ratio of transcription with Arc-SL35 to transcription without Arc-SL35. (*C*) DNAse I footprints of Arc-SL35 (75 nM) and RNA polymerase (7.5 nM) bound to the NC and the C promoters.

formed, heparin was added as a competitor to prevent reinitiation, NTPs were added to initiate transcription, and the rate

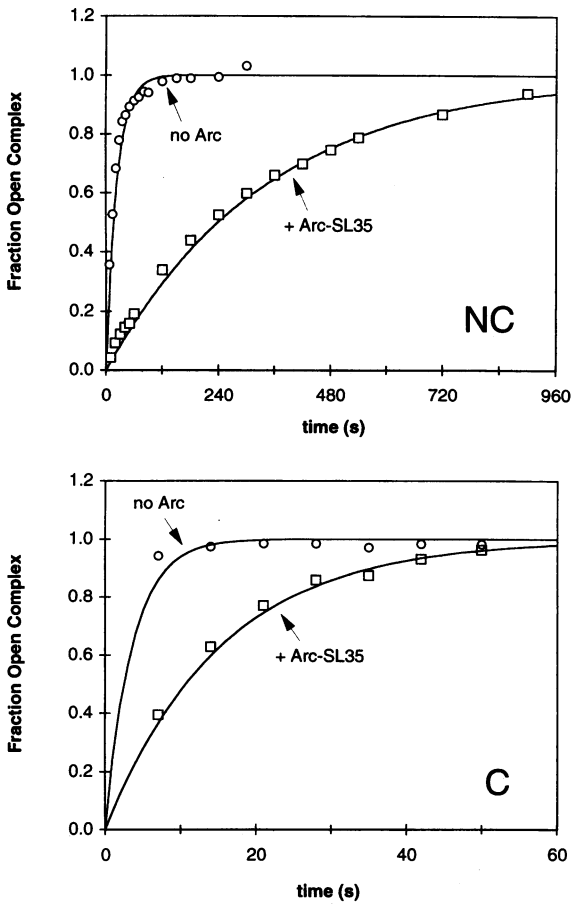

FIG. 2. Kinetics of open-complex formation by RNA polymerase with the NC and C promoters in the presence and absence of Arc-SL35. The solid lines are single exponential fits of the data. The rate constants from these fits are 2.6 min^{-1} (NC), 0.16 min^{-1} (NC plus Arc-SL35), 18 min^{-1} (C), and 3.9 min^{-1} (C plus Arc-SL35).

of promoter clearance was determined by measuring either the disappearance of the open-complex DNAse I footprint or the accumulation of completed transcripts as a function of time. An example of a footprinting experiment for the C promoter is shown in Fig. 3*A*. In this experiment, Arc-SL35 was preincubated with the DNA before the addition of RNA polymerase. The Arc-SL35 footprint can be seen in the absence of RNA polymerase (lane 2) and following promoter clearance (lanes 10–12). At time 0 (lanes 8 and 9), the RNA polymerase footprint completely overlaps that of Arc, making it difficult to

Table 1. Apparent rates* of open-complex formation and promoter clearance

	Intrinsic rates, min^{-1}		Rates + Arc-SL35, min^{-1}	
Promoter	Open complex	Clearance	Open complex	Clearance
NC	2.4 ± 0.2	0.27 ± 0.07†‡	0.13 ± 0.02†	0.84 ± 0.29‡
		0.35 ± 0.02†§		0.82 ± 0.27§
C	≥16	0.08 ± 0.01†‡	3.5 ± 0.70	0.25 ± 0.05†‡
		0.10 ± 0.02†§		0.22 ± 0.02†§

Rates of open-complex formation were determined using 7.5 nM RNA polymerase.

*Rates are expressed as the mean ± SD ($n \geq 3$).

†The rate-limiting step in transcription initiation in the presence or absence of Arc-SL35 is shown.

‡Rates for promoter clearance were determined from footprinting experiments.

§Rates for promoter clearance were determined from single-round run-off transcription experiments.

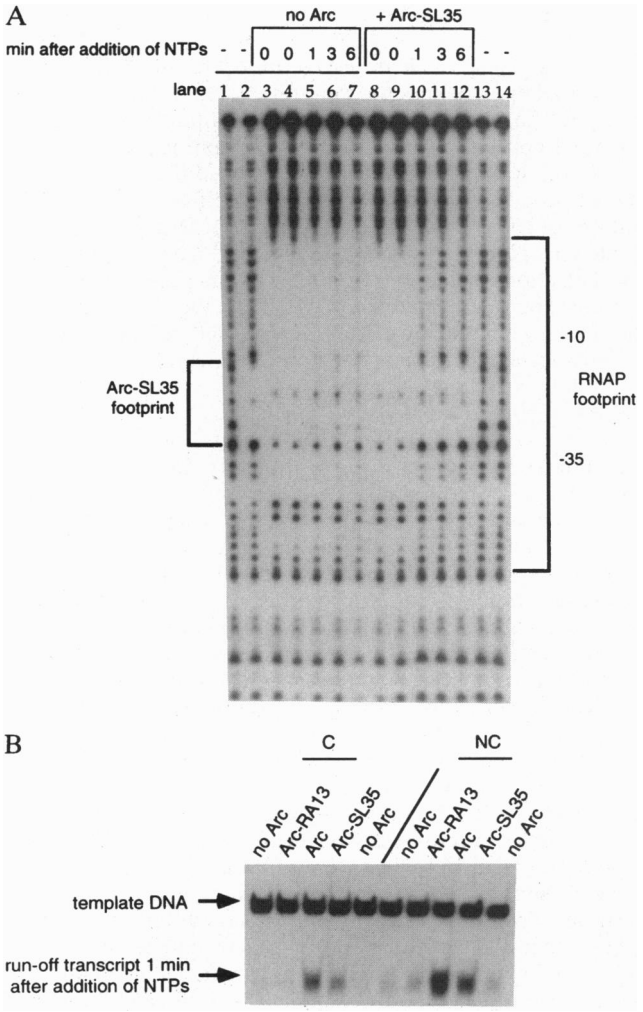

FIG. 3. (*A*) Clearance of RNA polymerase from the C promoter assayed by footprinting. Footprints 1, 3, and 6 min after the addition of NTPs to preformed open complexes are shown in lanes 5–7 (without Arc-SL35) and lanes 10–12 (with Arc-SL35). Control lanes: 1, no protein with heparin; 2, Arc-SL35 with heparin; 3 and 8, open complex without NTPs but with heparin for 10 s; 4 and 9, open complex without NTPs but with heparin for 10 min; 13 and 14, 1 and 10 min after RNA polymerase was added to premixed heparin and DNA. The clearance rates for this experiment were 0.08 min^{-1} without Arc-SL35 and 0.20 min^{-1} with Arc-SL35. (*B*) Promoter clearance of preformed open complexes assayed by run-off transcription at a single time point with Arc-SL35, wild-type Arc, and the operator-binding defective mutant Arc-RA13.

know whether both proteins are bound simultaneously to the promoter (see *Discussion*). Controls show that premixing heparin and template DNA prevents initiation by RNA polymerase (lanes 13–14), and that NTP-independent dissociation of RNA polymerase from the promoter is not significant during the time course of the reaction (lanes 3, 4, 8, and 9). In the absence of Arc, the open-complex footprint of RNA polymerase is still visible 6 min after initiation (lane 7). In the presence of Arc-SL35, the open-complex footprint is significantly diminished 1 min after initiation (lane 10).

As shown in the run-off transcription assays of Fig. 3*B*, the ability of Arc-SL35 to stimulate promoter clearance is shared by wild-type Arc but not by a mutant (RA13) that is stably folded but cannot bind the operator because a key DNA contact residue has been altered (6). In the experiments of Fig. 3*B*, the Arc proteins were added after formation of open complexes, while in the previous experiments, they were added before open-complex formation. Thus, the order of addition of

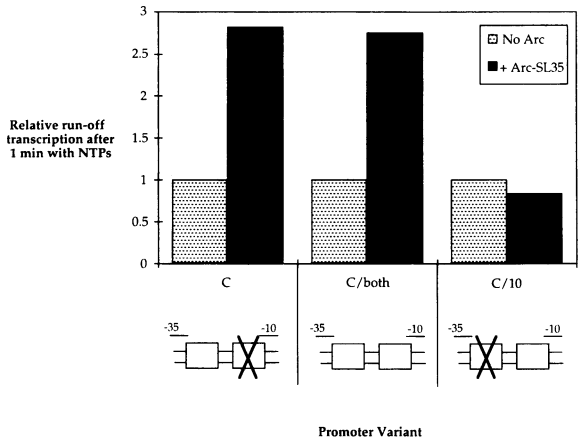

FIG. 4. Run-off transcription promoter clearance assays with the C, C/both, and C/10 promoters in the presence and absence of Arc-SL35. C/both is identical to the C promoter, except for the sequence TCTA at −17 to −14, which restores the *arc* subsite proximal to the −10 region. C/10 has the following changes from the C promoter: AGGCACGT at −28 to −21 and TCTA at −17 to −14, which inactivate the −35 proximal *arc* subsite and restore the −10 proximal subsite.

Arc to the reactions is not critical. Because the binding-defective RA13 mutant does not stimulate promoter clearance, it appears that Arc must be bound to the operator to accelerate promoter clearance. The same conclusion is supported by the finding that Arc-SL35 did not affect clearance from a promoter without operator sites (not shown).

To investigate the subsite requirements for acceleration of promoter clearance, two additional promoter variants were constructed (Fig. 4). In run-off transcription assays, Arc-SL35 accelerated promoter clearance approximately 3-fold from the C/both variant, which contains both active subsites of the wild-type *arc* operator (Fig. 4). This value is similar to that observed with the C promoter that contains only the −35 proximal subsite. By contrast, Arc-SL35 did not accelerate clearance from the C/10 variant, in which the single active subsite is proximal to the −10 region of the promoter (Fig. 4), even though the affinity of this subsite for an Arc dimer is similar to that of the −35 proximal subsite (4). These data indicate that Arc bound at the −35 proximal *arc* subsite is both necessary and sufficient for enhancement of promoter clearance.

Arg-23 Participates in Promoter-Clearance Acceleration. In the Arc-operator cocrystal (5), the exposed portion of helix A provides a potential surface for interactions with bound RNA polymerase. Fig. 5 shows promoter clearance assays from the C/both promoter for a set of Arc mutants with helix-A substitutions (6). The RA23 mutant shows significantly reduced acceleration of promoter clearance. Although Arg-23 contacts a phosphate in the operator complex and has a mild DNA-binding defect (5, 6), DNA mobility shift experiments

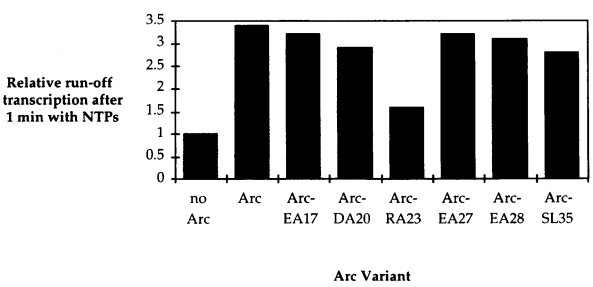

FIG. 5. Run-off transcription promoter clearance assays with the C/both promoter and variants of Arc.

show that the RA23 mutant is fully bound to the operator at the protein concentration (300 nM) used in these experiments (not shown). Moreover, increasing the concentration of RA23 to 1 μM did not result in greater activity in the clearance assay (not shown). We conclude that Arg-23 plays a role in acceleration of promoter clearance by Arc. The four additional helix-A mutants tested showed wild-type levels of clearance activity.

DISCUSSION

The results presented here demonstrate that Arc has two activities in transcription initiation, slowing open-complex formation and accelerating promoter clearance. Only a few other transcription factors have been shown to activate promoter clearance. These include the prokaryotic cAMP receptor protein at the *malT* promoter (11), the eukaryotic cAMP response-element binding protein PBP (12), and the basal transcription factors TFIIE and TFIIH (13).

The finding that Arc accelerates clearance whether it is added before or after open-complex formation suggests that it can bind in close apposition to RNA polymerase in the open complex and affect clearance of the enzyme through direct contacts or indirect effects mediated through DNA structure. For example, by introducing unfavorable electrostatic or steric contacts, Arc might weaken the binding of RNA polymerase to the DNA and thus lower the transition-state energy required for promoter clearance. The model in which Arc and RNA polymerase bind simultaneously is also supported by abortive-initiation experiments performed by Liao and McClure (14), which indicate that Arc slows open-complex formation by retarding isomerization rather than blocking formation of closed complexes. ^{35}S-labeled Arc also comigrates with open complexes of wild-type P_{ant} in DNA mobility shift experiments, but we have been unable to show rigorously that Arc is bound at the expected position because the Arc footprint is obscured by that of RNA polymerase with all reagents tested (unpublished results). The idea that enhancement of promoter clearance requires specific contacts between Arc and RNA polymerase is also consistent with the findings that the wild-type Arg-23 side chain is required for full stimulation of clearance and that only an Arc dimer bound at the −35 proximal subsite stimulates clearance. In principle, some of the same interactions between Arc and RNA polymerase could even be responsible for slowing isomerization and for stimulating promoter clearance.

During growth of bacteriophage P22, Arc establishes repression at P_{ant} promoters that are being actively transcribed (2). Arc's ability to slow open complex formation and speed promoter clearance may provide a mechanism that allows repression to be rapidly achieved even when RNA polymerase is bound at P_{ant}. For example, a single Arc dimer could bind to the −35 proximal subsite leading to acceleration of promoter clearance by the bound polymerase molecule and retardation of the rate at which a new open complex with another polymerase molecule could form. This would free the −10 proximal subsite, allowing cooperative binding of a second Arc dimer and efficient repression of further transcription.

The dual activities of Arc allow it to act as either a repressor or an activator in transcription assays *in vitro*. Which activity is observed for a specific promoter will depend on a number of factors. These include the rate of open-complex formation, which may change depending on the promoter and the RNA polymerase concentration; the rate of promoter clearance, which may vary depending on the promoter and the NTP concentration; how Arc increases or decreases these rates; and how other factors such as ionic strength, temperature, and supercoiling affect these rates. For a standard set of reaction conditions (Table 1), transcription initiation from the NC

promoter is limited by the rate of promoter clearance in the absence of Arc-SL35 ($\approx$0.27 min^{-1}) and by the rate of open-complex formation in the presence of Arc-SL35 ($\approx$0.13 min^{-1}). Under these conditions, negative regulation of the NC promoter involves a change in the rate-limiting step. The overall reduction in the rate of the slowest step is about 2-fold, a value consistent with the magnitude of repression of the NC promoter by Arc-SL35 (Fig. 1*B*). Transcription from the C promoter is limited by the rate of promoter clearance both in the absence ($\approx$0.08 min^{-1}) and in the presence of Arc-SL35 ($\approx$0.25 min^{-1}) (Table 1). This increase in rate is consistent with activation of the C promoter by Arc-SL35 (Fig. 1*B*). The dual activities of Arc make it difficult to predict the regulatory consequences of changes in promoter sequence or environmental conditions. For example, using fusions of the NC and C promoters to *lacZ*, we found that Arc-SL35 repressed both promoters approximately 2-fold *in vivo* (unpublished results). Since Arc-SL35 activates the C promoter *in vitro*, the relative rates of open-complex formation and clearance for this promoter are probably different under the conditions used *in vitro* than *in vivo*.

The intrinsic promoter strengths of the NC and C promoters *in vitro* are consistent with the observed rates of open-complex formation and promoter clearance (Table 1). The C promoter is weaker than the NC promoter because clearance from both promoters is rate limiting and is 2- to 3-fold slower from the C promoter (Table 1). Changing position -8 from a nonconsensus to a consensus base pair does not increase promoter strength, even though the rate of open-complex formation increases, because this step is not rate limiting. This finding is consistent with previous results with consensus or near-consensus promoters and suggests that RNA polymerase may bind more tightly in the open complex with the C promoter (15, 16).

Like Arc, the regulatory protein MerR binds to an operator positioned between the -35 and -10 promoter elements. In the absence of mercury, MerR represses transcription by retarding isomerization to the open complex. In the presence of mercury, MerR becomes an activator and accelerates open-complex formation by a mechanism that apparently involves a conformational change in the protein-DNA complex (17). Although the steps at which Arc and MerR affect transcription are not identical, the similarities in the positions of the operators and the ability to both repress and activate transcription when bound to a single site suggest that proteins that bind between the -35 and -10 elements may be especially well suited to have dual functions in regulating transcription initiation.

Regulatory proteins that have opposing effects on distinct steps in transcription could be poised to act as regulatory switches when the rate-limiting step in transcription initiation is altered. In the cell, many variables presumably affect which step is rate-limiting, including temperature, DNA superhelicity, ionic strength, nutrient conditions, the concentration of free RNA polymerase, mutations, and the binding of transcription factors (13, 16, 18–22). Based upon the results presented here, any variable that changes the rate-limiting step in transcription of a specific promoter could potentially transform a repressor into an activator or vice versa.

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1. McClure, W. R. (1985) *Annu. Rev. Biochem.* **54,** 171–204.
2. Susskind, M. M. & Youderian, P. (1983) in *Lambda II*, eds. Hendrix, R. W., Roberts, J. W., Stahl, F. W. & Weisberg, R. A. (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 347–363.
3. Vershon, A. K., Liao, S.-M., McClure, W. R. & Sauer, R. T. (1987) *J. Mol. Biol.* **195,** 323–331.
4. Brown, B. M. & Sauer, R. T. (1993) *Biochemistry* **32,** 1354–1363.
5. Raumann, B. E., Rould, M. A., Pabo, C. O. & Sauer, R. T. (1994) *Nature (London)* **367,** 754–757.
6. Brown, B. M., Milla, M. E., Smith, T. L. & Sauer, R. T. (1994) *Nat. Struct. Biol.* **1,** 164–168.
7. Milla, M. E., Brown, B. M. & Sauer, R. T. (1993) *Protein Sci.* **2,** 2198–2205.
8. Smith, T. L. & Sauer, R. T. (1995) *J. Mol. Biol.* **249,** 729–742.
9. Hager, D. A., Jin, D. J. & Burgess, R. R. (1990) *Biochemistry* **29,** 7890–7894.
10. Smith, T. L. & Sauer, R. T. (1996) *J. Mol. Biol.*, in press.
11. Menendez, M., Kolb, A. & Buc, H. (1987) *EMBO J.* **6,** 4227–4234.
12. Narayan, S., Widen, S. G., Beard, W. A. & Wilson, S. H. (1994) *J. Biol. Chem.* **269,** 12755–12763.
13. Goodrich, J. A. & Tjian, R. (1994) *Cell* **77,** 145–156.
14. Liao, S.-M. (1988) Ph.D. Thesis (Carnegie-Mellon University, Pittsburgh, PA).
15. Ellinger, T., Behnke, D., Bujard, H. & Gralla, J. D. (1994) *J. Mol. Biol.* **239,** 455–465.
16. Ellinger, T., Behnke, D., Knaus, R., Bujard, H. & Gralla, J. D. (1994) *J. Mol. Biol.* **239,** 466–475.
17. Summers, A. O. (1992) *J. Bacteriol.* **174,** 3097–3101.
18. Adhya, S. & Garges, S. (1990) *J. Biol. Chem.* **265,** 10797–10800.
19. Botsford, J. L. & Harman, J. G. (1992) *Microbiol. Rev.* **56,** 100–122.
20. Leirmo, S., Harrison, C., Cayley, D. S., Burgess, R. R. & Record, M. T., Jr. (1987) *Biochemistry* **26,** 2095–2101.
21. Pruss, G. J. & Drlica, K. (1989) *Cell* **56,** 521–523.
22. Tsung, K., Brissette, R. E. & Inouye, M. (1990) *Proc. Natl. Acad. Sci USA* **87,** 5940–5944.