Enhancer-dependent activator proteins, which act upon the bacterial RNA polymerase containing the σ^{54} promoter specificity factor, belong to the AAA superfamily of ATPases. Activator-σ^{54} contact is required for the σ^{54}-RNAP to isomerize and engage the DNA template for transcription. How ATP hydrolysis is used to trigger changes in σ^{54}-RNA polymerase and promoter DNA that lead to RNA polymerase opening is poorly understood. Here, band shift and footprinting assays were used to investigate the DNA binding activities of σ^{54} and σ^{54}-RNA polymerase in the presence of the activator protein PspF bound to ADP. ATP hydrolysis results in the transition-state analogue ADP-AlF_{6}^{-}. Results show that different nucleotide-bound forms of PspF can change the interactions between σ^{54}, σ^{54}-RNA polymerase, and a DNA fork junction structure present within closed promoter complexes. This provides evidence that in the activation transduction pathway, several functional states of the activator, prior to ATP hydrolysis, can serve to alter the fork junction binding activity of σ^{54} and σ^{54}-RNA polymerase that precede full DNA opening. A sequential set of nucleotide-dependent transitions in σ^{54}-RNA polymerase promoter complexes needed for productive open complex formation may therefore depend upon different nucleotide-bound forms of the activator.

Transcription initiation is a major point at which gene expression is regulated. Within the three kingdoms of life, structurally conserved multisubunit DNA-dependent RNAPs catalyze transcription and require auxiliary factor(s) to facilitate transcription. In bacteria, a core RNAP (σ70-E) associates with one of several sigma (σ) factors to form a functional holoenzyme. The σ subunit confers promoter specificity to the bacterial RNAP (reviewed in Refs. 1 and 2). In Escherichia coli and many other bacteria, two types of RNAP holoenzymes exist: one that utilizes the vegetative σ factor σ^{70}, or one of the five alternative σ^{70} like factors, and an enhancer-dependent one that uses the σ^{54} factor (reviewed in Refs. 3 and 4). The key difference between the σ^{70} and the σ^{54} types is the conversion step from closed promoter-RNAP complexes to productive open complexes. For the σ^{70} family, this conversion can occur spontaneously without the involvement of activators. For the enhancer-dependent σ^{54}-RNAP, the transition from closed complex to open complex strictly relies upon the mechaenochemical activity of enhancer-binding activators that belong to the AAA class of proteins, which is a major point at which enhancers can serve to alter the fork junction binding activity of σ^{54} and σ^{54}-RNA polymerase that precede full DNA opening. A sequential set of nucleotide-dependent transitions in σ^{54}-RNA polymerase promoter complexes needed for productive open complex formation may therefore depend upon different nucleotide-bound forms of the activator.

Received for publication, February 6, 2003, and in revised form, March 13, 2003
Published, JBC Papers in Press, March 20, 2003, DOI 10.1074/jbc.M301296200

Wendy Cannon‡, Patricia Bordes§, Siva R. Wigneshweraraj‡, and Martin Buck§

From the Department of Biological Sciences, Sir Alexander Fleming Building, Imperial College London, South Kensington Campus, London SW7 2AZ, United Kingdom

© 2003 by The American Society for Biochemistry and Molecular Biology, Inc. Printed in U.S.A.

This paper is available online at http://www.jbc.org

---

* This work was supported by a Wellcome Trust project grant (to M. B.) The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ These authors contributed equally to this work.

§ To whom correspondence should be addressed. Tel.: 44-207-594-5442; Fax: 44-207-594-5419; E-mail: m.buck@imperial.ac.uk.

1 The abbreviations used are: RNAP, RNA polymerase; AAA, ATPase associated with various cellular activities; PspF, phage shock protein F; OP-Cu, ortho-copper phenanthroline; ATPγS, adenosine 5'-β,γ-imido-triphosphate; AMP-PNP, adenosine 5'-β-thio-triphosphate; GTPγS, guanosine 5'-γ-thio-triphosphate; ADPAlF_{6}^{-}, aluminum fluoride.

The amino-terminal region 1 of σ^{54} makes a major contribution to binding the fork junction structure at σ^{70} (7, 8, 10–12). This nucleoprotein organization constitutes the direct binding target for AAA activators and is known as the regulatory center (12, 13). The AAA activators of the σ^{54}-RNAP couple the energy derived from ATP hydrolysis to remodel the regulatory center, relieving the inhibitory interactions with the template strand of the fork junction structure at σ^{70} and promoting new interactions with the adjacent non-template strand required for forming open complex (5, 8, 11, 14).

Open complex formation by the σ^{70}-RNAP and σ^{54}-RNAP proceeds via several intermediate states involving large conformational changes in the RNAP (15). However, very little information exists on the mechanisms that trigger changes in RNAP and promoter DNA that lead to transcription. Analysis of the mechanisms of transcription initiation by the σ^{54}-RNAP will extend our understanding further on the functioning of the RNAP as a complex molecular machine. Recently, we demonstrated that it is possible to "trap" σ^{54} or the σ^{54}-RNAP bound to promoter DNA with the E. coli AAA activator phage shock protein F (PspF) in the presence of ADP aluminum fluoride (ADP-AlF_{6}^{-}), an analogue of ATP at the point of hydrolysis (13). Using heteroduplex forms of the Sinorhizobium meliloti nifH promoter probes containing fork junction structures at -12 and, for experimental simplicity, only the AAA domain of PspF (PspF_{1-275}) (Table I), we now report
that nucleotide-dependent action of AAA activators on σ^{54} or σ^{64}-RNAP significantly changes the interactions made with fork junction DNA at −12. Altered activator-dependent promoter DNA binding activities of σ^{54} and σ^{64}-RNAP were observed in the presence of poorly or non-hydrolyzed forms of ATP (adenosine 5′-(γ-thio)triphosphate (ATPγS) and ADP-AlF₄⁻, respectively), suggesting that several nucleotide bound states of the AAA activator, each capable of remodeling a DNA promoter, are present. Completion of the ATP hydrolysis cycle, or persistence of ATP hydrolysis, per se is not linked to partial remodeling of the regulatory center. Evidence that a mixed nucleotide-bound (ATP and ATPγS) state of the activator resulted in altered remodeling activity was also obtained.

Overall the results provide clear evidence that discrete functionalities are associated with different nucleotide bound states of PspF, which orchestrate open complex formation by the σ^{54}-RNAP during the ATP hydrolysis cycle by remodeling σ^{54}-RNAP-fork junction interactions. Hence by inference we suggest that other AAA proteins will have several distinct nucleotide-dependent functional states.

**EXPERIMENTAL PROCEDURES**

**DNA and Proteins**—The main DNA molecules and proteins used in this work are listed in Table I. The Klebsiella pneumoniae σ^{4} protein (amino acids 1–477) and a derivative lacking region 1 (amino acids 57–477, Δσ^{4}) were prepared as described before (16). Mutant σ^{4} proteins (amino acids 1–477) were purified as described before (10). The intact AAA domain of PspF lacking the C-terminal DNA-binding domain, PspF₁₋₉₋₇, was used in activation assays (17). E. coli core RNAP (E) was from Epicentre Technologies. Wild type homoduplex or mismatched S. meliloti nifH wild-type homoduplex or mismatched S. meliloti nifH promoter DNA heteroduplexes were prepared by annealing 88-bp oligonucleotides as described (11, 18), one of them being 5′-32P-end-labeled using T4 polynucleotide kinase and [γ-32P]ATP (3000 Ci/mmol).

**Native Gel Mobility Shift Assays**—Binding reactions were conducted at 30 °C in STA buffer (25 mM Tris acetate, pH 8.0, 8 mM Mg-acetate, 100 mM KCl, 1 mM diithiothreitol and 3.5% (v/v) PEI-GOOG-6000). Where indicated, σ^{4}, Δσ^{4}, or mutant σ^{4} were present at 1 μM and the σ^{54}-RNAP holoenzyme at 100 nM (formed with 1:4 ratio of core RNAP to σ^{4}). σ^{4} or σ^{54} proteins or σ^{54}-RNAP were incubated for 5 min with 16 nM promoter probe and nucleotides as required: ADP-AlF₄⁻ (formed in situ by the addition of 0.2 mM AlCl₃ to a mixture containing 0.2 mM ADP and 5.0 mM NaF; Ref. 15), ATP (2 μM), ATPγS (2 μM, unless otherwise stated), or ATP-SβATP/mismatch (0.2/2 mM, unless otherwise stated). After addition of 10 μM PspF₁₋₉₋₇ (unless otherwise stated) the reactions were incubated for a further 10 min prior to resuspending on a 4.5% native polyacrylamide gel. Native gels were run in 25 mM Tris, 200 mM glycine buffer (pH 8.6). Complexes were detected by PhosphorImager analysis. The error range for the amount of DNA band shifted was ±5%, depending upon the σ^{4} and ATPγS preparations used. For comparative purposes when using variants in PspF or DNA the same σ^{4} and ATPγS preparations were used.

**DNA Footprinting Assays**—Binding reactions (10 μl) were conducted as described above but with 100 nM promoter DNA probe and in STA buffer without diithiothreitol. Footprinting reagents were added as described (11, 14), reactions were terminated, and bound and unbound DNAs were separated on native polyacrylamide gels. DNA was then excised, processed, and analyzed on a denaturing 10% polyacrylamide gel. For ortho-copper phenanthroline (OP-Cu) footprinting, 0.5 μl of a solution of 4 mM ortho-phenanthroline, 0.92 mM CuSO₄, and 0.5 μl of 0.116 M mercaptoethanol acid were added to the reactions and incubated for 2 min. The reactions were terminated by the addition of 1 μl of 28 mM 2,9-dimethyl-1,10-phenanthroline. For DNase I footprints, 1.75 × 10⁷ units of enzyme (Amersham Biosciences) was added to the reactions for 1 min, followed by addition of 10 μM EDTA to stop cutting. For KMnO₄ footprinting, 4 mM fresh KMnO₄ was added for 30 s, followed by 50 mM β-mercaptoethanol to quench DNA oxidation. Gel-isolated DNA was eluted into 0.1 mM EDTA (pH 8) (for DNase I footprinting) or H₂O (for OP-Cu footprinting and KMnO₄ footprinting) overnight at 37 °C. KMnO₄-oxidized DNA was cleaved with 10% (v/v) piperidine at 90 °C for 20 min. Recoveries of isolated DNA were determined by dry Cerenkov counting and equal numbers of counts were loaded onto 10% sequencing gels. Dried gels were visualized and quantified using a PhosphorImager.

**RESULTS**

**ADP-AlF₄⁻ Form of PspF₁₋₉₋₇ Changes the DNA Binding Activities of σ^{54} and σ^{54}-RNAP**

DNA footprinting data suggest that the stable binding between the ADP-AlF₄⁻, associated form of PspF₁₋₉₋₇ (PspF₁₋₉₋₇-ADP-AlF₄⁻) and promoter-bound σ^{54} or σ^{54}-RNAP changes the DNA interactions made by σ^{54} or σ^{54}-RNAP (13). To further explore these alterations in DNA interactions we analyzed the ability of σ^{54} and σ^{54}-RNAP to bind a range of S. meliloti nifH promoter probes (that mimicked the conformation of the DNA within closed promoter complexes) by native-PAGE in the absence and presence of PspF₁₋₉₋₇-ADP-AlF₄⁻. The promoter probes differed in the template strand sequence in the region of heteroduplex at the −12/−11 fork junction (Fig. 1a).

As shown in Fig. 1a, σ^{54} does not detectably bind to promoter probes with mutant or missing template strand sequences at position −12 (probes 2–9; Ref. 11). In contrast, strong binding of σ^{54} and σ^{54}-RNAP was evident on probe 1, which contains wild-type template strand sequences within the heteroduplex segment (Table Ia and Fig. 1, a and b, respectively). Strikingly, in the presence of PspF₁₋₉₋₇-ADP-AlF₄⁻, the binding activity of σ^{54} to probes 2, 7, and 8 was significantly increased, suggesting that the stable complex formation between σ^{54} and PspF₁₋₉₋₇-ADP-AlF₄⁻ (hereafter called the trapped σ^{54}) has altered the DNA-binding activity of σ^{54} (Fig. 1a).

**DNA Sequences Around Position −1 Are Important for the Binding of Trapped σ^{54} and σ^{54}-RNAP to Promoter Probe 2**

DNA footprinting data on promoter probe 1 have established that compared with the binary σ^{54}-DNA complexes, ADP-AlF₄⁻-dependent trapped σ^{54}-DNA complexes have extended DNase I footprints toward the transcription start site, as do ATP hydrolysis-dependent isomerized binary σ^{54}-DNA complexes (11, 19). This indicates that the extended σ^{54}-DNA interactions occur in response to interactions with certain nucleotide-bound forms of the activator. We used the promoter probe with the mutant template strand sequence (GT at positions −12 and −11 instead of TG; probe 2), to which trapped σ^{54} and σ^{4}.

W. Cannon and M. Buck, unpublished data.
RNAP bound most efficiently (Fig. 1) to further examine the increased DNA binding functionality of trapped $\sigma^{54}$ and $\sigma^{54}$-RNAP. By using a series of shortened variants of promoter probe 2 (probes 2A–2F; Table II), we explored whether the binding of trapped $\sigma^{54}$ (ADP-AlF$_3$-dependent complex between PspF$_{1,275}$ and $\sigma^{54}$); b, Eo$\sigma^+$ and trapped Eo$\sigma^+$ (as in A except with Eo$\sigma^+$) to $^{32}$P-labeled promoter probes harboring a range of mutations at positions −12 and −11. The wild-type promoter sequence is represented at the top of the figure. Boxed are the consensus GC (−14/−13) and the −12/−11 nucleotides; mutant sequence(s) are indicated in gray, a deleted residue by Δ; the schematics shows which strand contains the mutant sequences. The amount of promoter complexes (%) was determined by PhosphorImager analysis and is indicated.

**FIG. 1. Binding of $\sigma^{54}$, Eo$\sigma^+$, and PspF$_{1,275}$-ADP-AlF$_3$-bound forms thereof to S. meliloti nifH promoter probes.** Gel mobility shift assays showing the binding of $\sigma^{54}$ and trapped $\sigma^{54}$ (ADP-AlF$_3$-dependent complex between PspF$_{1,275}$ and $\sigma^{54}$); b, Eo$\sigma^+$ and trapped Eo$\sigma^+$ (as in A except with Eo$\sigma^+$) to $^{32}$P-labeled promoter probes harboring a range of mutations at positions −12 and −11. The wild-type promoter sequence is represented at the top of the figure. Boxed are the consensus GC (−14/−13) and the −12/−11 nucleotides; mutant sequence(s) are indicated in gray, a deleted residue by Δ; the schematics shows which strand contains the mutant sequences. The amount of promoter complexes (%) was determined by PhosphorImager analysis and is indicated.

### Binding of Trapped $\sigma^{54}$ and $\sigma^{54}$-RNAP to Promoter Fork Junction DNA at −12

OP-Cu is a minor groove-specific DNA cleaving reagent that has been widely used to study $\sigma^{54}$-RNAP interactions with the −12 promoter region (6, 19). In closed promoter complexes formed with the $\sigma^{54}$-RNAP on homoduplex promoter probe (Table Ia), the minor groove at the −12 position is distorted and thus is susceptible to cleavage by OP-Cu (6). OP-Cu-mediated DNA cleavage at this position is not evident in open promoter complexes suggesting that the distortion distinguishes closed and open promoter complexes (6). We used OP-Cu footprinting to probe whether binding of trapped $\sigma^{54}$ and trapped $\sigma^{54}$-RNAP to promoter probe 2 changes $\sigma^{54}$ interactions with the fork junction structure at −12. To do so, we formed promoter complexes with $\sigma^{54}$ and $\sigma^{54}$-RNAP on probe 2 in the absence and presence of PspF$_{1,275}$-ADP-AlF$_3$, treated them with OP-Cu, and separated the probe 2 bound and unbound complexes by native-PAGE. Promoter complexes were excised from native gels, the DNA was isolated and analyzed by denaturing-PAGE.

As shown in Fig. 2, OP-Cu treatment of naked promoter probe 2 revealed a hypersensitive site around position −12 (lane 2), as expected because of the heteroduplex segment at this position (Table Ia). This hypersensitive site was also evident in the weak complexes formed between $\sigma^{54}$ or $\sigma^{54}$-RNAP and probe 2 in the absence of PspF$_{1,275}$-ADP-AlF$_3$, (Fig. 2, lanes 5 and 6). This is striking because the OP-Cu-mediated DNA cleavage at −12 is significantly reduced in complexes formed between $\sigma^{54}$ or $\sigma^{54}$-RNAP and probe 1 (which contains wild-type template strand sequences in the heteroduplex segment; Table Ia) because of the tight binding of $\sigma^{54}$ or $\sigma^{54}$-RNAP to this heteroduplex segment (19). Interestingly, analysis of the complexes formed between trapped $\sigma^{54}$ or trapped $\sigma^{54}$-RNAP and probe 2 showed that the DNA cleavage around position −12 is...
Thus, the footprinting data establishes that lanes 12
the functional states of PspF1
II). Thus, these initial results demonstrate that a new
upon promoter sequences between positions
ATP
2 (Fig. 3 and data not shown). The complex (hereafter called the
Strikingly, the presence of the ATP
or AMP-PNP in assays using promoter probes 1 and 2 (Fig. 3).

Table I
DNA probes and proteins used in this work

| a | S. melliloti σ70 promoter probes* |
|---|----------------------------------|
| | -26 | -14 | +1 |
| Homoduplex | CAGCGGTCGGACATCGAGCGGACCCC... | GTCGCCGACGCTGCTGAAAATGCTGATGCGGACCCC... | +28 |
| Probe 1 | CAGCGGTCGGACATCGAGCGGACCCC... | GTCGCCGACGCTGCTGAAAATGCTGATGCGGACCCC... | +28 |
| Probe 2 | CAGCGGTCGGACATCGAGCGGACCCC... | GTCGCCGACGCTGCTGAAAATGCTGATGCGGACCCC... | +28 |

| b | Proteins |
|---|----------------------------------|
| σ54 | His, tagged |
| Δ1σ54 | His, tagged |
| σ54 Region 1 mutants | His, tagged |
| PspF1–275 | His, tagged |
| PspF1–275 mutants | His, tagged |
| Core RNA polymerase (E) | Epicentre Technologies |

* The consensus GG and GC of the σ54 binding site is indicated in bold. Boxed are the mismatched regions at positions −11 and −12 relative to the transcription start site (+1).

strongly reduced (Fig. 2, compare lanes 3 and 4 with 5 and 6). Thus, the footprinting data establishes that σ54 and σ54-RNA interactions near the −12 promoter region are altered in the presence of PspF1–275-ADP-AlF₃.

Interaction of ATPγS Bound PspF1–275 with σ54 Results in a Novel Promoter Complex

To increase our understanding of how new functional states of PspF1–275 are created by nucleotide interactions, we explored whether changes in DNA-binding activity of σ54 and σ54-RNA in the presence of PspF1–275-ADP-AlF₃, are seen with other nucleotides. The poorly or non-hydrolyzable forms of ATP, ATPγS, and adenosine 5’-(γ-thiotriphosphate (GTP-S), or ATPγS were present demonstrated that ATPγS-complex formation was specific to the yS forms of the trinucleotide (data not shown). As shown in Fig. 4a, the amount of ATPγS-complex formation increased with ATPγS, and saturated at 1 mM ATPγS (20% ATPγS-complex). Increased σ54-
DNA binding was also observed when ATPγS was substituted with GTPγS (data not shown), although ATPγS was the most effective in promoting binding of σ54 to promoter probe 2 (5-fold better than GTPγS). The amount of ATPγS-complex formation also titrated with PspF1–275 and σ54 (Fig. 4, b and c, respectively). Increasing amounts of DNA did not increase the fraction of DNA in the ATPγS-dependent complex, suggesting that PspF1–275-ATPγS acts on free σ54 and not on its promoter DNA-bound form (data not shown). Furthermore, ATPγS-complex formation was dependent upon the integrity of the GAPTGA motif of PspF1–275 (residues 83–88), a signature motif in the AAA domain of σ54 activators, which is involved in direct binding interactions with σ54 (11, 13, 17). No ATPγS-complexes were detected when PspF1–275 harboring the T86A, T86S, F85A, or F85L mutations within the GAPTGA motif were used (data not shown). Because these PspF1–275 mutants were not defective for nucleotide binding, we conclude that the failure to form the ATPγS-complex arises from a defective interaction with σ54 and not defects in nucleotide binding per se. As expected, attempts to form the ATPγS-complex with PspF1–275 harboring mutations within motifs involved in nucleotide interactions or in ATP hydrolysis (Walker A and B motifs and the putative “arginine finger” residue) failed (data not shown).

Interaction of σ54 with a Mixed Nucleotide-bound Form of PspF1–275 Results in a Novel σ54-DNA Complex

Activators of the σ54-RNA must form oligomeric structures to hydrolyze ATP (5, 21, 22). The state of the bound nucleotide within each promoter of the oligomer during active ATP hydrolysis is not known. However, we considered that differences could exist between promoters, and attempted to form the ATPγS-complex in the presence of increasing amounts of ATPγS plus either 4 mM ATP or AMP. As shown in Fig. 5a, we observed that the amount of ATPγS-dependent complex formation was significantly increased in the presence of ATP, espe-
Nucleotides Orchestrate $\sigma^{54}$ Activator Functionality

### Table II

| Probe 2 variants | Probe name | $\sigma^{54}$-DNA$^b$ | Trapped $\sigma^{54}$-DNA$^b$ | $\sigma^{54}$-DNA$^c$ | Trapped $\sigma^{54}$-DNA$^c$ | ATP$\gamma$S complex$^c$ |
|------------------|------------|-----------------------|--------------------------|-----------------|--------------------------|--------------------------|
| −60 to +28       | Probe 2    | <3                    | 22                       | 26              | 37                       | 9                        |
| −35 to +6        | Probe 2A   | <3                    | 12                       | 10              | 37                       | 6                        |
| −35 to +3        | Probe 2B   | <3                    | 9                        | 11              | 30                       | 3                        |
| −35 to +1        | Probe 2C   | <3                    | 8                        | 18              | 31                       | <3                       |
| −35 to −1        | Probe 2D   | <3                    | 12                       | 24              | 32                       | <3                       |
| −35 to −2        | Probe 2E   | <3                    | 4                        | 24              | 15                       | <3                       |
| −35 to −3        | Probe 2F   | <3                    | 4                        | 27              | 11                       | <3                       |
| −35 to T-3$^d$   | Probe 2G   | <3                    | 4                        | 18              | 7                        | ND$^d$                   |
| −35 to B-1$^d$   | Probe 2H   | <3                    | 4                        | 17              | 10                       | ND$^d$                   |

$^a$ Expressed as % promoter probe complexed with $\sigma^{54}$ and $\sigma^{54}$-RNAP and trapped versions thereof.

$^b$ This promoter probe contains a double-stranded segment from −35 to −3 and a single-stranded top strand segment from −1 to −3.

$^c$ This promoter probe contains a double-stranded segment from −35 to −3 and a single-stranded bottom stranded segment from −1 to −3.

$^d$ ND, not determined.

### Fig. 2

**OP-Cu footprints of ADP·AlF$_3$-dependent complexes formed between PspF$_{1-275}$ and $\sigma^{54}$ or E in S. meliloti nifH promoter probe 2.** The hypersensitive sites at positions −11 and −12 are indicated by arrows. The non-template strand is $^{32}$P-labeled. The cleavage products were treated as described under Experimental Procedures and were run on a 10% sequencing gel.

### Fig. 3

**The interaction between ATP$\gamma$S-bound PspF$_{1-275}$ and $\sigma^{54}$ results in a novel promoter complex on promoter probe 2.** Gel mobility shift assays showing the ATP$\gamma$S-bound PspF$_{1-275}$-dependent formation of a $\sigma^{54}$-DNA complex (lane 13) using $^{32}$P-labeled promoter DNA probes. This ATP$\gamma$S-dependent complex relies upon the S. meliloti nifH promoter probe 2 (compare lanes 5 and 13) and migrates faster than the trapped-$\sigma^{54}$ complex and DNA (probe 1, lane 4, or probe 2, lane 12). Where indicated, 0.2 mM ATP$\gamma$S or AMP-PNP were added.
Nucleotides Orchestrate σ\(^{54}\) Activator Functionality

**Fig. 4.** Effect of increasing concentrations of ATPγS (a), PspF\(_{1-275}\) (b), and σ\(^{54}\) (c) on ATPγS-complex formation on promoter probe 2. *S. meliloti* nifH was \(^{32}\)P-labeled. When kept constant the concentrations of σ\(^{54}\), ATPγS, and PspF\(_{1-275}\) used were: 1 μM σ\(^{54}\) (a and b), 2 mM ATPγS (b, filled symbols) and 0.2 mM ATPγS (b, unfilled symbols) and 10 μM PspF\(_{1-275}\) (a and c). ATPγS complex formation was assayed by gel shift analysis and the amount of ATPγS-complex formation (%) was determined by PhosphorImager analysis.

plex on native gels was changed in the presence of PspF\(_{1-275}\)-ATPγS and PspF\(_{1-275}\)-ATPγS/ATP (Fig. 5d, compare lane 2 with lanes 3 and 4). Control reactions with PspF\(_{1-275}\)-bound to AMP or AMP-PNP confirmed that the mobility change of σ\(^{54}\)-RNAP occurred in response to PspF\(_{1-275}\)-ATPγS or PspF\(_{1-275}\)-ATPγS/ATP (data not shown).

Action of PspF\(_{1-275}\)-ATPγS and PspF\(_{1-275}\)-ATPγS/ATP on σ\(^{54}\) and σ\(^{54}\)-RNAP Leads to Altered Interactions with the Fork Junction at σ\(^{54}\)

We first attempted to characterize the interactions between the fork junction promoter structure and σ\(^{54}\) or σ\(^{54}\)-RNAP in the PspF\(_{1-275}\)-ATPγS and PspF\(_{1-275}\)-ATPγS/ATP complexes with probe 2 by OP-Cu and potassium permanganate (KMnO\(_4\)) footprinting techniques (Fig. 6, a and b). KMnO\(_4\) is a single-stranded thymine-reactive DNA oxidizing agent that is widely used to detect local DNA melting. Treatment of naked probe 2 by OP-Cu or KMnO\(_4\) revealed a hypersensitive region around position −12 (Fig. 6, a, lane 2, and b, lane 5). This is because of the heteroduplex segment at −12/11 (for OP-Cu-mediated DNA cleavage) and to the unpaired thymine at position −11 (for KMnO\(_4\)-mediated DNA cleavage) (Table Ia).

**σ\(^{54}\) Interactions**—The hypersensitive site at position −12 is evident in reactions containing only σ\(^{54}\) and probe 2 (Fig. 6, a, lane 3, and b, lane 1). This is consistent with σ\(^{54}\) being weakly bound to probe 2 in the absence of any nucleotide-bound forms of PspF (see above). Strikingly, in the presence of PspF\(_{1-275}\)-ATPγS or PspF\(_{1-275}\)-ATPγS/ATP, the hypersensitivity site at −12 almost completely disappeared in the OP-Cu reactions, demonstrating altered interactions with the −12 promoter region (Fig. 6a, lanes 4 and 5). In contrast, the hypersensitive site at position −12 was still evident within the ATPγS and ATPγS/ATP complexes when probed with KMnO\(_4\) (Fig. 6b, lanes 2 and 3). Yet, the intensity of the cleavage at the −11 thymine is reduced in the ATPγS-complex when compared with the ATPγS/ATP-complex (Fig. 6b, compare lanes 2 and 3). This difference in cleavage intensity was also observed on the probe 2 version of the σ\(^{54}\)-dependent *E. coli* glnHp2 promoter (data not shown), and again indicates changed interactions with the −12 promoter region.

**σ\(^{54}\)-RNAP Interactions**—As before (Fig. 2), the binding of σ\(^{54}\)-RNAP to probe 2 did not affect the inherent hypersensitive site at the −12 position to cleavage by OP-Cu (Fig. 6a, lane 6). However, in the presence of PspF\(_{1-275}\)-ATPγS or PspF\(_{1-275}\)-ATPγS/ATP, σ\(^{54}\)-RNAP interactions with probe 2, particularly around the −12 position, are altered as demonstrated by the reduction of OP-Cu-mediated cleavage (Fig. 6a, compare lanes 3 and 6 with 4 and 5 and 7 and 8, respectively). The progressive increase in the cleavage intensity at −12 in the presence of PspF\(_{1-275}\)-ATPγS, PspF\(_{1-275}\)-ATPγS/ATP, and PspF\(_{1-275}\)-ATP, respectively (Fig. 6a, compare lanes 7, 8, and 9), supports the view that σ\(^{54}\)-RNAP interactions with the −12 promoter region are altered in response to interactions with certain nucleotide-bound forms of PspF.

To determine whether the DNA has locally melted in response to PspF\(_{1-275}\)-ATPγS and PspF\(_{1-275}\)-ATPγS/ATP, we probed complexes formed between the σ\(^{54}\)-RNAP and probe 2 in the presence of PspF\(_{1-275}\)-ATPγS and PspF\(_{1-275}\)-ATPγS/ATP, respectively, by KMnO\(_4\). No local DNA melting toward the transcription start site was detected (data not shown). However, consistent with the OP-Cu footprinting data, binding of σ\(^{54}\)-RNAP to probe 2 in the absence of nucleotide-bound forms of PspF did not protect the thymine at −11 from oxidation by KMnO\(_4\) (data not shown). Furthermore, differences in the cleavage pattern at the −11 thymine were observed in the complexes formed between σ\(^{54}\)-RNAP and probe 2 in response to PspF\(_{1-275}\)-ATPγS and PspF\(_{1-275}\)-ATPγS/ATP on the probe 2 version of the *S. meliloti* nifH and *E. coli* glnHp2 promoters (data not shown). These differences were very similar to those seen with σ\(^{54}\) (see above and Fig. 6b). Overall, the OP-Cu and KMnO\(_4\) footprinting data strongly suggest that different nucleotide-bound forms of PspF have different effects on the −12 promoter region interactions made by the σ\(^{54}\)-RNAP, but that local DNA melting does not occur.

**Action of PspF\(_{1-275}\)-ATPγS and PspF\(_{1-275}\)-ATPγS/ATP on σ\(^{54}\) and σ\(^{54}\)-RNAP Leads to Altered Interactions with Promoter DNA**

Next, we attempted to differentiate the interactions created between the promoter DNA and σ\(^{54}\) or σ\(^{54}\)-RNAP in the PspF\(_{1-275}\)-ATPγS and PspF\(_{1-275}\)-ATPγS/ATP complexes with probe 2 by DNase I footprinting (Fig. 6c). Binding of σ\(^{54}\) or σ\(^{54}\)-RNAP to the *S. meliloti* nifH homoduplex probe or probe 1 (Table Ia) protects the DNA between positions −34 and −5. These protections extend further in the downstream direction in open promoter complexes formed by the σ\(^{54}\)-RNAP on the homoduplex probe, indicating isomerization of the σ\(^{54}\)-RNAP in
response to ATP hydrolysis by the activator (11, 14, 19).

$\sigma^{54}$-RNAS Interactions—Complexes were formed between the $\sigma^{54}$-RNAP and probe 2 in the presence and absence of various nucleotide-bound forms of PspF. As expected, in the absence of PspF the DNA is protected between positions −34 and −1 by the $\sigma^{54}$-RNAP (Fig. 6c, lane 3). The presence of PspF$_{1-275}$-ATPγS leads to an extended footprint in the downstream direction (Fig. 6c, lane 4); indicating isomerization of the $\sigma^{54}$-RNAP-probe 2 complex in response to PspF$_{1-275}$-ATPγS. Interestingly, such an extension is not evident in the presence of PspF$_{1-275}$-ATPγS/ATP (Fig. 6c, compare lanes 4 and 5). In striking contrast to the extended footprint seen on the homoduplex probe, the $\sigma^{54}$-RNAP footprint on probe 2 appears to shorten in the downstream direction in response to the action of PspF$_{1-275}$-ATPγS/ATP (Fig. 6c, lane 6). It seems that ATPγS, ATPγS/ATP, and ATP each cause changes in $\sigma^{54}$-RNAP-promoter interactions.

$\sigma^{54}$ Interactions—Consistent with the gel-mobility shift assay (Fig. 1), no strong interaction between $\sigma^{54}$ and probe 2 was detected in the absence of PspF$_{1-275}$. However, at higher concentrations of probe 2, a weak $\sigma^{54}$-probe 2 complex could be detected. Analysis of this complex by DNase I footprinting revealed that the $\sigma^{54}$ footprint was short (between −34 and −10; data not shown), in contrast to that seen with probe 1 (between −34 and −5; Refs. 11, 14, and 19). In the presence of PspF$_{1-275}$-ATPγS, the $\sigma^{54}$ footprint on probe 2 is extended in the downstream direction (beyond position −1) as seen with the $\sigma^{54}$-RNAP (Fig. 6c, compare lanes 7 and 4). Interestingly, in the presence of PspF$_{1-275}$-ATPγS/ATP, $\sigma^{54}$ footprints the DNA between −34 and −5, as seen in complexes formed between $\sigma^{54}$ and probe 1 or the homoduplex probe (Fig. 6c, lane 8 and data not shown).

Overall, these footprinting results suggest that different sets of activator-dependent DNA interactions (within and outside the −12 promoter region) are made by $\sigma^{54}$ and $\sigma^{54}$-RNAP depending on the nucleotide bound states of the activator and involves promoter sequences downstream of −12 to about −1 (the promoter region where DNA melting is seen in normal open complexes).

Role of $\sigma^{54}$ Region 1 in PspF$_{1-275}$-ATPγS and PspF$_{1-275}$-ATPγS/ATP mediated Complex Formation

Previous data have indicated that region 1 of $\sigma^{54}$ and the promoter sequences at −12 are intimately involved in the activator responsiveness of the $\sigma^{54}$-RNAP-closed complex (7, 8, 11). The interaction $\sigma^{54}$ region 1 makes with the −12 promoter sequence generates a nucleoprotein target for the activator (12, 13). We investigated the role of $\sigma^{54}$ region 1 in PspF$_{1-275}$-ATPγS and PspF$_{1-275}$-ATPγS/ATP mediated binding of $\sigma^{54}$ to probe 2.

$\Delta 1$σ$^{54}$—The binding of wild-type $\sigma^{54}$ and a mutant form of $\sigma^{54}$ lacking its amino-terminal residues 1–56 ($\Delta 1$σ$^{54}$) to probe 2 were compared. In contrast to the wild-type $\sigma^{54}$, $\Delta 1$σ$^{54}$ bound the promoter probe 2 well (Fig. 7a, compare lane 2 and 6), suggesting that region 1 inhibits the initial binding activity of $\sigma^{54}$ to promoter probe 2. To examine whether formation of the ATPγS- and/or the ATPγS/ATP-complex requires $\sigma^{54}$ region 1,
we added PspF1–275-ATP5S or PspF1–275-ATP5S/ATP to the Δσ54-promoter probe 2 complex. As shown in Fig. 7a, no ATP5S-complex or ATP5S/ATP-complex was detected (compare lanes 3 and 7 and 4 and 8, respectively). Similarly, the addition of PspF1–275-ADP·AlF4 to the Δσ54-probe 2 complex did not result in trapped complex formation (data not shown). Therefore, we conclude that the increased binding of Δσ54 to probe 2 is likely to be because of PspF1–275-ATP5S, PspF1–275-ATP5S/ATP, or PspF1–275-ADP·AlF4-mediated conformational change of Δσ54 region 1. Overall, it appears that in the absence of activation or in the presence of sequences within the fork junction that prevent tight binding (as in probe 2), region 1 of Δσ54 acts negatively so as to limit DNA binding.

Region 1 Ala Mutants—By using a series of triple alanine substitution mutants (Ala mutants) in Δσ54 region 1 (10), we attempted to identify region 1 residues that (i) prevented binding of Δσ54 to probe 2 and (ii) allowed ATP5S- and ATP5S/ATP-complex formation. Initially, we tested the binding activity of the Ala mutants to probe 2. In contrast to Δσ54, none of the alanine mutations resulted in significantly increased binding of Δσ54 to probe 2 (Fig. 7b). This suggests that several sequences in region 1 prevent initial binding of Δσ54 to probe 2. Based on the ability of the Ala mutants to form the ATP5S-complex, two classes of mutants can be distinguished: Class 1 includes the Ala mutants 6–8, 9–11, 12–14, 15–17, 21–23, 24–26, 30–32, 33–35, 36–38, 42–44, 45–47, and 48–50 that did not show increased probe 2-binding activity in response to PspF1–275-ATP5S (Fig. 7b). Class 2 essentially includes the wild-type and...
greatly stimulated the probe 2-binding activity of the Ala mutants. As shown in Fig. 7c, a further two classes can be distinguished: Class 1 essentially includes the wild-type and all Ala mutants between residues 6 and 14 (Fig. 7c, 6–8, 9–11, and 12–14) and 27–50 (Fig. 7c, 27–29, 30–32, 33–35, 36–38, 39–41, 42–44, 45–47, and 48–50). Class 2 includes the Ala mutants between residues 15 and 26 (Fig. 7c, 15–17, 18–20, 21–23, and 24–26) that did not form the ATPγS/ATP-complex effectively. Strikingly, this ATPγS/ATP-complex formation pattern is consistent with previous observations in which the \( \sigma^{54} \)-RNAP formed with the Ala mutants (15–17, 18–20, 21–23, and 24–26) failed to efficiently catalyze transcription in vitro in response to subsaturating PspF concentrations (10). Given that the Ala 21–23 mutant fails to stably bind the PspF1-275-ADP-AlF₃ (13) and residue 20 (Gln-20) localizes proximal to the +12 promoter region within \( \sigma^{54} \)-RNAP closed complexes (19), it appears that residues 15–26 are required for the PspF1-275-ATPγS/ATP-dependent tight binding of \( \sigma^{54} \) to probe 2. Importantly, the differences in probe 2-binding activity observed with the Ala mutants highlights the fact that different \( \sigma^{54} \) region 1 residues are involved in interacting with different nucleotide-bound forms of the activator that consequently leads to different sets of promoter interactions.

**DISCUSSION**

Many cellular processes involving DNA manipulation like replication, transposition, transcription, and restriction may all be regulated through the control of interactions made with fork junction structures. A conserved location for a DNA fork junction, where DNA opening originates, is evident within transcription complexes formed with the enhancer-dependent \( \sigma^{54} \)-RNAP and enhancer-independent \( \sigma^{54} \)-RNAP (8). We have shown that the ADP-AlF₃-bound form of the AAA domain of \( \sigma^{54} \)-dependent activator PspF is able to change fork junction DNA interactions made by \( \sigma^{54} \) and its RNAP. This property of altering DNA-binding activity is also shared by PspF bound by ATPγS, and by mixed nucleotide bound states (ATPγS and ATP). We propose that an early nucleotide-dependent step in activator-dependent \( \sigma^{54} \)-open complex formation occurs prior to ADP or P, release, and is associated with changing the \( \sigma^{54} \)-fork junction interactions that are needed for subsequent interactions made by the RNAP to form the productive open promoter complex.

**Mechanochemical Functions of PspF**—The ways in which PspF uses ATP binding and hydrolysis to promote open complex formation by \( \sigma^{54} \)-RNAP are not well understood. The effects of ADP-AlF₃, ATPγS, and ATPγS/ATP-bound forms of PspF have on \( \sigma^{54} \)-DNA interactions begin to address this issue. Several lines of evidence have shown that the \( \sigma^{54} \)-RNAP makes use of a fork junction DNA structure to limit DNA opening prior to activation (7, 8, 11). An extension of this view is that changed interactions at the fork junction are required to allow open complexes to form. Variations in promoter sequences suggest that a range of natural DNA fork junctions will exist, and that although we have used artificial fork junctions in our work, closely related structures will exist in many natural \( \sigma^{54} \)-dependent promoter complexes. The DNA band shift results establish that the functional state of the activator required for stable binding to the \( \sigma^{54} \)-RNAP was created by interaction with ADP-AlF₃, an analogue of ATP at the transition state for hydrolysis. This stable binding of the activator to the \( \sigma^{54} \)-RNAP leads to altered interactions between \( \sigma^{54} \) and the fork junction structure at −12. The amino-terminal region 1 of \( \sigma^{54} \) is required for creating the fork junction structure and activator binds directly to region 1. This suggests that the activator is able to couple events in ATP hydrolysis to changes in interactions between \( \sigma^{54} \) and promoter DNA through re-
structuring the $\sigma^{54}$ determinant, region 1, that creates and maintains the fork junction structure. This view is consistent with the chaperone-like property of AAA proteins and predicted changes in activator protomer structure during ATP binding and hydrolysis (5, 23). Our data indicate functional significance for different nucleotide bound states of PspF.

New Binary $\sigma^{54}$ and $\sigma^{32}$-RNAP Promoter Complexes—Results with ATP$\gamma$S and ATP$\gamma$S/ATP suggest that a set of interactions between the activator and $\sigma^{54}$ or $\sigma^{34}$-RNAP can occur in response to changes in the DNA binding properties of $\sigma^{54}$. Again, the critical property of the DNA probe used to demonstrate changes in DNA binding was the presence of a fork junction structure. In contrast to the results with ADP-AlF$\_x$, no stable ternary $\sigma^{54}$-DNA complex was seen with ATP$\gamma$S or ATP$\gamma$S/ATP and activator, suggesting that more than one nucleotide-bound form of activator can transiently interact with $\sigma^{54}$ and $\sigma^{34}$-RNAP to change its DNA binding properties. This ATP$\gamma$S-dependent transient interaction is presumably not dependent upon rapid hydrolysis of the triphosphate, but could involve sensing of the $\gamma$-phosphate prior to hydrolysis or the formation of some intermediate in a reaction related to normal ATP hydrolysis. Because the ADP-AlF$\_x$-bound form of the activator also changed DNA binding properties of the $\sigma^{54}$ and $\sigma^{34}$-RNAP at the same fork junction probe, it seems that activator bound by ATP$\gamma$S, ATP$\gamma$S/ATP, or ADP-AlF$\_x$ have some shared functionality. It seems that ATP$\gamma$S can cause the activator to overcome an energetically unfavorable interaction between $\sigma^{54}$ and the DNA fork junction that is otherwise inhibitory for $\sigma^{54}$-DNA binding interactions. The inhibition appears to be caused by region 1 of $\sigma^{54}$ and region 1 also seems to be needed for the formation of the ATP$\gamma$S- and ATP$\gamma$S/ATP-dependent binary complexes.

Nucleotide Hydrolysis-independent Isomerization of $\sigma^{54}$—There are remarkable contrasts to previous results in which region 1 of $\sigma^{54}$ was required to bind to a set of heteroduplex probes carrying certain ~12 fork junction structures and where ATP hydrolysis was necessary to remodel and isomerize the complex (probe 1 and variants thereof; Refs. 11 and 14, and Table Ia). In this work $\sigma^{54}$ region 1 inhibits binding to the heteroduplexes with alternative fork junctions and poorly hydrolyzed NTPs are used to remodel the $\sigma^{54}$ without stable DNA melting (probe 2; Table Ia). DNA heteroduplexes with opposing requirements for ATP versus ATP$\gamma$S for altered binding of $\sigma^{54}$ may reflect two alternate states of a natural promoter: one in which unfavorable interactions caused by region 1 of $\sigma^{54}$ need to be overcome for binding (e.g. on probe 2; Table Ia and this work), and one in which a strong initial set of interactions that rely upon region 1 need to be changed (e.g. on probe 1; Table Ia, and Refs. 11 and 14). The extent to which the proposed alternate states of the promoter contribute to activation is likely to be DNA sequence-specific, and therefore promoter-specific. Several lines of evidence indicate the ATP$\gamma$S-dependent reaction relates closely to the normal activation of $\sigma^{54}$-dependent promoters. These are the common reliance upon (i) region 1 of $\sigma^{54}$, (ii) the integrity of the GAPTGA sequences in PspF, (iii) the “arginine” finger in PspF, and (iv) similar concentration dependences upon $\sigma^{54}$, PspF, and nucleotide.

Nucleotide-dependent Activation of Transcription—Because a common core RNAP is used by the enhancer-independent class of $\sigma$ factors, it would appear that the special features of $\sigma^{54}$-dependent transcription relate closely to the activator targeting an unusually stable $\sigma^{54}$-RNAP-fork junction complex. Some of the $\sigma^{54}$-RNAP-DNA interactions that activator change seem to have a modest energetic cost, as demonstrated by the action of the ADP-AlF$\_x$- and ATP$\gamma$S-bound forms of the activator. Others, notably the DNA opening per se seems to correlate to ATP hydrolysis and appear to have a higher energetic cost. A feature of AAA proteins is the use of certain amino acid sequences that function to “sense” the state and the presence of the $\gamma$-phosphate of ATP, and potentially relay this information to cause conformational changes required for their biological output. The shared property of ADP-AlF$\_x$ and ATP$\gamma$S-bound forms of the activator in causing changes in DNA binding by $\sigma^{54}$ and its RNAP may well be related to a $\gamma$-phosphate sensing process upon binding to these ATP. The failure of ADP, ATP, and AMP-PNP to behave like ADP-AlF$\_x$ and ATP$\gamma$S can be explained by differences or absences of $\gamma$-phosphate interactions among the various nucleotides tested. For ATP$\gamma$S or ADP-AlF$\_x$, the sensing of the $\gamma$-phosphate of the ATP is implicated as critically changing the functionality of the activator, a common theme for AAA proteins where nucleotide binding and hydrolysis control the binding interactions needed for substrate remodeling. It seems that the nucleotide-dependent changes in $\sigma^{54}$ and DNA structure lead to the delivery of the promoter DNA into the DNA binding cleft of the RNAP where stable melting occurs.

Overview—Our results can be viewed as providing snapshots of potential intermediates in the activation process. The use of DNA probes that mimic the proposed states of promoter DNA, and the ATP analogues provide the useful tools. However, proof of the mechanism will require detailed time resolved analyses of ATP-dependent conformational changes in the native protein and DNA components. The effects of ATP$\gamma$S and ATP together upon the activity of PspF is interesting and suggests new or increased functionality through different bound nucleotides and creation of a mixed nucleotide bound state. Structures of the AAA proteins HslU and p97 have shown that binding of different nucleotides leads to conformational changes in HslU and p97 (24, 25). Here the content of the nucleotide binding pocket determines HslU conformation by bringing the $\alpha$S and $\alpha$-helical domains of HslU together. In so doing, the I-domain of HslU is moved. The $\sigma^{54}$ binding site in PspF is believed to be equivalent to the I-domain of HslU (5, 17), and so the combined effects of ATP$\gamma$S and ATP or ADP might be explained by their effects upon the $\sigma^{54}$ binding site in PspF. In relation to ATP hydrolysis, structural differences between the ATP and ADP bound states may be a key element in how PspF acts on $\sigma^{54}$ and its RNAP. Large conformational changes associated with ATP binding as opposed to hydrolysis are common in ATP-dependent molecular machines (25). It seems a range of nucleotide-bound promoters of PspF will contribute to full activation, some contributing more toward particular steps than others, and some preceding hydrolysis and product release. The precise nucleotide dependence seems to be DNA-dependent, indicating that energy coupling in $\sigma^{54}$-dependent systems critically involves key promoter sequences that communicate via the $\sigma$ factor to the activator. The interaction of $\sigma^{54}$ with the fork junction DNA is clearly subject to regulation and given the similarity between bacterial and eukaryotic multisubunit RNAPs, a range of control proteins may act to regulate transcription through targeting the fork junction structure. Interestingly TATA-binding protein DNA binding is regulated in an ATP-dependent fashion by MotI (26).

Acknowledgments—We thank Jörg Schumacher for PspF proteins, Pampa Ray for $\sigma^{43}$ protein, Paul Casaz for mutant $\sigma^{54}$ proteins, and Patricia Burrows for comments on the manuscript.

REFERENCES

1. Westen, M. M. (1998) FEBS Microbiol. Rev. 22, 127–150
2. Gross, C. A., Chan, C., Dombroski, A., Gruber, T., Sharp, M., Tupy, J., and Young, B. (1998) Cold Spring Harbor Symp. Quant. Biol. 63, 141–155
3. Merrick, M. J. (1993) Mol. Microbiol. 10, 903–915
4. Buck, M., Gallegos, M. T., Studholme, D. J., Gui, Y., and Gralla, J. D. (2000) J. Bacteriol. 182, 4129–4136
5. Zhang, X., Chaney, M. K., Wig什neshwaraj, S. R., Schumacher, J., Bordes, P.,
Nucleotides Orchestrate $\sigma^{54}$ Activator Functionality

Cannon, W., and Buck, M. (2002) Mol. Microbiol. 45, 895–903
6. Morris, L., Cannon, W., Claverie-Martin, F., Austin, S., and Buck, M. (1994) J. Biol. Chem. 269, 11563–11571
7. Guo, Y., Wang, L., and Gralla, J. D. (1999) EMBO J. 18, 3736–3745
8. Guo, Y., Lew, C. M., and Gralla, J. D. (2000) Genes Dev. 14, 2242–2255
9. Fenton, M. S., Lee, S. J., and Gralla, J. D. (2000) EMBO J. 19, 1130–1137
10. Casaz, P., Gallegos, M. T., and Buck, M. (1999) J. Mol. Biol. 282, 229–239
11. Cannon, W., Gallegos, M. T., and Buck, M. (2000) Nat. Struct. Biol. 7, 594–601
12. Wigneshweraraj, S. R., Chaney, M. K., and Buck, M. (2001) J. Mol. Biol. 306, 681–701
13. Chaney, M., Grande, R., Wigneshweraraj, S. R., Cannon, W., Casaz, P., Gallegos, M. T., Schumacher, J., Jones, S., Elderkin, S., Dago, A. E., Morett, E., and Buck, M. (2001) Genes Dev. 15, 2282–2294
14. Cannon, W., Gallegos, M. T., and Buck, M. (2001) J. Biol. Chem. 276, 386–394
15. Murakami, K. S., Masuda, S., Campbell, E. A., Muzzin, O., and Darst, S. A. (2002) Science 296, 1285–1290
16. Cannon, W., Missalidis, S., Smith, C., Cottier, A., Austin, S., Moore, M., and Buck, M. (1995) J. Mol. Biol. 248, 781–803
17. Bordes, P., Wigneshweraraj, S. R., Schumacher, J., Zhang, X., Chaney, M. K., and Buck, M. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 2278–2283
18. Cannon, W., Gallegos, M. T., Casaz, P., and Buck, M. (1999) Genes Dev. 13, 357–370
19. Wigneshweraraj, S. R., Kuznedelov, K., Severinov, K., and Buck, M. (2003) J. Biol. Chem. 278, 3455–3465
20. Popham, D. L., Szeto, D., Keener, J., and Kustu, S. (1989) Science 243, 629–635
21. Wyman, C., Rombel, I., North, A. K., Bustamante, C., and Kustu, S. (1997) Science 276, 1658–1661
22. Rombel, I., North, A., Hwang, I., Wyman, C., and Kustu, S. (1998) Cold Spring Harbor Symp. Quant. Biol. 63, 157–166
23. Vale, R. D. (2000) J. Cell Biol. 150, 13–19
24. Wang, J., Song, J. J., Seong, I. S.; Franklin, M. C., Kamtekar, S., Eom, S. H., and Chung, C. H. (2001) Structure 9, 1107–1116
25. Rouiller, I., DelalBarre, B., May, A. P., Weis, W. I., Brunger, A. T., Milligan, R. A., and Wilson-Kohalek, E. M. (2002) Nat. Struct. Biol. 9, 950–957
26. Darst, R. P., Dasgupta, A., Zhu, C., Hsu, J.-Y., Vroom, A., Muldrow, T., and Auble, D. T. (2003) J. Biol. Chem. 278, 13216–13226
Nucleotide-dependent Triggering of RNA Polymerase-DNA Interactions by an AAA Regulator of Transcription
Wendy Cannon, Patricia Bordes, Siva R. Wigneshweraraj and Martin Buck

J. Biol. Chem. 2003, 278:19815-19825.
doi: 10.1074/jbc.M301296200 originally published online March 20, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M301296200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 26 references, 15 of which can be accessed free at http://www.jbc.org/content/278/22/19815.full.html#ref-list-1