Escherichia coli single-stranded DNA-binding protein is a supercoiled template-dependent transcriptional activator of N4 virion RNA polymerase

Peter Markiewicz,^{1,4} Cherie Malone,^{1,5} John W. Chase,^{3,6} and Lucia B. Rothman-Denes^{1,2,7}

Departments of ¹Molecular Genetics and Cell Biology and ²Biochemistry and Molecular Biology, The University of Chicago, Chicago, Illinois 60637 USA; ³Department of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York 10461 USA

Coliphage N4 is a double-stranded DNA virus that requires the sequential activity of three different RNA polymerases during infection. The N4 virion RNA polymerase, which is carried in the virion and is injected with the DNA at the start of infection, is responsible for the synthesis of N4 early RNAs. In vitro, the virion RNA polymerase can transcribe double-stranded N4 DNA accurately and efficiently but only when the DNA is denatured. We have shown previously that the activity of DNA gyrase is required for in vivo early N4 transcription. We report here that *Escherichia coli* single-stranded DNA-binding protein (SSB) is also required for N4 early transcription. In vitro, linear or relaxed templates cannot be activated by SSB; however, supercoiled template and SSB allow the virion polymerase to recognize its promoters on duplex DNA and activate transcription. The effects of supercoiling are limited to transcript initiation and are not required for transcript elongation. The activation is specific for SSB; no other single-stranded DNA-binding proteins can substitute. Therefore, SSB is one of a small number of proteins that function to stimulate both replication and transcription. The basis for the specificity of SSB, the mechanism of transcriptional activation by SSB and template supercoiling, and their role in the N4 transcriptional program during development are discussed.

[Key Words: Coliphage N4; RNA polymerase; single-stranded DNA-binding protein; transcriptional activation; template supercoiling]

Bacteriophage N4 is a double-stranded DNA virus specific for *Escherichia coli* K12 strains. Transcription of its genome is regulated through the sequential activity of three distinct RNA polymerases (for review, see Kiino and Rothman-Denes 1988). Early N4 transcripts are synthesized by a rifampicin-resistant, N4-coded RNA polymerase, which is carried within the virion and injected into the cell at the start of infection (Falco et al. 1977). N4 middle transcripts are synthesized by a second N4-coded RNA polymerase (N4 RNA polymerase II) (Zehring and Rothman-Denes 1983; Zehring et al. 1983). Late in infection, the N4-coded, single-stranded DNA-binding protein directs the host RNA polymerase to transcribe the N4 structural genes (Zivin et al. 1981; N. Baek, M. Choi and L. Rothman-Denes, in prep.)

Purified virion RNA polymerase shows peculiar template specificity. It is unable to use native duplex genomic N4 DNA as a template (Falco et al. 1978, 1980), however; and unlike all other DNA-dependent RNA

polymerases, it transcribes denatured or single-stranded, promoter-containing templates accurately and efficiently (Haynes and Rothman-Denes 1985; Glucksmann et al. 1992). This result suggests that the structure of N4 DNA must be altered upon entry into the cell to allow transcription by the virion RNA polymerase. It is likely that the N4 virion RNA polymerase utilizes a supercoiled template because active host DNA gyrase is required for N4 early RNA synthesis (Falco et al. 1978). However, in vitro, supercoiled, promoter-containing templates do not support virion RNA polymerase activity. We report that the *E. coli* single-stranded DNA-binding protein (SSB) is required for in vivo early N4 transcription. Addition of SSB activates supercoiled templates for in vitro transcription by the N4 virion RNA polymerase. Other single-stranded DNA-binding proteins cannot substitute. The characteristics of template activation by SSB argue for specific interactions between SSB and DNA.

Results

Early N4 RNA synthesis requires active SSB

The N4 virion RNA polymerase, which is injected into the cell upon infection, is resistant to the antibiotic rif-

Present addresses: ⁴Department of Biology, University of California at Los Angeles, Los Angeles, California 90024 USA; ⁵Department of Microbiology, University of Iowa, Iowa City, Iowa 52242 USA; ⁶ U.S. Biochemical Co., Cleveland, Ohio 44128 USA.

⁷Corresponding author.

ampicillin at concentrations that inhibit *E. coli* RNA polymerase activity (Falco and Rothman-Denes 1979). Consequently, the *in vivo* activity of the virion polymerase can be quantified by measuring [^3H]uridine incorporation into acid-insoluble material in cells pretreated with rifampicin and chloramphenicol (Rothman-Denes and Schito 1974). Although the rate of rifampicin-resistant [^3H]uridine incorporation in wild-type parent cells increases after infection (Fig. 1A,C), no such increase is observed upon infection of a strain containing the temperature-sensitive mutation *ssb-1*, which maps in the amino-terminal portion of SSB (Y55H) (Williams et al. 1984) (Fig. 1B). The *ssb-1* mutant strain is restrictive for N4 infection at temperatures as low as 33°C. These results indicate that SSB is required for early N4 transcription. In contrast, strains containing the temperature-sensitive mutation *ssb-113*, which alters the carboxy-terminal domain of SSB (P176S) (Chase et al. 1984), support normal early transcription (Fig. 1D) under conditions either permissive or restrictive for cell viability. The implications of this result will be discussed later.

Supercoiled template and SSB allow N4 virion RNA polymerase to accurately initiate and terminate transcription

Although the N4 virion polymerase accurately recognizes its promoters on denatured DNA, the enzyme is incapable of utilizing linear duplex template and cannot

bind to duplex, promoter-containing DNA (Haynes and Rothman-Denes 1985). Figure 2A shows the rate of virion RNA polymerase transcription on supercoiled pBRK DNA, which contains the left-most 2.2 kb of the N4 genome, including the early promoters P1 and P2, followed by their respective terminators T1 and T2 (Fig. 2B). It was expected that recognition of the transcription termination signal in this N4 fragment would allow the synthesis of discrete transcripts, comparable to those observed *in vivo* (Haynes and Rothman-Denes 1985). In the absence of SSB, the supercoiled, promoter-bearing template does not support significant transcription. Addition of SSB activates transcription at least 40-fold. Total incorporation decreases as the ratio of SSB over DNA is increased (Fig. 2A), with optimal stimulation at substoichiometric (20-fold below saturation) ratios and at, or near, the reported intracellular SSB concentration (0.5 μM ; Williams et al. 1984). The same template, in nicked circular or linear form, cannot be activated by SSB to support N4 virion RNA polymerase transcription (Fig. 2A). Analysis of the products by gel electrophoresis demonstrates that discrete RNAs are produced (Fig. 3A). To determine the exact site of initiation, we sequenced the 5' ends of the RNAs synthesized on SSB-activated, supercoiled pBRK with [$\gamma\text{-}^{32}\text{P}$]GTP (Fig. 3B). Recovery of end-labeled 550- and 1100-base transcripts allowed unambiguous identification of the transcript start positions at the (+)582 G on the N4 *HpaI* K sequence (P2, Fig. 3B) (Ohmori et al. 1988). This initiation site is identical to

Figure 1. *In vivo* N4 virion RNA polymerase activity in *E. coli* strains containing SSB-1 or SSB-113 mutant proteins. [^3H]Uridine incorporation was measured as described elsewhere (Rothman-Denes and Schito 1974). (○) Infection at 33°C; (●) infection at 43°C. A and B are parent and *ssb-1* strains, respectively; C and D are parent and *ssb-113* strains, respectively.

Figure 2. SSB activation of N4 virion RNA polymerase transcription on supercoiled pBRK template, demonstrating specific initiation and termination. (A) Supercoiled (●), relaxed (○), or *Pst*I-restricted (□) pBRK DNAs (0.5 μg) were preincubated with SSB at various protein–DNA (weight/weight) ratios in a transcription assay mixture at 37°C for 5 min; polymerase and nucleotides were subsequently added. Total acid-precipitable material was measured. (B) Diagram of pBRK DNA indicating positions of promoters (P1 and P2), terminators (T1 and T2), and transcripts.

that used both in vitro on single-stranded template and in vivo (Haynes and Rothman-Denes 1985). The pattern of Southern blot hybridization (not shown) indicated that the 550- and 1000-base RNAs initiate at P2 and extend to the termination signal T2 and the GC-rich linker at the junction of pBR322 and N4 sequences, respectively. Synthesis of the 1600-base RNA (arrowhead) was too low to generate an unambiguous RNA sequence, but its size and hybridization pattern was consistent with initiation at P1, bypass of T1, and termination at T2. Longer RNAs originate at P2 and proceed past the junction into pBR322 sequences. These results show that supercoiled, SSB-activated template allows the N4 virion RNA polymerase to initiate transcription with in vivo fidelity on duplex DNA.

Supercoiling is required for initiation but not for elongation by N4 virion RNA polymerase

Supercoiling could be necessary for the formation of the initiation complex and/or the elongation process. To distinguish between these two possibilities, supercoiled SSB-activated template (pBRK) was incubated with vir-

ion RNA polymerase and GTP to form an initiation complex. The template was then restricted with *Rsa*I, and the remaining ribonucleoside triphosphates were added to allow elongation. Such treatment resulted in the appearance of RNAs of ~100 bases in length, the size expected for transcripts initiating at P1 and P2 and terminating at distal *Rsa*I sites (Fig. 4A), demonstrating that supercoiling is necessary only for transcription initiation. These results are reminiscent of the in vitro activity of the enzyme on DNA–membrane complexes in infected cell extracts. During isolation, the DNA in the complex is severely nicked, eliminating supercoiling. In these complexes, virion RNA polymerase activity is detected but there are no new rounds of transcription initiation [Falco and Rothman-Denes 1979].

As shown in Figure 4B, transcripts produced by the virion polymerase on SSB-activated, supercoiled pBRK are sensitive to S1 digestion. These results demonstrate that the RNA product is released when synthesized on a supercoiled, SSB-activated template. In contrast, RNAs synthesized on denatured pBRK, in the presence or absence of SSB, are resistant to S1. Most likely these RNAs are in stable RNA–DNA hybrids [Falco et al. 1980].

Figure 3. Analysis of RNA species synthesized on SSB-activated, supercoiled pBRK DNA. (A) RNAs from reactions containing no SSB (lane 1) and SSB at SSB/DNA ratios of 1 (lane 2) and 3 (lane 3) on supercoiled pBRK DNA were resolved on an 8 M urea/12% polyacrylamide gel. (B) Sequencing of the 5' ends of the 550- and 1100-base [γ - 32 P]GTP-initiated RNAs from supercoiled pBRK in the presence of SSB at a 1 : 1 (weight/weight) ratio. After preincubation of the template with SSB, virion polymerase, unlabeled CTP, UTP, ATP (1 mM final), and 1 mCi of [γ - 32 P]GTP (>7000 Ci/mmol) were added, and the reaction was allowed to proceed for 5 min. Unlabeled GTP was then added to 1 mM to allow complete elongation of nascent RNAs. Samples were electrophoresed, and the 550- and 1100-base RNAs were extracted and sequenced as described in Materials and methods.

Transcriptional activation of supercoiled template is SSB specific

Single-stranded DNA-binding proteins are thought to mediate nonspecific, DNA-protein interactions, such as "melting" of secondary structure (Chase and Williams 1986). If SSB activation depends on nonspecific interactions, the effect might be mimicked in vitro by other single-stranded DNA-binding proteins.

Six different single-stranded DNA-binding proteins were tested in the in vitro activation assay. The proteins fell into two groups according to their effects on total RNA synthesis. The first group, which includes the T7-coded DNA-binding protein (Scherzinger et al. 1973), the N4 single-stranded DNA-binding protein (Lindberg et al. 1989), the T4 gene 32 product (Kowalczykowski et al. 1981b), and F-episome single-stranded DNA-binding protein (SSF) (Chase et al. 1983 a), had no effect on transcription (Fig. 5a). A second group, consisting of the T4 gene 32 product amino-terminal fragment 32*I (Lonberg et al. 1981), and fd gene V protein (Alberts et al. 1972) induced incorporation into acid-precipitable radiolabeled material (Fig. 5b). However, RNA transcripts were

shorter than 20 nucleotides and did not initiate at promoters or at specific sites on the template. These RNAs were synthesized even when the template did not contain N4 virion RNA polymerase promoters and hybridized to both strands of the template (not shown). Therefore, activation of the supercoiled template depends on specific features of the SSB molecule, rather than properties common to single-stranded DNA-binding proteins.

Transcriptional activation by SSB mutants

We tested the effect of the SSB mutants in vitro on transcription of plasmid CLM 363, which carries promoter P1 and the signal for transcription termination T2 present in the N4 *HpaI* K DNA fragment.

SSB exists in its active form as a stable tetramer composed of four identical subunits (Williams et al. 1983). The *ssb-1* mutation affects the stability of the tetramer at high temperatures (Bujalowski and Lohman 1991), as well as the formation of the tetramer at low temperatures (Williams et al. 1984). The temperature-sensitive

Figure 4. (A) The supercoiled template is required for initiation and not for elongation. Supercoiled pBRK (0.5 μ g) and SSB [1 : 1 ratio (weight/weight)] were incubated with 1 mM GTP and polymerase to form the initiation complex. *RsaI* was then added, and allowed to restrict for 5 min, after which the remaining nucleotides were added and the reaction was allowed to proceed. Products were then electrophoresed as described in Materials and methods. (B) Sensitivity of RNA synthesized on SSB-activated supercoiled template to S1 nuclease. Incubation, S1 reaction, and analysis are described in Materials and methods.

phenotype can be reversed *in vivo* by increasing the concentration of the mutant protein (Chase et al. 1983b). An optimum SSB to DNA ratio (0.75) and different SSB or SSB-1 concentrations were tested at 39°C (Fig. 6A). Although SSB-1 was deficient in activating transcription at low concentrations, activation was detected above 0.5 μ M, a concentration known to shift the equilibrium to the tetrameric form (Williams et al. 1984).

The *ssb-113* mutation codes for a protein with a slightly increased affinity for DNA (Chase et al. 1984). In contrast to SSB-1, SSB-113 is able to activate transcription at all concentrations tested (not shown). It is interesting to note that SSB-113 is a more efficient activator (Fig. 6B) than the wild-type protein. Similar results (not shown) were obtained with SSB_{chya}, a derivative of SSB resulting from the removal of the last 42 amino acids by chymotryptic digestion (Williams et al. 1983). The results of these experiments not only agree with the *in vivo* effects of the SSB mutations but also indicate that a native carboxy-terminal SSB domain is not required for transcriptional activation.

Discussion

The results presented in this paper indicate that transcription by N4 virion RNA polymerase requires SSB and supercoiling of the template. Although the requirement

for SSB is common in DNA synthesis and recombination, it is unprecedented in transcription. The characteristics of transcriptional activation of N4 virion RNA polymerase promoters by SSB argue for formation of a complex involving specific protein–DNA and/or protein–protein interactions.

Numerous cases exist of DNA polymerases being stimulated only by their cognate single-stranded DNA-binding proteins (Kowalczykowski et al. 1981a). This specificity is thought to reside in the specific template conformation provided by single-stranded DNA-binding proteins at the primer–template junction or through specific protein–protein interactions. Extensive genetic evidence indicates that the T4 gene 32 protein interacts with a large number of T4-coded proteins involved in recombination and replication (Mosig et al. 1979). Formosa et al. (1983) showed biochemically a large range of heterologous gene 32 protein–protein interactions. Similar experiments with SSB and proteins present in uninfected cells yielded disappointing results (Perrino et al. 1988). At present, we have no evidence for specific virion RNA polymerase–SSB interactions.

SSB interactions with supercoiled template at the virion RNA polymerase promoters

It is well documented that negative supercoiling can modulate the activity of many eubacterial RNA polymerase promoters (Sansey 1979). Supercoiling activates the *Salmonella typhimurium* *leu* 500, as well as the *S. typhimurium* and *E. coli* *his* promoters (Margolin et al. 1985; Rudd and Menzel 1987) while it represses the expression of the *E. coli* *gyrA* and *gyrB* genes (Menzel and Gellert 1983). An increase or decrease in superhelical turns changes the relative position of functional groups, alters the affinity of proteins to DNA (von Hippel et al. 1984), and changes the structure of regulatory complexes (Borowiec et al. 1987; Richet and Raibaud 1991). The

Figure 5. Effect of various DNA-binding proteins on N4 virion RNA polymerase transcription of supercoiled pBRK template. (a) (●) SSB; (○) SSF; (△) T7 gene 2.5 protein; (■) T4 gp32; (□) N4 single-stranded DNA-binding protein. (b) (●) SSB; (○) fd gene V protein; (□) T4 gp32*1.

Figure 6. Differential ability of SSB-1 (A) and SSB-113 (B) to activate supercoiled template for N4 virion polymerase transcription. Standard polymerase assay conditions were used except that incubation was at 39°C. In A, SSB and SSB-1 were present at 0.75 (weight/weight) protein/template ratio, and the concentration (μM) of SSB and SSB-1 was varied. In B the DNA was maintained constant and the ratio of SSB or SSB-113/DNA was varied. Other conditions are as in Materials and methods.

dependence of N4 virion RNA polymerase on supercoiled template for promoter recognition differs, however, from all cases studied in that template supercoiling is an absolute prerequisite.

A number of structural features distinguish supercoiled from relaxed templates. Introduction of negative supertwists into DNA leads to regions of single-strandedness (Kohwi-Shigematsu and Kohwi 1985), the formation of cruciforms (Lilley 1980; Panayotatos and Wells 1981), or sections of left-handed Z DNA (Peck et al. 1982; Singleton et al. 1982; Jaworski et al. 1991), which would not be found in a linear or relaxed molecule (for review, see Wells 1988). What DNA sequence features in the N4 early promoters might contribute to structures that allow specific protein binding to the supercoiled template? Two pairs of inverted repeats are present in the consensus sequence of the virion RNA polymerase promoters (Haynes and Rothman-Denes 1985), one within the promoter and the second immediately downstream of the site of transcription initiation. Site-directed mutagenesis of the promoter sequences indicates that the integrity of the inverted repeats within the promoter is essential for transcriptional activity (Glucksmann et al. 1992). Recent results also indicate that these inverted repeats are extruded as cruciforms upon the introduction of negative supercoils (X. Dai, unpubl.).

Why is SSB required for promoter recognition? We speculate that supercoil-induced cruciform extrusion might direct the binding of SSB to the promoter region and stabilize the promoter in an active conformation (Glucksmann et al. 1992). It has been shown that negative supercoiling provides specific regions for SSB bind-

ing on a double-stranded template. *E. coli* SSB binds to specific sites on supercoiled, but not linear, plasmid DNA and renders it susceptible to S1 nuclease (Gilkin et al. 1983). The template determinants of SSB binding, however, were not investigated. In our case, we propose that the formation of a SSB-DNA complex is directed by the inverted repeats at the promoter. Although the possible stem-loop structures may be too small to be intrinsically stable, a dynamic equilibrium of rapidly interconverting structures (Lilley and Markham 1983) might be stabilized by SSB and subsequently recognized by virion RNA polymerase.

Why is activation of N4 virion RNA polymerase promoters dependent exclusively on SSB? All single-stranded DNA-binding proteins share the property of binding nonspecifically and with high affinity to single-stranded DNA and, in most cases, with positive cooperativity (Chase and Williams 1986); however, they differ substantially in their mode of binding. Of the several proteins that we have tested, the T4 gene 32 product and N4 single-stranded DNA-binding protein are active as monomers, bind to DNA with high cooperativity, and force the DNA into an extended conformation (Delius et al. 1972; Kowalczykowski et al. 1981b; Lindberg et al. 1989). The fd gene V product is a dimer that, upon binding to DNA, forces two protein-covered DNA strands into a helical rod-like structure (Alberts et al. 1972). The binding mode of the above-mentioned proteins is independent of solution conditions. In contrast, SSB exists in solution as an extremely stable tetramer that interacts with single-stranded DNA in a variety of binding modes, depending on solution conditions (for review, see Loh-

man and Bujalowski 1990). Under experimental conditions similar to ours, the SSB tetramer has a DNA-binding site of 56 nucleotides (Bujalowski et al. 1988). Analysis of SSB–single-stranded DNA complexes by electron microscopy has revealed a “beaded” morphology in which the apparent contour length of DNA has been reduced by ~75% (Chrysogelos and Griffith 1982). Micrococcal nuclease digestion and cross-linking studies indicate that single-stranded DNA wraps around the SSB tetramer. We suggest that the specificity of SSB activation is the result of the unique mode of DNA binding of SSB. Although surprising, this specificity is not unprecedented. SSB specifies the site of priming activity of the *dnaG* primase in phage G4 DNA replication (Benz et al. 1983) and of *E. coli* RNA polymerase in phage M13 DNA replication (Geider and Kornberg 1974). In both cases, specific priming occurs at sites that are characterized by stem-loop structures (Hiasa et al. 1989, 1990), and other single-stranded DNA-binding proteins cannot substitute (G. Nigel Godson, pers. comm.).

The results presented in this paper indicate that SSB is a member of a small number of bifunctional DNA-binding proteins that both interact with single-stranded DNA and also regulate gene expression. The adenovirus-coded DNA-binding protein (DBP) binds preferentially to single-stranded DNA in a sequence-independent fashion (Schechter et al. 1980) and is involved in DNA replication (Tsuji et al. 1991). In addition, DBP regulates adenovirus gene expression (Klessig and Grodzicker 1979; Handa et al. 1983; Chang and Shenk 1990) and increases the affinity of binding of the cellular transcription factor NF1 to its specific binding sites at the ends of the adenovirus genome (Cleat and Hay 1989). Additionally, the N4-coded, single-stranded DNA-binding protein (Lindberg et al. 1989) is required for activation of the *E. coli* RNA polymerase- σ^{70} holoenzyme at N4 late promoters (N. Baek, M. Choi and L.B. Rothman-Denes, in prep.), as well as being required for N4 DNA replication (Guinta et al. 1986; Lindberg et al. 1989).

N4 is one of a small number of bacteriophages that synthesizes its own RNA polymerases (Chamberlin et al. 1970; Clark et al. 1974). It has been hypothesized that these phages make their own RNA polymerases to become independent of their hosts for transcription (Rabussay and Geiduschek 1977). N4 could be seen as an extreme example of this strategy because injection of the virion-associated RNA polymerase bypasses the need for the host transcriptional machinery early in infection. The results presented in this paper demonstrate that N4 relies, however, on the activity of at least two host proteins, SSB and DNA gyrase, for early transcription. What is the reason for such dependence on two host functions? It has been shown that the level of supercoiling inside the cell is influenced by [ATP]/[ADP] ratios (McClellan et al. 1990; Hsieh et al. 1991a,b). We suggest that the dependence of N4 early RNA synthesis on gyrase activity allows the phage to monitor the ability of the host cell to support phage growth. Under unfavorable conditions, N4 would establish a pseudodysogenic condition, or carrier state, during which the host and phage ge-

nomes undergo asymmetric segregation in the absence of viral replication (Satta et al. 1969).

Materials and methods

Restriction enzymes were purchased from BRL and used according to the manufacturer's recommendations. S1 nuclease and RNA sequencing enzymes were from PL Biochemicals. [α - 32 P]GTP was from Amersham. [γ - 32 P]GTP was synthesized by use of [32 P]orthophosphate (>7000 Ci/mmol; New England Nuclear) according to the method of Johnson and Walseth (1979). [3 H]Uridine (28 Ci/mmol) was from New England Nuclear. Rifampicin and chloramphenicol were from Sigma Biochemicals.

Bacterial strains and plasmids

The *E. coli* strains used were N99 (*rpsL*, *galK*) and M5248 (N99, *bio275*, *cl857*, *lpd*); HM591 (W3110 *rha*, *thy*) and KLC792 (HM591, *ssb-113*) (Chase et al. 1984). The plasmids used were pKC30 (Rosenberg et al. 1983), pACYC184 (Chang and Cohen 1978), and Δ R/pLL10 (Rothstein et al. 1979). KLC1039/pKAC25 was used for expression of the SSB-1 mutant (Williams et al. 1984).

High expression of the SSB-113 mutant was achieved by cloning the *EcoRI* DNA fragment from pKAC20 (Chase et al. 1984) into the unique *EcoRI* site of pACYC184. The orientation of the DNA fragment that gave the greatest expression of wild-type protein was identified, and the plasmid was designated pKAC21. Expression of SSB-113 was carried out in a *ssb-113* mutant strain, KLC792. From the purification of the protein, it was estimated that a 60-fold overexpression was achieved (Chase et al. 1983b).

To further increase expression of the wild-type *ssb* gene, the gene with 26 nucleotides of its ribosome binding site was placed under λ pL regulation in pKC30. The plasmid was designated pKAC27 and was transformed into M5248 for temperature-dependent expression. We estimate the expression of SSB obtained from this construction to be ~200-fold above the normal cellular level of SSB (data not shown).

Purification of proteins

The N4 virion RNA polymerase was purified as described previously (Falco et al. 1980). Wild-type SSB was purified as described previously (Chase et al. 1980), with certain modifications (Chase et al. 1984). SSB-113 and SSB-1 were purified as described (Chase et al. 1984; Williams et al. 1984). The partial proteolysis product of SSB produced by digestion with chymotrypsin (SSBchyA) was prepared as described (Williams et al. 1983). DNA-binding protein SSF was purified according to Chase et al. (1983a). The T4-gene 32 protein, its amino-terminal fragment 32*I, and bacteriophage fd gene V protein were provided by Ken Williams (Yale University, New Haven, CT). T7 DNA-binding protein was provided by Paul Sadowski (University of Toronto, Canada).

DNA templates

The subcloning of N4 virion RNA polymerase promoters and their characterization is described elsewhere (Glucksmann et al. 1992). pBRK, a plasmid containing the N4 *HpaIK* fragment (Malone et al. 1988), was isolated from W3350 (F⁻, Cou^R, *gal*, *lac*). CLM 363 is an M13 mp9 derivative that carries the PM103 *Bam*HI fragment containing the P1 promoter at its *Bam*HI site

and the T2 terminator signal for N4 virion RNA polymerase at its *HincII* site. Both promoter and terminator sequences are in the same orientation as found in the N4 genome. CLM 363 replicative form I DNA was purified from JM103 according to Messing (1983).

RNA polymerase assay

Reactions were carried out in a 50- μ l volume, with 2–5 μ l of enzyme (Falco et al. 1980), 0.5 μ g of template DNA, 0–10 μ g of SSB or other DNA-binding proteins, 10 mM Tris-HCl (pH 8.0), 10 mM MgCl₂, 1 mM EDTA, 1 mM dithiothreitol, 50 mM NaCl, and 1 mM of each unlabeled ribonucleoside triphosphates except GTP. Either [α -³²P]GTP or [γ -³²P]GTP was added along with the enzyme to initiate the reaction. When maximum incorporation at the 5'-terminal nucleotide was required for RNA sequencing, only [γ -³²P]GTP was added. Otherwise, unlabeled GTP was added to 0.1 mM and the reaction was allowed to proceed for another 5 min before the GTP concentration was increased to 1 mM to ensure complete elongation of the RNAs. Reactions were terminated by ethanol precipitation, resuspended in formamide loading buffer (80% formamide, 50 mM Tris-HCl at pH 8.0, 20 mM EDTA, 0.5% bromophenol blue, and xylene cyanol), and electrophoresed on 8 M urea/6–8% polyacrylamide gels. An aliquot of the reaction was reserved for trichloroacetic acid (TCA) precipitation and counting.

RNA sequencing

[γ -³²P]GTP-initiated RNAs produced as described above were passed through Sephadex G25 to remove unincorporated label and electrophoresed on 5% acrylamide gels. RNAs were localized on the gel by autoradiography. The gel slice was removed, and RNA was allowed to elute by diffusion in 100 μ l of phenol-saturated water with 5 μ g of tRNA, overnight, at room temperature. After ethanol precipitation, the purified RNA was resuspended in 10 μ l of H₂O and subjected to enzymic sequencing reactions by use of PL Biochemicals RNA sequencing kit. Products were electrophoresed on 7 M urea/20% polyacrylamide gels, and the radioactivity was visualized by autoradiography.

S1 nuclease assays

After a standard RNA synthesis reaction, as described above, and cooling to room temperature, 200 units of S1 nuclease in 1 μ l of dilution buffer (20 mM sodium acetate at pH 5.5, 0.5 M NaCl) was added directly to the reaction mixture. This quantity of S1 was sufficient to degrade single-stranded oligonucleotides in the reaction at pH 8.0 within 5 min, without affecting duplex template. Reaction products were precipitated, resuspended, and electrophoresed.

Acknowledgments

We thank Kathy Hudson for technical assistance, Drs. Paul Sadowski and Ken Williams for generously providing some of the single-stranded DNA-binding proteins, Drs. Sidney Kustu, Carol Gross, Diane Kiino, and Tom Poche and Rick Carter, and all the members of the L.B.R.-D. laboratory for critical reading of the manuscript. Work was supported by National Institutes of Health grants AI 12575 to L.B.R.-D. and GM 11301 to J.W.C.

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

References

- Alberts, B.M., L. Frey, and H. Delius. 1972. Isolation and characterization of gene 5 protein of filamentous bacterial viruses. *J. Mol. Biol.* **68**: 139–152.
- Benz, E.W. Jr., J. Sims, D. Dressler, and J. Hurwitz. 1983. Tertiary structure is involved in the initiation of DNA synthesis by the dnaG protein. In *Mechanistic studies of DNA replication and genetic recombination* (ed. B. Alberts), pp. 279–291. Academic Press, New York.
- Borowiec, J.A., L. Zhang, S. Sasse-Dwight, and J.D. Gralla. 1987. DNA supercoiling promotes the formation of a bent repression loop in *lac* DNA. *J. Mol. Biol.* **196**: 101–111.
- Bujalowski, W. and T.M. Lohman. 1991. Monomer-tetramer equilibrium of the *Escherichia coli* *ssb-1* mutant single strand binding protein. *J. Biol. Chem.* **266**: 1616–1626.
- Bujalowski, W., L.B. Overman, and T.M. Lohman. 1988. Binding mode transitions of *Escherichia coli* single strand binding protein-single-stranded DNA complexes. *J. Biol. Chem.* **263**: 4629–4640.
- Chamberlin, M.J., J. McGrath, and L. Waskell. 1970. New RNA polymerase from *Escherichia coli* infected with bacteriophage T7. *Nature* **228**: 227–231.
- Chang, A.C.V. and S.N. Cohen. 1978. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the p15A cryptic miniplasmid. *J. Bacteriol.* **134**: 3215–3227.
- Chang, L.-S. and T. Shenk. 1990. The adenovirus DNA-binding protein stimulates the rate of transcription directed by adenovirus and adeno-associated virus promoters. *J. Virol.* **64**: 2103–2109.
- Chase, J.W. and K.R. Williams. 1986. Single-stranded DNA binding proteins required for DNA replication. *Annu. Rev. Biochem.* **55**: 130–136.
- Chase, J.W., R.F. Whittier, J. Auerbach, A. Sancar, and W.D. Rupp. 1980. Amplification of the single-stranded DNA binding protein in *E. coli*. *Nucleic Acids Res.* **8**: 3215–3227.
- Chase, J.W., B.M. Merrill, and K.R. Williams. 1983a. F sex factor encodes a single-stranded DNA binding protein (SSB) with extensive homology to *E. coli* SSB. *Proc. Natl. Acad. Sci.* **80**: 5480–5484.
- Chase, J.W., J.B. Murphy, R.F. Whittier, E. Lorensen, and J.J. Sninsky. 1983b. Amplification of the *ssb-1* mutant single-stranded DNA binding protein in *E. coli*. *J. Mol. Biol.* **164**: 193–211.
- Chase, J.W., J.J. L'Italien, J.B. Murphy, E.K. Spicer, and K.R. Williams. 1984. Characterization of the *E. coli* SSB-113 mutant single-stranded DNA binding protein: Cloning of the gene, DNA and protein sequence analysis, HPLC peptide mapping and DNA binding studies. *J. Biol. Chem.* **259**: 805–814.
- Chrysogelos, S. and J. Griffith. 1982. *Escherichia coli* single-strand binding protein organizes single-stranded DNA in nucleosome like units. *Proc. Natl. Acad. Sci.* **79**: 5803–5807.
- Clark, S., R. Losick, and J. Pero. 1974. New RNA polymerase from *B. subtilis* infected with PBS2. *Nature* **252**: 21–24.
- Cleat, P.H. and R.T. Hay. 1989. Co-operative interactions between NFI and the adenovirus DNA binding protein at the adenovirus origin of replication. *EMBO J.* **8**: 1841–1848.
- Delius, H., N.J. Mantell, and B. Alberts. 1972. Characterization by electron microscopy of the complex between T4 bacteriophage gene 32 protein and DNA. *J. Mol. Biol.* **67**: 341–350.
- Falco, S.C. and L.B. Rothman-Denes. 1979. Bacteriophage N4-induced transcribing activities in *Escherichia coli* I. Detection and characterization in cell extracts. *Virology* **95**: 454.
- Falco, S.C., K. VanderLaan, and L.B. Rothman-Denes. 1977. Vir-

- ion-associated RNA polymerase required for bacteriophage N4 development. *Proc. Natl. Acad. Sci.* **74**: 520–523.
- Falco, S.C., R. Zivin, and L.B. Rothman-Denes. 1978. Novel template requirements of N4 virion RNA polymerase. *Proc. Natl. Acad. Sci.* **75**: 3220–3224.
- Falco, S.C., W. Zehring, and L.B. Rothman-Denes. 1980. DNA-dependent RNA polymerase from bacteriophage N4 virions. Purification and characterization. *J. Biol. Chem.* **255**: 4339–4347.
- Formosa, T., R.L. Burke, and B.M. Alberts. 1983. Affinity purification of bacteriophage T4 proteins essential for DNA replication and genetic recombination. *Proc. Natl. Acad. Sci.* **80**: 2442–2446.
- Geider, K. and A. Kornberg. 1974. Conversion of the M13 viral single-strand to the double-stranded replicative form by purified proteins. *J. Biol. Chem.* **249**: 3999–4005.
- Gilkin, G.C., G. Gargiulo, L. Rena-Descalzi, and A. Worcel. 1983. *E. coli* single-stranded DNA binding protein stabilizes specific denatured sites in superhelical DNA. *Nature* **303**: 770–774.
- Glucksmann, M.A., P. Markiewicz, C. Malone, and L.B. Rothman-Denes. 1992. Specific sequences and a hairpin structure in the template strand are required for N4 virion RNA polymerase-promoter recognition. *Cell* **70**: 491–500.
- Guinta, D., J. Stambouly, S.C. Falco, J.K. Rist, and L.B. Rothman-Denes. 1986. Host and phage-coded functions required for coliphage N4 DNA replication. *Virology* **150**: 33–44.
- Handa, H., R.E. Kingston, and P.A. Sharp. 1983. Inhibition of adenovirus early region IV transcription in vitro by a purified viral DNA binding protein. *Nature* **302**: 545–547.
- Haynes, L.L. and L.B. Rothman-Denes. 1985. N4 virion RNA polymerase sites of transcription initiation. *Cell* **41**: 597–605.
- Hiasa, H., K. Tanaka, H. Sakai, K. Yoshida, Y. Honda, T. Komano, and G.N. Godson. 1989. Distinct functional contributions of the three potential secondary structures in the phage G4 origin of complementary DNA strand synthesis. *Gene* **84**: 17–22.
- Hiasa, H., H. Sakai, T. Komano, and G.N. Godson. 1990. Structural features of the priming signal recognized by primase: Mutational analysis of the phage G4 origin of complementary strand synthesis. *Nucleic Acids Res.* **18**: 4825–4831.
- Hsieh, L.-S., R. Burger, and K. Drlica. 1991a. Bacterial DNA supercoiling and the ATP/ADP changes associated with transition to anaerobic growth. *J. Mol. Biol.* **219**: 443–450.
- Hsieh, L.-S., J. Rouviere-Yaniv, and K. Drlica. 1991b. Bacterial DNA supercoiling and the ATP/ADP ratio: Changes associated with salt shock. *J. Bacteriol.* **173**: 3914–3917.
- Jaworski, A., N.P. Higgins, R.D. Wells, and W. Zacharias. 1991. Topoisomerase mutants and physiological conditions control supercoiling and Z-DNA formation in vivo. *J. Biol. Chem.* **266**: 2576–2581.
- Johnson, R.A. and T.F. Walseth. 1979. The enzymatic preparation of [α -³²P]ATP, [α -³²P]GTP, [³²P]cAMP, and [³²P]cGMP, and their use in the assay of adenylate and guanylate cyclases and cyclic nucleotide phosphodiesterases. *Adv. Cyclic Nucleotide Res.* **10**: 135–167.
- Kiino, D.R. and L.B. Rothman-Denes. 1988. Bacteriophage N4. In *The bacteriophages* (ed. R. Calendar), vol. 2, pp. 457–474. Plenum Press, New York.
- Klessig, D.F. and T. Grodzicker. 1979. Mutations that allow human ad2 and ad5 to express late genes in Monkey cells map in the viral gene encoding the 72K DNA binding protein. *Cell* **17**: 957–966.
- Kohwi-Shigematsu, T. and Y. Kohwi. 1985. Poly(dG)-poly(dC) sequences, under torsional stress, induce an altered conformation upon neighboring DNA sequences. *Cell* **43**: 199–206.
- Kowalczykowski, S.C., D.G. Bear, and P.H. von Hippel. 1981a. Single-stranded DNA binding proteins. In *The enzymes. Nucleic acids Part A* (ed. P.D. Boyer), vol. XIV, pp. 374–444. Academic Press, New York.
- Kowalczykowski, S.C., N. Lonberg, J.W. Newport, and P.H. von Hippel. 1981b. Interactions of bacteriophage T4 gene 32 protein with nucleic acids: I. Characterization of the binding interactions. *J. Mol. Biol.* **145**: 75–104.
- Lilley, D.J.M. 1980. The inverted repeat as a recognizable structural feature in supercoiled DNA molecules. *Proc. Natl. Acad. Sci.* **77**: 6468–6472.
- Lilley, D.M.J. and A.F. Markham. 1983. Dynamics of cruciform extrusion in supercoiled DNA: Use of a synthetic inverted repeat to study conformational populations. *EMBO J.* **2**: 527–533.
- Lindberg, G.J., S.C. Kowalczykowski, J.K. Rist, A. Sugino, and L.B. Rothman-Denes. 1989. Purification and characterization of the coliphage N4-coded single-stranded DNA binding protein. *J. Biol. Chem.* **264**: 12700–12708.
- Lohman, T.M. and W. Bujalowski. 1990. *E. coli* single-strand binding protein: Multiple DNA-binding modes and cooperativities. In *The biology of nonspecific DNA-protein interactions* (ed. A. Revzin), pp. 131–170. CRC Press, Boca Raton, FL.
- Lonberg, N., L. Kowalczykowski, S. Paul, and P.H. Von Hippel. 1981. Interactions of bacteriophage T4 gene 32 protein with nucleic acids. III. Binding properties of two specific proteolytic digestion products of the protein gp32*^I and gp32*^{III}. *J. Mol. Biol.* **145**: 122–138.
- Malone, C., S. Spellman, D. Hyman, and L.B. Rothman-Denes. 1988. Cloning and generation of a genetic map of bacteriophage N4. *Virology* **162**: 328–336.
- Margolin, P., L. Zumstein, R. Sternglanz, and J.C. Wang. 1985. The *Escherichia coli* supX locus is topA, the structural gene for DNA topoisomerase I. *Proc. Natl. Acad. Sci.* **82**: 5437–5441.
- McClellan, J.A., P. Boublikova, E. Palecek, and D.M.J. Lilley. 1990. Superhelical torsion in cellular DNA responds directly to environmental and genetic factors. *Proc. Natl. Acad. Sci.* **87**: 8373–8377.
- Menzel, R. and M. Gellert. 1983. Regulation of the genes for *E. coli* DNA gyrase: Homeostatic control of DNA supercoiling. *Cell* **34**: 105–113.
- Messing, J. 1983. New M13 vectors for cloning. *Methods Enzymol.* **101**: 20–98.
- Mosig, G., A. Luder, G. Garcia, R. Dannenberg, and S. Bock. 1979. In vivo interactions of genes and proteins in DNA replication and recombination of phage T4. *Cold Spring Harbor Symp. Quant. Biol.* **43**: 501–515.
- Ohmori, H., L.L. Haynes, and L.B. Rothman-Denes. 1988. Terminally repeated sequences of the coliphage N4 genome. *J. Mol. Biol.* **202**: 1–10.
- Panayotatos, N. and R.D. Wells. 1981. Cruciform structures in supercoiled DNA. *Nature* **289**: 466–470.
- Peck, L.J., A. Nordheim, A. Rich, and J.C. Wang. 1982. Flipping of cloned d(pCpG)_n·d(pCpG)_n DNA sequences from right to left handed helical structure by salt, Co(III) or negative supercoiling. *Proc. Natl. Acad. Sci.* **79**: 4560–4564.
- Perrino, F.W., R.R. Meyer, A.M. Bobst, and D.C. Rein. 1988. Interaction of a folded chromosome-associated protein with single-stranded DNA binding protein of *E. coli* identified by affinity chromatography. *J. Biol. Chem.* **263**: 11833–11839.
- Rabussay, D. and E.P. Geiduschek. 1977. Regulation of gene action in development of lytic bacteriophages. *Compr. Virol.*

- 8: 1–196.
- Richet, E. and O. Raibaud. 1991. Supercoiling is essential for the formation and stability of the initiation complex at the divergent *malEp* and *malKp* promoters. *J. Mol. Biol.* **218**: 529–542.
- Rosenberg, M., Y. Ho, and A. Saltzman. 1983. The use of pKC30 and its derivatives for controlled expression of genes. *Methods Enzymol.* **101**: 123–138.
- Rothman-Denes, L.B. and G.C. Schito. 1974. Novel transcribing activities in N4-infected *Escherichia coli*. *Virology* **60**: 65–72.
- Rothstein, R.J., L.F. Lau, C.P. Bahl, S.A. Narang, and R. Wu. 1979. Synthetic adaptors for cloning DNA. *Methods Enzymol.* **68**: 98–109.
- Rudd, K.E. and R. Menzel. 1987. His operons of *Escherichia coli* and *Salmonella typhimurium* are regulated by DNA supercoiling. *Proc. Natl. Acad. Sci.* **84**: 517–521.
- Sansey, B. 1979. Modulation of gene expression by drugs affecting DNA gyrase. *J. Bacteriol.* **138**: 40–47.
- Satta, G., A. Pesce, and G.C. Schito. 1969. The carrier state of *E. coli* cells infected with N4 bacteriophage. *Giorn. Microbiol.* **17**: 151–161.
- Schechter, N.M., W. Davies, and C.W. Anderson. 1980. Adenovirus-coded DNA-binding protein. Isolation, physical properties and effects of proteolytic digestion. *Biochemistry* **19**: 2802–2810.
- Scherzinger, E., F. Litfin, and E. Jost. 1973. Stimulation of T7 DNA polymerase by a new phage-coded protein. *Mol. Gen. Genet.* **123**: 247–262.
- Singleton, C.K., J. Klysik, S.M. Stirdivant, and R.D. Wells. 1982. Left-handed Z DNA is induced by supercoiling in physiological ionic conditions. *Nature* **299**: 312–316.
- Tsuiji, M., P.C. Van der Vliet, and G.R. Kitchingman. 1991. Temperature sensitive mutants of Adenovirus single-stranded DNA binding protein. *J. Biol. Chem.* **266**: 16178–16187.
- von Hippel, P.H., D.G. Bear, W.D. Morgan, and J.A. McSwiggan. 1984. Molecular aspects of transcription *Annu. Rev. Biochem.* **53**: 389–446.
- Wells, R.D. 1988. Unusual DNA structures. *J. Biol. Chem.* **263**: 1095–1098.
- Williams, K.R., E.K. Spicer, M. LoPresti, R.A. Guggenheimer, and J.W. Chase. 1983. Limited proteolysis studies on the *E. coli* single-stranded DNA-binding protein: Evidence for a functionally homologous domain in both the *E. coli* and T4 DNA binding proteins. *J. Biol. Chem.* **258**: 3346–3355.
- Williams, K.R., J.B. Murphy, and J.W. Chase. 1984. Characterization and functional defect in the *E. coli* single-stranded DNA-binding protein encoded by the *ssb-1* mutant gene: Expression of the *ssb-1* gene under lambda pL regulation. *J. Biol. Chem.* **259**: 11804–11811.
- Zehring, W.A., S.C. Falco, C. Malone, and L.B. Rothman-Denes. 1983. Bacteriophage N4-induced transcribing activities in *E. coli*. III. A third cistron required for N4 RNA polymerase II activity. *Virology* **126**: 678–687.
- Zehring, W.A. and L.B. Rothman-Denes. 1983. Purification and characterization of coliphage N4 RNA polymerase II activity from infected cell extracts. *J. Biol. Chem.* **258**: 8074–8080.
- Zivin, R., W. Zehring, and L.B. Rothman-Denes. 1981. Transcriptional map of bacteriophage N4. Location and polarity of N4 RNAs. *J. Mol. Biol.* **152**: 335–356.

Escherichia coli single-stranded DNA-binding protein is a supercoiled template-dependent transcriptional activator of N4 virion RNA polymerase.

P Markiewicz, C Malone, J W Chase, et al.

Genes Dev. 1992, **6**:

Access the most recent version at doi:[10.1101/gad.6.10.2010](https://doi.org/10.1101/gad.6.10.2010)

References

This article cites 69 articles, 27 of which can be accessed free at:
<http://genesdev.cshlp.org/content/6/10/2010.full.html#ref-list-1>

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).

Focused on your science.