Proc. Nat. Acad. Sci. USA
Vol. 72, No. 3, pp. 784–788, March 1975

Nucleotide Sequence of an RNA Polymerase Binding Site at an Early T7 Promoter

(initiated complex/DNA·RNA hybridization/RNA sequence)

DAVID PRIBNOW

The Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138

Communicated by J .D. Watson, December 16, 1974

ABSTRACT ***Escherichia coli*** **RNA polymerase (EC 2.7.7.6), bound in a tight complex at an early T7 promoter, protects 41 to 43 base pairs of DNA from digestion by DNase I. The protected DNA fragment contains both the binding site for RNA polymerase and the mRNA initiation point for the promoter. The sequence of the DNA fragment and the sequence of the mRNA that it codes for are presented here. A seven-base-pair sequence, apparently common to all promoters, is implicated in the formation of a tight binary complex with RNA polymerase.**

The transcription of a gene begins at a specific site called a promoter (1). At such a site the RNA polymerase interacts with the DNA in order to initiate the synthesis of an RNA molecule. How does the polymerase *recognize* a promoter? The answer might be found in the nucleotide sequence of the DNA.

At 37°, in a low salt environment, the *Escherichia coli* RNA polymerase (holoenzyme)* (EC 2.7.7.6; nucleosidetriphosphate:RNA nucleotidyltransferase) forms tight binary complexes at a limited number of sites along a DNA template (2–8). At any particular site, the double-stranded region of the DNA bound up in a stable complex is "protected" by the polymerase from digestion with DNase (8–13). The end product of such DNase treatment is an intact complex consisting of a polymerase molecule bound to a specific DNA fragment (8, 14). What is the nature of the protected DNA fragment, and how does it relate to a promoter?

I have isolated a single RNA polymerase-protected fragment from the "early region" of bacteriophage T7 DNA. This DNA fragment contains the initiation point for an "early" T7 messenger RNA molecule. Here I report the nucleotide sequence of the promoter fragment.

METHODS AND RESULTS

E. coli RNA polymerase transcribes the first 20% (the early region) of the 25 × 10^6 molecular weight T7 genome *in vivo* (15–17) and *in vitro* (17–20). Most transcription is initiated at three strong, independent promoters (A1, A2, and A3) situated at the far left end of the T7 DNA molecule, preceding the first of the early genes (17, 20). RNA polymerase forms tight binary complexes at these three sites (4–7). In order to isolate and determine the sequence of a single T7 binding site, a way had to be found to bind the polymerase selectively to *one* T7 promoter.

Tight complexes between RNA polymerase and its binding sites are destabilized when the salt concentration in the binding mixture is raised (or when the temperature is lowered) (2–4, 6, 21–23). Under these conditions DNase can degrade *all* of the DNA. If, however, the polymerase has been allowed to initiate RNA synthesis, elevated salt concentrations do not disengage the polymerase (2, 21–23), and polymerase-protected DNA fragments can be obtained (13). I have been able to selectively "lock" RNA polymerase onto the T7 A3 promoter by forming a salt-stable, initiated complex exclusively at that site on the T7 genome. The key to the specification of the A3 site is the use of a dinucleotide (24, 25) to direct a limited initiation event. E. Minkley and I (20) have shown that particular dinucleotides (50–100 μm) can be used in the presence of very low concentrations of triphosphates to selectively "prime" transcription by RNA polymerase at specific T7 promoters. The polymerase makes use of a dinucleotide by adding RNA precursors onto its 3′-OH end (20, 26). When RNA polymerase and T7 DNA are mixed together with the dinucleotide CpA and the triphosphate CTP, the polymerase "initiates" synthesis, forming CpApC-OH, exclusively at the T7 A3 promoter. The formation of the phosphodiester bond between CpA and CTP renders the complex stable in 0.2 M KCl and insensitive to inactivation by the drug, rifampicin (5, 27). [The elongation of CpApC-OH primer in the presence of rifampicin produced only class A3 messengers (20).]

The A3 protected fragment was isolated by DNase treatment of the initiated complex. First, RNA polymerase and T7 [^{32}P]DNA (molar ratio of RNA polymerase to T7 DNA, 15:1) were incubated together for 10 min with CpA and CTP in 0.05 M KCl (37°) to allow initiation at the A3 site to take place, and then KCl was added to a final concentration of 0.2 M to dislodge noninitiated polymerase molecules from other binding sites. Next, the DNA was digested with DNase I (200 μg/ml) for 10 min at 37°. After the reaction was stopped with EDTA, the remaining A3 complexes were run directly on a 2 ml Sephadex G-100 column. Radioactive fractions excluded from the column were pooled and extracted with phenol to remove the polymerase. The [^{32}P]DNA was then precipitated with ethanol, resuspended, and electrophoresed on a nondenaturing, 10% polyacrylamide gel.

The double-stranded A3 DNA migrated on the gel as a single (although slightly diffuse) band in the size range of 40 to 50 base pairs. The DNA fragments were over 90% pure, since less than 10% as much DNA was recovered from a reaction where initiation was precluded by withholding CpA or

* Throughout this paper, "RNA polymerase" refers to the holoenzyme.

CTP. Nothing but small digestion products was obtained when polymerase was absent from the initiation reaction.

An advantage of obtaining the protected fragment from an initiated complex is that the fragment is expected to contain the sequence at which RNA polymerase normally initiates A3 mRNA synthesis. To verify this, I determined the initial sequence of the RNA molecule transcribed from the T7 A3 promoter and then asked whether the A3 protected fragment coded for that same RNA sequence.

In order to obtain RNA from the A3 promoter alone, I allowed RNA polymerase to initiate at the A3 site with CpA and [α-^{32}P]CTP (5 min, 37°) and added rifampicin to block any subsequent initiation. Then I added ATP, GTP, and UTP (all α-^{32}P-labeled) to a final concentration of 2.0 μM and allowed synthesis to proceed at 25° for 10 min before stopping the reaction with sodium dodecyl sulfate. In the presence of very low concentrations of triphosphates (1.0–5.0 μM), RNA polymerase "pauses" (for reasons which remain unclear) as it transcribes a template, giving rise to a series of *overlapping* products with a common initiation point (28). Thus, a radioautograph of a polyacrylamide gel on which the A3 RNA products were run showed a series of distinct, labeled RNA bands, whose sizes ranged from about 5 to 80 nucleotides. New T1 oligonucleotides appeared on the fingerprints as progressively longer RNAs were eluted from the gel, digested with RNase T1, and fingerprinted, establishing a unique oligonucleotide order extending from the 5′ end. When the sequences of the individual oligonucleotides were determined by subsequent pancreatic RNase digestion and alkaline hydrolysis, the sequence of the longest RNA product analyzed could be deduced. The first 19 bases of the CpA-primed A3 RNA are: 5′ C-A-C-A-U-G-A-A-A-C-G-A-C-A-G-U-G-A-G-OH 3′. Does the protected fragment from the A3 site code for any of this sequence?

I prepared complexes between RNA polymerase and the A3 DNA fragment by initiating synthesis with CpA and CTP and then treating with DNase, as described earlier. At the end of 10 minutes' DNase digestion, however, ATP, GTP, and UTP (all α-^{32}P-labeled, 3.5 μM) were added to the reaction, and RNA synthesis was allowed to proceed for 5 min at 37°. When the products of this "run-off" synthesis were electrophoresed on a polyacrylamide gel, a pattern of labeled bands identical to that obtained in the previous experiment was observed, except that no products longer than 21 or 22 nucleotides had been synthesized. The sequence of the longest "run-off" product, transcribed from the initiation point to the end of the DNA fragment, was found to be: 5′ C-A-C-A-U-G-A-A-A-C-G-A-C-A-G-U-G-A-G-N-N(N)-OH 3′ (the last two or three bases were not determined).

The two CpA-primed RNA sequences are identical, but RNA polymerase normally initiates synthesis at the T7 A3 promoter with ATP (17, 20). However, it is known that dinucleotide initiations do occur within a few bases of normal, ATP or GTP (29) initiations. CpA primes synthesis from the T7 A1 promoter (20), generating the sequence C-*A-U-C-G*-OH (D.P., unpublished results), and Kramer *et al.* (30) have shown that the *in vivo* A1 mRNA sequence begins ppp-*A-U-C-G*-OH; in addition, the dinucleotide GpA primes *lac* UV5 RNA synthesis, giving rise to the sequence G-*A-A-U-U-G*-OH (28), and the normal, ATP-initiated UV5 RNA sequence begins ppp*A-A-U-U-G*-OH (28). Preliminary evidence (R. Kramer, personal communication) indicates that

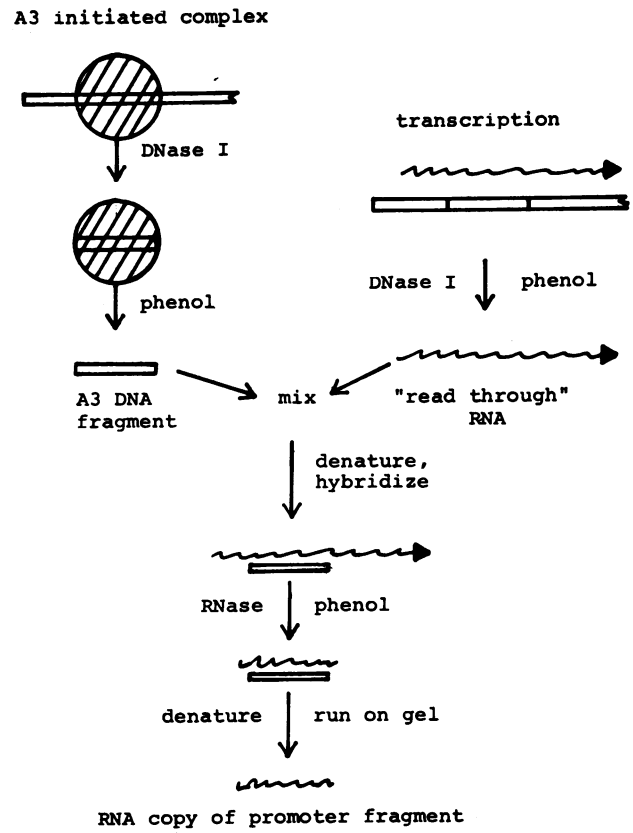

FIG. 1. Scheme for the isolation of an RNA whose sequence is complementary to the T7 A3 promoter fragment.

the initial T7 A3 mRNA sequence synthesized *in vivo* is ppp*A-U-G*-OH, suggesting that the polymerase normally initiates at the *A-U-G* in the CpA-primed RNA sequence (see Fig 2). Thus, the A3 promoter fragment, which is over 40 base pairs long, codes for 18 or 19 bases of A3 mRNA. Now, what is the sequence of the entire fragment?

Determination of the sequence of RNA is technically more manageable than determination of that of DNA. I, therefore, wanted to obtain an RNA sequence that was *complementary* to the A3 promoter fragment. Since RNA complementary to the entire *transcribed strand* (r-strand) (31) of the DNA fragment would contain the A3 "run-off" sequence at its 3′ end, I decided to isolate the RNA by *hybridizing denatured A3 DNA fragments to natural, r-strand transcripts which "read through" the A3 promoter*. RNase treatment of the hybridization mixture should leave no RNA intact except that which is directly hybridized to the r-strand of the DNA fragments. The scheme is outlined in Fig. 1 and is detailed below.

Since RNA chains initiated at the A1 and A2 promoters "read through" the A3 site (17, 20), a complete copy of the A3 promoter sequence is contained within these transcripts (see Fig. 2). Therefore, in one reaction I synthesized high specific activity [^{32}P]RNA from the A1, A2, and A3 sites using the dinucleotides CpA and CpC (20) to prime transcription. The synthesis was limited so that transcripts (average length about 900 bases) did not proceed too far beyond the A3 promoter. In a separate reaction, I prepared A3 promoter fragments by initiating RNA polymerase on T7 DNA with CpA and CTP, raising the salt concentration, and treating the mixture for 10 min with DNase I. The two reactions con-

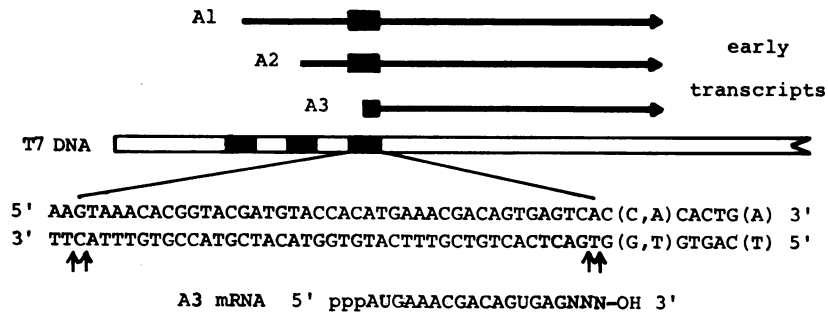

FIG. 2. DNA and mRNA sequences at the T7 A3 promoter. Hyphens have been omitted. The ends of the polymerase-protected DNA sequence are indicated with *arrows*.

taining the [^{32}P]RNA and the polymerase–DNA complexes were each extracted with phenol and ether and separately dialyzed into 0.30 M NaCl/0.030 M sodium citrate. After extensive dialysis, Mg^{++} was added to the RNA solution; it was treated with DNase I to eliminate the endogenous template and was again extracted with phenol and ether. Aliquots of labeled RNA and unlabeled promoter fragments were then mixed (molar ratio of RNA to DNA molecules about 2:1), boiled for 2 min to denature the DNA, and hybridized for 1 hr at 60°. The DNA·RNA hybrids were cooled to room temperature and treated with RNase T1 (or RNase A) for 1 hr to digest *all but the hybridized RNA*. After a last phenol extraction, the hybrids were precipitated with ethanol, resuspended in Tris-borate buffer (pH 8.3) containing urea, and run on a 40 cm 12% acrylamide-7 M urea gel (32). Fig. 3 shows a radioautograph of part of such a gel.

Bands A and B are labeled RNA species that are complementary in sequence to the T7 A3 promoter fragment. In the first column is a sample derived from a T1 RNase digest of labeled transcripts that had been hybridized to A3 DNA fragments. In the second column is a control sample in which the hybridization was carried out in the absence of DNA fragments. A similar control pattern was obtained when: (*i*) A3 fragments were added *after* the 60° hybridization, (*ii*) the hybridization was carried out at 0°, and (*iii*) the preparation of DNA fragments was done without initiation—no CTP was present in the binding reaction.

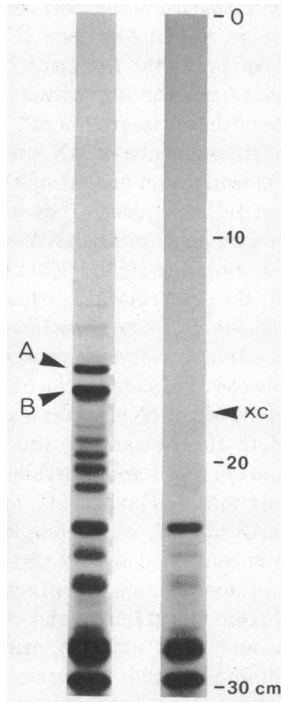

FIG. 3. Radioautograph of a polyacrylamide gel showing: RNA products (bands A and B) complementary to the A3 promoter fragment (*left*); and the RNA products obtained in a control hybridization (*right*). Less radioactivity was present in the control. (xc, xylene cyanol dye marker.)

The lengths of bands A and B, estimated from a calibration curve provided by T. Maniatis, are 52 and 49 bases, respectively. Since both bands represent RNA copied from the same DNA strand, band B must be a slightly shorter piece of band A RNA. This is borne out by the sequence determined for each RNA.

The sequence of the longer band (A) is: 5′ A-A-G-U-A-A-A-C-A-C-G-G-U-A-C-G-A-U-G-U-A-C-*C-A-C-A-U-G-A-A-A-C-G-A-C-A-G-U-G-A-G*-U-C-A-C(C,A)C-A-C-U-G(A)-OH 3′. Band B has exactly the same sequence except that it does not contain the 5′ oligonucleotide, A-A-G. These sequences were determined by standard techniques (33), including RNase T1 and RNase A fingerprints, digestion of T1 oligonucleotides with RNase A or RNase U2, digestion of RNase A oligonucleotides with RNase T1, and alkaline hydrolysis to determine nearest neighbors (the RNA was labeled separately with each of the four triphosphates). The italicized portion of the RNA sequence given above is identical to the A3 "run-off" sequence, proving that the hybrid RNA does in fact contain the sequence of the A3 promoter fragment.

The DNA sequence of the promoter fragment, inferred directly from the RNA sequence, is presented in Fig. 2. It is slightly longer than the sequence of nucleotides physically protected from DNase by the polymerase. This is a consequence of the specificity of the RNase T1 (which cuts after *G*) used to digest the A3 DNA·RNA hybrid, since RNA extending beyond either end of the hybrid is conserved up to the point of a T1 cleavage site.

The probable ends of the A3 protected fragment are indicated in Fig. 2 by arrows. (Although the arrows represent the ends of the DNase-resistant sequence in the bottom strand, the ends of the upper strand are expected to lie within a few bases of these points.) The A-A-G at the 5′ end of the hybrid RNA can be removed, albeit slowly, by the T1 enzyme. This places the left end of the A3 DNA fragment in the immediate vicinity of the *G* in the oligonucleotide, A-A-G. When the DNA·RNA hybrid was digested with RNase A (which cuts after *U* and *C*), the sequence A-A-G-U-A... was conserved at the 5′ end of the RNA, showing that the

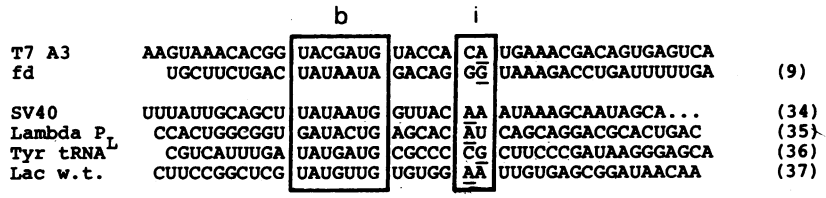

FIG. 4. Comparison of promoter sequences (see *text*). *b*, Homologous sequence probably engaged by RNA polymerase; *i*, mRNA initiation point (*underlined*). Hyphens have been omitted. SV40, simian virus 40; w.t., wild type.

U-A phosphodiester bond was inaccessible to the enzyme. Thus, the probable left end of the DNA fragment is after the A-T base pair or after the G-C base pair, as indicated in Fig. 2. Since "run-off" synthesis apparently proceeds all of the way to the end of the bound DNA, the right end of the protected fragment is established by the length of the "run off" product. The CpApC-primed "run-off" RNA is 21 or 22 nucleotides long, placing the right end of the DNA fragment immediately after the C-G base pair or the A-T base pair, again indicated by arrows. The actual length of the polymerase-protected A3 DNA fragment is therefore 41 to 43 base pairs.

Whereas the DNA sequence (Fig. 2) is that of a promoter fragment obtained from an "initiated" complex, RNA polymerase does form a tight, but noninitiated complex at the A3 promoter (5–7). What is the relationship between the positions occupied by the polymerase at the A3 promoter in these two alternative states? To answer this question, I first incubated RNA polymerase and T7 [^{32}P]DNA together in low salt (0.05 M KCl, 10 min at 37°), enabling complexes to form at all early T7 promoters (no dinucleotides or triphosphates were present); then the mixture was treated for 10 min with DNase I, still in low salt, and the digestion was stopped with EDTA. When the radioactive DNA fragments were subsequently run on a polyacrylamide gel, they were seen to migrate as a single band in the same position as purified A3 promoter fragments (obtained from initiated complexes). Thus, RNA polymerase protects the same amount of double-stranded DNA when it is initiated at the A3 promoter and when it is bound in a noninitiated conformation at any early T7 promoter.

To determine what *sequence* at the A3 promoter is protected from DNase by noninitiated polymerase molecules, I did a hybridization experiment similar to that described earlier (Fig. 1), but using DNA fragments that were obtained in low salt from DNase-treated, noninitiated complexes. When the [^{32}P]RNA complementary to the protected fragments was run on a polyacrylamide gel, several distinct bands in the size range of 40 to 50 nucleotides were observed. Two of these bands migrated identically to the A3 bands A and B shown in Fig. 3. These two RNAs were eluted from the gel and fingerprinted, and it was determined that they had exactly the same sequences as the hybrid RNAs A and B. The other RNA species in the same size range on the gel came from hybrids with protected fragments from other early T7 promoters (D.P., unpublished results). The tight binding site occupied by a noninitiated polymerase molecule at the A3 promoter is therefore the same as the site occupied by a polymerase molecule that has initiated synthesis with CpA and CTP.

Given that the A3 fragment contains the tight binding site and the mRNA initiation point, can the polymerase recognize it as a functional promoter? I prepared A3 promoter fragments several times by the DNase I protocol outlined earlier, followed by phenol extraction to eliminate polymerase molecules. The fragments were dialyzed into fresh buffers (0.05–0.15 M KCl); and, under a variety of conditions, attempts to get added RNA polymerase to synthesize A3 mRNA from the fragments failed. Apparently, the A3 promoter fragment is missing some critical information. Details of the sequence analysis will be published later.

DISCUSSION

How does the T7 A3 fragment compare with other promoter fragments? Heyden *et al.* (8) have shown that at a low molar ratio of RNA polymerase to bacteriophage fd DNA, the polymerase will preferentially bind to and protect one fd promoter from digestion by DNase I. Schaller *et al.* (14) have determined the nucleotide sequence of the resulting 42-base-pair DNA fragment, which codes for 19 bases of fd mRNA. J. Gralla (personal communication) has isolated a 42- to 43-base-pair protected fragment from the *E. coli lac* UV5 promoter, and this fragment codes for 19 bases of *lac* mRNA. In these respects, the fd and *lac* UV5 promoter fragments are virtually identical to the T7 A3 fragment. I conclude that, in general, when RNA polymerase is bound at any initiation site on a natural DNA template, it protects a stretch of nucleotides about 42 base-pairs long from digestion by DNase I. The protected DNA fragment contains an RNA polymerase binding site and an mRNA initiation point, and it codes for about 20 bases of mRNA.

There must be some specific *sequence* within all promoters that is involved in the stable binding of polymerase molecules, and that sequence is at least largely contained within the protected DNA fragments. In addition to the sequences of the fd and T7 A3 protected DNAs, sequences extending at least 20 base pairs on either side of the mRNA initiation points are known for simian virus 40 (34), lambda P_L (35), and the *E. coli* Tyr-tRNA (36) and *lac* (37) genes. These sequences are presented in Figure 4 (as inferred protected sequences), along with the fd and T7 sequences, and are written as RNA sequences so as to facilitate a comparison. Transcription is oriented from left to right, and the mRNA initiation point is underlined in each case. The sequences are written with the initiation points essentially in vertical alignment, since the polymerase binds promoters in a fixed position relative to the initiation points.

Among the promoter sequences, there is a homologous, 7-base sequence lying to the left of the initiation points. I feel that the DNA sequence

5′ T-A-T-Pu-A-T-G 3′
3′ A-T-A-Py-T-A-C 5′

is implicated in the formation of a tight binary complex with RNA polymerase. Essentially the same conclusion has been

reached by Schaller *et al.* (14). The difference between two *lac* promoter sequences demonstrates the importance of this common sequence. The wild-type *lac* promoter sequence requires an auxiliary protein, catabolite activator protein (38), for efficient function. J. Gralla (personal communication) has shown that the UV5 promoter mutation (39) changes the *lac* sequence so that it exactly matches the proposed binding sequence, and the UV5 promoter functions efficiently without the effector protein (40, 41). The fact that the 7-base-pair sequence is A-T-rich is probably significant in that formation of a tight polymerase-DNA complex involves "local melting" of the DNA strands (42).

In functional and molecular terms, a promoter is a region along a DNA template at which RNA polymerase first "recognizes" some sequence, then "melts out" a section of the DNA (42), forming a tight binary complex, and then initiates an RNA chain with ATP or GTP (29) as it begins transcribing the template. [A "weak" binary complex, demonstrated by Mangel and Chamberlin (6), may be formed at that recognition sequence.] Several arguments suggest that some sequence information required for promoter function defined in these terms is not contained in the polymerase-protected DNAs. Attempts to rebind RNA polymerase to isolated fd (14) and T7 A3 promoter fragments and to synthesize mRNA from these fragments have been unsuccessful. In addition, Maurer *et al.* (43) find that polymerase cannot form a stable complex with a large piece of lambda DNA, the left end of which is about 33 base pairs to the left of the lambda P_L initiation point (as oriented in Fig. 4). Furthermore, a strong lambda promoter mutation, *sex* (44), and two *lac* promoter mutations (45) fall in a region about 35 base pairs to the left of the lambda P_L (35) and *lac* (37) mRNA initiation points. Therefore, it is likely that before the RNA polymerase can form the tight pre-initiation complex, it must interact with some sequence about 35 base pairs upstream from the initiation point, adjoining the region of the promoter that it protects from digestion by DNase.

I am very grateful to Bob Simpson for the RNA polymerase used in these experiments, to Allan Maxam for teaching me the RNA sequencing technology, and to Wally Gilbert for helping me put together an intelligible manuscript. This work was supported by USPHS Grant GM09541 from the National Institute of General Medical Sciences.

1. Epstein, W. & Beckwith, J. R. (1968) *Annu. Rev. Biochem.* **37**, 411–436.
2. Stead, N. W. & Jones, O. W. (1967) *J. Mol. Biol.* **26**, 131–135.
3. Hinkle, D. & Chamberlin, M. (1970) *Cold Spring Harbor Symp. Quant. Biol.* **35**, 65–72.
4. Hinkle, D. C. & Chamberlin, M. J. (1972) *J. Mol. Biol.* **70**, 157–185.
5. Chamberlin, M. & Ring, J. (1972) *J. Mol. Biol.* **70**, 221–237.
6. Mangel, W. F. & Chamberlin, M. (1974) *J. Biol. Chem.* **249**, 3007–3013.
7. Bordier, C. & Dubochet, J. (1974) *Eur. J. Biochem.* **44**, 617–624.
8. Heyden, B., Nusslein, Ch. & Schaller, H. (1972) *Nature New Biol.* **96**, 9–12.
9. Matsukage, A., Murakami, S. & Kameyama, T. (1969) *Biochim. Biophys. Acta* **179**, 145–157.
10. LeTalaer, J. & Jeanteur, Ph. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 3211–3215.
11. Okamoto, T., Sugiura, M. & Takanami, M. (1972) *Nature New Biol.* **237**, 108–109.
12. Ruger, W. (1971) *Biochim. Biophys. Acta* **238**, 202–211.
13. Sentenac, A., Ruet, A. & Fromageot, P. (1968) *FEBS Lett.* **2**, 53–56.
14. Schaller, H., Gray, C. & Herrmann, K. (1975) *Proc. Nat. Acad. Sci. USA* **72**, 737–741.
15. Siegel, R. B. & Summers, W. C. (1970) *J. Mol. Biol.* **49**, 115–123.
16. Hyman, R. W. (1971) *J. Mol. Biol.* **61**, 369–376.
17. Dunn, J. J. & Studier, F. W. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 1559–1563.
18. Millette, R. L., Trotter, C. D., Herrlich, P. & Schweiger, M. (1970) *Cold Spring Harbor Symp. Quant. Biol.* **35**, 135–142.
19. Maitra, U., Lockwood, A. H., Dubnoff, J. S. & Guha, A. (1970) *Cold Spring Harbor Symp. Quant. Biol.* **35**, 143–156.
20. Minkley, E. G. & Pribnow, D. (1973) *J. Mol. Biol.* **77**, 255–277.
21. Jones, O. W. & Berg, P. (1966) *J. Mol. Biol.* **22**, 199–209.
22. Richardson, J. P. (1966) *J. Mol. Biol.* **21**, 83–114.
23. Mangel, W. F. & Chamberlin, M. (1974) *J. Biol. Chem.* **249**, 3002–3006.
24. Downey, K. M. & So, A. G. (1970) *Biochemistry* **9**, 2520–2525.
25. Downey, K. M., Jurmark, B. S. & So, A. G. (1970) *Biochemistry* **10**, 4970–4975.
26. Hoffman, D. J. & Niyogi, S. K. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 574–578.
27. Sipple, A. & Hartman, G. (1968) *Biochim. Biophys. Acta* **157**, 218–219.
28. Maizels, N. M. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 3585–3589.
29. Maitra, U., Nakata, Y. & Hurwitz, J. (1967) *J. Biol. Chem.* **242**, 4908–4918.
30. Kramer, R., Rosenberg, M. & Steitz, J. A. (1974) *J. Mol. Biol.* **89**, 767–776.
31. Summers, W. C. & Szybalski, W. (1968) *Virology* **34**, 9–16.
32. Gilbert, W. & Maxam, A. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 3581–3585.
33. Brownlee, G. G. (1972) *Laboratory Techniques in Biochemistry and Molecular Biology: Determination of Sequences in RNA*, eds. Work, T. S. & Work, E. (North Holland, Amsterdam/American Elsevier, New York), pp. 54–201.
34. Dhar, R., Weissman, S. M., Zain, B. S. & Pan, J. (1974) *Nucleic Acids Res.* **1**, 595–614.
35. Maniatis, T., Ptashne, M., Barrell, B. G. & Donelson, J. E. (1974) *Nature* **250**, 394–397.
36. Sekiya, T. & Khorana, G. H. (1974) *Proc. Nat. Acad. Sci. USA* **71**, 2978–2982.
37. Dickson, R., Ableson, J., Barnes, W. & Reznikoff, W. (1975) *Science* **187**, 27–35.
38. Beckwith, J., Grodzicker, T. & Arditti, R. (1972) *J. Mol. Biol.* **69**, 155–160.
39. Arditti, R., Scaife, J. & Beckwith, J. (1968) *J. Mol. Biol.* **38**, 421–426.
40. Silverstone, A. E., Arditti, R. R. & Magasanik, B. (1970) *Proc. Nat. Acad. Sci. USA* **66**, 773–779.
41. Eron, L. & Block, R. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 1828–1832.
42. Saucier, J.-M. & Wang, J. C. (1972) *Nature New Biol.* **239**, 167–170.
43. Maurer, R., Maniatis, T. & Ptashne, M. (1974) *Nature* **249**, 221–223.
44. Gottesman, M. E. & Weisberg, R. A. (1971) in *The Bacteriophage Lambda*, ed. Hershey, A. D. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.), pp. 113–138.
45. Hopkins, J. D. (1974) *J. Mol. Biol.* **87**, 715–724.