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Characterization of the β-lactamase promoter of pBR322

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ABSTRACT

The β-lactamase promoter of pBR322, derived from Tn3, has been characterized using several techniques. The transcription initiation site is located 35 base pairs from the translation initiation codon of β-lactamase. The mRNA produced *in vitro* has a 5' pppGpA terminus. RNA polymerase bound at this start site protects a region from about -50 to +20 from DNAase I cleavage using the footprinting technique. RNA polymerase binds rapidly to the β-lactamase promoter. The half-time of association is less than one-half minute. The half-time of dissociation is approximately 6 hr. A study of the binding of RNA polymerase at different temperatures showed a large change between 11° and 15°C. Comparison of these parameters with those reported for other promoters is discussed.

INTRODUCTION

Genes specifying ampicillin resistance are contained on several transposons and naturally occuring plasmids. The cloning vector, pBR322, carries an ampicillin resistance gene that has been derived from Tn3 (1,2). The protein encoded by this gene, a TEM 1 β-lactamase, has been studied and partially sequenced (3,4). Little has been reported concerning the initiation of transcription of the β-lactamase gene, even though the β-lactamase promoter is presumed to be the source of transcription which allows expression of eukaryotic genes cloned into pBR322 at the *Pst* I site, such as human leukocyte interferon (5), rat proinsulin (6,7), dihydrofolate reductase, (8) and corticotropin-β-lipotropin (9).

The entire β-lactamase gene of pBR322 including the presumptive promoter region has been sequenced by Sutcliffe (10). On the basis of sequence homology with other promoters, Sutcliffe proposed a possible location of the β-lactamase promoter 9 nucleotides upstream from the translation start signal (10). A comparison of the DNA sequence around the proposed start site with a model promoter sequence of Rosenberg and Court (11) indicates several promoter site candidates.

Although the precise location of this promoter has not been reported, some relevant information is known. β-lactamase activity is not inducible by its substrate (3), nor is it affected by the DNA gyrase inhibitor novobiocin (12). Ampicillin resistance due to β-lactamase activity is also not affected by cAMP levels (3) in contrast to cAMP sensitivity found for the chloramphenicol resistance gene of Tn9 (13). Because of its constant level of expression, β-lactamase levels have been used to estimate plasmid copy number (14). West et al., studied the promoters of pBR322 by nitrocellulose filter binding experiments (15). A Hae III fragment spanning the presumptive β-lactamase promoter region was found to bind RNA polymerase selectively and the binding was not sensitive to high salt concentrations.

This paper describes the location of the β-lactamase promoter and aspects of the RNA polymerase-β-lactamase promoter interaction. The position of transcription initiation was identified by measuring the length of RNA produced from in vitro transcription and determining the 5' terminal nucleotides of the synthesized β-lactamase mRNA. The RNA polymerase-promoter complex was probed using the DNAase I footprinting technique (16), which shows the region of the DNA which is protected from DNAase I digestion by the bound protein.

The association rate and stability of RNA polymerase-promoter complexes have been studied by the filter binding technique (17,18,19). The filter binding studies of the β-lactamase promoter described here report on the rate of formation and dissociation of the RNA polymerase-promoter complex and the effects of temperature on formation of the complex. A comparison of these binding parameters among different promoters may yield a better understanding of the nature of the general RNA polymerase-promoter DNA interaction.

METHODS

PEI cellulose plates were obtained from Brinkmann Instruments, Westbury, N. Y. α-^{32}P-GTP and α-^{32}P-ATP (specific activity 450 Ci/mmole) and γ-^{32}P-ATP (specific activity 4000 Ci/mmole) were obtained from ICN, Irvine, CA. Taq I, Mbo II, and Hha I were obtained from New England Biolabs, Beverly, MA. Cfo I was obtained from Bethesda Research Laboratories, Bethesda, MD. Alkaline phosphatase, DNAase I, and polynucleotide kinase were obtained from Boehringer Mannheim, Indianapolis, IN. The BspR I preparation was reported previously (20). E. coli RNA polymerase was prepared by the procedures of Burgess and Jendrisak (21) and Lowe, et al. (22). The RNA polymerase holoenzyme was judged to be pure by SDS-polyacrylamide gel electrophoresis (23). A stock of the

pBR322 sequenced by Sutcliffe was obtained from the Plasmid Reference Center, Stanford, CA. The isolation of plasmid DNA was by Brij 58, sodium deoxycholate lysis as described previously (20).

Polyacrylamide gels, photography, autoradiography, and elution of restriction fragments were as reported previously (20). End labeling DNA with γ-^{32}P-ATP, the use of 7 M urea denaturing gels, and dimethyl sulfate sequencing reactions have been described (24,25).

Nitrocellulose filters, BA85, were from Schleicher and Schuell, Keene, NH. The filters were pretreated by soaking in 1N KOH from 10 min, washing extensively with H_2O, and soaking in binding buffer until use (26).

DNA trapped on the filters was eluted by incubating the filter in 0.4 ml elution buffer (0.5 M ammonium acetate, 0.01 M magnesium acetate, 0.1% sodium dodecyl sulfate, 0.1 mM EDTA) (24) at 37° with shaking for 1 h and precipitating the supernatant with ethanol. Typically 90-95% of the radioactivity was recovered.

In vitro transcription was carried out in 20 mM Tris-Cl, pH 7.4, 4 mM magnesium acetate, 0.1 mM EDTA, 0.1 mM dithiothreitol, 40 mM KCl, 3 pmol DNA template, and 1.5 µg RNA polymerase holoenzyme. The reaction mix was preincubated 10 min at 37° and then 3 unlabeled nucleotide triphosphates (40 µM each) and 0.4 µM of α-^{32}P-labeled nucleotide triphosphate was added and the incubation continued an additional 15 min. 100 µg tRNA was added and the reaction was stopped by phenol extraction and ethanol precipitation. The precipitated RNA was resuspended and electrophoresed on a 20% polyacrylamide, 7 M urea gel. RNA was located by autoradiography and eluted from the gel by cutting the band into small pieces and shaking at 37° with 0.3 M sodium acetate, 4 µM magnesium acetate and 5 µg tRNA. The solution was then filtered through siliconized glass wool and ethanol precipitated (24). Alkaline hydrolysis was performed by adding an equal volume of 1N NaOH to the RNA solution, folowed by overnight incubation of 37°. Aliquots of hydrolyzed RNA were spotted on PEI-cellulose plates and developed for approximately 5 hours at 65° using a 2.3% hydrolyzed RNA, 7 M urea homo mix C (27). The plates were dried and the position of radioactive nucleotides determined by autoradiography.

DNAase I footprinting was by the procedure of Schmitz and Galas (16). The DNA binding mix consisted of 20 mM Tris-Cl, pH 8, 10 mM $MgCl_2$, 1 mM EDTA, 1 mM dithiothreitol, 5% glycerol, 40-100 mM KCl, 0.2 mg/ml bovine serum albumin, 5 mM $CaCl_2$ and 5 pmol ^{32}P end-labeled DNA. RNA polymerase holoenzyme (10 µg) was added to 200 µl of binding mix and allowed to bind for 5 min at

37°. DNAase I (0.05 µg) was added, incubated for 45 seconds, and the reaction was stopped by adding 20 µl of 3 M ammonium acetate, 0.25 M EDTA, and 0.15 mg/ml sonicated calf thymus DNA. tRNA (25 µg) was added and the reaction mix was ethanol precipitated and resuspended in 0.05 M NaOH, 4 M urea. The samples were electrophoresed in 8-20% polyacrylamide, 7 M urea gels. A DNAase I digestion of the fragment under the above conditions but which contained no RNA polymerase, gave a control pattern of partially digested DNA. The positions of DNAase I cleavage on the fragment were determined relative to base locations deduced from DNA sequencing reactions.

Formation of RNA polymerase-promoter DNA complexes was studied by trapping the complexes on nitrocellulose filters (17, 19). The binding mixture contains 10 mM Hepes, pH 7.4, 10 mM $MgCl_2$, 0.1 mM EDTA, 100 mM KCl, 50 µg/ml bovine serum albumin, 1 mM dithiothreitol and 0.05 pmol ^{32}P end-labeled DNA per assay. The level of RNA polymerase required to saturate DNA fragments was determined empirically. All experiments used a RNA polymerase/DNA ratio in excess of this saturating amount.

Association of RNA polymerase-DNA complexes was measured by adding RNA polymerase to a DNA binding mix preincubated at 37°. At various times after addition, 30 µl aliquots were removed and added to 170 µl of a stop mix containing 100 µg/ml Heparin in 10 mM Hepes, pH 7.4, 10 mM $MgCl_2$, 0.1 mM EDTA, and 100 mM KCl. After 5 min incubation 180 µl was filtered with gentle suction through nitrocellulose filters and washed with 1 ml of 10 mM Hepes, pH 7.4, 10 mM $MgCl_2$, 0.1 mM EDTA, and 100 mM KCl. The filter was then dried and counted in a toluene-based scintillation cocktail. A background blank taken before addition of RNA polymerase (t = 0) was subtracted. Total DNA input was measured by spotting 27 µl of the DNA binding mix on a filter or by spotting 180 µl of the DNA binding mix plus stop mix on a filter and counting. The background at t=0 was typically $\leq 7\%$ of total input. All results are plotted as the percent of total input after subtracting background.

The rate of dissociation of the RNA polymerase-DNA complex was measured by allowing the complexes to form for 5 min and adding 170 µl stop mix per 30 µl of binding mix to complex any free RNA polymerase. 180 µl aliquots were removed at various times by filtering as described above. A background assay was obtained by adding the stop mix prior to RNA polymerase addition and then removing 180 µl aliquots at the same times as above.

Binding assays at different temperatures were carried out by equilibrating 30 µl DNA binding mix at various temperatures for at least 10 min, then adding RNA polymerase and incubating an additional 5 min. 170 µl of

stop mix at room temperature was added, incubated 1 min, and then 180 µl was filtered as above.

RESULTS

Location of transcription initiation site

The β-lactamase promoter has been partially located by the sequencing of pBR322 (10) and the filter binding experiments of West et al. (15). These reports suggest the promoter must be between 4155 and 4344, using Sutcliffe nomenclature (1). In order to locate the promoter more precisely, a filter binding experiment was performed using small restriction fragments from this region of pBR322. A restriction fragment was isolated that spanned the region from Taq I (4017) to BspR I (4344) (see Fig. 1). This 327 bp fragment was end labeled with ^{32}P and subjected to RNA polymerase binding. The RNA polymerase-promoter complex was trapped on nitrocellulose filters, eluted and electrophoresed on a polyacrylamide gel, and visualized by autoradiography. In Fig. 2 the filter-bound DNA (lane B) compared with the control DNA (lane A), indicates the 327 bp fragment is bound by RNA polymerase. In lanes C and D this 327 bp fragment is cleaved by Cfo I prior to filter binding. Lane D is the material eluted from the filter. A comparison of lanes C and D suggests that the 242 bp Cfo I - Taq I fragment must contain a RNA polymerase binding site. Lanes E and F show the results of an analogous procedure using Mbo II instead of Cfo I which suggest that the 195 bp fragment contains a promoter site (Fig. 1). Therefore the β-lactamase promoter must be located on a 110 bp

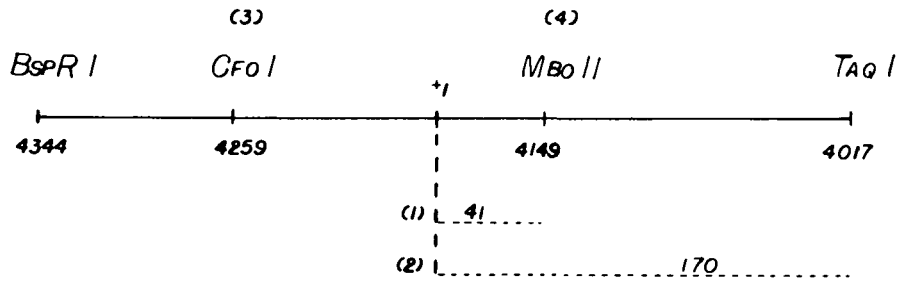

Figure 1. Restriction map of the β-lactamase promoter region. The BspR I-Taq I fragment corresponds to the positions indicated on pBR322 and 3759 to 4086 of Tn 3 (2). (1) indicates the RNA produced by in vitro transcription of the 110 bp Cfo I-Mbo II fragment. (2) indicates the RNA produced by in vitro transcription at the 242 bp Cfo I - Taq I fragment. (3) indicates the position of ^{32}P end-labeling of the top strand for DNAase I footprinting. (4) indicates the position of ^{32}P end-labeling of the bottom strand for DNAase I footprinting.

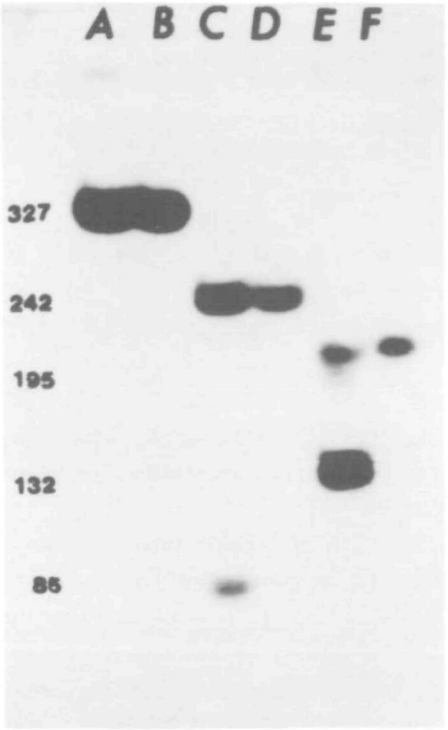

Figure 2. The β-lactmase promoter was localized on different restriction fragments by filter binding assays. ^{32}P end-labeled restriction fragments (see Figure 1) were electrophoresed on a 7% polyacrylamide gel and visualized by autoradiography. (A) 327 bp restriction fragment (B) DNA eluted from filter after RNA polymerase binding of 327 bp DNA (C) 327 bp fragment cleaved with Cfo I (D) DNA eluted from filter after RNA polymerase binding of Cfo I cleaved 327 bp DNA (E) 327 bp fragment cleaved with Mbo II (F) DNA eluted from filter after RNA polymerase binding of Mbo II cleaved 327 bp DNA.

Cfo I - Mbo II fragment between 4148 and 4258 on pBR322. Other filter binding experiments have shown that the Cfo I - Mbo II 110 bp fragment does bind RNA polymerase selectively.

To identify the site of transcription initiation, two different restriction fragments, both containing the β-lactamase promoter region, were transcribed in vitro using α-^{32}P-GTP or α-^{32}P-ATP (Fig. 1). The transcribed RNA was then electrophoresed on a denaturing gel with a dimethylsulfate sequencing reaction as size markers to determine the length of the transcribed RNA. Fig. 3 shows the RNA produced from transcription of the 110 bp Cfo I - Mbo II restriction fragment is approximately 41 nucleotides long. When the

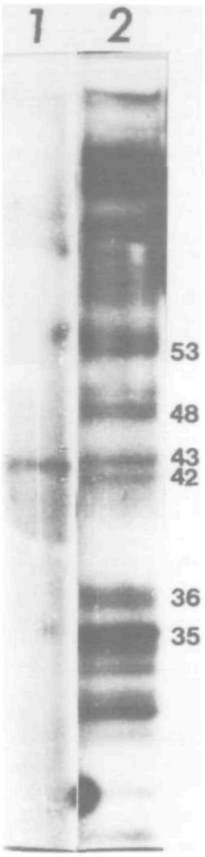

Figure 3. The in vitro transcription product of 110 bp Cfo I - Mbo II restriction fragment labeled with α-^{32}P-GTP was electrophoresed on a 20% polyacrylamide, 7 M urea gel. Lane 1 shows the in vitro synthesized RNA of approximately 41 nucleotides. Lane 2 is a dimethylsulfate sequencing reaction used to deduce the approximate length of the RNA.

242 bp Cfo I - Taq I fragment is transcribed, the resulting RNA is approximately 170 nucleotides in length. The lengths of these RNAs suggest the transcription must proceed toward Mbo II and Taq I and must start in the region of 4185 to 4190.

Two preliminary experiments offer information about the nature of the 5' terminus of the RNA. Transcription of the above fragments with α-^{32}P-ATP or α-^{32}P-GTP produced labeled RNA while transcription with γ-^{32}P-ATP produced no major labeled RNA. Also the RNA from a transcription using α-GTP was shown to release $^{32}P_i$ upon treatment with phosphatase. These results suggest the β-lactamase RNA may begin with pppG.

To precisely determine the 5' terminus of the transcribed RNA, α-^{32}P-ATP labeled RNA was isolated from a single band of 41 nucleotide RNA from a denaturing gel. An aliquot of this RNA was treated with alkaline phosphatase

to remove the 5' triphosphate. The dephosphorylated RNA sample and an untreated aliquot were then hydrolyzed with alkali. The alkaline hydrolyzates were separated by homochromatography on a PEI plate (Fig. 4). Lane 2 shows 2 slow-moving spots in the hydrolyzed RNA. The minor spot runs with the same mobility as the pppAp standard in lane 3. The major spot runs with a slower mobility as predicted for pppGp on ion exchange resins (28,29). Since the RNA

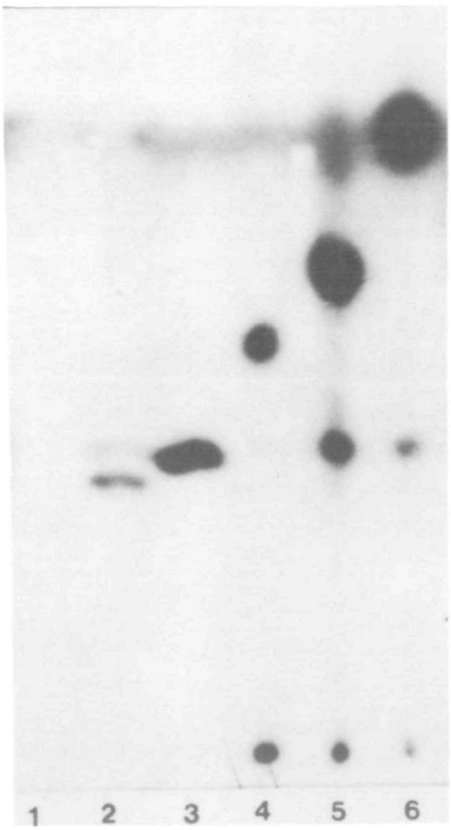

Figure 4. The 5' terminus of *in vitro* transcribed RNA was determined by homochromatography on PEI-cellulose Plates. 41 nucleotide α-^{32}P-ATP labeled RNA was isolated and aliquots were treated and separated by homochromatography at 65° for 5 hours.

The faster moving 3'-phosphate-mononucleotides have run off the PEI plate so only the slower moving nucleoside tri- and tetra-phosphates are visible. (1) RNA labeled with α-^{32}P-ATP treated with phosphatase and then hydrolyzed with 0.5 N NaOH. (2) RNA labeled with α-^{32}P-ATP hydrolyzed with 0.5 N NaOH. (3) pppAp standard product, by alkaline hydrolysis of pppApG, (4) pppApG, (5) pppG, (6) pppA.

is labeled with α-^{32}P-ATP the major spot must arise from RNA containing pppG-^{32}pA. An analogous experiment with α-^{32}P-GTP labeled RNA shows only one spot with a mobility expected for pp^{32}pGp An examination of the published sequence reveals only 1 possible GpA sequence within 10 bp on either side of the region of DNA suggested for an initiation site by the length of the transcribed RNA. Therefore, the most probable site of β-lactamase transcription initiation is at G (4189 of pBR322), with a minor start site at A (4188). This places the site of initiation 35 bp upstream from the translation start signal for β-lactamase.

RNA Polymerase Protection of a region of DNA from DNAase I

The RNA polymerase-promoter complex can be probed in vitro by DNAase I footprinting (16). This method involves the formation of a RNA polymerase-promoter complex using a DNA fragment labeled only at one end. The complex is then subjected to partial DNAase I digestion such that the DNA is, on the average, nicked once per fragment. The partially cleaved DNA is separated by size on a denaturing gel to allow the position of the DNAase I nicks to be located. Comparison of RNA polymerase-bound DNA digestion patterns with unbound DNA control patterns shows a region of DNA which differs in its susceptibility to DNAase I. The perturbation of this digestion pattern by RNA polymerase binding of the lac UV5 promoter has been reported (16). The DNAase I footprint patterns of both strands of the β-lactamase promoter are shown in Figures 5 and 6. DNA in which the top strand (anti-sense strand or RNA homologous strand) was probed contained a ^{32}P-label at the Cfo I site (see Fig. 1). The bottom strand (sense strand) was labeled at the Mbo II site (see Fig. 1). DNAase I digestion patterns at either 40 or 100 mM KCl looked the same.

The results of DNAase I footprinting of both strands are reported in Fig. 5.A and 5.B and summarized in Fig. 6. The solid lines indicate strong protection from DNAase I digestion, while the broken lines indicate less protection. The arrows mark the location of enhanced DNAase I digestion. The general features of the β-lactamase promoter pattern are very similar to that reported for the lac UV5-RNA polymerase footprint. Both promoters are protected from approximately -50 to +20 (relative to +1 for the site of initiation). The region of enhancement between -20 to -27 is also present in both promoter patterns.

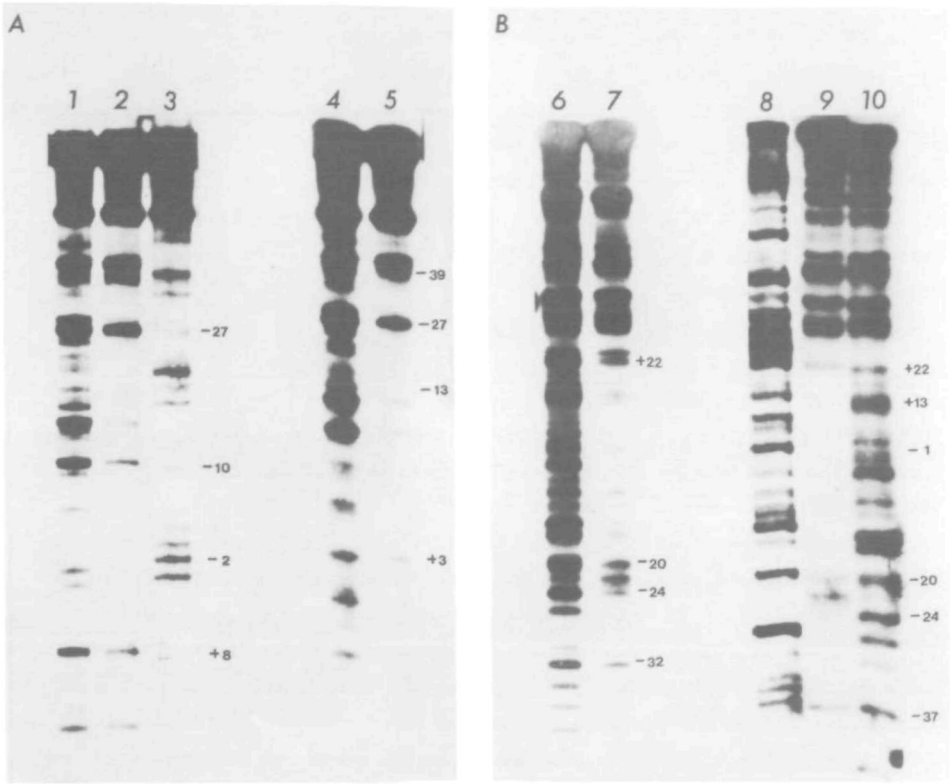

Figure 5. DNAase I footprinting of the RNA polymerase-β-lactamase complex. End labeled DNA was incubated with RNA polymerase to form complexes, DNAase I was added to partially digest exposed DNA, and the resulting digestion was electrophoresed on denaturing gels. Lanes (1) through (7) were electrophoresed on 8% polyacrylamide, 7 M urea gels. Lanes (8) through (10) were electrophoresed on a 20% polyacrylamide, 7 M urea gel. (A) Bottom strand labeled at Mbo II site of 110 bp Cfo I - Mbo II restriction fragment. (1) DNAase I digestion of Cfo I - Mbo II fragment (see Fig. 1), 40 mM KCl. (2) DNAase I digestion of RNA polymerase-promoter complex, 40 mM KCl. (3) G>A sequencing reaction. (4) Same conditions as (1), except 100 mM KCl. (5) Same conditions as (2), except 100 mM KCl. (B) Top strand labeled at Cfo I site and cleaved at either Mbo II or Taq I (6) DNAase I digestion of 242 p Cfo I - Taq I restriction fragment, 100 mM KCl (See Fig. 1). (7) DNAase I digestion of RNA polymerase-promoter complex, 100 mM KCl. (8) G > A sequencing reaction of 110 bp Cfo I - Mbo II fragment. (9) DNAase I digestion of RNA polymerase-promoter complex, 40 mM KCl. (10) DNAase I digestion of 110 bp fragment, 40 mM KCl.

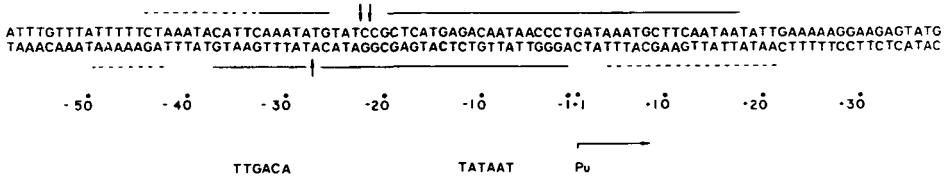

Figure 6. The summary of DNAase I footprinting experiments probing RNA polymerase-β-lactamase promoter complexes. The DNA sequence from -60 to +41 (Mbo II cleavage site) is shown. Hyphens are omitted for clarity. Numbering is relative to the transcription initiation site, +1. The solid lines indicate complete protection from DNAase I digestion as shown by an absence of DNAase I cleavage bands on the gels in Fig. 5. Broken lines indicate regions of partial protection. Arrows indicate enhanced digestion by DNAase I. A portion of the model promoter sequence of Rosenberg and Court (11) is shown below the β-lactamase promoter sequence to indicate partial sequence homology.

Filter binding experiments

The rates of formation and dissociation of the RNA polymerase-β-lactamase promoter and the effect of temperature on the formation of the complex were studied. The conditions used here are modifications of several filter binding assays previously reported (19,30).

As a control for specific promoter binding, two restriction fragments lacking promoter sequences were subjected to filter binding assays. One of these was the 85 bp restriction fragment from Cfo I (4258) to BspR I (4344) and the other was a 260 bp fragment from a region of DNA inside the β-lactamase coding sequence. Neither of these DNA fragments showed filter bound complexes higher than the general background level of 7%. Approximately 65% of the β-lactamase promoter fragments were bound by RNA polymerase and trapped on the filter under similar conditions. In these experiments, heparin was used to remove RNA polymerase from unstable, nonspecific complexes. Addition of heparin prior to RNA polymerase prevents the RNA polymerase from forming detectable complexes. Single-stranded DNA was shown to give similar results when used to complex free RNA polymerase (18).

Binding experiments and association rate measurements in 100 mM KCl or 40 mM KCl produced the same results. No salt dependence of binding of the β-lactamase promoter was also observed in experiments of West (15). The rate of complex formation using 100 mM M KCl is shown in Fig. 7. The β-lactamase promoter appears to bind RNA polymerase very quickly; the half-time of saturation is $\leq$ 1/2 min.

The rate of complex dissociation was measured by allowing complexes to

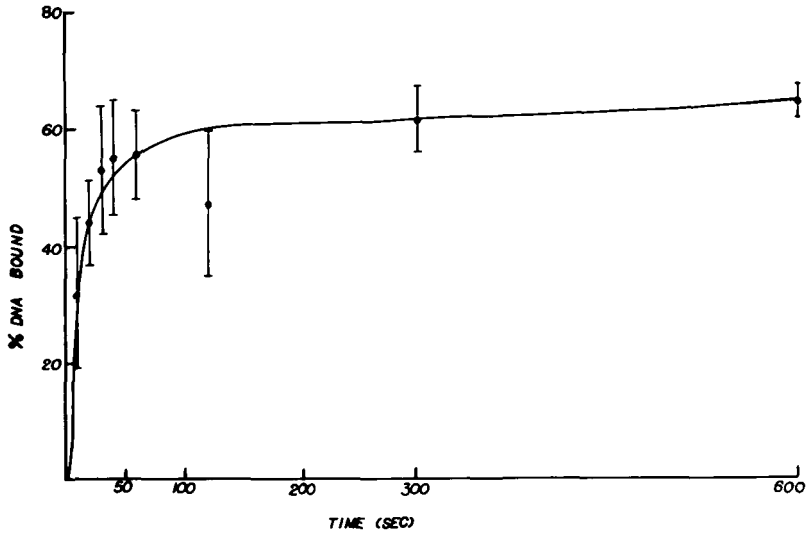

Figure 7. Binding curve for rate of formation of RNA polymerase-β-lactamase promoter complexes. Percent DNA bound is plotted as the average of triplicate assays. Error bars represent one standard deviation from the mean.

form and then adding heparin. Only radioactively labeled DNA which remained bound to RNA polymerase would be trapped on the filter since any RNA polymerase which dissociated would be complexed by the added heparin before it could rebind the DNA fragment. Filtering aliquots of the mixture at various times allows the portion still bound to be directly determined and the dissociation rate can be calculated. In preliminary experiments it was found that after longer times of incubation the background counts of DNA trapped on the filter increased to significant levels. To eliminate differences in background, a control was assayed at each time point. The composition of the control was identical to the dissociation mix except the heparin was added prior to adding the RNA polymerase. After subtracting the background control each assay point was expressed as the percent of CPM remaining bound compared to the CPM of DNA bound at the earliest time of t=0.5 min. Each point in Fig. 8 represents the average of duplicate assays. The data suggest that the half-time of dissociation is approximately 6 h.

The effect of temperature on complex formation was measured by preincubating the DNA binding mix at various temperatures and then adding RNA polymerase and incubating an additional 5 min. The heparin stop mix was then added and the mixture was filtered at room temperature. The results of 3 to 5

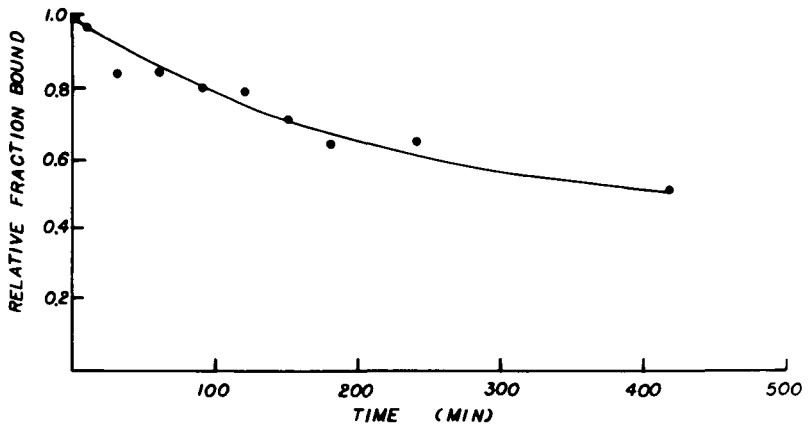

Figure 8. Binding curve for the rate of dissociation of RNA polymerase-β-lactamase promoter complexes. Relative fraction bound is the amount bound at different times/amount bound at earliest time sampled t=0.5 min. Data is plotted as average of duplicate assays.

assays are shown in Fig. 9. A gradual increase is observed between 2° to 11°C and 19° to 37°C. A sharp increase occurs between 11° and 19° with the greatest change observed between 11° and 15°C.

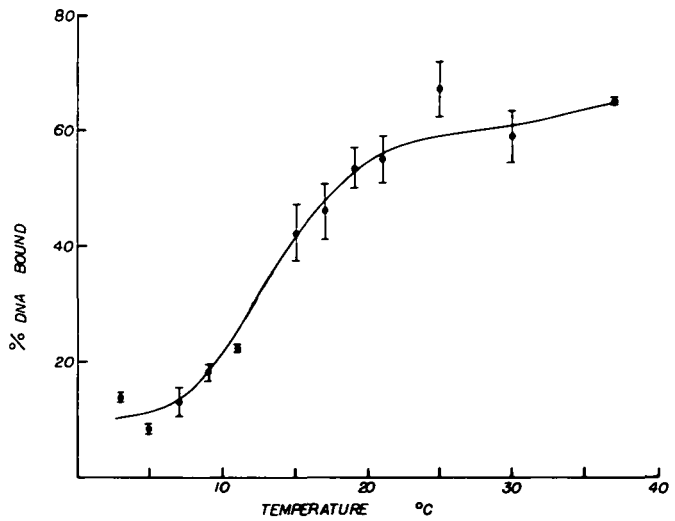

Figure 9. Binding curve of RNA polymerase-β-lactamase promoter complex formation at different temperatures. Each point is the average of 3 to 5 assays. The error bars represent one standard deviation from the mean.

DISCUSSION

Gene expression in prokaryotes is regulated primarily at the level of transcription initiation. In order to better understand this regulation, studies of the interaction of RNA polymerase with various promoters have been undertaken. DNA sequences of many promoters have been determined and comparisons based on the frequency of appearance of each of the four nucleotides at particular promoter positions have led to proposals of model promoter sequences (11,31,32).

The DNA region containing the β-lactamase promoter has been sequenced (10) and, on the basis of sequence similarilty to model promoter sequences, Sutcliffe proposed a possible location for mRNA initiation site 9 base pairs from the translation start signal. An examination of the region 100 bp upstream from the AUG (10) reveals that it is very A-T rich (68%) and contains at least 8 regions with some homology with the model sequence of Rosenberg and Court (11).

The results of *in vitro* transcription experiments indicate that only one of these potential start sites is used. The major site of transcription initiation of β-lactamase in RNA synthesis is at a G, position 4189 of pBR322 (3915 of Tn 3) with a small amount of initiation at the adjacent A residue. The mRNA transcript would contain 35 nucleotides preceding the AUG coding for the N-terminal amino acid of the β-lactamase leader. The β-lactamase promoter sequence as identified from the *in vitro* transcription start site, contains 4 of 6 nucleotides homologous to the model promoter sequence (11) in both the -35 and -10 regions (Fig. 6).

The DNAase I footprinting technique was used to identify the region surrounding the transcription start site that is within the RNA polymerase-promoter initiation complex. Steric blockage of DNAase I cleavage by the bound RNA polymerase over a segment of DNA from approximately -50 to +20 was observed. Also an area of enhanced cleavage by DNAase I within the complex (-21 to -27) is noted (Fig. 6). The protection envelope as well as the position of enhanced cleavage relative to the initiation site is almost identical to that reported for *lac* UV5 (16). It is observed that the area of increased sensitivity to DNAase I occurs in a region of the promoter in which no sequence homology between promoters is found (11). This information suggests that the DNA segment (-21 to -27) may be distorted or exposed in the complex while the two sequence specific regions are held in an inaccessible form by the interacting RNA polymerase.

The stability of RNA polymerase-DNA complexes allows them to be trapped

on nitrocellulose filters in order to be quantified. Filter binding assays have been used to measure the rates of formation and dissociation of RNA polymerase-promoter complexes on promoters such as T7 (17), fd (18), lac UV5 (30,33) and trp (34). The effects of temperature and salt conditions on complex stability have also been studied (18,19,35,36). Filter binding assays were used to measure the rate of association of the β-lactamase promoter with RNA polymerase and the dissociation rate of the stable (open) complex. The association rate of RNA polymerase with the β-lactamase promoter was found to be very fast, half-time of saturation < 0.5 min. The rate of association is of some interest to understanding what feature of the RNA polymerase-promoter interaction makes a promoter strong or weak. Maquat and Reznikoff (30) compared in vitro binding rates and in vivo expression levels of mutants and concluded that the level of expression (promoter activity) in vivo correlated well with the rate of association. Stefano et al. reached a similar conclusion in their study of in vitro constructed lac promoters (37). However this conclusion may not provide an adequate indication of expression between different classes of promoters. The trp promoter transcribes well in vitro and produces high level expression in vivo (38), and yet has a rather slow rate of association (34).

The rate of dissociation of the β-lactamase promoter-RNA polymerase complex is relatively slow (half-time ~ 6 h). This is faster than the reported rate of T7 (17) but several fold slower than for trp, (half-time ~ 1h (34)). The relevance of very slow dissociation rates to in vivo effects is unclear since the RNA polymerase would either begin transcription long before much dissociation had taken place or replication and cell division would occur which might be expected to cause clearing of RNA polymerase-DNA complexes.

The major effect of salt on the formation of stable RNA polymerase-DNA complexes has been high salt inhibition of formation of the complex; certain promoters are particularly sensitive (eg. Tc (15), $tRNA^{tyr}$ (39), and T7 A1 + D (19)) while other promoters are not as influenced by increasing salt concentrations (β-lactamase (15, this paper), λoop (39)). The temperature of the binding reaction seems to be critical, and a large change in the portion of fragment which can be bound by saturating levels of RNA polymerase is observed over a relatively small temperature range. For the β-lactamase promoter a sharp increase was noted between 11°-15°C. This is several degrees lower than the transition reported for T7 A1 + D promoters (19) and for entire T7 DNA (35,36). This is perhaps a consequence of the very high A-T rich character of the DNA around the β-lactamase promoter.

In vitro the reaction of RNA polymerase with the β-lactamase promoter is very rapid and exhibits a rather low transition temperature. From reported gene expression studies it is difficult to compare the in vitro activity of the β-lactamase promoter to other promoters. A systematic study correcting in vitro binding and initiation parameters of various promoters with in vivo expression in a standardized system should allow a detailed dissection of the promoter sequence as it relates to function and control of gene expression.

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