JOURNAL OF VIROLOGY, Dec. 1987, p. 3694–3700
0022-538X/87/123694-07$02.00/0
Copyright © 1987, American Society for Microbiology
Vol. 61, No. 12

Mapping the *B* and *D* Gene Promoters of Bacteriophage S13 by Footprinting and Exonuclease III Analysis

MONIQUE ARQUINT† AND JOHN H. SPENCER*

Department of Biochemistry, Queen's University, Kingston, Ontario, Canada K7L 3N6

Received 13 April 1987/Accepted 19 August 1987

The region of the bacteriophage S13 genome which contains the *B*, *K*, and *C* genes and most of the *A*, *D*, and *E* genes (map positions 627 to 2198) was analyzed for *Escherichia coli* RNA polymerase-binding sites by combined DNase I footprinting, exonuclease III analysis, and DNA sequencing. Two high-affinity binding sites that correspond to the *B* and *D* gene promoters were mapped at positions 936 to 995 and 1779 to 1848, respectively. Positions 936 to 995 are in gene *A, preceding the *B* gene, and positions 1779 to 1848 are in gene *C*, just preceding the *D* gene. In addition, two lower-affinity binding sites were identified at positions 1527 to 1538 and 1705 to 1756 preceding the *C* and *D* genes, respectively. The footprinting studies described here, in combination with previous studies on transcription, provide definitive evidence on the position of the *B* and *D* gene promoters in S13 and the closely related phage φX174.**

In the icosahedral bacteriophages S13 and φX174, the negative strand of the replicative form I DNA is the template used by *Escherichia coli* RNA polymerase for transcription of polycistronic mRNAs initiated at three promoter sites (9). The promoter upstream of the *A* gene has been localized very precisely by a combination of mutational studies (37), RNA polymerase binding analyses (23, 24, 38), and hybridization of in vitro transcripts to restriction fragments and correlation of an eight-base sequence at the 5′ end of an in vitro transcript to the DNA sequence of φX174 (3, 28, 35, 36).

The promoters upstream of the *B* and *D* genes have been localized with less accuracy. Determinations have been based on the approximate sizes of in vivo transcripts (12), RNA polymerase binding studies (23, 24, 38), and the hybridization of in vitro transcripts to restriction fragments (3, 35). Sequences obtained from the 5′ termini of in vitro transcripts consisted of as few as four or five bases (36), which resulted in some ambiguity in the precise locations of transcription starts on the φX174 sequence (28). Protection of restriction sites by *E. coli* RNA polymerase in both S13 and φX174 phage DNAs (25, 32) and in vivo transcription from a DNA fragment of φX174 cloned in a promoter vector (8) have narrowed down the area encompassing the *B* gene promoter. However, the exact location and number of promoters in the *B* and *D* gene region of these two phages remains to be determined. We analyzed the areas upstream of the *B* and *D* genes by DNase I footprinting (10) and exonuclease III protection analysis (5, 31) coupled with DNA sequencing, a combination of techniques which allows accurate mapping of DNA-binding sites.

In this paper we report the precise mapping of the *B* and *D* gene promoters, as well as the presence of two RNA polymerase-binding sites of lower affinity upstream of gene *D*.

MATERIALS AND METHODS

Materials. S13 replicative form I DNA was isolated and purified as described by Lau and Spencer (16). *E. coli* RNA polymerase was a generous gift of Maurice Ringuette, and its isolation, purification, and characterization have been described elsewhere (25). T4 polynucleotide kinase and exonuclease III were obtained from New England Nuclear Corp. (Montreal, Canada), and DNase I and calf intestinal alkaline phosphatase were from Bethesda Research Laboratories, Inc. (Gaithersburg, Md.). Restriction enzymes were purchased from Boehringer Mannheim Biochemicals (Montreal, Canada) or Bethesda Research Laboratories and used according to the specifications of the manufacturers. DEAE membrane was from Schleicher & Schuell, Inc. (Keene, N.H.).

Preparation of 5′-end-labeled restriction fragments. S13 replicative form I DNA was cleaved with *Ava*I, *Ava*II, or *Hin*dIII, each of which have a single cleavage site in S13 DNA in the region of the *B* and *D* genes (Fig. 1). The restricted DNA was dephosphorylated with 0.005 U of calf intestinal alkaline phosphatase per μg of DNA at 37°C for 30 min. The solution was extracted twice with phenol and precipitated with ethanol. The DNA fragments were then 5′ end labeled with T4 polynucleotide kinase and 150 μCi (50 pmol) of [γ-^{32}P]ATP by the method of Maniatis et al. (18). Fragments with a unique 5′ ^{32}P-end-labeled terminus were generated by cleavage with a second restriction enzyme and separated by electrophoresis in a 4% preparative polyacrylamide gel. The appropriate bands were excised, and the DNA was electroeluted and precipitated with 2.5 volumes of ethanol at −20°C.

DNase I footprinting and exonuclease III analysis. The procedure used for DNase footprinting was that of Galas and Schmitz (10) with minor modifications. 5′-end-labeled DNA fragment (0.05 to 0.3 pmol) was incubated with 0.01 to 5 pmol of *E. coli* RNA polymerase in 50 μl of binding buffer (10 mM Tris hydrochloride [pH 7.9], 10 mM $MgCl_2$, 0.1 mM EDTA, 0.1 mM dithiothreitol, 0 to 50 mM KCl, 50 μg of bovine serum albumin per ml). The optimal polymerase/DNA ratio was determined for every fragment. Polymerase and DNase I were each diluted with RNA polymerase storage buffer (0.1 M NaCl, 10 mM Tris hydrochloride [pH 7.9], 0.1 mM EDTA, 0.1 mM dithiothreitol, 50% glycerol). To avoid adverse effects on binding caused by glycerol concentrations above 10%, the combined volume of polymerase and DNase I added was kept constant at 10 μl/50 μl of final reaction volume. Binding of RNA polymerase to

* Corresponding author.
† Present address: Medical Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada M5G 1X5.

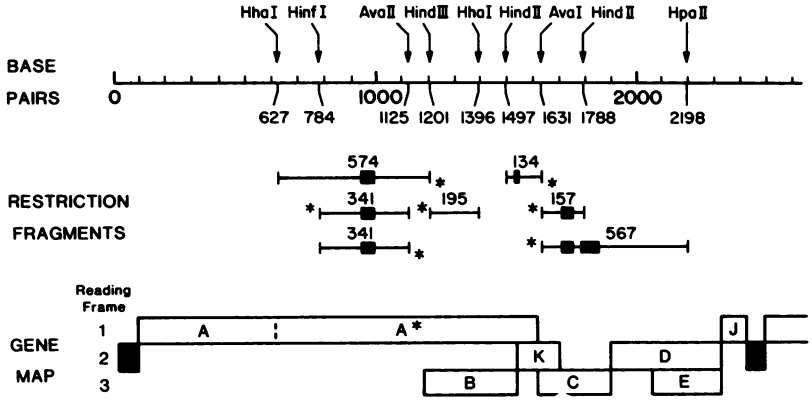

FIG. 1. Restriction fragments of S13 DNA used for DNase I and exonuclease III analysis. The restriction map identifies the sites used to generate the restriction fragments. The size in base pairs of each fragment is given above the fragment; the ^{32}P-labeled ends are marked by asterisks. The mapped binding sites are shown as solid boxes.

DNA was for 20 min at 37°C. The four nucleoside triphosphates were added to control reactions to final concentrations of 40 μM each, or alternatively, heparin was added to a final concentration of 80 μg/ml, and the incubation continued for a further 5 min. For footprinting, DNase I was added to a final concentration of 6 ng/ml, and the solution was incubated for 10 min at 25°C. For exonuclease III protection analyses, exonuclease III was added to a final concentration of 1 U/μl, and the solution was incubated for 3 h at 37°C. Reactions were stopped by the addition of an equal volume (50 μl) of a solution containing 0.2% sodium dodecyl sulfate, 30 mM EDTA, and 40 μg of sheared calf thymus DNA per ml and immediately treated with phenol. The DNA was then precipitated with 2.5 volumes of ethanol at −70°C and dissolved in 2 to 3 μl of 10 mM Tris hydrochloride [pH 8.0]–10 mM NaCl–2 mM EDTA, and 10 μl of loading buffer containing 95% formamide, 10 mM NaOH, 1 mM EDTA, 0.1% (wt/vol) xylene cyanol, and 0.1% (wt/vol) bromophenol blue was added. The solution was heated at 90°C for 2 to 3 min, chilled quickly in an ice bath, and loaded on a 6 or 8% polyacrylamide–8 M urea sequencing gel that had been preelectrophoresed at 30 mA for 1 h. After electrophoresis at 30 mA for 2 to 4 h, the gel was exposed at −70°C to Kodak XAR-5 or Cronex X-ray film with an intensifying screen. DNA sequenced by the procedure of Maxam and Gilbert (19) was run in parallel with the footprinting and exonuclease III analyses.

RESULTS

DNA fragments used for exonuclease III and DNase I analysis. The restriction fragments of S13 replicative form I DNA chosen for analysis and their location on the genetic map are diagrammed in Fig. 1. Two fragments covering the region 627 to 1201 contain the area occupied by the *B* gene promoter (8, 25, 35). Fragment 1201 to 1396 is representative of the region spanning positions 1000 to 1500 in which no RNA polymerase binding has previously been observed (23–25, 32, 38). The three fragments spanning positions 1497 to 2198 cover the region which includes the *D* gene promoter (3, 25, 32, 35).

***B* gene promoter region.** Exonuclease III analysis of the 341-base-pair (bp) *Hin*fI-*Ava*II fragment labeled on the noncoding strand at position 1125 revealed a major protected band of approximately 170 bases (Fig. 2A, lane 4). This mapped the left-hand boundary of the binding site to position 959 on the genetic map, determined precisely by comparison with the Maxam-Gilbert sequence run in parallel (data not shown). A second protected band of approximately 180 bases corresponds to position 947. A third band of approximately 190 bases was more prominent in similar experiments (data not shown) and at a higher polymerase/DNA ratio (lane 5) and indicates partial protection at position 939. Several larger protected bands also present in lane 5 were due to nonspecific binding, especially at the end of the fragment, routinely observed in the presence of excess RNA polymerase (31) and clearly absent at the lower poly-

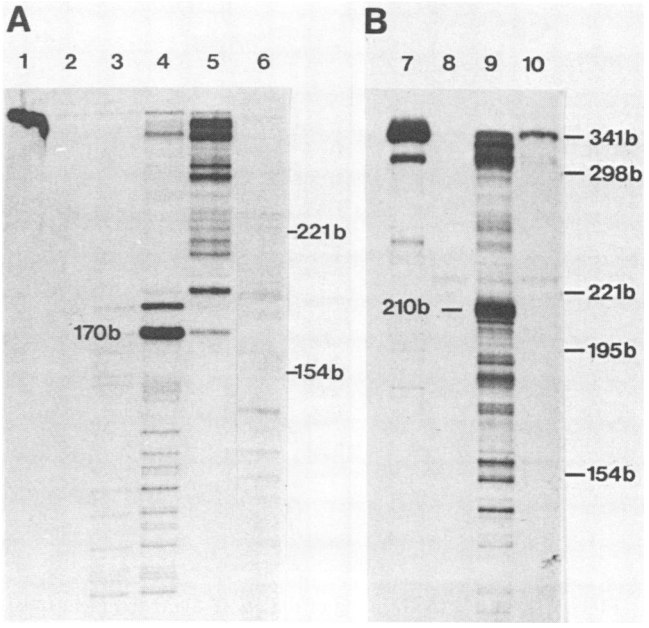

FIG. 2. Exonuclease III analysis of the binding of *E. coli* RNA polymerase to the *Hin*fI-*Ava*II fragment (positions 784 to 1125). (A) The fragment ^{32}P labeled at position 1125. (B) The fragment ^{32}P labeled on the opposite strand at position 784. Lanes 1 and 7, No exonuclease added; lanes 2 and 8, no polymerase added. Polymerase/DNA ratios: lane 3, 1:5; lanes 4, 6, 9, and 10, 1:1; lane 5, 5:1. Lanes 6 and 10, The four nucleoside triphosphates were added after binding of polymerase. Lanes 2 to 6 and 8 to 10, 1 U of exonuclease III per μl. The positions of λ DNA size markers are shown on the right of panels A and B.

merase/DNA ratio in lane 4. In the control reaction no protection was observed after addition of the four nucleoside triphosphates to the binding reaction (lane 6). Exonuclease III analysis of the same fragment labeled on the coding strand at postion 784 resulted in a major band of 210 bases (Fig. 2B, lane 9), mapping the right-hand end of the binding site to position 995. Minor bands ranging in size from 140 to 190 bases visible in lane 9 occurred only at lower polymerase/DNA ratios.

Exonuclease III mapping of the 574-bp *Hha*I-*Hin*dIII fragment labeled on the noncoding strand at position 1201 resulted in a band approximately 250 bases in size (not shown), placing the left-hand boundary of the binding site around position 950 on the genetic map. This confirmed the result with the 341-bp *Hin*fI-*Ava*II fragment. Binding at this site was resistant to heparin but sensitive to KCl concentrations of 100 mM or above, indicating that the half-life of the binding complex in 100 mM KCl was considerably shorter than the time of exonuclease III digestion. Increases in ionic strength are known to increase drastically the rate of dissociation of RNA polymerase from promoters (13).

DNase I footprinting of the 341-bp *Hin*fI-*Ava*II fragment labeled at position 1125 on the noncoding strand mapped the boundaries of the major binding site to positions 936 and 995 (Fig. 3), providing additional confirmation of the results of the exonuclease III analysis. Figure 3 also shows the existence of a DNase I-hypersensitive site at position 957-958. A footprint of the opposite strand confirmed these results (data not shown).

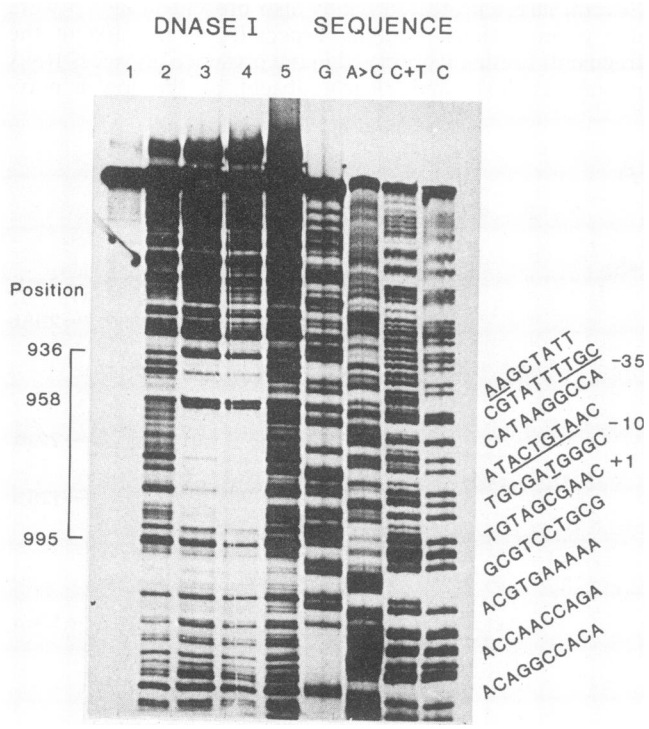

FIG. 3. DNase I footprinting of the *Hin*fI-*Ava*II fragment (positions 784 to 1125). The fragment was 5′ ^{32}P labeled at position 1125. Lane 1, No DNase I added; lane 2, no polymerase added. Polymerase/DNA ratios: lanes 3 and 5, 1:1; lane 4, 2.5:1. Lane 5, The four nucleoside triphosphates were added after binding of polymerase. Lanes 2 to 5, 6 ng of DNase I per ml. The lanes on the right are the four reactions of the Maxam-Gilbert sequence. The DNA sequence from positions 940 to 1037 is shown on the right. The complementary sequence of the canonical promoter sequences at positions 946 to 951 (−35) and 970 to 975 (−10) and the putative transcription start at 982 (+1) of the *B* gene are underlined. The positions of the footprint and a hypersensitive site are shown on the left.

Exonuclease III analysis of the 195-bp *Hin*dIII-*Hha*I fragment labeled at position 1201 on the coding strand revealed protected bands corresponding to regions throughout the whole fragment. The number of bands increased at higher polymerase/DNA ratios, a pattern characteristic of nonspecific binding. In the presence of heparin, very faint bands became visible only at high polymerase/DNA ratios, also indicating nonspecific binding (data not shown). This confirmed the absence of a specific binding site in this region.

***D* gene promoter region.** The relatively large region from 1497 to 2198 (Fig. 1) was chosen for analysis because an area of strong binding revealed by RNA polymerase protection of restriction sites at 1755, 1779, 1788, and 1821 had been identified in a previous study from this laboratory (25). Weaker binding sites were also present, shown by the partial protection of restriction sites at positions 1497, 1522, 1629, and 1638 (25).

Footprint analysis of the 134-bp *Hin*dII-*Ava*I fragment 5′ end labeled on the noncoding strand at position 1631 (Fig. 4A) revealed a protected area from 1527 to 1583 (lanes 3 and 4), the upper end corresponding exactly to the prominent 105-nucleotide band protected from exonuclease digestion (lane 7). The binding was sensitive to heparin (compare lanes 8 and 9) and required a high RNA polymerase/DNA ratio. A partial footprint observed at the lower end of the gel in lanes 3 and 4 was due to fragment end binding.

DNase I footprinting of the 157-bp *Hin*dII-*Ava*I fragment labeled at 1631 on the coding strand (Fig. 4B) mapped one binding site between positions 1705 and 1745-1756 (lanes 3 and 4). The variability in the protection from 1745 to 1756 correlates with the appearance of multiple protected bands in the exonuclease III analysis (lane 7). A second footprint partly visible at the lower end of the gel was due to fragment end binding (lane 4). Binding to the 157-bp fragment was heparin sensitive (lanes 9 and 10) and occurred at a polymerase/DNA ratio larger than 1.

DNase I footprinting of the 567-bp *Ava*I-*Hpa*II fragment 5′ end labeled on the coding strand at 1631 (Fig. 5) revealed a binding site from positions 1779 to 1848, possibly extending to 1860 (lanes 2 and 3). The footprint contained a strong DNase-hypersensitive site at position 1803, very close to the location of a 170-base band protected from exonuclease digestion (lane 9). The discrepancy between the exonuclease III protection and the footprint data indicated that binding at this site does not block exonuclease digestion efficiently. Thus, instead of a strongly protected exonuclease band defining the 3′ end of the site, several faint bands were observed in the middle of the binding site (lane 8). Furthermore, contrary to the footprint analysis, which showed heparin sensitivity of the binding at this site (lane 5), exonuclease III digestion revealed residual protection in the middle of the binding site (in the presence of heparin) (lane 9). A second binding site starting at position 1756 (lower end of lane 4) corresponded to the lower-affinity binding site previously mapped on the 157-bp *Hin*dII-*Ava*I fragment (Fig. 4B). Also, there was an indication of a third footprint around position 2070. Binding at this site was confirmed by the presence of the 440-base exonuclease III band in lanes 8 and 9.

DISCUSSION

The strong RNA polymerase-binding site mapped precisely by DNase I footprinting to positions 936 to 995 and by

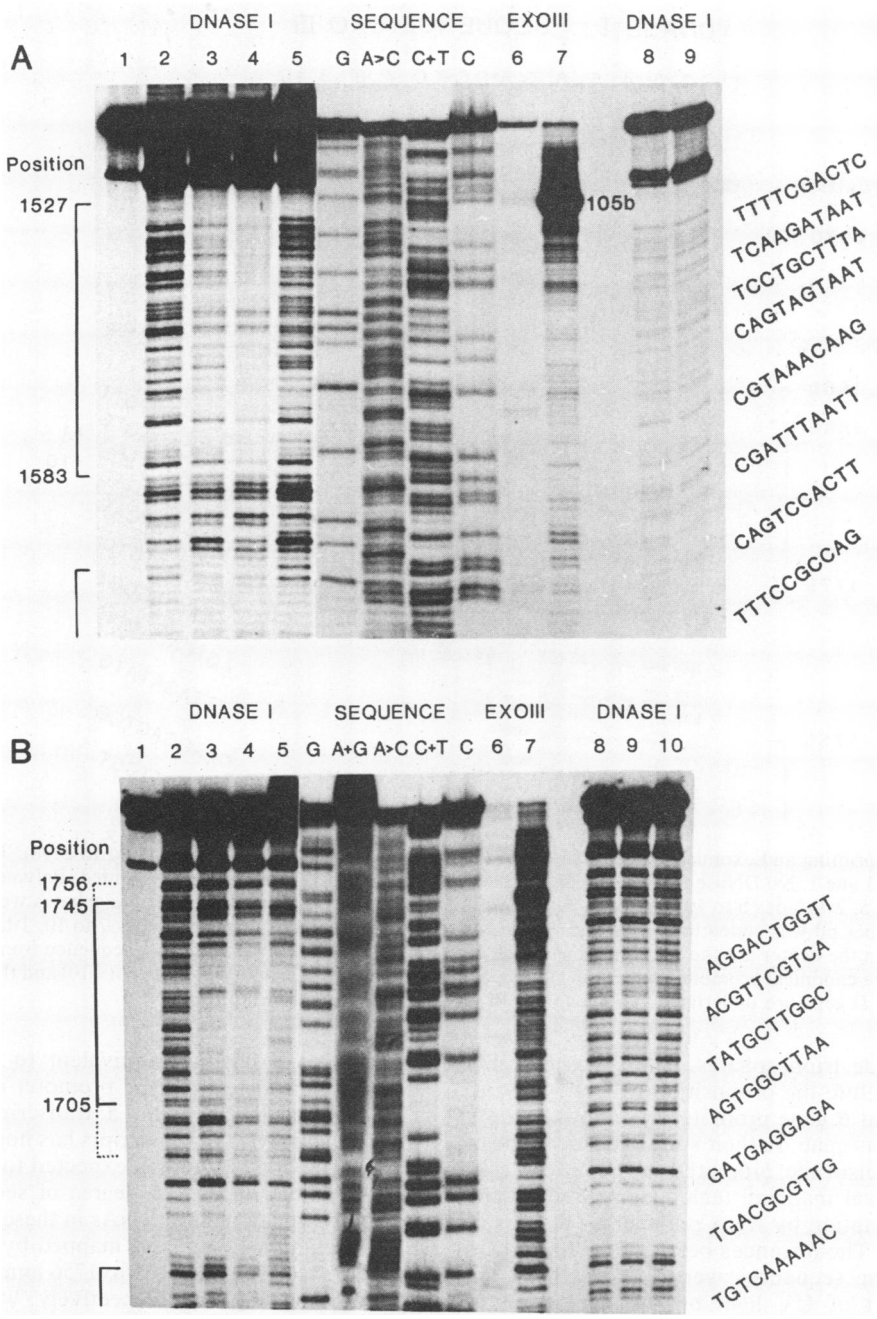

FIG. 4. DNase I footprinting and exonuclease III analyses. (A) The *Hin*dII-*Ava*I fragment (positions 1497 to 1631). The fragment was 5′ ^{32}P labeled at position 1631. Lane 1, No DNase I or exonuclease III added. Lanes 2, 6, and 8, no polymerase added. Polymerase/DNA ratios: lanes 3, 5, 7, and 9, 5:1; lane 4, 15:1. After binding of polymerase, the four nucleoside triphosphates were added to lane 5 and 80 μg of heparin per ml was added to lanes 8 and 9. Lanes 2 to 5, 8, and 9, 6 ng of DNase I per ml; lanes 6 and 7, 1 U of exonuclease III per ml. The quantity of DNA in lanes 8 and 9 was 10% of that in lanes 1 to 5. (B) The *Ava*I-*Hin*dII fragment (positions 1631 to 1788). The fragment was 5′ ^{32}P labeled at position 1631. Conditions were as described for panel A except for the extra lane (lane 10), which had a polymerase/DNA ratio of 15:1 and 80 μg of heparin per ml. Also, the quantity of DNA in lanes 8 to 10 was identical to that in lanes 1 to 5. The sequence lanes in the center are the four reactions of the Maxam-Gilbert sequence. The DNA sequences from positions 1522 to 1601 (A) and 1747 to 1678 (B) are shown on the right; the positions of the footprints are on the left.

exonuclease III analysis to positions 959 to 995 lies within a broader area of strong binding previously mapped by restriction site protection (25). Several features of this site indicate that it is the *B* gene promoter. At positions 946 to 951 and 970 to 975 it contains sequences homologous to the −35 and −10 canonical promoter sequences found upstream of all procaryotic transcription starts (11, 26, 33). Position 982, which is seven bases downstream of the Pribnow box sequence (1), corresponds to the putative transcription start site of the *B* gene in bacteriophage φX174. This was the preferred of two start sites proposed by Sanger et al. (28) on the basis of limited in vitro mRNA sequence data (36). In control experiments we observed transcription from the binding site as shown by the loss of binding protection after the addition of

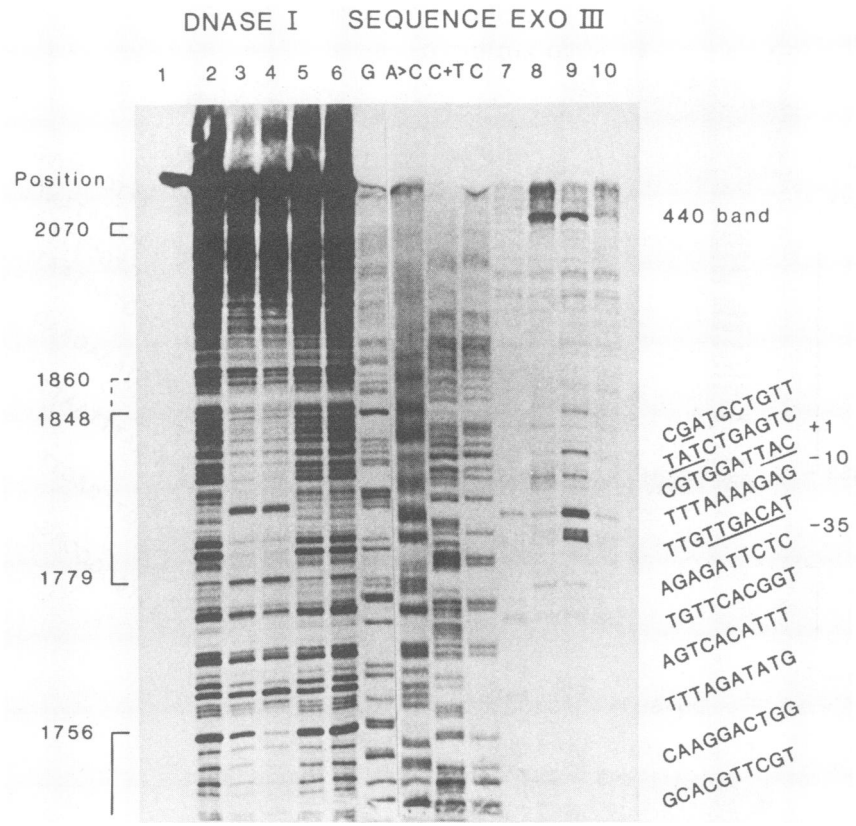

FIG. 5. DNase I footprinting and exonuclease III analysis of the *Aval-Hpa*II fragment (positions 1631 to 2198). The fragment was 5′ ^{32}P labeled at position 1631. Lane 1, No DNase I or exonuclease III added. Lanes 2 and 7, No polymerase added. Polymerase/DNA ratios: lanes 3 and 6, 1:2; lanes 4 and 5, 2:1; lanes 8 to 10, 1:3. After binding of polymerase, the four nucleoside triphosphates were added to lanes 6 and 10 and 80 μg of heparin per ml was added to lanes 5 and 9. Lanes 2 to 6, 6 ng of DNase I per ml. Lanes 7 to 10, 1 U of exonuclease III per μl. The sequence lanes in the center are the four reactions of the Maxam-Gilbert sequence. The DNA sequence from positions 1726 to 1835 is shown on the right. The canonical promoter sequences at positions 1789 to 1794 (−35) and 1813 to 1818 (−10) and the putative transcription start at 1827 (+1) of the *D* gene are underlined. The positions of the footprints are shown on the left.

the four ribonucleoside triphosphates. The present results neither support nor refute the proposition by Pollock et al. (21) that the functional *B* gene promoter lies approximately 2,000 bp upstream of its gene. In their study, the locations of the three major polycistronic promoters in S13 were estimated by a radiological mapping technique, based on the termination of transcription by RNA polymerase at sites of UV-induced damage. The distances between the promoters and the protein-coding sequences were deduced from the severity of the effect of UV light on the expression of individual proteins, using the *D* transcription unit as the point of reference. One explanation for the unexpected estimates of the promoter locations is that different transcripts when truncated at sites of UV damage may be degraded at different rates, introducing errors in promoter mapping by this technique. Also, in vitro binding assays such as those in the present study may not reflect in vivo conditions used by Pollock et al. (21).

The major footprint upstream of the *D* gene mapped to positions 1779 to 1848-1860 correlates well with the strong but broad area of binding located by restriction site protection (25) and narrowed down the binding to a precise site. The site lies close to the *D* gene translation start at 1859 (16) and contains sequences at 1789 to 1794 and 1813 to 1818 homologous to the −35 and −10 canonical promoter sequences. An in vitro transcription start was mapped in ϕX174 on the basis of very short 5′-terminal mRNA sequences to a position equivalent to 1827 in S13, at the expected distance from the promoter consensus sequences (36). As for the *B* gene, a transcription start based on sequence analysis of transcripts has not been determined for the *D* gene of S13, but it is expected to coincide with that in ϕX174 owing to the high degree of sequence homology between the bacteriophage DNAs in these two regions (16, 29).

Two minor binding sites mapped by footprinting to positions 1527 to 1583 and 1705 to 1756 immediately downstream of the *B* and *K* genes, respectively, were only observed at RNA polymerase/DNA ratios two- to fourfold higher than those required to detect the major footprints upstream of the *B* and *D* genes. It is not known whether these sites have any functional significance or whether the binding merely reflects nonspecific affinity. Studies are under way to determine whether these sites support transcription in vivo.

A third minor binding site was faintly visible as a footprint, and its 3′ end was mapped approximately by exonuclease analysis to position 2070, within the *D* gene. In ϕX174, at a position equivalent to 2054 of S13, a prominent *Micrococcus luteus* polymerase transcription start has been detected by runoff transcription and RNA 5′-end fingerprinting (7). Although Ernst et al. (7) showed that *E. coli* RNA polymerase holoenzyme did not transcribe from this site, our data indicate that it is able to bind to it.

In line with other footprinting analyses (34), the 5′ boundaries of the *B* and *D* gene binding sites are at −46 and −48

and the 3′ boundaries are at +14 and +22 relative to the putative transcription starts at +1. The sizes of the binding sites, 59 bp and at least 69 bp, also lie within the range of values observed in other studies (10, 34). The larger size of the *D* gene polymerase-binding site and the divergence of the DNase I footprinting and exonuclease III mapping data may indicate flexible boundaries at this site. This correlates with the observations by Axelrod (2) and Smith and Sinsheimer (36) that transcription at the *D* promoter can start at several adjacent nucleotide residues.

The mapping data obtained by exonuclease III analysis agreed closely with the footprinting data for the 3′ end of the *B* gene site and the 5′ end of the minor site at 1527 to 1583. However, the two techniques resulted in variant values for the 5′ end of the *B* gene site and the 3′ end of the *D* gene site. The divergence may in part be due to the difference in digestion conditions used for DNase I and exonuclease III. Although the 3-h digestion time used for exonuclease III is much longer than that for DNase I, it is well below the half-life of 6 to 20 h reported for the binding of RNA polymerase to several procaryotic promoters (4, 14, 27). It is possible that during extended incubations with exonuclease, the polymerase-DNA complex partly detaches at points of weaker interaction while remaining attached at points of stronger contact. A comparison between the exonuclease and DNase data indicates that there might be a weaker area of interaction around the −35 region of the *B* gene promoter. In contrast, at the *D* gene promoter, the interactions at the Pribnow box and transcription start site appear less stable than the contact at the −35 region.

It may be significant that the two variant sites of exonuclease III protection (see above) coincide almost exactly with the strong hypersensitive sites in the middle of the *B* and the *D* gene promoter footprints (positions 959 and 1801). Sites of DNase hypersensitivity are commonly observed in footprints (22, 30) and are thought to occur at points of strong contact between the bound protein and one or several nucleotide residues. This contact may cause an increased helical twist at the site of interaction, a feature proposed by Lomonossoff et al. (17) to be preferentially attacked by DNase. Thus, it appears from our data that the close contact of RNA polymerase with the DNA template, which causes the hypersensitivity at the *B* and *D* gene binding sites, also stops the linear progression of exonuclease abruptly.

The response to heparin varied at the different sites. The two minor binding sites and the *D* gene promoter site were sensitive, but the *B* gene promoter site was resistant. This general pattern of heparin sensitivity correlates well with that reported for ϕX174 by Smith and Sinsheimer (35), who found that in the presence of heparin, transcription decreased from the *D* promoter and slightly increased from the *B* promoter. Heparin was originally thought to affect polymerase binding indirectly by inactivating the enzyme after its release from short-lived binding complexes (39). However, the heparin sensitivity of the *D* promoter need not indicate that binding at this site is short lived. Rather, heparin may directly attack RNA polymerase bound to this promoter site, as seen with other procaryotic promoters (6, 15, 20).

We found the combined use of DNase I and exonuclease III advantageous for the precise mapping of binding sites. Exonuclease is a sensitive binding probe and provides evidence for binding even if only a small proportion of DNA fragments contain bound RNA polymerase. In the same situation, a clear footprint is not easily recognizable; thus, exonuclease proved useful in preliminary experiments for the rapid determination of the optimun polymerase/DNA ratio. On the other hand, the observation of a clear DNase I footprint represents more definitive evidence for binding than protection from exonuclease digestion because the appearance of multiple protected bands in exonuclease III analyses occasionally causes difficulty in interpretation. Finally, DNase I allows the visualization of one or several complete sites on a fragment, whereas exonuclease allows mapping of one border of a binding site only. Drawbacks associated with one binding assay can therefore be overcome by the use of the second assay.

In summary, our data provide evidence for the precise location in S13 of the *B* gene promoter at positions 946 to 982 and of the *D* gene promoter at 1789 to 1827 and for the presence of two lower-affinity binding sites at 1527 to 1583 and 1705 to 1756.

ACKNOWLEDGMENTS

This work was supported by a grant from the Medical Research Council of Canada. M.A. was supported by a Franklin Bracken scholarship from Queen's University.

We thank Diane Fredenburgh for technical assistance, George Mackie and Peter Davies for valuable advice on techniques, Hans Metz for photography and art work, and Theresa Brennan for preparation of the manuscript.

LITERATURE CITED

1. **Aoyami, T., and M. Takanami.** 1985. Essential structure of *E. coli* promoter. II. Effect of the sequences around the RNA start point on promoter function. Nucleic Acids Res. **13**:4085–4096.
2. **Axelrod, N.** 1976. Transcription of bacteriophage ϕX174 *in vitro*: selective initiation with oligonucleotides. J. Mol. Biol. **108**:753–770.
3. **Axelrod, N.** 1976. Transcription of bacteriophage ϕX174 *in vitro*: analysis with restriction enzymes. J. Mol. Biol. **108**: 771–779.
4. **Bertrand-Burggraf, E., J. F. Lefevre, and M. Daune.** 1984. A new experimental approach for studying the association between RNA polymerase and the *tet* promoter of pBR322. Nucleic Acids Res. **12**:1697–1706.
5. **Chan, P. T., and J. Lebowitz.** 1983. The coupled use of "footprinting" and exonuclease III methodology for RNA polymerase binding and initiation. Application for the analysis of three tandem promoters at the control region of colicin EI. Nucleic Acids Res. **11**:1099–1116.
6. **DeLorbe, W. J., S. Surzycki, and G. Gussin.** 1979. Inactivation of *E. coli* RNA polymerase by polyriboinosinic acid: heterogeneity of RS complexes. Mol. Gen. Genet. **173**:51–59.
7. **Ernst, H., G. R. Hartmann, and H. Domdey.** 1982. Species specificity of promoter recognition by RNA polymerase and its transfer by the sigma factor. Eur. J. Biochem. **124**:427–433.
8. **Fedchenko, V. I., A. V. Chestukhin, and M. F. Shemyakin.** 1983. Dependence of the activity of ϕX174 B promoter in the expression of *Escherichia coli gal* operon on the number of its copies and their orientation. Mol. Biol. **17**:418–429.
9. **Fujimura, F. K., and M. Hayashi.** 1978. Transcription of isometric single-stranded DNA phage, p. 485–505. *In* D. T. Denhardt, D. Dressler, and D. S. Ray (ed.), The single-stranded DNA phages. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
10. **Galas, D. J., and A. Schmitz.** 1978. DNase footprinting: a simple method for the detection of protein-DNA binding specificity. Nucleic Acids Res. **5**:3157–3170.
11. **Hawley, D. K., and W. R. McClure.** 1983. Compilation and analysis of *Escherichia coli* promoter DNA sequences. Nucleic Acids Res. **11**:2237–2255.
12. **Hayashi, M., F. K. Fujimura, and M. Hayashi.** 1976. Mapping of *in vivo* messenger RNAs for bacteriophage ϕX174. Proc. Natl. Acad. Sci. USA **73**:3519–3523.
13. **Hinkle, D., and M. J. Chamberlin.** 1972. Studies of the binding

of *Escherichia coli* RNA polymerase to DNA I. The role of sigma subunit in site selection. J. Mol. Biol. **70**:157–185.
14. **Kadesch, T. R., S. Rosenberg, and M. J. Chamberlin.** 1982. Binding of *Escherichia coli* RNA polymerase holoenzyme to bacteriophage T7 DNA. Measurements of binding at bacteriophage T7 promoter A_1 using a template competition assay. J. Mol. Biol. **155**:1–29.
15. **Küpper, H., R. Contreras, H. G. Khorana, and A. Landy.** 1976. The tyrosine tRNA promoter, p. 473–484. *In* R. Losick and M. Chamberlin (ed.), RNA polymerase. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
16. **Lau, P. C. K., and J. H. Spencer.** 1985. Nucleotide sequence and genome organization of bacteriophage S13 DNA. Gene **40**:273–284.
17. **Lomonossoff, G. P., P. J. G. Butler, and A. Klug.** 1981. Sequence-dependent variation in the conformation of DNA. J. Mol. Biol. **149**:745–760.
18. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning, a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
19. **Maxam, A. M., and W. Gilbert.** 1977. A new method for sequencing DNA. Proc. Natl. Acad. Sci. USA **74**:560–564.
20. **Pfeffer, S. R., S. J. Stahl, and M. J. Chamberlin.** 1977. Binding of *Escherichia coli* RNA polymerase to T7 DNA. Displacement of holoenzyme from promoter complexes by heparin. J. Biol. Chem. **252**:5403–5407.
21. **Pollock, T. J., I. Tessman, and E. S. Tessman.** 1978. Radiological mapping of functional transcription units of bacteriophages ϕX174 and S13. The function of proximal and distal promoters. J. Mol. Biol. **124**:147–160.
22. **Queen, C., and M. Rosenberg.** 1981. A promoter of pBR322 activated by cAMP receptor protein. Nucleic Acids Res. **9**:3365–3377.
23. **Rassart, E., and J. H. Spencer.** 1978. Localization of *Escherichia coli* RNA polymerase binding sites on bacteriophage S13 and ϕX174 DNA: alignment with restriction enzyme maps. J. Virol. **27**:677–687.
24. **Rassart, E., J. H. Spencer, and M. Zollinger.** 1979. Localization of *Escherichia coli* RNA polymerase binding sites on bacteriophage S13 and ϕX174 DNAs by electron microscopy. J. Virol. **29**:179–184.
25. **Ringuette, M., and J. H. Spencer.** 1987. Localization of *Escherichia coli* RNA polymerase binding sites on bacteriophage S13 replicative form I DNA by protection of restriction enzyme cleavage sites. J. Virol. **61**:2297–2303.
26. **Rosenberg, M., and D. Court.** 1979. Regulatory sequences involved in the promotion and termination of RNA transcription. Annu. Rev. Genet. **13**:319–353.
27. **Russell, D. R., and G. N. Bennett.** 1981. Characterization of the β-lactamase promoter of pBR322. Nucleic Acids Res. **9**: 2517–2533.
28. **Sanger, F., G. M. Air, B. G. Barrell, N. L. Brown, A. R. Coulson, J. C. Fiddes, C. A. Hutchinson III, P. M. Slocombe, and M. Smith.** 1977. Nucleotide sequence of bacteriophage ϕX174. Nature (London) **265**:687–695.
29. **Sanger, F., A. R. Coulson, T. Friedmann, G. M. Air, B. G. Barrell, N. L. Brown, J. C. Fiddes, C. A. Hutchison III, P. M. Slocombe, and M. Smith.** 1978. The nucleotide sequence of bacteriophage ϕX174. J. Mol. Biol. **125**:225–246.
30. **Schmitz, A., and D. J. Galas.** 1979. The interaction of RNA polymerase and lac repressor with the lac control region. Nucleic Acids Res. **6**:111–137.
31. **Shanblatt, S. H., and A. Revzin.** 1984. Kinetics of RNA polymerase-promoter complex formation: effects of non-specific DNA-protein interactions. Nucleic Acids Res. **12**:5287–5306.
32. **Shemyakin, M. F., and V. Y. Shumilov.** 1981. Protection of sites on the replicative form I of phage ϕX174 DNA recognized by the restriction enzymes HindII, BspRI, and AluI. Mol. Biol. **15**:1144–1157.
33. **Siebenlist, U., R. B. Simpson, and W. Gilbert.** 1980. *E. coli* RNA polymerase interacts homologously with two different promoters. Cell **20**:269–281.
34. **Simpson, R. B.** 1982. Evidence for close contacts between RNA polymerase and promoter DNA, p. 164–180. *In* R. L. Rodriguez and M. J. Chamberlin (ed.), Promoters. Structure and function. Praeger Publishers, New York.
35. **Smith, L. H., and R. L. Sinsheimer.** 1976. The *in vitro* transcription units of bacteriophage ϕX174. II. *In vitro* initiation sites of ϕX174 transcription. J. Mol. Biol. **103**:699–710.
36. **Smith, L. H., and R. L. Sinsheimer.** 1976. The *in vitro* transcription units of bacteriophage ϕX174 III. Initiation with specific 5′ end oligonucleotides of *in vitro* ϕX174 RNA. J. Mol. Biol. **103**:711–735.
37. **Vanderbilt, A. S., M. T. Borras, S. Germeraad, I. Tessman, and E. S. Tessman.** 1972. A promoter site and polarity gradients in phage S13. Virology **50**:171–179.
38. **Williams, R. C., and H. W. Fisher.** 1980. Electron microscopic determination of the preferential binding sites of *Escherichia coli* RNA polymerase to ϕX174 replicative form DNA. J. Mol. Biol. **140**:435–439.
39. **Zillig, W., P. Palm, and A. Heil.** 1976. Function and reassembly of subunits of DNA-dependent RNA polymerase, p. 101–125. *In* R. Losick and M. Chamberlin (ed.), RNA polymerase. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.