
Interaction between *E. coli* RNA polymerase and the *tetR* promoter from pSC101: homologies and differences with other *E. coli* promoter systems from close contact point studies

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ABSTRACT

The interaction between *E. coli* RNA polymerase and the *tetR* promoter from pSC101, was studied by protection and premodification experiments, using dimethyl sulfate, methylation of single stranded cytosines, and DNAase I footprinting. Whereas qualitative and quantitative results from the chemical approach conform to patterns already displayed by other promoter systems, hypersensitive sites to DNAase I attack differ from those of other promoters. Distribution and nature of the contacts suggest that regions of the promoter sequence participate differently in complex formation. The involvement of major and minor grooves of the double helix in the complex with the enzyme, differs along the promoter. After a comparison of the results from seven different promoters, a pattern of conserved contacts seem to appear. Comparison of temperature dependence of local unwinding around the transcription start site (detected by the appearance of single stranded cytosines), and DNAaseI footprinting, reveals that the process leading to stable complex formation can be achieved without disruption of base-pairing.

1. INTRODUCTION

Much work has been devoted to determine the elements characterizing DNA targets for *E. coli* RNA polymerase (RNAP) and to study the transcription initiation process and related control mechanisms (for reviews, see 1-4)

Although recent approaches have shed more light on the molecular bases of these processes, central problems remain yet unsolved, mainly the relationship between the chemical and physical properties of a precise sequence and its biological behaviour as an *E. coli* promoter.

In the last years, chemical and enzymatic methods for determining close contact points of specific proteins on DNA, have been applied in static approaches to study RNAP-promoter complexes. Earliest work on *lac UV5* and *A3* from bacteriophage *T7* promoters revealed that they have many common features between them, suggesting that RNAP binds promoters in a general and rather similar way (2).

Recently, dynamic approaches derived from the above techniques, gave more

information about the different steps preceding the transcription start, allowing the proposal of mechanistic models accounting for the *in vitro* properties of the studied promoter (5,6) including the effect of supercoiling (7).

We present here a complete static determination of close contact points of RNAP on the tetR promoter from pSC101, which controls the expression of the tetracycline repressor gene. Experiments were carried out by protection against alkylation and alkylation-interference (pre-modification) procedures, as well as by DNAase I footprinting.

Comparison of the present static results with those already published for other promoters, bring out some new elements about the involvement of conserved and non-conserved sequences of the promoter in the interaction process.

2. MATERIALS AND METHODS

2.1. Promoter fragments.

We have isolated a 126 bp fragment containing tetR promoter from pAT153, a derivative from pBR322 (8). The two strands were labelled separately by the following procedures :

After an EcoRI digestion (Boehringer, Mannheim), labelling at 3' ends was carried out by terminal deoxynucleotidyl transferase or DNA polymerase I, Klenow fragment (Boehringer, Mannheim) and ^3_2P -ddATP (Amersham, England) or at 5' ends by polynucleotide kinase (Boehringer, Mannheim) and ^3_2P -ATP (Amersham, England). A secondary restriction with Hph I (New England, Biolabs) was introduced. The resulting Hph I - EcoRI fragment, 126 bp long, labelled at one end, was further purified by gel electrophoresis.

Notice that it was described for other tet repressor-resistance gene systems, that the tetR promoter is doubled (9). In pSC101 the putative second tetR promoter (from sequence analysis) has its -10 box located at the EcoRI site, it would therefore be inactivated in our 126 bp fragment.

2.2. RNA polymerase.

E. coli RNA polymerase holoenzyme was prepared according to (10). The preparation contains 80-90 % σ subunit minimum and is at least 30 % active according to the test described in (11). From titration experiments using the abortive initiation assay, we found that our enzyme preparation contains a minimum of 44 % active molecules (12). Similar results were found by nitrocellulose binding assays (unpublished results).

2.3. Protection and interference experiments.

Protection experiments. Protection against dimethyl sulfate (DMS) attack was performed according to (13). Labelled DNA is incubated with RNAP, in conditions where the DNA will be involved in a stable, heparin-resistant complex with the enzyme (stoichiometry and association rate constant for complex formation were determined previously under the experimental conditions employed).

Once complex formation is achieved and after heparin challenge is performed in order to eliminate non-specific interactions, DMS is added. The alkylation procedure is done in conditions where on the average one purine per strand is alkylated at most (DMS methylates guanines in the major groove at the N7 position and adenines in the minor groove at the N3 position). The reaction is quenched and the reaction mixture is filtered through

nitrocellulose filters in order to reduce background by removing any free-DNA. The filter-retained fraction is eluted from filter, alkylated purines are removed by incubation at 90° and the sugar-phosphate backbone is split at the apurinic sites by a piperidine treatment, as described just as in the chemical DNA sequencing method (14).

Purines in close contact with the enzyme will be protected against methylation; in autoradiograms from polyacrylamide gel electrophoresis, the corresponding bands will appear as less intense than their respective control bands, corresponding to DNA treated in the same way without RNAP.

Interference experiments. Labelled DNA is methylated in conditions where at most one purine per strand is modified. Methylated DNA is then incubated with RNAP; after a heparin challenge, the mixture is filtered on nitrocellulose filters. DNA is fractionated in filter-bound and filtrate fractions. The latter will be enriched in DNA fragments bearing methylated purines at positions interfering with complex formation. Interfering purines will correspond to bands appearing with higher intensities in the filtrate lanes with respect to control ones. Interference experiments reveal not only the bases in contact with RNAP in the final, stable, heparin-resistant complex, but also transient contacts during complex formation.

Notice that interfering methylated purines do not necessarily correspond to protected sites and vice-versa.

Experimental procedures. a) Protection against DMS attack was carried out with 0.1 pmol of DNA fragment (50,000 ³²P cpm minimum) and 4 pmol RNAP (total concentration). After 5 minutes incubation at 30°, in a 100 µl solution containing 50 mM sodium cacodylate pH 8, 10 mM MgCl₂, 0.1 mM EDTA, 1 mM DTT, 100 mM KCl and 100 µg/ml bovine serum albumine (buffer M), heparin (50µg/ml final) is added (Sigma, Missouri). After 20" of heparin challenge, the DNA/RNAP complex is methylated for 50 seconds with 1 µl of 10.7 M DMS (Aldrich, Steinheim) at 30°. The reaction is quenched by adding 1 ml of buffer B (10 mM Tris-HCl pH 7.9, 10 mM MgCl₂, 100 mM KCl, 1 mM DTT, 0.1 mM EDTA and 10 mM 2-mercaptoethanol). The solution is filtered on nitrocellulose filters (BA85, Schleicher and Schüll, Ø 0.45 µ); filters are rinsed with 0.5 ml buffer B without 2-mercaptoethanol, and the filter bound fraction is eluted by incubation with 0.5 ml buffer E (10 mM Tris-HCl pH 7.5, 10 mM MgCl₂, 0.1 mM EDTA, 0.5 M ammonium acetate, 1 % sodium dodecyl sulfate, 10 µg/ml tRNA), for 1 hour at 37°. After two ethanol precipitations, the pellet is resuspended in 90 µl 20 mM ammonium acetate, 5 mM EDTA and treated as in the G>A procedure in the chemical sequencing method (15). Products are analyzed by electrophoresis on 7 M urea polyacrylamide gels. Control lanes are DNA methylated in the absence of RNAP.

b) The interference procedure has been adopted from that described in (14): 0.1 pmol DNA (100,000 (³²P) cpm) in 100 µl buffer M are methylated with 1 µl 10.7 M DMS, during 1 minute at 25°. After two ethanol precipitations, the pellet is resuspended in 100 µl buffer B without 2-mercaptoethanol. 4 pmol RNAP are added and the complex is incubated for 10 minutes at 37°. A heparin challenge is then performed at 50 µg/ml (final concentration) during 20 seconds. The mixture is diluted with 0.4 ml buffer B and filtered on nitrocellulose filters. The filter-bound fraction is eluted as in the protection procedure and both fractions, filter-bound and filtrate are analyzed as in a).

c) Controls: The 1:1 stoichiometry for the complex was checked by titrations and electrophoresis on non-denaturing polyacrylamide gels and nitrocellulose binding assays (not shown). Association rate constants and their dependence on temperature and enzyme concentration were determined by the nitrocellulose binding assay, to optimize the conditions for protection and interference experiments (manuscript in preparation).

Experiments performed without heparin challenge exhibit similar contact

point pattern, but with stronger background noise. DNA fragments bearing tetR promoter were examined for contact points far from the promoter: no modification with respect to control lanes was detected within the limits of confidence of our experiments (see results section).

Finally, the tetR promoter from pSC101 overlaps partially with the tetA promoter; we have verified by the abortive initiation assay (data not shown) that EcoRI restriction inactivates tetA, and conclude that in our fragment only tetR is active. This is furthermore confirmed by results from chemical and enzymatic protection experiments presented in this paper.

All experiments were done at least three times, the results were quantified by scanning autoradiographies (see below) and average histograms were drawn.

2.4. DNAase I footprinting.

Experiments were performed as described in (16) with the following modifications: 0.1 pmol of the 126 bp fragment ($5 \cdot 10^4$ (32P cpm) are incubated with 4 pmol RNAP in 100 μ l buffer C (10 mM Tris-HCl pH 7.9, 10 mM MgCl₂, 0.1 mM EDTA, 100 mM KCl, 1 mM DTT, 10 mM CaCl₂, 100 μ g/ml bovine serum albumine). After a given incubation time (depending on the temperature of the assay, see results section), heparin is added at 50 μ g/ml. The heparin challenge is performed for 20 seconds and then DNAase I (Sigma, Missouri) is added at 0.2 μ g/ml final. The digestion is stopped after different times, (depending on the incubation temperature, see results) by adding 10 μ l of a solution containing 100 mM EDTA, 5% SDS, and 10 μ g/ml tRNA. After two ethanol precipitations, the pellet is resuspended in 80 % formamide V/V, 0.1 (W/V) xylene-cyanol blue, 1 mM EDTA, 10 mM NaOH, the solution is heated 2 minutes at 90°, rapidly cooled and then loaded onto a sequencing gel.

2.5. Identification of unpaired cytosines.

These experiments were carried out according to the method described in (17). The local melting of the promoter in the complex with RNAP around the transcription start site, can be revealed by alkylation with DMS. The N3 of cytosines, normally engaged in a H-bond pairing can be methylated only when C's are unpaired. Cytosines alkylated at N3 are labilized with respect to non-alkylated ones; they can be cleaved by a mild hydrazine treatment followed by an alkaline treatment at 90°. They appear in a sequencing gel as extra-bands in the G lane.

Experimental procedure. 0.1 pmol of the tetR promoter fragment is incubated with 4 pmol RNAP in 50 μ l buffer H (25 mM HEPES, pH 8, 100 mM KCl, 10 mM MgCl₂, 1 mM dithiothreitol, 100 μ g/ml bovine serum albumine) for 15 minutes at 30°. After a heparin challenge as described above, 1 μ l of 10.7 M DMS was added for 40 seconds at 30°. Methylation was stopped with 200 μ l of 3 M ammonium acetate, 1 M 2-mercaptoethanol, 20 mM EDTA, 10 μ g/ml tRNA. After ethanol precipitation, the pellet is resuspended in 20 μ l H₂O, followed by 20 μ l of hydrazine (Aldrich, Steinheim) and allowed to react for 7 minutes at 4°. The reaction is quenched with 200 μ l 0.6 M sodium acetate at 4° followed by two ethanol precipitations. The pellet is dissolved in 100 μ l 1 M piperidine and treated as in the chemical sequencing method (14).

In the present procedure, the filtration step is omitted due to relative instability of the methylated cytosines (this step is anyway unnecessary since single stranded cytosines appear as extra-bands). No differences in the reactivity of the cytosines were found, in experiments performed with or without a heparin challenge.

2.6. Quantitative evaluation of the reactivity of the different residues.

Autoradiograms corresponding to the different assays were scanned in a Shimadzu CS-930 densitometer; areas under the peaks were integrated by a on-line computer. Histograms were constructed, the variance of mean values being within the fork found for G>A control patterns (see results section).

To determine the extent of adenine methylation, overexposed autoradiograms were scanned when necessary.

When normalization was required in order to compare test and control experiments, it was performed taking into account the area associated with unperturbed regions of DNA, far away from the promoter region.

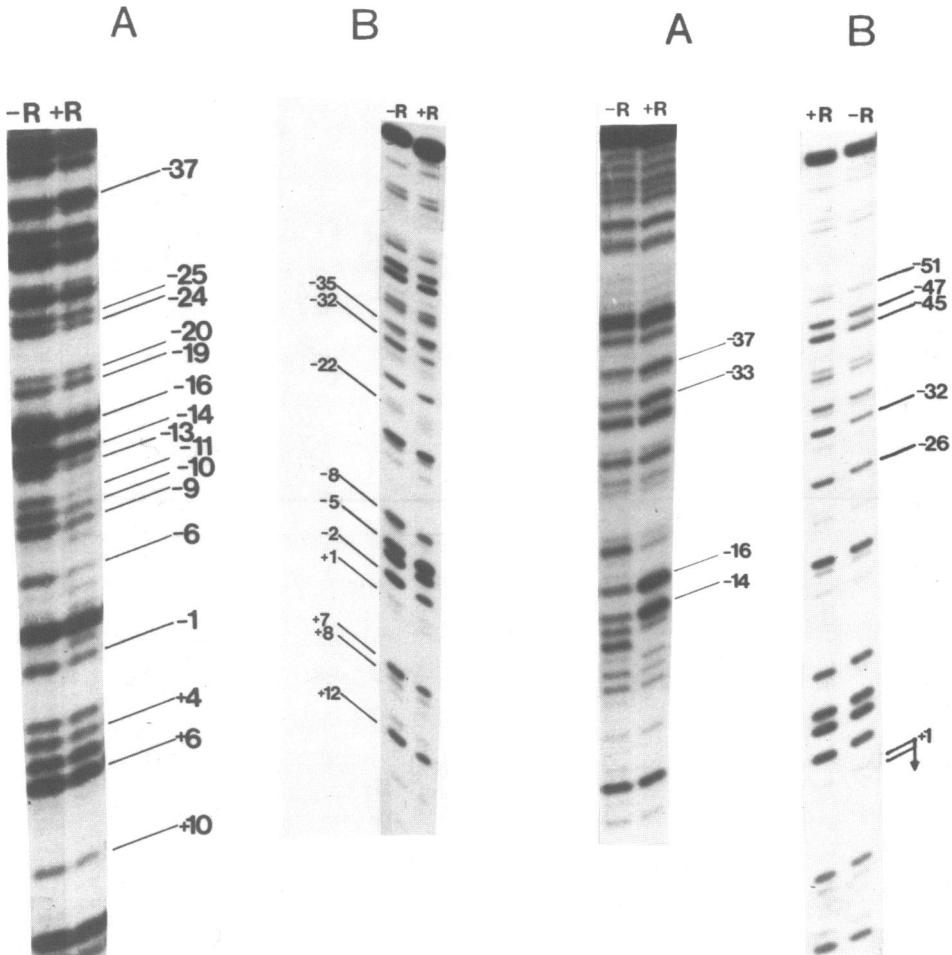
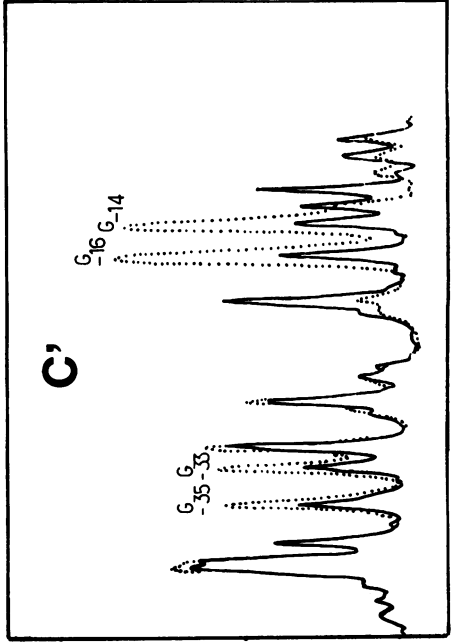
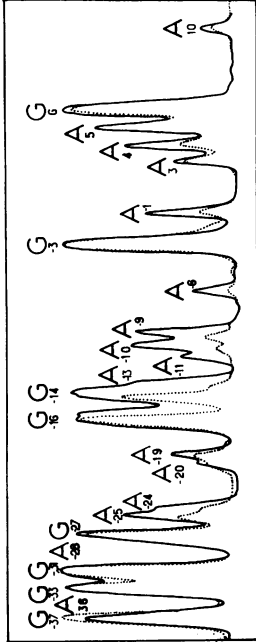
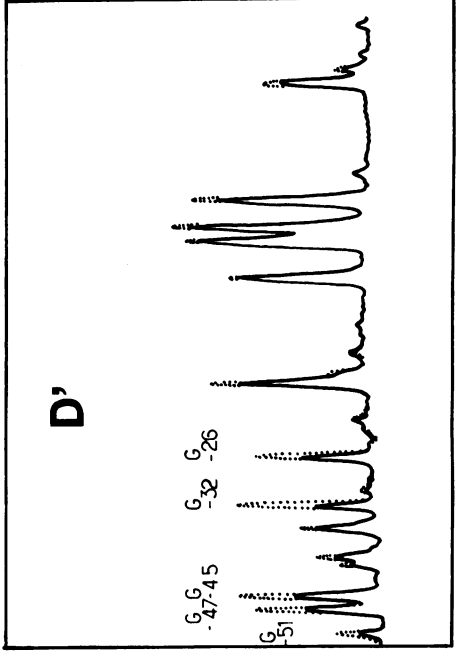
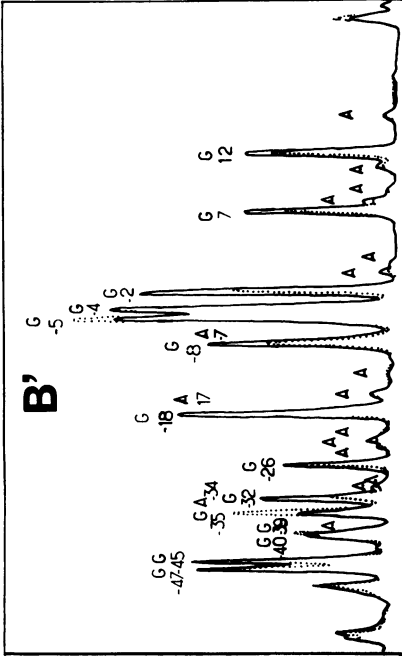


FIGURE 1. Autoradiograms from protection (left panel) and interference (right panel) experiments, showing purines of the *tetR* promoter involved in close contacts with RNAP. A: antisense strand; B: sense strand. For protection experiments: +R and -R correspond to samples methylated in the presence or and the absence of RNAP respectively. For interference experiments: +R and -R correspond to filtrate and control samples respectively. Protected, overmethylated and interfering purines in the complex are indicated. Nucleotides are numbered relative to transcription start (+1).



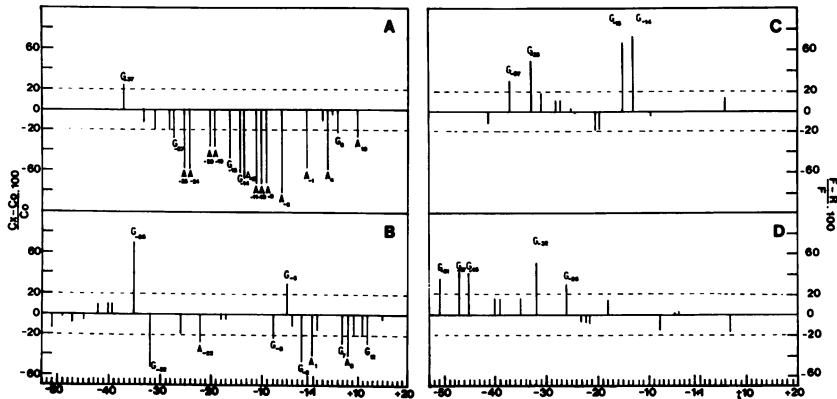


FIGURE 2. Quantitation of contacts. Panels on the left: superimposed densitometer tracings of autoradiograms corresponding to control (—) and protection (---) for the antisense (A') and the sense (B') strands; and control (—) and filtrate (----) DNA in interference experiments for antisense (C'), and sense strands (D'). The densitograms presented correspond to autoradiograms shown in figure 1.

Panels above: Histograms corresponding to an average of at least three different experiments. The variance doesn't exceed 10% of the mean values. A and B: protection experiments for the antisense (A) and the sense (B) strands. C and D: interference experiments, for the antisense (C) and the sense (D) strands. (C_x) and (C_o): intensity of bands in the presence (C_x) and in the absence (C_o) of RNAP in protection experiments. F: filtrate sample, and R: control sample, in interference experiments.

3. RESULTS

3.1. Close contact points of RNAP on the tetR promoter.

From densitograms, we have determined the variance of band intensities in G>A control experiments. It appears to be around 10 % of average values. From that, we will consider as a "contact" in what follows (in protection as well as in interference experiments) variations exceeding in 20 % the intensity of the corresponding control band. Nevertheless, it is conceivable that transient or weak contacts, could give rise to smooth modifications of the pattern, hardly distinguishable from the background using the present methodology.

Qualitative aspects. Figures 1 and 2 show results from protection and interference experiments. Purines protected by RNAP on tetR promoter are spread from -32 to +12 (transcription start = +1), whereas methylated purines interfering with complex formation are found between -51 and -14.

The general profile is similar to those already reported for lacUV5 and A3 from phage T7 promoters (2), i.e. a) most of the contacts are located on the antisense strand ; b) protections are centered around the Pribnow box and

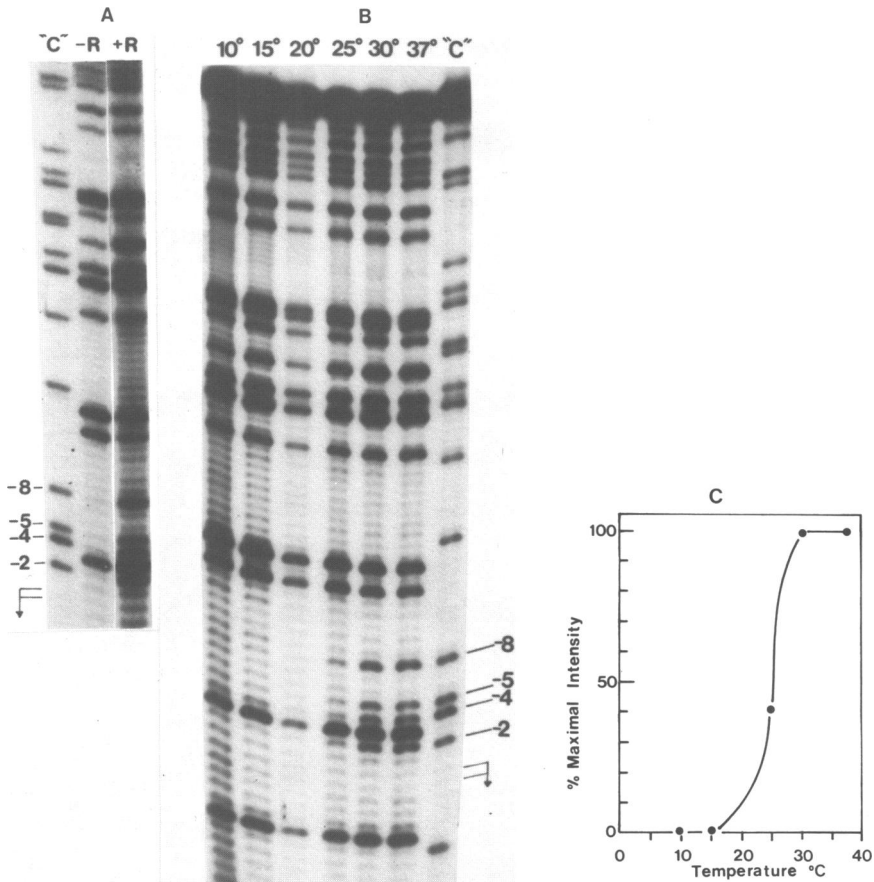


FIGURE 4. Single stranded cytosines in the *tetR* promoter (antisense strand) complexed with RNA polymerase. A) Autoradiograms showing the unpaired cytosines. +R lane: 126 bp fragment at $6.4 \cdot 10^{-9}M$, RNAP at $3.2 \cdot 10^{-7}M$; -R lane: control without RNAP. "C": cytosine sequence lane. Incubation was carried out at 30°C for 15 minutes, and methylation was performed during 40 seconds.

B) Autoradiograms showing the temperature dependence of local unwinding of the *tetR* promoter in the presence of RNAP. Concentrations of DNA fragment and enzyme are as in A); in all cases preincubation of the complex was carried out for 15 minutes; methylation times were: 2' at 10°C, 1.5' at 15°C, 1' at 20°C, 45'' at 25°C, 30'' at 30°C, and 20'' at 37°C.

C) Temperature dependence of cytosine methylation in the presence of RNAP, from quantitation of results shown in B), (see materials and methods section).

3.3 DNAase I footprinting.

DNAase I footprinting experiments show (see figure 5) that RNAP protects the 126 bp fragment bearing the *tetR* promoter, from positions -50 to +20 in both strands. Several sites highly sensitive to nuclease attack appear in the

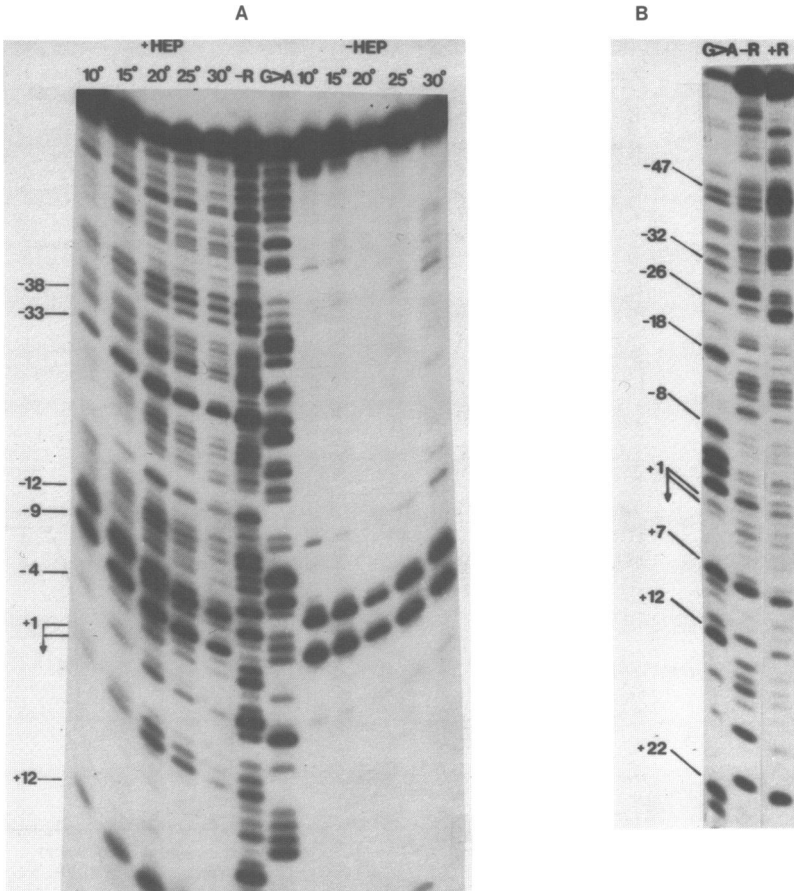


FIGURE 5. DNAase I footprinting. Autoradiograms showing footprintings of RNA polymerase on both strands of the 126 bp fragment. A: antisense and B: sense strands.

A) footprints performed at different temperatures, with and without an heparin challenge. -R: control lane (126 bp fragment digested by DNAase I in the absence of RNAP). A G>A sequence lane is also presented. The fragment was $8 \cdot 10^{-9}M$ and RNAP $4 \cdot 10^{-7}M$ in all experiments. Preincubation of the complex was carried out for 90' at 10°C, 75' at 15°C, 60' at 20°C, 45' at 25°C, and 30' at 30°C. Heparine challenge was performed at 50µg/ml (final concentration) for 20". DNAase I digestion was carried for 80" at 10°C, 60" at 15°C, 40" at 20°C, 30" at 25°C, and 20" at 30°C. The control experiment was performed at 30°C (no difference were found in control lanes within the temperature range explored, data not shown).

B) footprinting of RNAP on the 126 bp fragment, sense strand. R: 126 bp fragment $6.4 \cdot 10^{-9}M$, RNAP $3.2 \cdot 10^{-7}M$. After 30' of incubation at 30°C, a heparine challenge was performed as in A); DNAase I treatment was carried out for 20". -R: control experiment without RNAP. G>A: sequence lane.

complexed DNA. They are located at positions -20/-19, -13/-12, and -10/9, in the antisense strand and -60/-59, -36/-35, -35/-34, -34/-33, -27/-26, and -26/-25 in the sense strand.

Footprinting experiments performed at different temperatures, under conditions where roughly 100% of DNA molecules enter into heparine-resistant complexes as tested by nitrocellulose binding assays (control experiments not shown), reveal that the protection pattern remains unchanged between 10° and 30°. The intensities and the location of hypersensitive sites are also conserved (see figure 5).

Figure 5 also shows results from experiments performed with and without a heparin challenge. In the absence of heparin, at the RNAP concentration employed (see legend to figure 5), almost all the fragment is protected against nucleolytic attack; the presence of the same hypersensitive sites as in cases where DNAase I treatment is performed after the heparin challenge, reveals that the same specific complexes are present in both conditions and at all temperatures explored.

4. DISCUSSION

In order to draw out possible general features about E. Coli RNAP specific targets on DNA, we have compared the present results on the tetR promoter with close contact patterns already reported for six other E. coli promoters.

Comparison of close contact point maps of RNAP on promoters, raises a priori several questions. The very existence of a common "consensus" contact pattern of these promoters with RNAP is conjectural; it is conceivable that individual promoters adopt particular conformations when complexed to RNAP, or the latter could undergo promoter-specific transconformations, etc... Furthermore, the alignment of contact maps for comparison is increasingly ambiguous towards the "-35" regions, due to the fluctuating "-10" to "-35" spacer size (17±2 bp average) and to the frequently poor fit of the "-35" sequence with its consensus hexamer (TTGACA). In the case of tetR, the generally accepted "-35" sequence reads CTGACT, and is separated from the "-10" hexamer by 21 bp. Contacts were plotted on sequences aligned as if promoters conform to homogeneous B-helices; alternance of different helix conformations in promoters (or at least important stacking differences) cannot be excluded (see, for instance, (23) and (24).

Comparison is also made difficult because of the different, and sometimes unspecified, experimental procedures used in obtaining the various maps. Protected bases have their gel band intensities reduced to various extents,

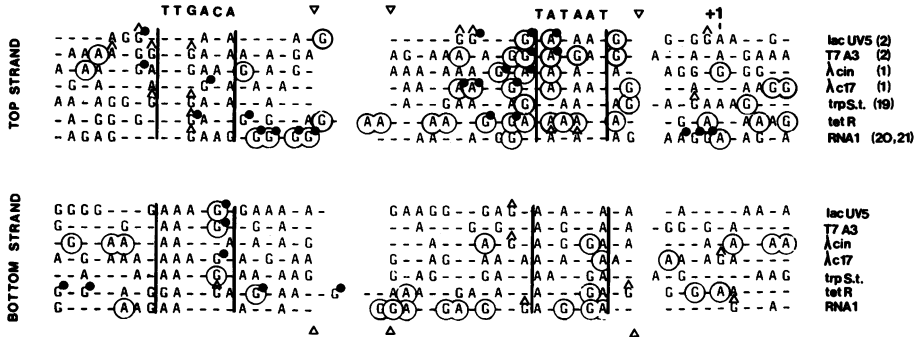


FIGURE 6. Compilation of dimethyl sulfate protection and interference results for different *E. coli* promoters, presented as in (22). Promoters are aligned as in (2) and (22), with respect to conserved hexamers and the transcription start site. (G) and (A) : protected purines; ● : methylated purines that interfere with binding of polymerase; ▲ : purines showing enhanced methylation in the complex. Data are taken from references given in the figure. We add to the original representation shown in (22), results from *tetR* and *RNA 1* promoters.

with respect to their control counterpart; their objective assignment needs quantitative evaluation, taking account of background noise, especially since at least the relative intensities of bands depend on temperature, RNAP concentration, time of preincubation, etc...

Figure 7a represents the superposition of the contact maps of seven different promoters, drawn to the best of our knowledge. Protected bases are mainly clustered within a 40 bp sequence (-32,+8) (corresponding to segment including bp-32 to +8, and the base positions are numbered as in figure 7); three promoters also contribute protections close to -40; for *tetR*, protections extend up to +12. Enhanced methylation of purines appears essentially in (-32,-38) and around -18 and +1. Most interferences accumulate in (-32,-38) and in (-10,-20). Interfering bases, or those showing enhanced methylation, are mainly located in the major groove, and are clustered in (-30, -40) and (-10,-20).

The main features between -32 and -40 are interferences and overmethylations; the former suggests that this region participates in early stages of complex formation, the latter that it is still affected (probably due to its conformation) in the final complex. According to (25), the β and β' subunits of RNAP are involved in close contacts with this region, for the *lacUV5* promoter.

The "-35" hexamer bears a single protection, G-32 on the sense strand

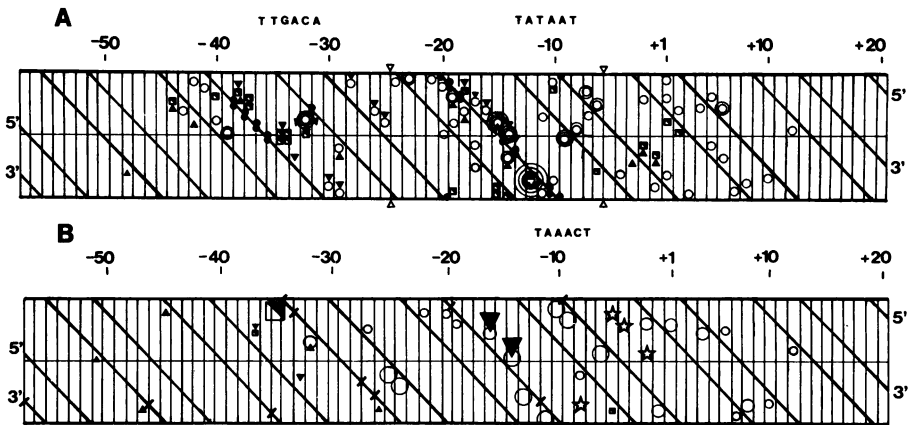


FIGURE 7.

A) Polymerase contacts on promoters showed in figure 6, drawn on a cylindrical projection of a B-DNA helix. \blacktriangle : interfering purines; \blacksquare : purines that polymerase enhances to DMS attack; \circ : purines that polymerase protects against DMS attack (concentrical circles correspond to the number of protections in the same position); \bullet : ethylated phosphates that interfere with complex formation (from (2)). Conserved sequences are indicated. Promoters were aligned as in figure 6; (Δ) indicated sites where sequences were squeezed in order to fit consensus elements of the different promoters. B) RNA polymerase contacts on the *tetR* promoter, depicted on a cylindrical projection of B-form DNA. Symbols are as in A). Other symbols: \star : cytosines methylated on N3 position; / : hypersensitive sites for DNAase I attack. Size of the symbols corresponds to the relative intensity of protections, overmethylation and interferences (from data presented in figure 2). Protections: \circ : 20-40%; \bigcirc : 40-60%; \bigcirc : >60%; enhancement of methylation: \square : 20-40%; \square : 40-60%; \blacksquare : >60%; interferences: \blacktriangle : 20-40%; \blacktriangle : 40-60%; \blacktriangle : >60% .

(three promoters); the same base appears overmethylated in one promoter and as an interfering site in four cases, an ethylation interference occurs on the neighbour phosphate -32/-31 in two promoters; G-34 (antisense strand) is overmethylated in four promoters, and is the target of one interference.

The (-21,-31) region has no overmethylated site ; no interference is seen in segment (-21,-24), which seems not at all, or poorly, affected by the enzyme in all steps of complex formation. For *tetR*, the major groove of (-17,-31) is almost completely unaffected.

The region (-8,-20) has many contacts with RNAP; for the *lacUV5* promoter, it was shown that β , β' and σ subunits are present at this place (25). We will analyse contacts in this domain into two arbitrary segments: (-14,-20), and (-8,-13), the latter containing the Pribnow box.

a) Region (-14,-20) presents an impressive concentration of interfering,

overmethylated and protected sites (for tetR, they are among the most intense, see figures 2 and 7b). Contacts are distributed along both grooves of the helix; many of them appear fairly "conserved", for instance on the antisense strand, guanines located at positions -14 and -15. Notice that several "up" and "down" mutations map in this region ((22) and references therein,(26),(27)), although no significant sequence conservation was detected.

b) The segment (-8,-13) (the Pribnow box), also harbours numerous contact points in both grooves, most of them being protected sites. In the case where quantitative information is available (A3T7, tetR), it appears that protections in this region are the strongest. A-12 in the antisense strand (conserved at 95%) is the most faithful contact in the Pribnow box (5 promoters). Interferences or overmethylations are rather uncommon, with the exception of A-12. It should be kept in mind that part or all of the Pribnow box, and the region downstream, to or beyond +1, are single stranded in the final complex, which could provide a rationale for the presence of interferences and overmethylations seen up and downstream at the edges of the unwound region.

As already mentioned, promoter alignments are ambiguous; it is possible, for instance, to take as alignment references protected and/or interfering guanine towards -32 in the sense strand and the first protected and interfering guanine just upstream the "-10" hexamer in the antisense strand. This alignment (not shown) reveals the major common contact points, but does not change the general picture seen in figure 7a.

Our results on the tetR promoter are in excellent agreement with the "average" close contact points picture (figure 7b); both the distribution of contacts and their nature appear to be rather similar, and the relative intensities of contacts on tetR correlate rather well with the statistical distribution in the integrated scheme: all seven promoters share approximately the same "hot spots", but local details are almost promoter specific. It should be remembered that the methods we used only point to protected, overmethylated or interfering purines; except for single stranded cytosines, no information on the fate of the pyrimidine half of the pairs is available.

DNAase I footprinting.

DNAase I footprinting experiments carried out at incubation temperatures from 10°C to 30°C, reveal two interesting features. First, at temperatures where no cytosine unpairing is detected, RNAP and the tetR promoter can form a stable closed complex, just as observed for the lac UV5 promoter (5). It would

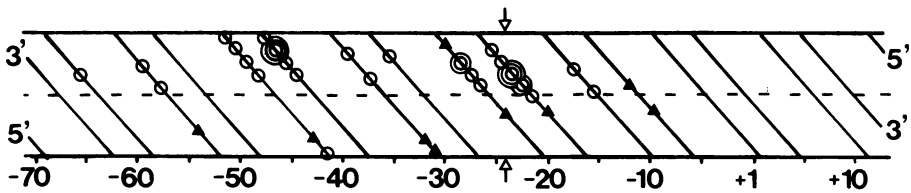
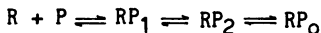


FIGURE 8. Comparison of hypersensitive sites to DNAase I attack described for nine different promoter-RNAP complexes with those found for the tetR system, depicted on a cylindrical projection of B-form DNA.

O : sites of enhanced cleavage described for nine different promoters (see (6) and reference therein). The number of concentric circles correspond to the number of hypersensitive sites described for a given position. ▲ : hypersensitive sites for the tetR promoter. The projection is rotated by 180° with respect to the projections shown in figure 7. Promoter alignment is as in figures 6 and 7. The arrow indicate the site where sequences were squeezed in order to fit the consensus elements of the different promoters. When the tetR promoter is aligned taking into account a 17 bp spacer only, the particular distribution of the sites of enhanced cleavage with respect to the other promoters is still present (not shown).

correspond to the intermediary step RP_2 postulated (28-30) between the unstable complex RP_1 and the open one, RP_0



where R stands for RNAP, and P for promoter. The $RP_1 \rightleftharpoons RP_2$ step is rate limiting for lacUV5 and λ_{PR} promoters (28,30). Secondly, the global persistence of promoter protection and hypersensitive sites, especially those observed downstream (-9/-10, -11/-12), over the 10°C to 30°C range, indicates that they remain unaffected upon promoter unwinding, inferring that conformation changes during the $RP_2 \rightleftharpoons RP_0$ step, if any, are not sensed by DNAase.

We also notice that in the tetR promoter region upstream of -25, which is almost unprotected by RNAP in the final complex, sites hypersensitive to DNAase I attack are distributed with a periodicity of about 10 bp; this is reminiscent of the DNAase I digestion pattern of DNA reclined unspecifically on some solid support, which could be in this case the rather bulky RNAP.

Comparing the DNAase I digestion map of the tetR promoter to those of several other E. coli promoters (see (6) and figures 5 and 8), it appears that, although the sizes of the areas protected by RNAP are comparable, the former is distinguishable by several criteria (figure 8). The total number of hypersensitive sites observed on the tetR promoter (nine) exceeds that of any

other promoter (≤ 6); also, the area over which the sites are found is largest in tetR (48 bp, compared to ≤ 37 bp), and this promoter bears two very strong hypersensitive sites which are the closest to +1 (-9/-10 and -11/-12, top strand). An interesting observation concerns the location of hypersensitive sites on the tetR promoter, which lie globally outside the helix area common to all other promoters (top half of the cylindrical projection, fig. 8). The mean axis of the hypersensitive sites carried by tetR is rotated 90° to 120° with respect to that of the other promoters. Since the tetR promoter conforms to the consensus contact pattern with RNAP, as seen by the chemical approach, we may assume that the position of RNAP along the double helix is fairly comparable for all promoters. Taken together with the fact that DNAase I is assumed to be a DNA conformation reporter (see (31,32) and (33) for a discussion), this would then infer that in the tetR promoter, DNA adopts a particular conformation which differs from that occurring in other promoters.

Notice also that in figure 8, the regions of DNAase I enhanced cleavage, mainly -20 to -30, and -40 to -50, coincide with promoter regions mostly devoid of contacts with RNAP (see fig. 7a).

Our results support the existence of a consensus contact pattern between RNAP and promoters in E. Coli; this suggests that the structure of the complexed enzyme is rather promoter independent. In contrast, the conformation of the complexed tetR promoter, as sensed by its hypersensitivity to DNAase I attack, is distinguishable from that of other promoters, as if the rather rigid RNAP could force promoters to adopt a fairly wide variety of conformations, depending on their sequences for instance. Ligand induced local distortions of DNA conformation have been observed (Eco R1 (34), repressors (35)); in the case of promoters, nature and map of the conformational changes possibly induced by RNAP remain however to be determined. We have mainly presented here a static study of the stable complex; a dynamic view of the various steps involved in complex formation (as obtained for lac UV5 and λ_{PR} (5,28-30)) and a better identification of the nature of the promoter signal are required in addition, to understand the molecular mechanism of transcription initiation. Work is in progress in our laboratory towards this goal.

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