Volume 6 Number 1 January 1979 Nucleic Acids Research

# The interaction of RNA polymerase and lac repressor with the lac control region

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Received 24 October 1978

ABSTRACT

We have examined the interactions of lac repressor and RNA polymerase with the DNA of the lac control region, using a method for direct visualization of the regions of DNA protected by proteins from DNAase attack. The repressor protects the operator essentially as reported by Gilbert and Maxam (1) with some small modifications. However, the evidence reported here concerning the binding of RNA polymerase to the DNA of the promoter mutant UV5 indicates that : 1) the RNA polymerase molecule binds asymmetrically to the promoter DNA, 2) RNA polymerase protects DNA sequences to within a few bases of the CAP binding site, suggesting direct interaction between polymerase and the CAP protein at this site, 3) RNA polymerase still binds to the promoter when repressor is bound to the operator, but fails to form the same extensive complex.

## INTRODUCTION

The molecular control of the expression of the lac operon is embodied in the interactions between the repressor protein and the operator, and the RNA polymerase and the promoter. In the absence of inducer the repressor binds to the operator and prevents the transcription of the three genes of the operon (2-4). Superimposed on this control is the modulating effect of the catabolite gene activating protein (CAP) which also binds to the lac control region (5, 6). There is substantial evidence that the binding of repressor to operator prevents the formation of an initiation complex by the RNA polymerase (7, 8), although it has not been determined whether the repressor prevents the binding of RNA polymerase to the promoter or in some way alters the character of the complex formed. The new evidence we present in

this report indicates that the RNA polymerase can still recognize the promoter when repressor is bound to operator, but fails to form the same extensive complex that occurs in the absence of repressor.

It is possible to obtain detailed information about the physical structure of protein-DNA complexes by examining the fragments produced by partial DNAase digestion of a complex in which the DNA is labelled on one end only (9). This method has been termed "footprinting". We have applied this technique to the lac system with the idea of directly visualizing the changes in the protection of the DNA caused by the interactions among the repressor, RNA polymerase, and the DNA of the lac operon control region. We have examined the patterns of protection from DNAase I on both strands using restriction fragments containing the DNA of the lac control region altered by the promoter mutation UV5 (a mutation that renders the promoter independent of CAP control and increases the binding strength of the promoter (10)). Fragments of DNA protected from extensive DNAase digestion have already been isolated and characterized in detail (1, 11). Therefore, it was possible to compare these data with the individual "footprints" observed for both the repressor and RNA polymerase, and also to see the shifts in each pattern caused by the interaction with the other protein.

## MATERIALS AND METHODS

Lactose repressor protein, from E. coli K 12 strains carrying the wild type I gene and the I12-X86 double mutation, respectively, was purified as described in reference 12. This mutant repressor has a highly increased affinity for both operator and non-operator DNA, as described previously (12).

Three preparations of RNA polymerase holoenzyme were used in these studies. Preparation A (kindly provided by B. Allet) was purified by the method of Burgess (13). Preparation B (kindly provided by H. Sommer) and C (New England Biolabs) were purified by the method of Burgess and Jendrisak (14). The RNA polymerase of all three preparations was judged to be at least

90% pure and at least 70% saturated with sigma protein by SDS-polyacrylamide gel electrophoresis (17). Preparations B and C give the same promoter protection patterns, which is slightly different from the results obtained with preparation A (see Results section).

The DNA of the lac control region was isolated from a plasmid provided by L. Johnsrud, as described in reference 12. A fragment of 285 base pairs was isolated after restriction enzyme fractionation of the pMB9-derived plasmid, pLJ3. The exact sequence of this fragment has been reported (15). The construction of the plasmid is described in reference 16. After labelling the 5' ends of this double-stranded fragment by the method described in reference 18, two segments of DNA containing lac control regions in the same orientation were separated by cleaving the 285 base pair fragment with the restriction enzyme Hae III (New England Biolabs). The resulting fragments of 117 and 168 base pairs were then isolated by polyacrylamide gel electrophoresis. Each of these pieces then contained the identical promoter-operator region, but were labelled on opposite strands.

Protection of Operator by Repressor : About 1 µg of a labelled DNA fragment in 100 µl of buffer A (10 mM Tris-HCl, pH7.9 , 125 mM KCl, 10 mM $MgCl_2$, 10 mM $CaCl_2$, 0.1 mM dithiothreitol, 25% glycerol*) was incubated for 15 min. at 38°C. Then 1 µl of purified I12-X86 repressor was added to give a final concentration of 50 µg/ml. Wild type repressor was used at a concentration of 80 µg/ml. After further incubation at 38°C for 10 min., 4 µl of DNAase I (Worthington, bovine pancreatic) in buffer A was added to give a final concentration of 0.13 µg/ml. The DNAase digestion was stopped after 30 seconds by adding 25 µl of a 3 M ammonium acetate, 0.25 M EDTA solution containing 0.15 mg/ml sonicated calf thymus DNA (Serva). The DNA in this

* Since glycerol has been shown to increase the affinity of repressor for operator (33), the experiments were also done in buffer A without glycerol, at both 125 mM and 10 mM KCl (data not shown). No difference was seen in the protection.

sample was then concentrated and desalted by ethanol precipitation and prepared for electrophoresis by resuspension in 50 mM NaOH, 0.5 mM EDTA and 5 M urea as described in reference 18. Electrophoresis was on a 20% polyacrylamide gel, as described by Maxam and Gilbert (18), except that the thickness of the gel was reduced to 0.35 mm.

Protection of Promoter by RNA polymerase : This experiment was carried out as described above for the repressor protection experiment, with the substitution of 7 μg (9 units) of purified RNA polymerase holoenzyme for repressor in the reaction mixture.

Competition Experiments : The control reactions containing only repressor or RNA polymerase and DNA were carried out as described above. In the competition reactions repressor was incubated with the DNA, as above, then the RNA polymerase was added and incubation continued for another 10 min. before the DNAase was added (or the proteins were added in reverse order).

Time course experiments carried out with RNA polymerase (in buffer A) showed that the protection pattern is identical after 5, 15 and 30 minutes incubation with polymerase. Experiments in which the polymerase was incubated with the DNA at 38°C in buffer A without glycerol showed that the pattern was unchanged. The same result was also obtained at 20°C with 25% glycerol (buffer A).

The possibility of artifacts caused by pre-nicking of the restriction fragment was ruled out by running a DNA sample from each preparation by itself, alongside the protection reaction sample. No hidden nicks or contaminating fragments were in evidence. The possibility that the repressor or RNA polymerase preparations contained any kind of nuclease activity was checked in a similar fashion on both strands with negative results.

## RESULTS

### The Protection Pattern of Lac Repressor on Lac Operator DNA

The isolation of a well-defined fragment of the operator that is protected by repressor from the action of DNAase would appear to define the binding site completely. However, if there

were flanking segments which were protected less completely, or if there were an interrupted protection pattern, this method would define only the largest section of the repressor binding site. Thus with the availability of a technique which avoids these limitations, it was possible to examine the pattern for repressor on both strands of the operator region. The double-stranded restriction fragments (117 and 168 base pairs) which contain the lac control region from the CAP-independent mutant UV5 (10) were labelled with $^{32}P$ at opposite 5' ends with respect to the operator orientation. Purified lac repressor was added to the reaction mixture containing one of the labelled DNA fragments, then the DNA was partially degraded with a small amount of DNAase I so that most of the DNA remained unbroken. The resulting set of end-labelled fragments, when run on a denaturing polyacrylamide gel and autoradiographed, should map out the region(s) protected by the repressor. By comparison with a reaction carried out in the absence of repressor, the missing bands will indicate the sites where specific blockage of the DNAase cleavage reaction is affected by repressor binding. This method has been discussed previously (9).

In figure 1 we show the autoradiographs for such a set of reactions for each DNA strand, side by side with base-specific cleavage reactions (18) to permit identification of the DNAase-produced fragments. Keep in mind that the fragments produced by chemical cleavage are terminated at the 3'end with a phosphate group, while the DNAase-produced fragments are terminated with a 3'hydroxyl group. The electrophoretic mobility is therefore slightly greater for the DNAase-produced fragments. It is immediately clear that the protected region is well-defined on both strands, and that cleavage by DNAase just at the right end of the protected region (upper strand) is enhanced by the presence of the repressor. The pattern is diagrammed in figure

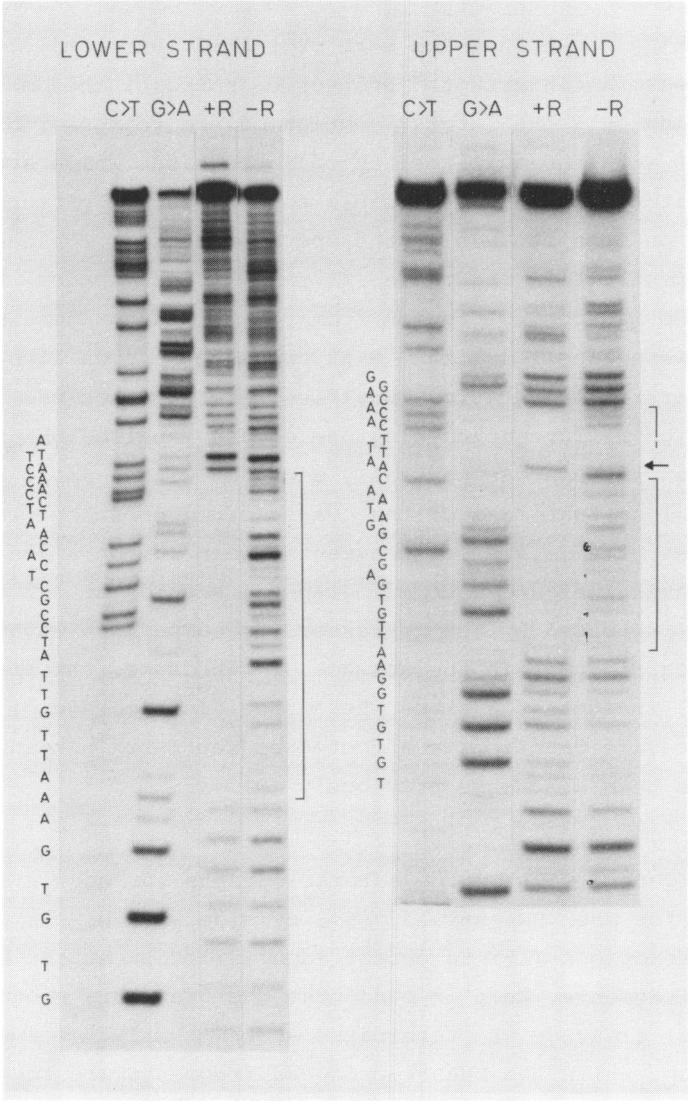



Figure 1. The footprint of repressor on both strands of operator DNA. Partial DNAase digestions of 5' end-labelled, operator-containing DNA fragments bound to I12-X86 lac repressor were run on a 20% denaturing polyacrylamide gel next to the indicated sequencing reactions (18), and autoradiographed. Reaction conditions are described in Materials and Methods. The lower strand footprint (left) was obtained using the 168 base pair fragment, and the upper strand footprint (right) was obtained using the 117 base pair fragment. The brackets indicate the protected regions and the arrow enhanced DNAase cleavage.

6a.* The only unexpected features here are the existence of a few protected bases† on the upper strand just beyond the main protected region on the 3' side, and the enhancement of cutting in the upper strand. The experiments shown in figure 1 were done with the I12-X86 repressor, which binds to the lac operator about $10^4$ times stronger than wild type repressor, while the affinity for the inducer isopropyl-B-D-thiogalactoside (IPTG) is unaffected (6). Identical results, however, were obtained using wild type repressor. IPTG (1mM) abolished all of the protective pattern on the DNA by wild type repressor, but had no influence on the I12-X86 repressor protection (data not shown). This agrees with the observation that strains carrying this mutation are not induced for B-galactosidase even at high IPTG concentrations (12).

The Protective Pattern of RNA Polymerase on Lac Promoter DNA

Under the same conditions in effect for the experiments of figure 1 we preincubated each of the promoter-containing DNA fragments with RNA polymerase holoenzyme, then partially degraded the DNA. The pattern of missing bands in the resulting autoradiograph (lane 2 in figure 2a and b) show several segments of protection on both strands and a striking asymmetry between the strands. The protection pattern using RNA polymerase preparation A is diagrammed in figure 6b showing both those segments protected and those sites where enhancement of the DNAase cutting occurs.

As is the case for all the results reported here, the protection patterns were established and confirmed by at least three separate protection experiments. The reaction products were routinely run on gels for different times to resolve different fragment sizes, and two autoradiograms with different exposures were made from each gel to help bring the different band intensities within an optimal range. An example of a high reso-

* The protection patterns for all the experiments of this paper are summarized in the diagrams collected in figure 6.

† Because the three T's contiguous to these bases are not well-cleaved by DNAase I it is not possible to define the 5' end of this segment exactly.

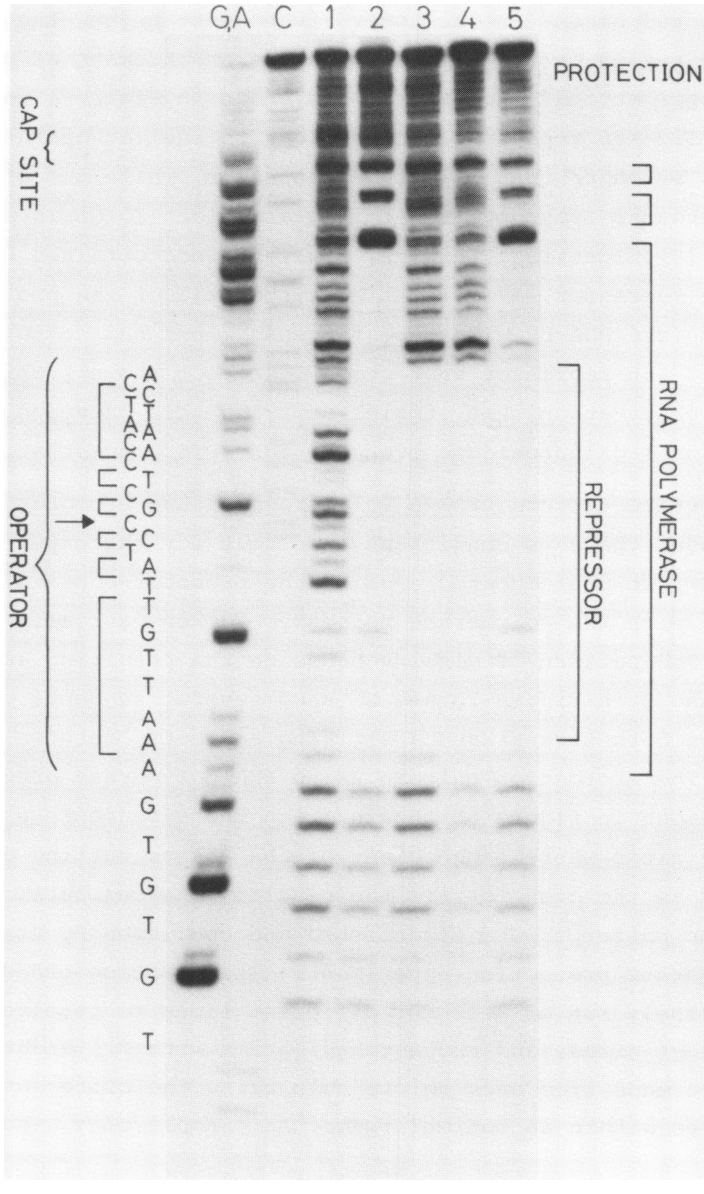



Figure 2a. Footprints of RNA polymerase and repressor, individually and in competition, on the lac control region DNA. The protection reactions were carried out as described in the text. a) (above) lower strand, using the 168 base pair fragments.

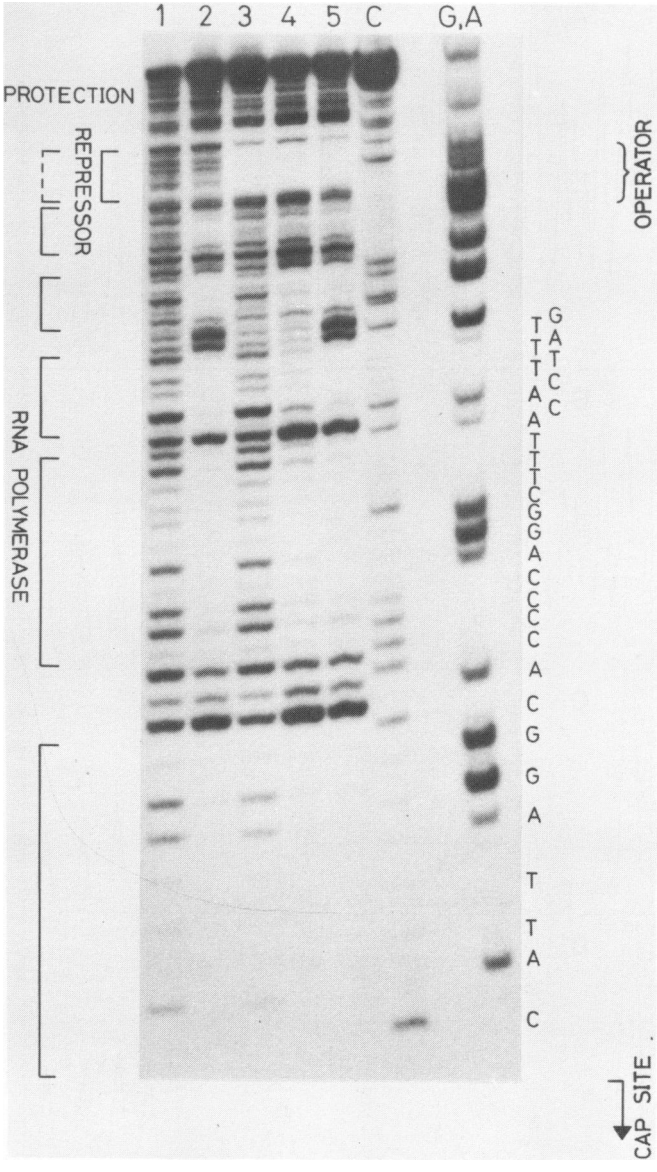



Figure 2b. The upper strand, using the 117 base pair fragment. The lanes in both autoradiographs are : 1, no added proteins; 2, RNA polymerase only; 3, repressor only (112-X86); 4, repressor added before polymerase; 5, polymerase added before repressor. The regions protected are indicated by the brackets on the right.

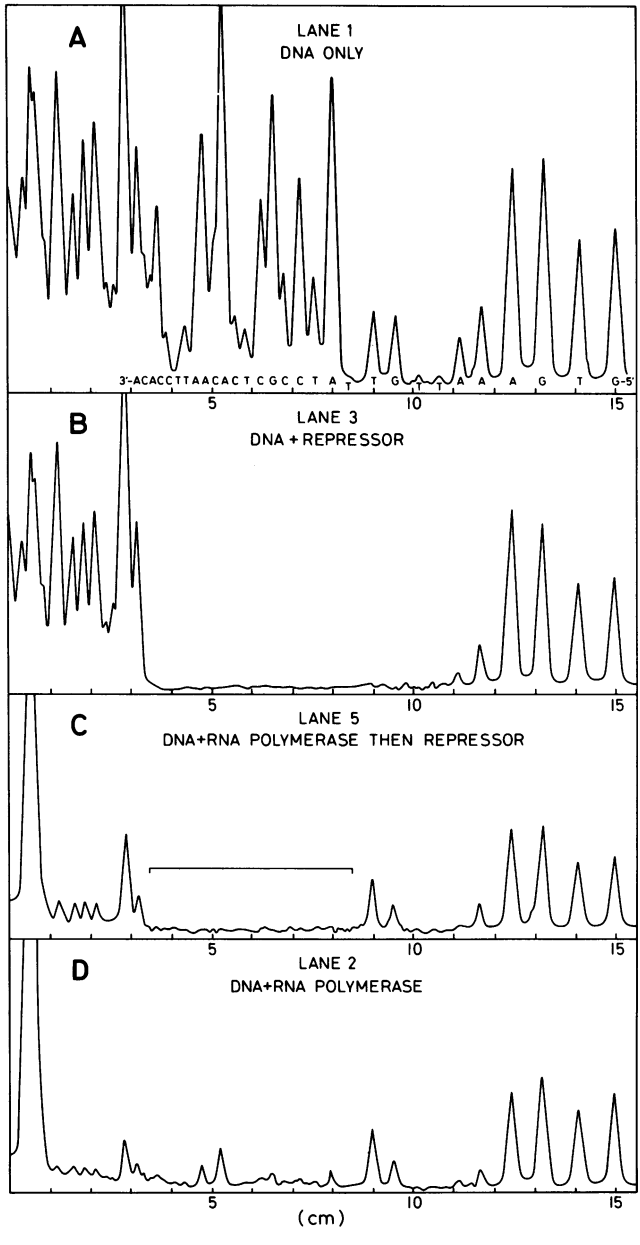



Figure 3. Densitometric scans of the autoradiographs shown in figure 2a, for the operator region. a) lane 1, no added proteins; b) lane 3, repressor only; c) lane 5, polymerase then repressor; d) lane 2, polymerase only. The bracket in (c) showed the region where repressor, added after polymerase, increases the protection.

lution gel for the region around base -15 on the upper strand is shown in figure 4. Detailed assignments of the protected bases were made from several gels.

The protected segment in the lower strand from base -24 to base +21 is the piece one would expect to isolate from an extensive DNAase I reaction, and this is essentially the segment isolated after RNA polymerase protection in such a reaction (11). This region has been picture as the RNA polymerase binding site for the initiation complex (11, 19, 20). It is similar in many respects to analogously isolated segments of other promoters (reviewed in references 11 and 19). The pattern shown in figure 6b (and in 6c, discussed below) however, extends much farther in one direction. The full protective envelope covers about 74 base pairs : from the CAP binding site (as defined by genetic and methylation modification experiments (6, 11, 30, 33)) to the far end of the operator (the repressor-protected fragment*). Recently methylation modification experiments have shown that RNA polymerase can affect bases as near the CAP site as base -37 (16).

It is not immediately evident from figure 3 that operator region of the upper strand is protected at all by RNA polymerase. Further experiments have shown, however, that the slight protection seen here is a real and repeatable effect. This was confirmed by repeating these experiments with another preparation of RNA polymerase (preparation B, see Materials and Methods) as shown in figure 5a. Here the protection of the upper DNA strand in the operator region is evident. This pattern, diagrammed in figure 6c, shows other minor differences from the protection by preparation A of RNA polymerase (figure 6b). A pattern identical to 6c was also found using preparation C (data not shown).

It is important to note that protection of a base by a protein may be manifested in changes in intensity of the band as well as in its complete elimination. In a protection pattern like that shown in figure 5a the levels of differential protec-

* The 5' end of the protection on the upper strand cannot be precisely defined. The diagram in figure 6b shows the limit we observe.

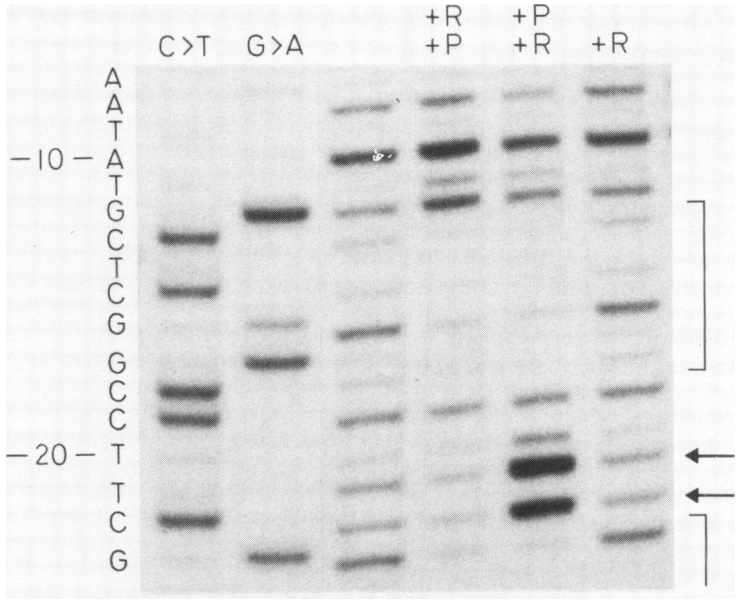



Figure 4. Autoradiograph of gel showing detail of protection for repressor-RNA polymerase competition. The reactions were the same as those in figure 2b. The sequencing reactions (18) are as indicated. The next lane (unlabelled) is for no added proteins. The next three lanes are for reactions with the proteins added as indicated (P for polymerase, R for repressor). The order of addition is indicated by the order of the letters. The base numbers are as in figure 6.

tion cannot be completely represented in diagrams like those in figure 6. These diagrams are schematic summaries of the data. In figures 5b and c densitometric scans of the autoradiographs of figure 5a (upper strand) are displayed. A comparison of the unprotected pattern (5b) with the protected pattern (5c) clearly shows the differential nature of this protection. The most striking thing about this pattern, however, like that of figures 2 and 3, is that it extends to the CAP site.

## The Competition Between Repressor and RNA Polymerase

The details of the protection patterns of repressor and RNA polymerase having been elucidated, it is possible to study

the effect of one protein on the other through their ability to alter these patterns. It is clear at the outset that some change is expected because these molecules overlap in the regions they protect. By simple order-of-addition experiments we studied the effect of repressor on the formation of the RNA polymerase-promoter complex, and on a pre-formed complex; and the effect of RNA polymerase on the binding of repressor to operator. In figures 2 a and b we show the results of these experiments for both DNA strands. In lanes 1, 2 and 3 (in both figures) are shown the array of fragments for 1) no additions, 2) polymerase alone added, and 3) repressor alone added. In lanes 4 and 5 (both figures) both repressor and polymerase were present. In lane 4 we see the result of adding repressor first, then polymerase, and then performing the partial DNAase reaction. The reverse order is shown in lane 5 : the polymerase complex was allowed to form before the addition of repressor.

The comparison between lane 3 (repressor only) and lane 4 (repressor then polymerase) for both strands can, in principle, answer the question of whether repressor prevents polymerase binding. Figure 2 (lower strand) seems to imply that it does : the pattern is the same as for repressor alone. Figure 3 (upper strand) implies that it does not prevent binding but alters it in a minor way. The effect of repressor on the formation of the polymerase complex thus appears to be highly strand-specific.

The inverse question; that is, does polymerase prevent the binding of repressor to operator, can be addressed by a comparison of lane 2 (polymerase only) with lane 5 ( repressor added after polymerase). Again, figure 2a seems to imply it does prevent binding and figure 2b implies it doesn't. This apparently indecisive result actually may provide an important clue to the nature of polymerase binding. On examining figure 2a carefully one can see that the operator region of lanes 2 and 5 is not protected in precisely the same way, thus there is some effect of the repressor on both strands. This is clearly shown by the densitometric scans of the autoradiograph in the operator region (figure 3). Note that a segment of the bands sup-

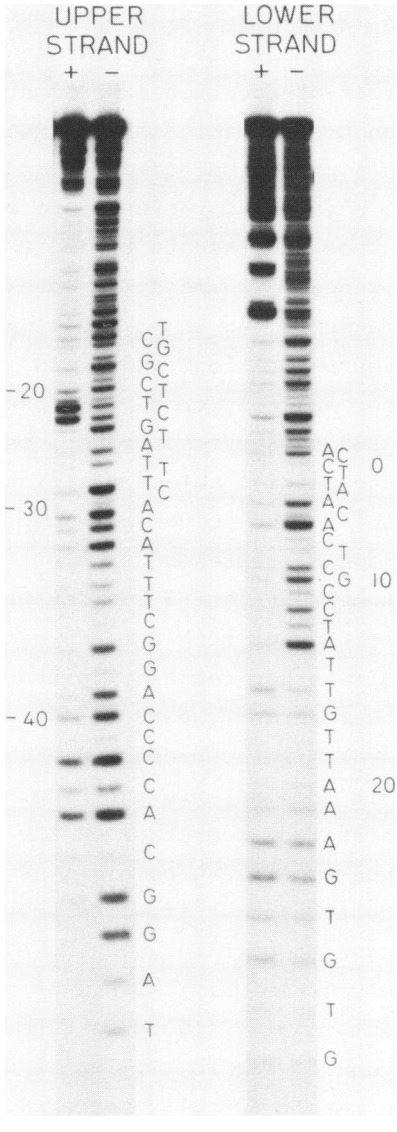
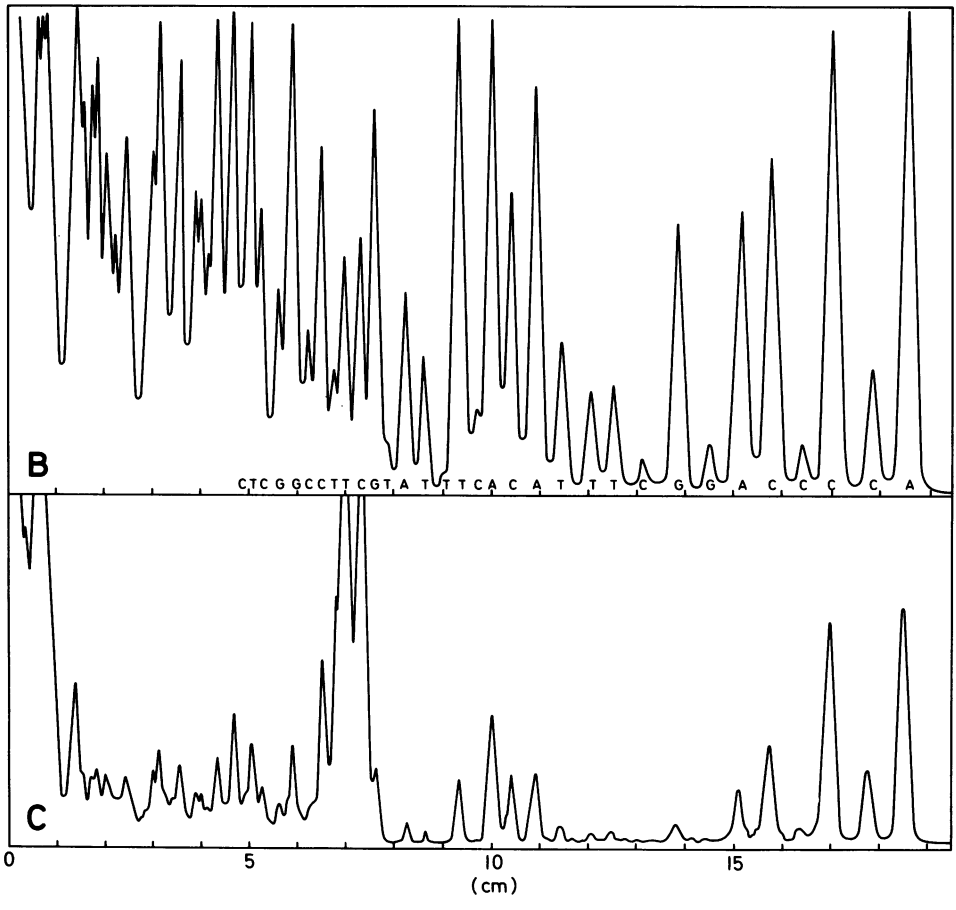
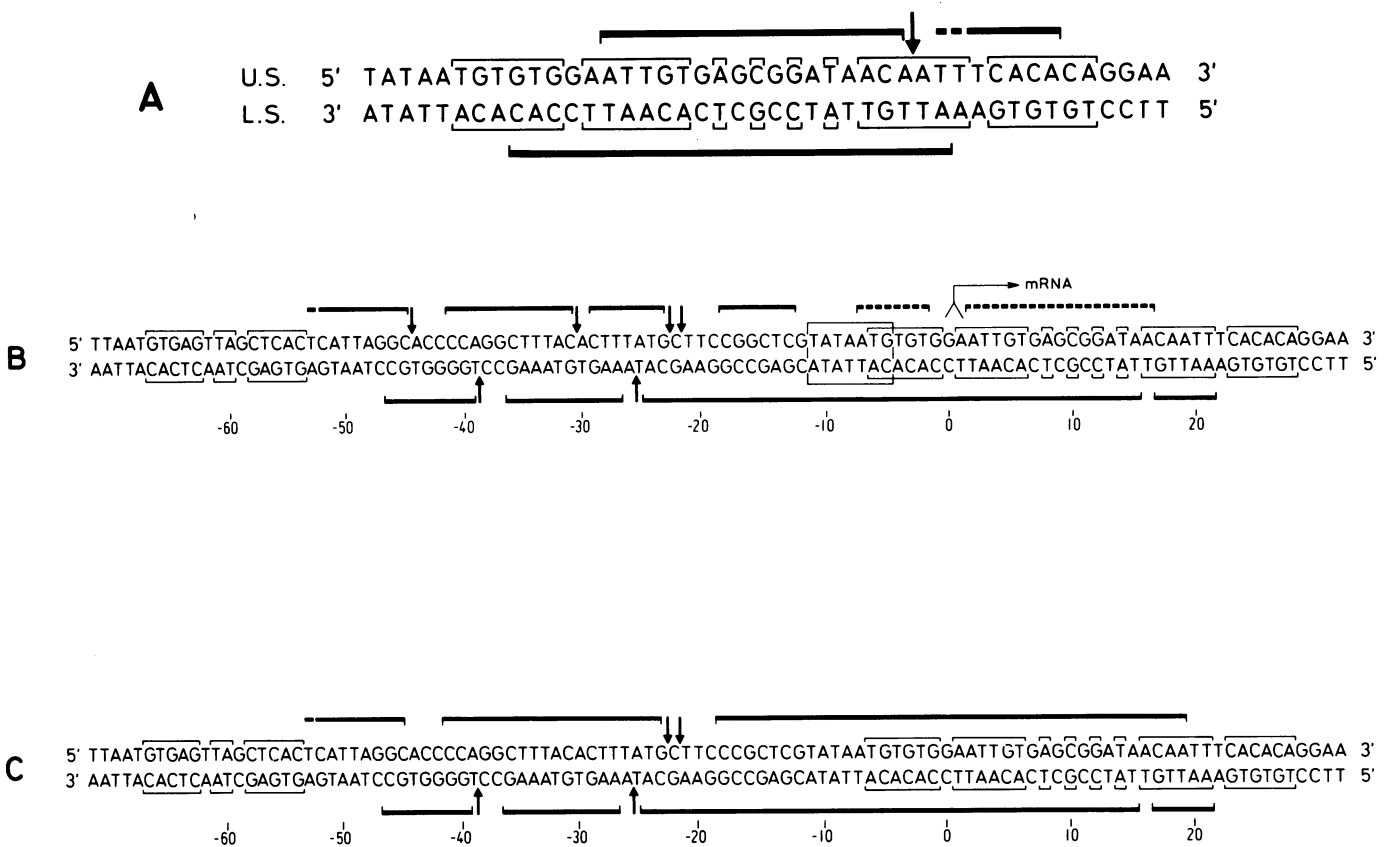



Figure 5. Footprint of RNA polymerase (preparation B, see Materials and Methods) on the promoter. a) autoradiographs of gels for both strands. + indicates RNA polymerase is present, - indicates it is not. Reactions were the same as for figure 2. b) densitometric scan of the (-) lane for the upper strand: c) scan of the (+) lane for the upper strand. The sequence is labelled according to the same convention used in figure 2.




pressed by RNA polymerase binding (3d) are further suppressed by the addition of repressor (3c). This additional protection, indicated by the bracket in 3c, is not the same as the protection of repressor by itself, and indicates that the repressor can interact with the DNA of the operator region in the presence of polymerase, but only in an altered manner.

To obtain a complete and detailed picture of the protection pattern it was necessary to combine the information from several gels in which the upper bands were resolved better than in figure 2. An example of such a gel is shown in figure 4 where each band in the region from -24 to -9 on the upper strand is


A
U.S. 5' TATAATGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAA 3'
L.S. 3' ATATTACACACCTTAACACTCGCCTATTGTTAAAGTGTGTCCTT 5'
B
mRNA
5' TTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAA 3'
3' AATTACACTCAATCGAGTGAGTAATCCGTGGGGTCCGAAATGTGAAATACGAAGGCCGAGCATATTACACACCTTAACACTCGCCTATTGTTAAAGTGTGTCCTT 5'
-60 -50 -40 -30 -20 -10 0 10 20
C
5' TTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCCGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAA 3'
3' AATTACACTCAATCGAGTGAGTAATCCGTGGGGTCCGAAATGTGAAATACGAAGGCCGAGCATATTACACACCTTAACACTCGCCTATTGTTAAAGTGTGTCCTT 5'
-60 -50 -40 -30 -20 -10 0 10 20

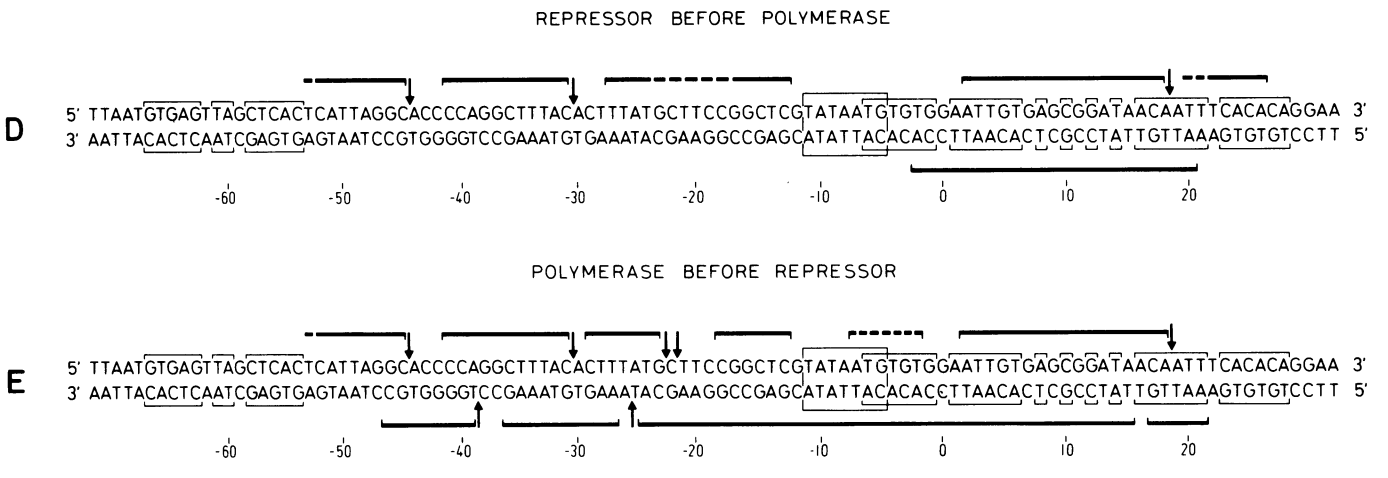



Figure 6. Diagrams of protected sequences in the lac control region. These diagrams are each summaries of the protection data obtained in several experiments. The solid brackets represent protected regions, the broken line brackets represent less well-protected regions, and the arrows represent sites of enhanced cleavage by DNAase I. The fine-line brackets around base +10 indicate the symmetry of the operator, and those around base -60 indicate the symmetry of the CAP site. a) Protection pattern of repressor; b) protection pattern of RNA polymerase, preparation A; c) protection pattern of RNA polymerase, preparations B and C; d) protection pattern of repressor followed by polymerase; e) protection pattern of RNA polymerase followed by repressor. The convention adopted here (and throughout the paper) is that a base is protected if the phosphodiester bond to its 5' side is protected.

well-resolved. All the data is combined and diagrammed in figures 6 d and e.

It is notable that the pattern of figure 6d, where the repressor was bound before polymerase was added, contains within it precisely the same pattern seen when only repressor was added. Likewise, the pattern in 6e, where polymerase was bound first, contains the complete pattern of polymerase-only protection.

## DISCUSSION

We have analyzed the patterns of protection of the lac control region from DNAase attack by lac repressor alone, RNA polymerase alone and both together in sequential binding experiments. The method used enabled us to monitor the intensity of DNAase cutting at almost every base in the region of interest. It was therefore possible to observe in detail aspects of the interactions not previously seen.

### a) Methodology

It is useful to view the footprinting technique as a method for sampling the vulnerability to DNAase attack of each phosphodiester linkage of the DNA, and therefore for sampling the protective effect of a DNA-bound protein. In these experiments the preincubation time of the DNA with the protein was long enough to allow equilibrium to be established. The extent of the DNAase reaction was such that most DNA fragments were uncut. The equilibrium would therefore remain undisturbed. To interpret the results it is necessary to consider the possible mechanisms by which DNA can be protected from attack by DNAase. The most direct sort of protection, and by far the simplest to interpret, is by direct physical shielding of the DNA from contact with the active site of DNAase. The DNAase I molecule is roughly 1/5 the size of the repressor tetramer and 1/14 the size of the RNA polymerase holoenzyme, but blockage of the active site need not even require a "standoff distance" comparable to the radius of the molecule. The geometry of the DNAase molecule and of the protein-DNA complexes considered here are completely unknown. In a favorable geometry, access to the DNA could possibly

be afforded by a very small exposed sequence. Detailed interpretations of the molecular configurations that are implied by our results will have to await physical studies of these molecules. A second possible mechanism for depression of DNAase activity at specific sites on the DNA is by the influence of some conformation change of the DNA induced by the presence of a protein covering a nearby site.

There is some sequence specificity of DNAase I (21) as can be seen by a glance at the variations in intensity in figures 1, 2, 3 and 4 and there is no way of strictly ruling out similar effects acting at long-range, which are induced by protein binding. However, we do not favor the idea that this mechanism is an important contributor to the patterns we observe. It is important to note that the variations in cutting due to sequence specificity are characteristically smaller than the changes due to protein protection. It seems more reasonable to interpret the protection patterns as reflecting the physical presence of the proteins in the protected region. With this assumption it is possible to construct a self-consistent picture from our results.

b) The Protection of Lac Operator by Lac Repressor

The footprint of the lac repressor on the operator can be seen at high resolution in the autoradiographs of figure 1. It is identical for the double mutant I12-X86 (12) and the wild type protein. Two features of this pattern carry information not obtainable by protected-fragment-isolation methods : the enhancement of DNAase I cutting at the place indicated by the arrow in figures 1 and 6a, and a few extra protected bases on the upper strand separated from the main segment (see figure 6a). At first glance at the figure (6a) it may be difficult to understand how these extra bases can be protected by the repressor since they are separated from the main segment by a few bases which can be cut by DNAase. This difficulty is easily resolved, however, by examining the position of the segment on the three-dimensional double helix (in the B-form). It is located on the same side of the helix and near to a protected sequence, GTTA, in the lower

strand. The repressor could easily block the access of DNAase to these bases, while permitting access to the region about half a turn away on the other side. In figure 7, the data is presented so that the three-dimensional structure of the protected regions can be appreciated. The B-form for the DNA is assumed here. The two views, from opposite sides of the helix, show a distinct asymmetry not obvious from the linear display.

This is in general agreement with the results of phosphate alkylation experiments (W. Gilbert, personal communication) and the recent results of Goeddel et al. (22) that imply that the specific binding of the repressor favors one side of the helix. The limited unwinding of the operator implied by the work of Wang et al. (23) should not substantially alter this picture.

The overall protection is more complete in the upper half of the operator (distal to the z gene). This agrees with the conclusion from various data (summarized in reference 22) that this half of the operator binds more strongly to repressor.

c) The Extent of RNA Polymerase Protection

The CAP protein, which binds to the lac control region at a specific site (when complexed with cAMP), has the effect of enhancing the ability of the RNA polymerase to initiate transcription (5, 6). It has been suggested that the CAP protein may act by altering the DNA in some way : by promoting melting or some unspecified structural change of the DNA at some distance (perhaps 30 to 50 base pairs) from its binding site (20, 30) Another scheme (11) has the CAP protein interacting directly with the polymerase on the DNA, and holding it in position thereby enhancing the formation of the "initiation complex"*. Our results strongly support such a model since it seems that the effective size of the RNA polymerase on the DNA is much greater

*We have adopted the term "initiation complex" to describe the complex we see in these experiments. There is a variety of terms, based on operational criteria, used to describe RNA polymerase-DNA complexes (e.g. see 20, 29, 32).

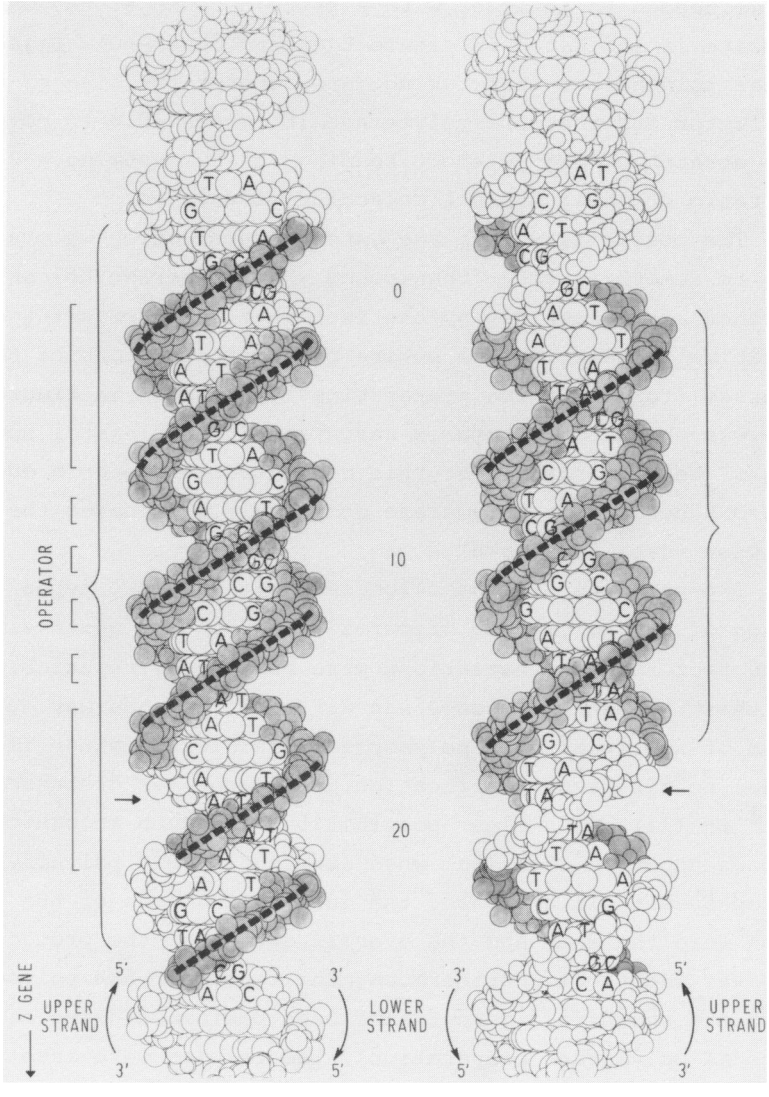



Figure 7. Drawing of the protection pattern of repressor (both wild type and I12-X86) on operator DNA in the B-form. The two views are front and back (180° rotation) of the same DNA segment. The darkened backbone represents the regions protected from DNAase attack. The square brackets indicate the operator symmetry, and the curved brackets the protected region on the facing side of the DNA as seen in each view. The arrow indicates the site of enhanced cleavage.

that the isolated fragment indicates, and is, in fact, precisely the size needed to interact with a protein bound at the CAP site. (This site is indicated in figure 6 around base -60). Gilbert (11) has pointed out that taking into account the size of the sigma factor and the core polymerase it is possible to cover such a stretch of DNA (without bending it) by assuming a 2:1 axial ratio of their overall molecular dimensions.

The possibility that the extended protection we observe is due to something other than bound RNA polymerase holoenzyme is further argued against by the fact that the "footprint" is insensitive to heparin. The entire protection pattern of RNA polymerase (for all three preparations), as shown in figure 6b and c, was shown to be heparin resistant for at least 1 hour (20 $\mu$g/ml heparin, 38$^{o}$C). At this concentration heparin added to the DNA before RNA polymerase completely eliminates the protection (data not shown).

The patterns of protection seen with the two types of preparation of RNA polymerase (figures 6b and c) are quite similar, but the fact that the variations were completely repeatable implies that the RNA polymerase was different in some way. The problem of subtly altered polymerase molecules or minor impurities due to different purification procedures, as discussed by Chamberlin (31), is a clear possibility here even though the preparations appeared identical when examined by SDS polyacrylamide electrophoresis. In any case, the general features of the protection were the same and the overall extent of the protected region was identical. Furthermore, the repressor-RNA polymerase experiments shown in figure 2 have been performed using all three of the RNA polymerase preparations. The results were identical with the exception of the minor differences in the polymerase protection pattern already noted.

d) Recognition and Binding to the Promoter

The existence of a recognition sequence for the RNA polymerase somewhere in the neighborhood of base -35 is strongly implicated (11, 24, 25,26). This region is now well within the

protective envelope of the RNA polymerase complex, indicating that this sequence may be bound by the polymerase in the initiation complex and not just when it first recognizes the promoter. This is in accord with the methylation-modification results of Johnsrud (16) on the same promoter, who found an effect of polymerase binding at base -37.

Evidence that the polymerase may bind to the "recognition" region before the formation of the initiation complex comes from the experiment in which the formation of the initiation complex is blocked by the repressor. The pattern obtained in this experiment, in which polymerase is added after repressor, is shown in figure 6d. When considered together with the polymerase-only pattern (figure 6b) this result suggests to us the following picture of the binding of RNA polymerase. When the DNA is held tightly by the repressor and the polymerase is thereby held physically away from the DNA in the operator region, part of the RNA polymerase multimer can still bind to the recognition sequence. It does not form an initiation complex because of the repressor, but the binding to the recognition region is still sufficiently strong to show up as a protection pattern (bases -54 to -31). The fact that this pattern is part of the pattern of the initiation complex indicates that in forming this complex the polymerase does not have to move at all.*

This picture then represents a significant modification of the view that the repressor excludes polymerase binding (7, 11). It certainly excludes full binding, in what we are calling the initiation complex, but still permits a binding of the recognition region which keeps the polymerase in position, ready to initiate when the repressor comes off the operator.

e) The Structure of the RNA Polymerase-Promoter Complex

The initiation complex of RNA polymerase with the promoter, figures 6b and c, has been shown by these experiments to have

* We cannot rule out completely the possibility that there are two polymerase molecules bound to the promoter to produce the protection we observe, but we consider it unlikely.

some unexpected features. The extensive region of protection and the interrupted nature of the pattern were unobservable by previous techniques. In viewing this intermittent protection pattern it is useful to consider the 3-dimensional configuration of the promoter DNA. Although the configuration of the DNA in the initiation complex is largely unknown, if we assume for the moment a B-form structure, the unprotected segments form a consistent pattern. Consider the segments around bases -43, -30 and -21, on the upper strand, and bases -38 and -25 on the lower strand. The segments at -43 and -38 are close together on the same side of the helix and form an intrusion into the protected region on this side. The segments at -30, -25 and -21 form another contiguous region roughly on the opposite side of the helix.

Much indirect evidence has accumulated that there is some local opening of the DNA strands by the polymerase (28, 29). Recently, more direct evidence of this has been obtained (31), and estimates range from 5 to 15 base pairs opened. The results presented here, while completely consistent with such a structure, provide no further evidence of which base pairs are involved. Further experiments using a single-strand-specific DNAase may resolve this issue.

As described in the results section, we have evidence that the repressor can bind to the operator, but in a different way, when the RNA polymerase is already bound to the promoter. That the results shown in figure 2 (lane 5) are not due to the repressor simply binding all the DNA fragments not already bound by the polymerase, is argued by the following facts :
1) The protection pattern shows some bands in the region protected only by polymerase which are almost completely suppressed, thus most of the fragments of DNA must be bound by polymerase.
2) The pattern of repressor protection inferred from the densitometer scans of figure 2b, c and d and the autoradiographs of figure 3 (plus repeat experiments, not shown) is not the same as for repressor alone. We conclude therefore that the repressor is binding to the operator even in the presence of polymerase, but in a different fashion. One possible explanation is that the

polymerase binds weakly to the operator region in this complex and thermal motion of the molecules permits the repressor to gain a tenuous foothold on the operator, thus excluding DNAase even more completely. Clearly more evidence is required to clarify the nature of this effect.

The results diagrammed in figure 6d, showing that RNA polymerase can protect the DNA in the "recognition" region when repressor is bound to operator seems to indicate some flexibility in the binding properties of RNA polymerase to promoter DNA. The physical structure of these complexes remains unknown, however.

Much detailed information about this model system for DNA-protein interaction can be obtained by the methods used in this work. To summarize the principal results : The RNA polymerase complex has been shown to be longer (as measured along the promoter DNA) than previously thought. The strand asymmetry in the protection patterns for both the repressor and the polymerase can partly be attributed to the 3-dimensional structure of the DNA. It appears that the polymerase binds to the promoter even in the presence of the repressor-operator complex. More information will be required to describe with confidence the detailed molecular events of initiation complex formation and the competition with repressor. Studies in progress will include the effect of the CAP protein on the protection patterns, and the effect of using DNA from the wild type promoter. Studies of the DNAase footprint patterns of RNA polymerase in other promoters can reasonably be expected to reveal larger regions of protection, and should shed further light on the mechanisms of polymerase-promoter interaction and transcription control.

ACKNOWLEDGEMENTS

We are grateful to Dr. Jeffrey Miller for support and encouragement and for useful discussions throughout the course of this work. We also thank Drs. Miller, Bernard Allet, Walter Gilbert, Lorraine Johnsrud and U. Siebenlist for their comments on the manuscript. The expert rendering of the figures by Mr. O. Jenni is greatly appreciated. This work was supported by a grant

from the Swiss National Science Fund (F.N. 3.179.77).

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