
Protein-DNA cross-linking at the *lac* promoter

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ABSTRACT

We report the results of photo-cross-linking of RNA polymerase and the cyclic AMP receptor protein (CRP) to the *lac* UV5 promoter region carried on either a linear fragment or a supercoiled plasmid. We have devised a protocol that allows the localisation of bases in contact with the protein. RNA polymerase makes contacts within the -10 and -35 regions of the promoter, essentially on the non-template strand. The CRP contact points found in a binary complex are affected by the formation of the ternary complex containing RNA polymerase. Supercoiling has no effect on the position of contacts in any of the complexes. These conclusions were derived from experiments performed using a generally applicable, non-interfering technique that reveals *direct* contacts between proteins and nucleic acids in nucleoprotein complexes.

INTRODUCTION

The initiation of transcription in *E. coli* requires the recognition of a promoter by the RNA polymerase holoenzyme. This process involves the formation of several intermediates leading to an open complex in which the DNA contains a locally melted region around the site of initiation of transcription [1]. Once in an open complex RNA polymerase is poised to enter into a series of elongation complexes and commence transcription (for a review see [2]). Information about these nucleoprotein complexes can be obtained from structural and kinetic studies. It is essential that the probes employed act rapidly and do not disturb the complex that they are designed to examine. Classical methods use chemical reagents or enzymes that modify or cut the nucleic acid component of the complexes. Unfortunately, this generally perturbs the nucleoprotein complex and is often relatively slow. Although footprinting experiments allow the localization of the protein on the nucleic acid, they yield little information about direct contacts between the protein and the nucleic acid.

Proteins can form covalent zero length cross-links to DNA following short, powerful impulses of laser UV radiation [3, 4, 5]. The two main advantages of this technique are that the modification process is rapid (irradiation times of 5 nsec, and subsequent photochemical reaction faster than 1 μ sec) and only reports interactions between residues which are in direct, intimate contact. UV irradiation, however causes substantial damage to

both the nucleic acid and, to a lesser extent, the protein [5, 6, 7]. Furthermore, laser UV cross-linking has not, to date, furnished information concerning which residues are in contact in the nucleoprotein complexes. We present here an approach that allows the determination of specific nucleic acids that are cross-linked by UV radiation under conditions that do not perturb the nucleoprotein complex prior to cross-linking. This approach also takes advantage of the UV induced modification of the DNA to deduce information about the nature of the protein-nucleic acid interaction (cf. [8]).

The nucleoprotein complex that we have investigated is the open complex formed between RNA polymerase and the *lac* UV5 promoter. This strong promoter allows efficient transcription in the presence or absence of the complex between cyclic AMP and its receptor protein (CRP) [9]. Footprinting techniques using nucleases such as DNase I have shown that RNA polymerase occupies a region of around 60 base pairs on *lac* UV5 [10, 11]. On another level, the phosphate 'contacts' between the DNA backbone and RNA polymerase were determined by methylation and ethylation interference studies [1, 12].

The locally melted region of the open complex with *lac* UV5 has been studied in several promoters using a range of chemical and enzymatic reagents. This open complex contains a region of 13 base pairs that are locally melted [1, 13, 14, 15]. The two DNA strands in the open region are not equally reactive to the majority of reagents used. Furthermore the extent of reactivity of a given base is a function of its position within the locally separated region, bases at the centre being more reactive than at the extremes [15]. These footprinting and interference experiments do not, however, allow unambiguous assignment of direct contacts between RNA polymerase and the DNA in open complexes.

In an attempt to provide a more detailed picture, chemically induced cross-linking of RNA polymerase to depurinated *lac* UV5 has been carried out [16]. This technique revealed many contact points between bases and the RNA polymerase subunits on both strands, principally in the -35 and -10 regions of the promoter. However the approach was intrinsically slow and extremely perturbing to the nucleoprotein complex.

The substitution of thymines by bromouracil allowed photo-induced cross-linking of these residues notably to the σ and β subunits of RNA polymerase in open complexes with the *lac*UV5 promoter [17]. Because the formation of such cross-links rendered

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the DNA chemically labile, Simpson was able to demonstrate that bases at positions -3 and $+3$ (relative to the start site of transcription) on the non-template strand were in intimate contact with the σ and β subunits respectively. Direct UV irradiation of open complexes of the *lac* UV5 or *deo* P1 promoter showed cross-linking of position T+1 on the template strand, presumably with a lysine residue of the β subunit [18]. A major drawback to these latter two approaches is the distortion induced in the DNA either by the substitution of thymines by bromouracil or to the long exposure times to levels of UV radiation that invariably lead to thymine dimer formation, modification and nicking reactions. All of these have potential effects upon the conformational integrity of the nucleoprotein complexes before covalent cross links are formed.

In this study we have examined the reactivity of the *lac* UV5 promoter alone and in complexes with RNA polymerase and CRP to laser induced UV radiation. We present a methodology that probes the nature of the protein-nucleic acid complexes in a rapid, non perturbing fashion.

MATERIALS AND METHODS

DNA. fragments of DNA were isolated from plasmids as described in [19]. 17 mer oligonucleotide primers were synthesised on a solid phase synthesizer (Pharmacia) on a 0.2 μ M scale. Fragments and oligonucleotides were 5' labelled with γ - 32 P dATP using T4 polynucleotide kinase. Fragments of *lac* UV5 were also 3' labelled using the Klenow fragment (DNA polymerase I large fragment) [20].

Protein. RNA polymerase was isolated by the method of [21] modified as described in [22]. The cyclic AMP receptor protein (CRP) was prepared as described in [23].

Nucleoprotein complexes. Open complexes were formed between RNA polymerase (20 nM) and DNA (4 nM) by incubating both at 37°C for 30 minutes in a buffer containing 100 mM KCl, 10 mM MgCl₂, 20mM HEPES (pH 8.4), 10 mM dithiothreitol, 3% glycerol and 100 μ g/ml BSA. When present CRP was added to a concentration of 50 nM in the presence of 50 μ M cAMP.

Laser irradiation. Samples (10 μ l) containing material to be irradiated were placed vertically in a thermostatically controlled Eppendorf tube. High intensity laser light was generated by a NdYAG laser producing light with a wavelength of 1064 nm [5]. An optical system consisting of two frequency doubling crystals and a series of dichroic mirrors allowed an incident beam of 266 nm to be directed onto the sample. Under optimal operating conditions a single pulse of radiation at an intensity of approximately 0.5×10^{11} W/m² of 5ns duration was used. The estimated energy dose per experiment was of the order of 100 J/m².

SDS PAGE of cross-linked complexes. Irradiated samples containing end labelled radioactive DNA were suspended directly in loading buffer (final concentration = 3% SDS, 5% glycerol, 31 mM Tris HCl pH 6.8) heated at 90°C for 3 minutes and applied to 6% acrylamide gels run at 200V. Gels were dried and autoradiographed.

Determination of protein contact points on the DNA. The technique employed consisted of heat denaturing the nucleoprotein complex, annealing with a primer that was complementary to

one of the two DNA strands, and extending this primer using the DNA polymerase I large fragment. Irradiated samples (10 μ l) containing non-radioactively labelled DNA fragments (4–5nM) were denatured by heating at 95°C for 15 minutes and chilled on ice for 2 minutes. To each sample was added 2.5 μ l of 10 \times buffer (500 mM Hepes, pH 8.0, 100 mM MgCl₂, 10 mM DTT) and an aqueous solution of a 5' end labelled 17 mer primer to a final volume of 25 μ l. An aliquot of 2.5 μ l of a stock solution of 10 mM deoxyribonucleotide triphosphates was added to each tube to give a final concentration of 500 μ M. The tubes were incubated at 48°C for 5 minutes and 2 units of Klenow enzyme were added to each. The reaction was terminated after 10 minutes either by precipitation in ethanol or by the addition of formamide containing 10 mM EDTA. Samples were heated and applied to 8% sequencing gels then dried and autoradiographed. For a quantitative analysis of the results, the autoradiograms were scanned using a Perkin-Elmer scanning densitometer equipped with a Shimadzu integrator.

RESULTS

UV induced cross-linking

End labelled DNA fragments of 203 base pairs containing the *lac* promoter were irradiated with a high energy laser pulse at 266 nm in the presence or absence of RNA polymerase. When these samples were subjected to electrophoresis on non denaturing gels, their migration was identical to that observed for non irradiated complexes. The irradiated nucleoprotein complexes exhibited the classical retardation of migration typical of open complexes between RNA polymerase and a 203 bp DNA fragment. However if open complexes between RNA polymerase and *lac* UV5 203 bp fragments both irradiated and non-irradiated were incubated on ice, frozen and stored at -20°C overnight

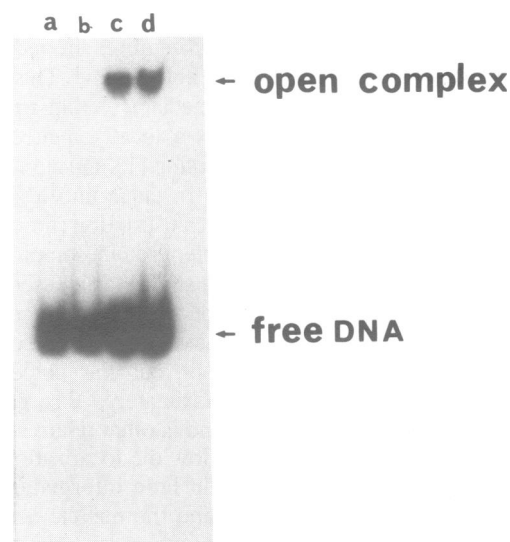


Figure 1: Band shift assay for the formation of cross-links between RNA polymerase and *lac* promoter fragments. Open complexes between RNA polymerase and a 203 bp fragment end labelled with 32 P were formed and irradiation using a NdYAG UV laser at 266 nm was carried out as described in Materials and Methods. Samples were frozen and thawed 3 times then stored at 4°C overnight prior to application to native 4% acrylamide gels. Gels were dried and autoradiographed. **a:** free DNA. **b:** non-irradiated open complex subsequent to freeze/thaw. **c:** open complex irradiated for 5 ns prior to freeze/thaw. **d:** open complex irradiated for 10 ns prior to freeze/thaw.

prior to separation on native gels the typical retardation of the DNA produced by RNA polymerase was observed *only* in the irradiated sample (Figure 1). Thus irradiation has covalently cross-linked RNA polymerase to the DNA fragment.

RNA polymerase was cross-linked to DNA in which the upper non template strand was 3' end labelled with α^{32} PdATP. Applying samples (heated at 90°C for 3 minutes) to a denaturing 6% acrylamide SDS gel and subsequent autoradiography gave the result shown in figure 2. The DNA was melted during the initial preheating step and subunits of RNA polymerase that were covalently cross-linked to the single strands of DNA migrated slower than free subunits and were labelled by the associated DNA (figure 2b). Western blots were carried out by electrotransfer to nitrocellulose and subsequent immunodecoration with specific polyclonal antibodies and 125 I labelled protein A. With antibodies directed against the σ subunit a similar pattern of retarded bands as that seen in Figure 2b was obtained. There is a heterogeneity of cross-linked σ subunits. This is probably due to cross-links formed between different sites on the primary sequences of both the protein and the DNA.

The use of antibodies against other subunits of RNA polymerase did not give detectable signals following Western blot analysis. Either these other subunits do not cross-link or the amount of protein in each band is below the detection threshold of the Western blot analysis. Under our conditions, approximately 15 to 20% of open complexes formed between RNA polymerase and DNA were cross-linked as judged from gels of the type shown in Figures 1 and 2. This corresponds to approximately 4 ng of RNA polymerase per gel lane which is at the limit of detection by our Western blot analysis. Consequently, only the major protein present in the cross-linked complexes will be detected by our assay. Thus, we conclude that the predominant cross-linked protein is the sigma subunit although we can not exclude that other subunits are also cross-linked to the DNA.



Figure 2: SDS PAGE of complexes between RNA polymerase and *lac* promoter fragment. Non irradiated and irradiated samples prepared as described in Materials and Methods were taken up in sample buffer containing 3% SDS, heated for 3 minutes at 90°C, and applied to discontinuous buffer 6% polyacrylamide gels in SDS and run at 200V at room temperature. Gels were dried and autoradiographed. a: non-irradiated open complex. b: open complex irradiated for 5 ns.

A native gel analysis on CRP-DNA cross-linked fragments subsequent to the addition of heparin (100 μ g/ml) in order to dissociate non-covalently linked complexes showed that around 1% of the complexes were cross-linked.

Location of radiation induced modification sites on the DNA

In order to determine the sites of cross-linking of proteins on the DNA we used the primer extension technique on the assumption that modified bases (including bases covalently attached to a polypeptide) should interfere with the polymerisation reaction of the Klenow fragment. Thus we carried out primer extension using DNA polymerase I large fragment along the denatured DNA strands of the promoter sequences [14, 24]. Subsequent to irradiation, heat denatured samples were hybridised with 32 P 5' end labelled 17 nucleotide oligomers complementary to the 3' extremity of each strand of the *lac* UV5 promoter fragment and were primer extended using DNA polymerase I large fragment as described in Materials and Methods. The products were heated in formamide and separated on an 8% polyacrylamide sequencing gel (Figure 3).

DNA alone

In the absence of UV irradiation the DNA polymerase I large fragment successfully completed polymerisation along the 203 bp fragment to form the fully extended product with no detectable pause or stop sites on either of the two strands. When the DNA fragment alone was irradiated, a pattern of terminations was observed on both strands (Figure 3 lanes 1, 3 and 5). The sites of termination were determined by comparison with a dideoxy sequencing reaction carried out using the same primer fragments. Primer extension ceased after incorporation of the relevant dNTP opposite numerous positions on both strands. The major termination sites in DNA alone corresponded to positions immediately preceding two or more consecutive thymine residues.

Table 1. Termination frequency of primer elongation on the upper, non-template strand of *lac* UV5 after irradiation.

Base position	Sequences (5' XXX3') acting as terminators	DNA alone % total	RP ₀ % total
-130	TTA	1.54	1.40
-107	TTC	4.55	4.32
-71	TTA	1.15	1.31
-56	TCA	2.73	2.08
-52	TCA	2.73	2.30
-49	TTA	2.64	2.10
-41	CCA	4.65	4.49
-34	TTT	3.77	0.50
-33	TTA	4.44	9.89
-27	TTT	4.47	6.49
-26	TTA	5.46	7.94
-20	TTT	4.31	5.53
-19	TCC	3.68	5.60
* -16	GGC	3.30	1.32
-13	TCG	2.12	2.35
* -7	AAAT	0.25	1.87
* -5	TGT	1.84	0.70
* -4	GTG	0.63	1.26
* -3	TGT	1.20	0.94
* -2	GTG	0.66	1.49
* -1	TGG	0.70	1.43
* +1	GGA	5.03	1.80

* Terminations not associated with pyrimidine dimer formation

Values were calculated by scanning gels of the type shown in Figures 3 and 4 using a Perkin Elmer densitometer and Shimadzu densitometer as outlined in Materials and Methods.

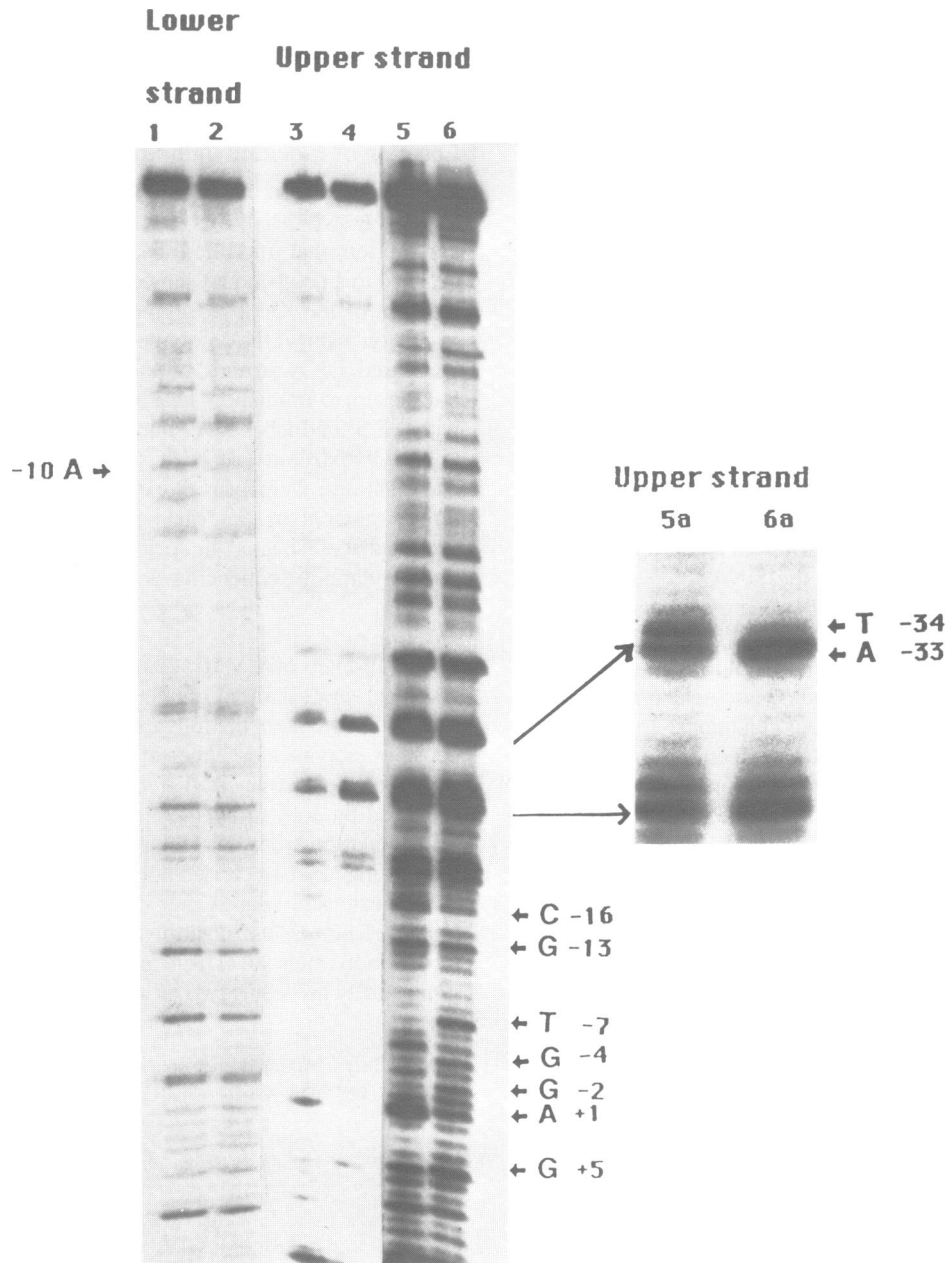


Figure 3: Primer extension pattern on irradiated DNA fragments and open complexes between RNA polymerase and linear fragments. Primer extension using the DNA polymerase I large fragment on irradiated and non-irradiated open complexes between RNA polymerase and 203 bp fragment was carried out as described in Materials and Methods. The bases are numbered according to their position with respect to the start site of transcription on the *lac* promoter. In lanes 1 and 2 the primer complementary to the lower, template strand was extended; in lanes 3 to 6 the primer complementary to the upper, non-template strand was extended. Lanes 1, 3, 5 and 5a represent primer extensions along naked DNA subsequent to irradiation. Lanes 2, 4, 6 and 6a represent primer extensions along irradiated DNA in open complexes with RNA polymerase. Lanes 5 and 6 show increased exposure of lanes 3 and 4 respectively. Lanes 5a and 6a shows the enlarged region around bands -34 to -25.

Lanes 5 and 6 in figure 3 represent longer exposures of lanes 3 and 4. Furthermore lanes 5a and 6a represent an enlarged region around position -20 to +35. The DNA polymerase I large fragment appears to terminate elongation immediately prior to putative thymine dimers whose formation is a result of a single pulse of UV radiation. The quantum yield for thymine dimer formation under conditions similar to those used here is around 4×10^{-2} [25]. The primer extension technique reports an average of 3.5% of termination events on the DNA at residues immediately preceding putative thymine dimers following a single

dose of UV radiation. A compilation of the relative frequency of terminations at each of the major sites of modifications on the upper strand of *lac* UV5 in DNA alone and in a binary complex is given in Table 1. Increasing the number of laser pulses did not lead to an observable increase in the frequency of terminations at these positions.

Nucleoprotein complexes

RNA Polymerase-DNA Complex: When an open complex was formed with RNA polymerase there was a distinctive alteration

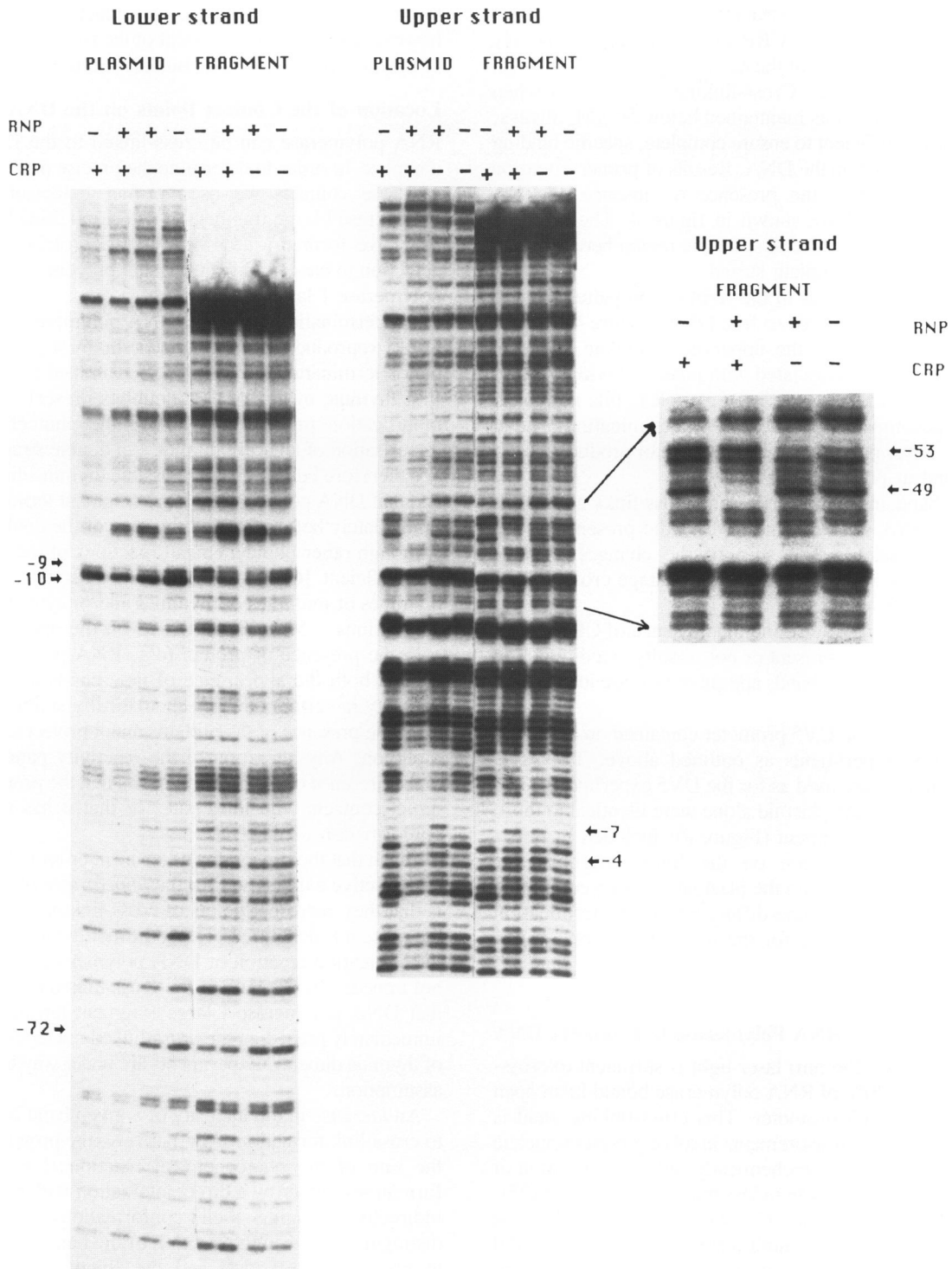


Figure 4: Primer extension pattern on irradiated DNA fragments, plasmids and complexes between RNA polymerase, CRP and plasmid contained *lac* promoters. Open complexes formation, irradiation and subsequent primer extension were carried out as described in Materials and Methods. Bases are numbered according to their position with respect to the start site of transcription on the *lac* promoter. An enlargement of the region around position -49 on the upper non-template strand of the *lac* fragment is included.

in the pattern of termination of primer extensions on both strands (Figure 3, lanes 2, 4 and 6). On the upper (non-template) strand at least three bands are strongly enhanced, notably at positions G -2, G -4 and T -7. The intensity of bands at positions A -33 and G +5 increases and the intensity of bands at positions T -34, C -16, T -5, and A +1 decreases (Figure 3 lanes 4 and

6). On the lower (template) strand, termination of extension subsequent to irradiation of the naked DNA produced the profile shown in figure 3 lane 2). No new termination positions appear in an open complex with RNA polymerase but there was a strong diminution in the intensity of the band at position -10 (fig 3 lane 2).

CRP-DNA Complex: The same strategy was applied here as that used for the RNA polymerase. CRP alone cross-links relatively poorly (1%) with a fragment of the *lac* UV5 promoter as shown by native gel electrophoresis. Cross-linking occurred only when the cAMP concentration was maintained below 50 μ M, always, however, at a level sufficient to ensure complete, specific binding of the complex to its site on the DNA. Results of primer extension on the UV5 fragment in the presence or absence of RNA polymerase and/or CRP are shown in figure 4. The enlarged portion of the figure is intended to show the region between -60 and -30 on the upper template strand.

There are clear differences in the termination pattern due to the presence of CRP compared to free DNA (Figure 4). A new termination site appears on the upper non-template strand at position C-53 that is not associated with putative thymine dimer formation. Furthermore, in a ternary complex, this new band persists but is accompanied by the loss of a termination band at position A-49. RNA polymerase alone does not produce loss of the intense band at position -49.

Changes in band intensities represent cross-links as well as changes in the DNA structure induced by the presence of the protein (cf. Discussion). The sum of the changes in band intensities is therefore greater than the percentage cross-linking determined by other assays.

On the lower non-template strand the presence of CRP, either when RNA polymerase is present or not, results in a diminution in intensity of A-72. No new bands appear or increase in intensity.

Supercoiled DNA: The *lac* UV5 promoter contained on a plasmid was used for UV experiments as outlined above. The same primers and strategy were used as for the UV5 experiments. The results of irradiation of the plasmid alone were identical to those obtained for the linear fragment (Figure 4); included in figure 4 are patterns of termination on the linear fragment for comparison with the patterns on the plasmid. In open complexes with RNA polymerase the same differences in the termination profile were seen as reported for the linear fragment.

DISCUSSION

Laser Cross-Linking of RNA Polymerase to Promoter DNA

A single pulse of UV (266 nm) laser light is sufficient to cross-link between 15 and 20% of RNA polymerase bound in an open complex to the *lac* UV5 promoter. This cross-linking yield is consistent with previous measurements involving protein-nucleic acid complexes [5]. The photochemically induced formation of covalent cross-links is complete in less than a microsecond [25]. This eliminates the possibility of rearrangements within the complex subsequent to a first modification. The irradiation did not alter the overall integrity of the complex as judged by migration on a non-denaturing gel. End labelled irradiated DNA runs as a single band on denaturing polyacrylamide gels demonstrating that no strand breaks are introduced into the DNA. We could clearly identify cross-links between the σ subunit and the DNA by Western blot analysis. Detection of cross-links to other subunits may be below the detection limit of our Western blot analysis. Based upon the migration of cross-linked RNA polymerase to promoter sequences other authors have suggested contacts between DNA and the σ , β , and β' subunits of RNA polymerase [16,17, 26, 27]. These assignments should however be accepted with some caution since, in our conditions, cross-reactivity of antibodies with bands exhibiting very large changes in mobility could only be observed with those directed against

the σ subunit. The existence of contacts with other subunits is however likely due to the extent of the footprint and the geometry of the quaternary structure, but has still to be firmly established.

Location of the Contact Points on the DNA

RNA polymerase can be cross-linked to the DNA in an open complex. In order to determine the precise position on the DNA of those contacts we used primer extension by the DNA polymerase I large fragment to detect modified bases (e.g. bases that have formed a cross-link to the protein). This limits our detection to base modifications that prevent elongation by DNA polymerase I large fragment.

The termination pattern of DNA polymerase I large fragment is very reproducible and characteristic for a given sequence. The major termination sites are located immediately preceding the first thymine of all the T-T doublets present. A very efficient modification process of DNA is the photochemical induced dimerisation of adjacent thymines in double stranded DNA [28]. We therefore believe that all possible thymine dimers are formed and that DNA polymerase I large fragment terminates at the base immediately before the first thymine of the doublet (Figure 5a). Although other photochemical reactions of the naked DNA are less efficient [6] we do observe bands that do not precede thymines or multiples of thymines and/or cytosines (for example at positions -5, -13, and -16 on the upper strand).

In the presence of protein (e.g. RNA polymerase and CRP) we see both the appearance of new bands and a difference in intensity in certain others (schematically summarized in Figure 5b). The presence of the protein cannot protect a given base from a photon. Any alteration of the reactivity pattern of the DNA in the presence of the protein implies that the protein either makes direct contacts with those sites or that it has altered the local conformation of the DNA.

Given that the protein forms covalent cross-links with the DNA an attractive explanation for the *appearance* of termination sites is that they represent points of cross-linking to the DNA. Since the protein is denatured prior to primer extension we expect that the elongation reaction of DNA polymerase I large fragment is not impeded by the bulk of the cross-linked protein. We assume that DNA polymerase I large fragment terminates at the base immediately preceding the cross-linked nucleotide (as in the case of thymine dimers). Experiments are under way to test this critical assumption.

An *increase* in the intensity of a given band could also be due to cross-link formation. Alternatively, the protein could increase the rate of the photochemical reaction (e.g. thymine dimer formation), either by a direct interaction with the residues or by indirectly changing their conformations. It is difficult to distinguish between the fraction of the band intensity that is due to cross-link formation and the fraction that is due to the photochemical modification of the base.

A *decrease* in the intensity of a given band must be due to an alteration of the reactivity of that base. The presence of the protein could change the conformation of the DNA thus altering the spatial disposition of the reactive species. This may well be the case for changes in the degree of formation of thymine dimers [29].

Contact Points in the *lac* UV5 Promoter

The changes observed in the termination pattern of DNA polymerase I large fragment are summarized in Figure 5b. All alterations of the primer extension pattern in an open complex (compared to naked DNA) fall within the region of interaction

of RNA polymerase with the *lacUV5* promoter as defined by a variety of 'footprinting' techniques [10, 11]. Three new bands appear on the upper (non-template) strand at positions -7, -4, and -2. We interpret these as cross-links to the protein formed from the DNA residues A-8, T-5, and T-3. All of those bases are in the locally separated region of the open complex.

The intensity of two bands, at positions +5 and -33, increases in the open complex. Interpretation is here rendered more difficult since these nucleotides are preceded by multiples of thymine that invariably form dimers in the naked DNA. The sum of the intensities of bands that appear or increase is approximately 15-20% of the total intensity in a lane. This number is in

agreement with the value of 15-20% cross-linking obtained from SDS and native gels and supports the interpretation given above.

A number of bands (positions -34, -5, +1 on the upper strand, and -10 on the lower strand) decrease in intensity in the open complex. There, the protein makes contacts or changes the DNA conformation such that the photochemical reaction pattern normally seen on the naked DNA is altered. The termination site at -10 in the lower (template) strand, for example, is no longer present in the open complex. The thymidine doublet (positions -9 and -8) that follows in the sequence is in the locally melted region of the open complex. It is therefore likely that the thymines are no longer in an optimal geometrical arrangement for the formation of dimers. In contrast, the thymine doublet at positions +1 and +2 of the lower strand appears to maintain their alignment, and no change in reactivity is observed in the open complex.

It is interesting that we find the majority, if not all, of the cross-linking to be associated with the non-template strand. Following others, we have previously suggested, based on chemical reactivity of the DNA to specific reagents [15] that the non template strand of *lac UV5* is in intimate contact with RNA polymerase in an open complex. This has also been put forward for another promoter [30] and is confirmed by the results shown here.

The pattern for CRP can also be explained by the formation of cross-links and conformational changes in the DNA. One band substantially increases (position C-53 on the upper strand). This may result either from a cross-link of T-54 with the protein, or from an alteration in the conformation of the pyrimidine bases at -55 and -54. Another band disappears (-72 on the lower strand). CRP binding might distort the conformation of the thymine doublet at positions -71, -70 preventing the formation of the dimer by UV.

An interesting change in pattern is seen at position -49 on the upper strand (located in front of a thymine doublet) which disappears in the presence of both CRP and polymerase, but is present when either of the individual proteins are bound. This indicates a difference in the binding interaction or the conformation of the DNA in the presence of both proteins. CRP might contact a larger site (now including both the thymines at positions -50 and -51) or the DNA between the two proteins may be distorted in the presence of both proteins. An interaction between CRP and polymerase (direct or indirect) is suggested since the disappearance of the band depends on the presence of both proteins. In contrast, none of the signals within the RNA polymerase binding domain are altered by the presence of CRP.

Experiments are under way in our laboratory to further characterize the interactions that give rise to the individual bands. We are currently isolating cross-linked complexes in order to verify the identity of the assigned cross-linking positions and the nature of the cross-linked subunits. Using the combination of a rapid mixing device and single pulse laser cross-linking we are also investigating the kinetics of open complex formation [31].

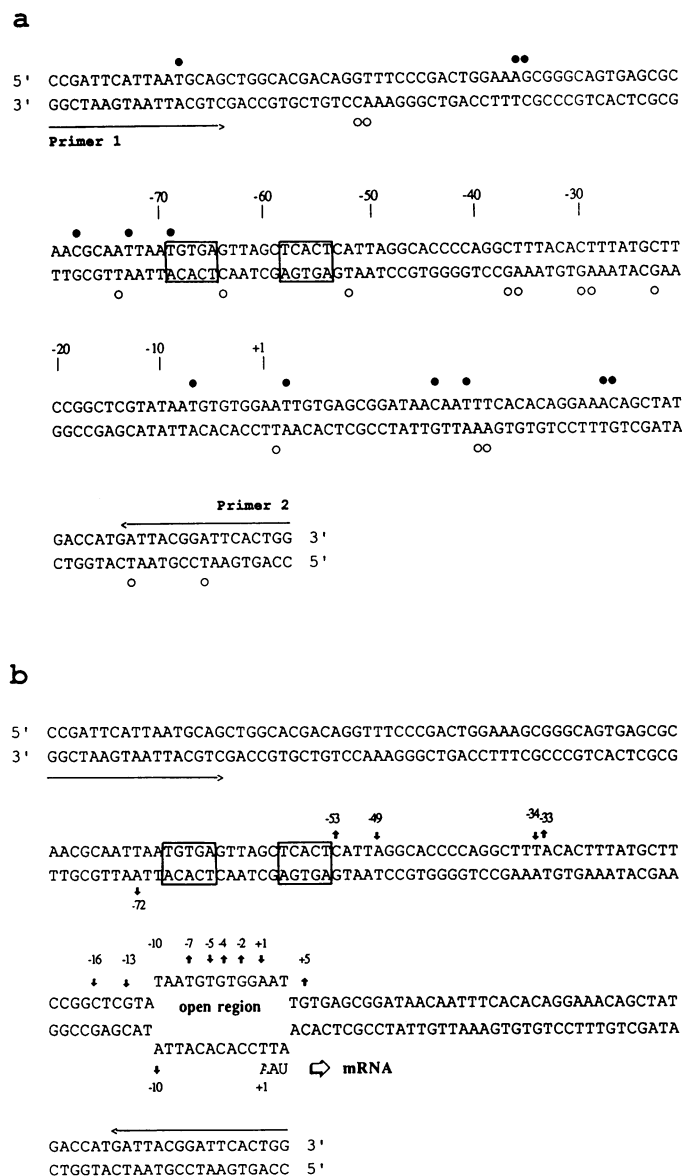


Figure 5: A summary of termination events on the irradiated *lac UV5* promoter DNA in the absence and presence of RNA polymerase and CRP. **a)** Primer extension along DNA alone. Open circles represent positions at which termination of extension of primer 1 occurs. Closed circles represent positions at which termination of extension of primer 2 occurs. **b)** Primer extension along DNA in the presence of RNA polymerase and CRP. Upward or downward pointing arrows indicate increases or decreases respectively in band intensity relative to naked DNA. The palindromic recognition site for CRP is boxed. The locally melted region characteristic of the open complex is indicated by strand separation.

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