TOPOGRAPHY OF INTERACTION OF *ESCHERICHIA COLI* RNA POLYMERASE SUBUNITS WITH *lac* UV5 PROMOTER

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1. Introduction

The structure of the complex of RNA polymerase with the DNA of promoter regions remains obscure. The use of chemical modification and crosslinking of the complex has revealed many contacts with RNA polymerase in the promoter (review [1]).

Here we describe the location of contacts in *lac* UV5 promoter with RNA polymerase subunits. The enzyme was crosslinked through its ϵ-amino groups of lysine residues to the partly depurinated promoter. This approach has been developed to determine the linear arrangement of histones along DNA in nucleosomes [2]. The full details of these experiments appeared in [3].

2. Material and methods

Escherichia coli RNA polymerase and *lac* UV5 promoter cut out from plasmid pLJ3 with restriction endonucleases *Eco*RI and *Hae*III were isolated as in [3]. Both strands of the promoter DNA were specifically labelled at each terminus with ^{32}P using polynucleotide kinase (at the 5′-terminus) or DNA polymerase (at the 3′-terminus). The end-labelled promoter was methylated with 50 mM dimethyl sulfate in 100 mM sodium cacodylate (pH 7.0) at 20°C for 7 min. Methylated purine bases were excised in 10 mM NaCl, 0.1 mM EDTA, 10 mM sodium cacodylate (pH 7.0) at 65°C for 1 h. The depurinated promoter (10 μg/ml) was crosslinked to RNA polymerase (100 μg/ml) in 50 mM NaCl, 5 mM $MgCl_2$, heparin (1 μg/ml, only for holoenzyme), 50 mM sodium cacodylate (pH 7.0) at 37°C for 2 h followed by reduction with $NaBH_4$ [3]. Polymerase subunits containing crosslinked double-stranded DNA were separated by electrophoresis in 5% polyacrylamide gel with SDS and then eluted from the gel. The 3′-^{32}P-labelled promoter fragments of each subunit were heat-denatured and directly electrophoresed in 25% polyacrylamide gel with 7% urea, whereas the crosslinked 5′-^{32}P-labelled promoter fragments were first released from proteins by digestion with pronase and then electrophoresed.

3. Results

The experimental strategy for locating contacts between the polymerase subunits and the promoter labelled with ^{32}P at the 3′- or 5′-termini of each DNA strand is based on the crosslinking of their complex [2]. The promoter is partially depurinated by its methylation with dimethyl sulfate and scission of methylated purine bases at neutral pH. The prepared complex of RNA polymerase with depurinated promoter retains all the main features of that with the unmodified promoter [3]. The ϵ-amino groups of lysine residues in the protein molecule form aldimine crosslinks with the aldehyde groups of depurinated DNA sites which are stabilized by reduction with $NaBH_4$. This makes one DNA strand split at the point of crosslinking at the sites of excised 'G' and 'A' bases and causes the attachment of the protein only to the 5′-terminal DNA fragment formed [2]. The points of contacts of RNA polymerase subunits with the promoter fixed through crosslinks can be determined by measuring the length of either the crosslinked 5′-terminal or unattached 3′-terminal DNA fragments [3]. Therefore, polymerase subunits containing the crosslinked double-stranded promoter were isolated by gel

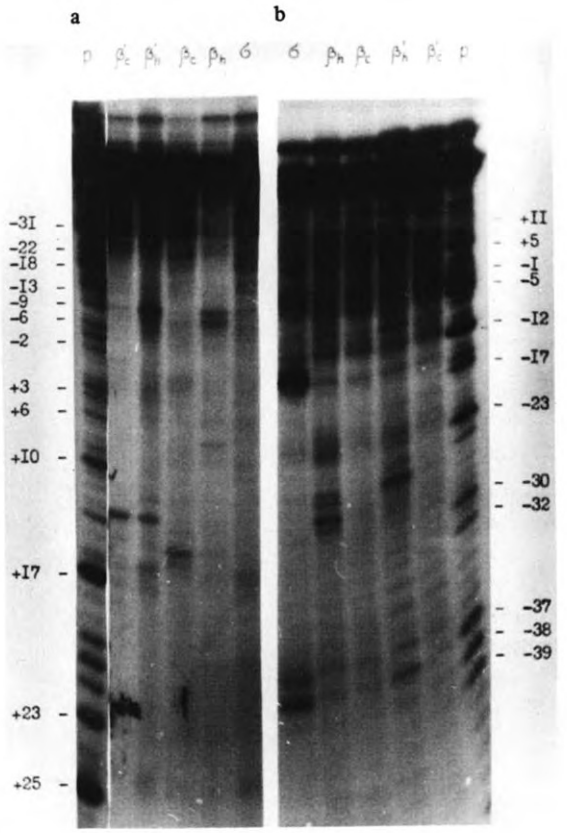

Fig.1. Autoradiograms showing the length of the 5′-terminal fragments of *lac* UV5 promoter crosslinked to each RNA polymerase subunits: (a) transcribed promoter strand; (b) non-transcribed strand; (p) fragments of promoter produced by its splitting at purine bases (G > A) according to [4] and used as standard fragments. $_c$ and $_h$ indicate the polymerase subunits within the core and holoenzyme, respectively.

electrophoresis and DNA was heat denatured. The length of ^{32}P-labelled promoter fragments was determined by gel electrophoresis in the presence of the fragments of the whole promoter also split at 'G' and 'A' bases [4] and used as standards; the protein-free 3′-terminal fragments were electrophoresed directly while the crosslinked 5′-terminal fragments were first released from proteins by pronase treatment and then electrophoresed.

Fig.1 shows the autoradiograms of gels separating the crosslinked 5′-^{32}P-labelled promoter fragments of transcribed and nontranscribed DNA strands. The crosslinked fragments are well recognized above the level of some background as intensive multiple bands shifted from the position of standard fragments although they all are also split at the 'G' and 'A' bases. This shifting and multiplication of the crosslinked fragments is likely to be due to the presence of a few crosslinked amino acids that still remain after pronase treatment. For example, these bands for transcribed promoter strand (fig.1a) are: +13.5 (β'_c); +13.5 and −9 (β'_h); +15.5 (β_c); −7, −9 (β_h); and for non-transcribed strand (fig.1b) are: −26 (β_c); −28, −27, −26.5 (β_h); −14, −37 (σ).

Fig.2 shows an example of gel electrophoresis of some labeled 5′- and corresponding 3′-terminal promoter fragments of a non-transcribed DNA strand. The position of these 3′-terminal fragments in the gel coincides with that of the standard fragments and enables us therefore to determine the points of contacts with higher precision than in the case of the 5′-fragments. The most significant bands of the 3′-fragments (fig.2b) are: −25, −30 (β'_h, β_h) and −17 (σ). The promoter splitting by crosslinking produces the 3′- and 5′-terminal fragments directly related to one another. For example, the 3′-fragment −30 in fig.2b (β_h) corresponds to the multiple and smeared 5′-fragments −28, −27, −26.5 in fig.1b (β_h).

Fig.3 summarizes the data of 8 such experiments done under different conditions with the both strands of the promoter labelled at the 5′- and 3′-ends since it is difficult to get a clear picture of all contacts in one gel. Moreover, although most of the contacts shown in fig.3 are well reproducable, some of them vary significantly, however, in different experiments: −47, −46, −23, +30 (non-transcribed strand), +25, +27 (transcribed strand) for holoenzyme and +30 (non-transcribed strand), +25, +27 (transcribed strand) for core enzyme.

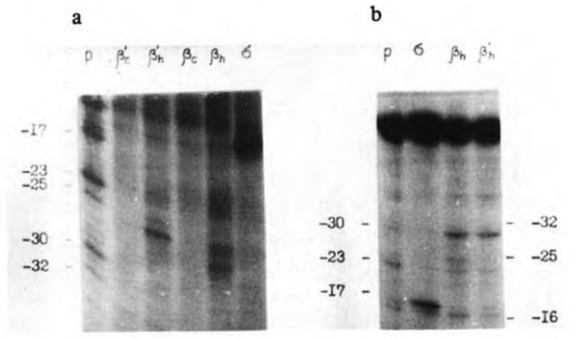

Fig.2. Autoradiograms showing the length of the 5′- (a) and 3′-terminal (b) fragments of non-transcribed DNA strand in the crosslinked polymerase–promoter complex. Designations are as in fig.1.

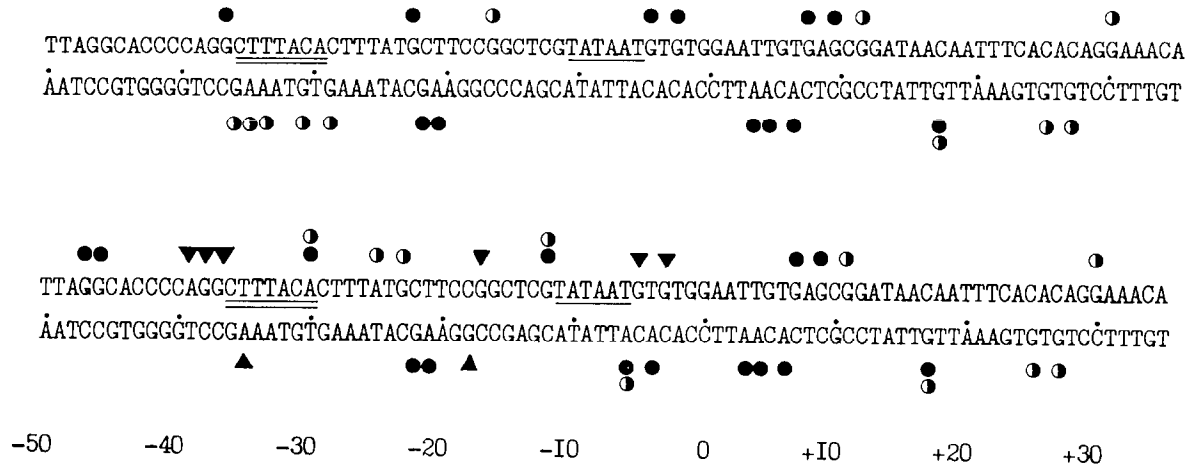

Fig.3. Crosslinking of RNA polymerase subunits with *lac* UV5 promoter in the core (a) and holoenzyme (b). Basepairs are numbered relative to the start of transcription at +1. Underlined are the 'Pribnow box' (——) and the '−35' region (═══): (●) β'; (◑) β; (▼) σ subunits.

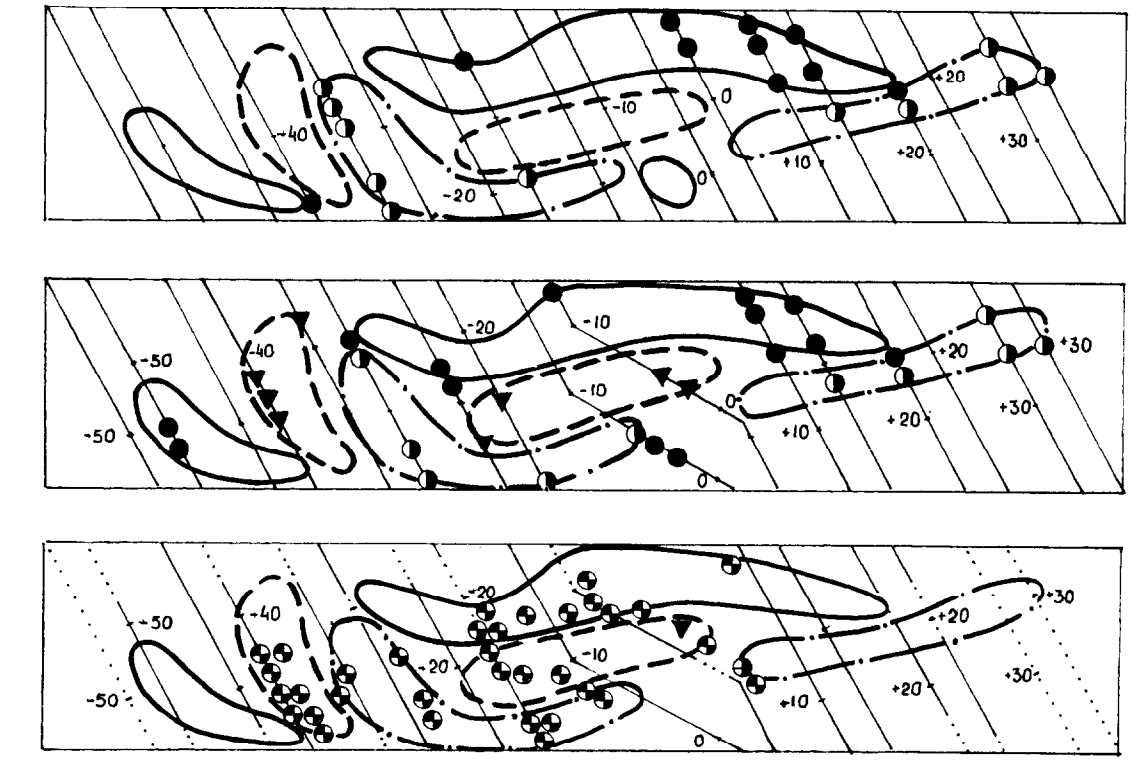

Fig.4. A model showing the planar representation of crosslinks of polymerase subunits in the core (a) and holoenzyme (b); (c) contacts of the promoter with the holoenzyme revealed by methylation of purine bases [9], promoter–polymerase UV-crosslinking [10] as well as by ethylation of DNA phosphates [1] and shown by signs (◓) between the DNA strands and on the DNA strands, respectively. The arrangement of polymerase subunits in fig.4c is made according to fig.4b. Promoter regions that are not protected by polymerase against DNase I digestion [4] are shown with dotted lines. Marked and outlined are the contacts of: β' (●, ——); β (◑, –·–); σ subunits (▼, – – –).

4. Discussion

These crosslinking experiments indicate that RNA polymerase makes contacts within the region of *lac* UV5 promoter located between +30 and –47 nucleotides (fig.3), which is in agreement with [1,5]. The core and holoenzyme show the same pattern of contacts with *lac* UV5 promoter only within the region between +30 and +3, but the pattern is essentially different between +1 and –37. Thus the 'Pribnow box' makes contacts with the β,β' subunits and only in the holoenzyme; the '–35' region is crosslinked to the β subunit in the core and to the σ subunit in the holoenzyme. Since the addition of the σ subunit to the core polymerase in the holoenzyme does not appear to affect the core structure [6], this difference in the interaction can be attributed to the change in the DNA conformation. Indeed, it has been shown that the holoenzyme, rather than the core polymerase [7], makes *lac* UV5 promoter unwind between +3 and –10 nucleotides [1] by ~260° [8].

Fig.4 shows a model for contacts of polymerase subunits on a planar representation of the cylindrical projection of the DNA molecule in the B form with 10.5 basepairs/turn. The DNA in the model for holoenzyme is unwound by 260° and extended by ~10 Å in the region between +3 and –10. This distortion of the DNA structure enabled us to arrange the contacts of polymerase subunits on the same side of the promoter [3] in a similar configuration for both the core and holoenzyme. The contacts between the polymerase and *lac* UV5 promoter in [1] are also shown for comparison in fig.4c and are fully discussed elsewhere [1]. It is worth mentioning however that there is a correspondence in the location on the promoter of the polymerase crosslinked sites and the phosphate groups that interact with the polymerase [1]. Both these sites are arranged in an area from +1 to –45 within 2 essential regions located between –2 and –20, and also between –30 and –40 nucleotides. By using the data of fig.4a,b, it is possible to assign the contacts on the promoter in fig.4c to the specific polymerase subunits.

Some differences between fig.4a,c are not surprising. DNA methylation [9] and UV crosslinks [10] make it possible to identify contacts between the protein and DNA within its grooves whereas our chemical crosslinks reveal mainly salt bridges between the polymerase lysine residues and DNA phosphates. Moreover, it seems that the ϵ-amino groups of lysines

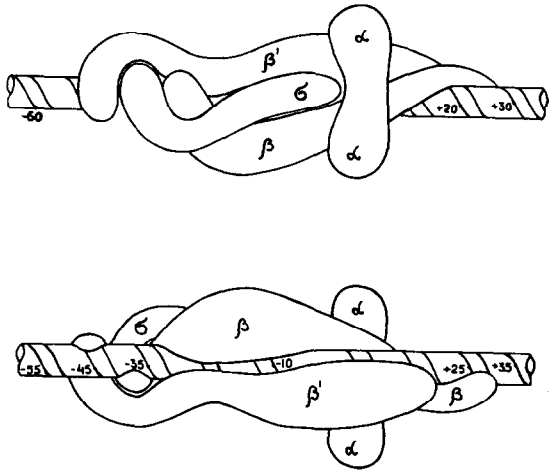

Fig.5. A model for the arrangement of RNA polymerase subunits on the *lac* UV5 promoter viewed from the opposite sides.

in DNA-bound proteins can oscillate between the adjacent phosphates located on the same or complementary DNA strands [2] and in such a way create the apparent multiplicity in the binding sites for the same lysine residue in crosslinking experiments.

The data of fig.4 provide the basis for a model of the quaternary structure of the RNA polymerase–promoter complex shown in fig.5. This model is consistent with the results of the subunit crosslinking by bifunctional reagents [11], the measured sizes of the subunits, and the distances between their mass centres [5,12]. The DNA in the model occupies, without any bending, the groove created on the border between the β and β' subunits; the σ subunit makes contacts with the promoter mainly from the same side as the β subunit in such a way that it can easily leave the polymerase after the initiation of transcription. The α subunits were not found to be crosslinked to the promoter, suggesting a lack of interaction between them, and it is therefore placed in rather an arbitrary position.

Acknowledgements

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