DISCRIMINATION BY tRNA OF TWO TYPES OF ISOLATED BINDING SITES FOR *E. COLI* RNA-POLYMERASE ON PHAGE LAMBDA DNA

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1. Introduction

Upon binding to a DNA template, the *E. coli* RNA-polymerase elicits the retention on nitrocellulose filters of the complex formed on one hand [1] and the protection against nuclease digestion of these DNA sequences which are covered by the polymerase on the other hand [2–10]. These fragments of protected DNA (referred to as pDNA) are therefore assumed to represent the binding sites for DNA-polymerase. The digested complex (pDNA–RNA polymerase) is itself retained on nitrocellulose membranes [3,4,7]. It seems legitimate to believe that these binding sites might contain the promotor sequences as genetically defined [11] or some specific sequences involved in their recognition. Initiation of transcription by RNA polymerase occurs at highly specific, discrete sites, the DNA sequences of which must have some unique features. However, it is well established that the number of binding sites for RNA-polymerase exceeds by far the number of genuine promoters [12]. Our endeavour has been to eliminate or greatly reduce the non-specific binding sites in the population of pDNA molecules. Our earlier results on λ [7] has shown that pDNA was enriched in A–T at low polymerase/DNA ratios (excess DNA) although at higher ratios pDNA did not depart from the base composition of total λ DNA.

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These results pointed to the existence of at least two types of sites with different affinities for the polymerase, as it had been already suggested by the competition experiments of Stead and Jones [13]. The analysis on acrylamide gels of pDNA has allowed the separation of two populations of molecules [8], one of which is considerably enriched in A–T (67%) with respect to total DNA and is only observed when sigma was present during the initial binding reaction. On grounds that sigma factor is a necessary element for promoter recognition [14], we have tentatively identified this sigma dependent A–T rich fraction of pDNA to the promoters themselves or some sequences involved in their recognition. We have now extended to T5 and T7 phages [15] our earlier results on λ [8], suggesting that the specific recognition of A–T rich DNA sequences by *E. coli* RNA-polymerase might be a general phenomenon.

The present work was aimed at finding another approach to the elimination of non-specific binding sites. Hinkle and Chamberlin [16] had observed that a very stable complex between RNA-polymerase and [^{3}H]T7 DNA was formed at 37° in the presence of sigma. When either one of these requirements were not met, the complexes formed underwent a rapid displacement by cold T7 DNA. By using a competitor polynucleotide, it seems therefore possible to displace RNA-polymerase molecules bound to non-specific sites (not sigma-dependent). We selected tRNA as such a competitor on account of its affinity for RNA-polymerase [17] as well as its easy availability.

2. Materials and methods

The purification of RNA-polymerase holoenzyme and core enzyme from *E. coli* A 19 was as previously described [7, 8] as was the preparation of [^{32}P]λ DNA. Pancreatic DNAase was from Worthington. Venom phosphodiesterase from Worthington or Calbiochem was treated according to Sulkowski and Laskowski [18] in order to remove any residual 5′-nucleotide activity. Stripped tRNA from yeast was obtained from General Biochemicals.

Binding mixtures contained 32 μg [^{32}P]λ DNA (11,900 cpm/μg), 16.8 μg RNA-polymerase (holoenzyme or core enzyme) in 0.8 ml 0.04 M Tris-HCl (pH 8.0)–10^{-2} M $MgCl_2$–10^{-3} M $CaCl_2$. Incubation was carried out for 15 min either at 37° or at 0° in ice. 0.35 ml aliquots were then transferred into tubes containing, in 0.15 ml of the above buffer, 100 μg DNAase, 100 μg phosphodiesterase and 500 μg of tRNA when indicated. Subsequent incubation was for 30 min at 37° in all cases. After addition of 5 ml of a cold solution of 0.01M Tris-HCl (pH 8.0)–0.05 M NaCl, the samples were filtered on millipore membranes (type HAWP, 0.45 μm pore size, 25 mm in diameter) under a vacuum of 5 cm Hg [1]. After washing with 40 ml of the same buffer and drying filters were counted in a toluene-based scintillation fluid using a Packard Tricarb scintillation counter. When pDNA was to be recovered from complexes retained on filters, the latter were not dried but eluted twice with 1 ml of 1% Na dodecyl sulfate for 15 min at 100°. Both eluates were pooled, adjusted to 0.1 M NaCl and precipitated by 2 vol of ethanol in the presence of 10 μg calf thymus DNA as a carrier. The nucleotide composition was determined as previously [7].

3. Results and discussion

The results presented in table 1 show that tRNA is able to displace RNA-polymerase from all its binding sites on λ DNA when either one of the following conditions are not met: presence of sigma factor and incubation at 37°. On the contrary, when incubation is carried out at 37° in the presence of sigma, a fraction of pDNA appears resistant to displacement by tRNA. In the above series of experiments, a ratio of polymerase/DNA equal to 0.45 was used. A large-scale experiment using a ratio of 2.42 was performed in order to analyse the pDNA retained on filter in the presence or in the absence of tRNA during the digestion step. This high ratio has been selected so as to give a lot of non-specific binding sites in the absence of tRNA

Table 1
Influence of tRNA on the protection of λ DNA under various conditions of polymerase binding.

Binding conditions	Digestion conditions	cpm retained	cpm retained corrected[a]	pDNA Retention[b] (%)
No polymerase	–tRNA	300	–	
	+tRNA	195	–	
0°, + σ	–tRNA	2,628	2,328	1.24
	+tRNA	175	0	0
37°, + σ	–tRNA	2,570	2,270	1.21
	+tRNA	514	319	0.17
0°, – σ	–tRNA	2,154	1,854	0.99
	+tRNA	223	28	0.02
37°, – σ	–tRNA	2,301	2,001	1.07
	+tRNA	213	18	0.01

[a] Corrected cpm are obtained by subtracting cpm retained in the absence of polymerase.
[b] Evaluated with respect to the amount of DNA in the incubation mixture.

Table 2
Base composition of protected λ DNA (pDNA).

Total	λ DNA (%)	pDNA −tRNA (%)	pDNA +tRNA (%)
C	25.5	25.0	20.8
A	25.0	24.9	29.1
G	24.5	24.7	21.2
T	25.0	25.4	28.9
A–T	50.0	50.3	58

resulting in a random nucleotide composition of pDNA [7]. Indeed the results presented in table 2 show that, without tRNA, the nucleotide-composition of pDNA is identical to that of total λ DNA. On the contrary, pDNA non displaced by tRNA appears significantly enriched in A–T (58%). This value is lower than the highest one obtained previously after purification on acrylamide gels [8]. However, it must be recognized that the present data suffer from two unfavourable conditions. First, the radioactivity analysed here included the background of mononucleotides retained on filter when no polymerase was present in the binding mixture. Correcting for this background of random composition (see table 1), it follows that DNA protected in the presence of tRNA actually contains 63% A–T. Second, the recovery of radioactivity after elution from filters did not exceed 80% in our hands, at variance with that claimed by Blattner [3]. Under the likely assumption that these mononucleotides are eluted more easily than pDNA, their contribution of randomly distributed counts have been underestimated in the above calculation, leading to a minimum value for A–T content.

In conclusion, the present work demonstrates the existence of at least two types of binding sites for *E. coli* RNA-polymerase on λ DNA. Sites of the first type, in small number and very rich in A–T, are specific for the holoenzyme (containing sigma) with which they can form at 37° complexes stable enough to withstand competition by tRNA. Sites of the second type, in large number of random composition, have a lower affinity making them susceptible to displacement by tRNA. Results are in full agreement with those of Stead and Jones on λ [13] and of Hinkle and Chamberlin on T7 [16, 19]. The A–T enrichment observed here confirms our former results on λ [7, 8] as well as recent ones from other groups on phage fd replicative form DNA [9, 10] and from our group on T5 and T7 [15]. Should the above technical drawbacks be eliminated, this simple procedure for isolation of sigma-dependent sites for DNA-polymerase would be of great interest. Whether the sites can be equated to those defined by their rifampicin-resistance [14] or to any of the promoter, entry or start sites as defined by Blattner et al. [20] cannot be decided as yet.

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