TEMPLATE SELECTION BY *E. COLI* RNA POLYMERASE HOLOENZYME

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1. Introduction

By what mechanism does *E. coli* RNA polymerase discriminate between specific promoter sites? Theoretically the enzyme could exist either as a single form with a uniquely defined specificity or in a small number of conformational states, each of which would have a preferred affinity for a particular class of promoter sites [1,2]. On the first model discrimination would depend solely on the relative affinity of the enzyme for particular promoters while on the second discrimination would also depend on the distribution of the enzyme between the different states.

These formal possibilities can be distinguished experimentally by in vitro template competition experiments. First an in vitro system is characterised in which RNA synthesis proceeds simultaneously from two types of promoter, e.g., phage and ribosomal RNA promoters. This system is then perturbed by the addition of increasing quantities of another DNA template. This DNA will cause a redistribution of polymerase molecules by competing for the enzyme. If polymerase exists as a single form with invariant specificity competition of RNA synthesised from different promoters should be independent of the competing template and depend only on the relative affinity of the enzyme for the respective promoters. By contrast if the enzyme exists in multiple forms each with differing initiation specificity the character of the competition observed should depend on the nature of the competing template.

A further implication of the multiple form model is that if one form of the enzyme has a high affinity for its preferred promoter, other forms of the enzyme will have a low affinity for the same promoter. This means that while all forms of the enzyme will bind to a given promoter the low affinity forms may have a slower rate of initiation than the high affinity form. Such interference could be alleviated and the rate of transcription from the promoter increased if the low affinity forms were preferentially bound by an added DNA template.

I show here that in a in vitro system synthesising phage T2 RNA and *E. coli* ribosomal RNA (rRNA) rRNA synthesis is preferentially competed by the addition of *E. coli* DNA. By contrast addition of phage T7 DNA results in a reduction of T2 RNA synthesis and a stimulation of rRNA synthesis. Further the residual rRNA synthesised in the presence of *E. coli* DNA is no longer preferentially sensitive to ppGpp, an effector which selectively reduces the affinity of polymerase holoenzyme for rRNA promoters [3] and thus affects promoter recognition.

2. Materials and methods

2.1. *RNA polymerase and DNA templates*

RNA polymerase holoenzyme was prepared from *E. coli* MRE 600 as described by Burgess and Travers [4]. Enzyme so prepared contained ∿ 0.7 mol σ-subunit/2 mol α-subunit. T2 DNA and $\phi 80\ d_3$ $rrnB^+\ ilv^+\ su^+7$ DNA were prepared by gentle phenol extraction of the corresponding purified phage followed by dialysis against 0.01 M Tris–HCl pH 7.9, 0.1 M potassium chloride, 0.0001 M EDTA. *E. coli* DNA was prepared as described by Travers [5]. The experiments were repeated with two preparations each of $\phi 80\ d_3$ $rrnB^+\ ilv^+\ su^+7$ DNA and *E. coli* DNA and three of RNA polymerase.

2.2. *RNA synthesis and analysis*

Reaction mixtures (200 μl) for synthesising RNA in vitro contained 0.04 M Tris–HCl pH 7.9 at 25°C, 0.01 M magnesium chloride, 0.006 M 2-mercaptoethanol, 0.0001 M EDTA, 0.05 M potassium chloride, 0.25 mM each of ATP, CTP and GTP, 0.003 mM [^{3}H]UTP (specific activity 49 Ci/mmol) 2.1 μg φ80 d_3 rrnB$^+$ ilv$^+$ su$^+$7 DNA, 0.3 μg T2 DNA, *E. coli* DNA and T7 DNA as indicated. This reaction mixture was incubated for 5 min at 30°C and RNA synthesis started by the addition of 0.8 μg RNA polymerase holoenzyme. After 30 min at 30°C the reaction was terminated by the addition of 200 μl 0.6 M sodium chloride, 0.06 M sodium citrate followed by the addition of 400 μl phenol saturated with 0.3 M sodium chloride, 0.03 M sodium citrate. In this system total RNA synthesis was linear for at least 60 min. Addition of heparin at 400 μg/ml at either 5 min or 30 min after addition of enzyme resulted in a reduction in the rate of RNA synthesis by 2 min and in complete cessation after a further 12 min.

The aqueous phase from the phenol extraction was used as a source of RNA for hybridisation. Total RNA synthesis was determined by trichloracetic acid precipitation of duplicate 25 μl aliquots of this aqueous phase. rRNA synthesis was determined by hybridisation of triplicate 25 μl aliquots of extracted RNA to 240 μg/ml denatured *E. coli* DNA in the presence and absence of an 10 μg/ml of an equimolar mixture of 16 S and 23 S *E. coli* rRNA. T2 RNA synthesis was determined by hybridisation of duplicate 25 μl aliquots of extracted RNA to 20 μg/ml denatured T2 DNA. All hybridisations were for 5 h at 67°C in 0.3 M sodium chloride, 0.03 M sodium citrate in a final volume of 300 μl. The hybridisation efficiencies of rRNA, *E. coli* RNA (less rRNA) and T2 RNA were 21%, 12% and 34% respectively and were determined by hybridisation of in vivo ^{32}P-labelled rRNA, in vitro ^{3}H-labelled *E. coli* RNA and in vitro ^{3}H-T2 RNA to the appropriate denatured DNA species.

3. Results

Simultaneous in vitro RNA synthesis from T2 and rRNA promoters was chosen as a suitable system for assaying the effect of template competition since RNA synthesis from these promoters responds differently to the nucleotide ppGpp [3]. φ80 d_3 rrnB$^+$ ilv$^+$ su$^+$7 DNA [6] was the template for rRNA synthesis and T2 DNA the template for T2 RNA synthesis. Figure 1 shows that addition of small quantities of *E. coli* DNA to such a system resulted in strong competition of rRNA synthesis but little competition of T2 RNA synthesis. Thus 0.2 μg *E. coli* DNA competed rRNA and T2 RNA synthesis by 59% and 13% respectively. Additional quantities of *E. coli* DNA further reduced T2 RNA synthesis while the level of rRNA synthesis was slightly increased. This

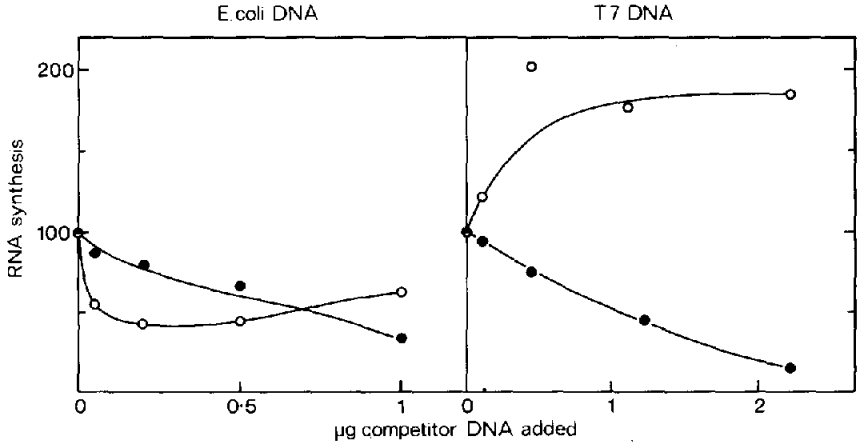

Fig.1. Competition of rRNA (o-o-o) and T2 RNA (•-•-•) synthesis by *E. coli* DNA and T7 DNA. The ordinate is normalised to rRNA and T2 RNA synthesis in the absence of added competitor DNA.

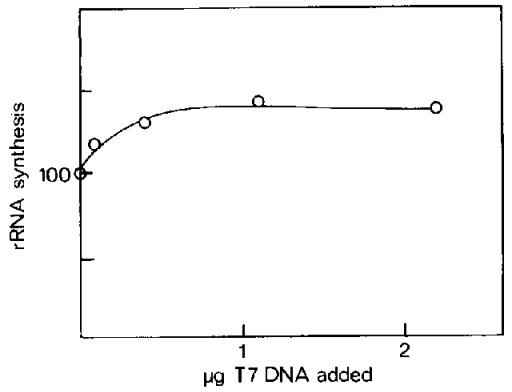

Fig.2. Stimulation of rRNA synthesis from $\phi 80\ d_3$ DNA by T7 DNA. In the absence of competitor total RNA synthesis was 43585 cpm and rRNA in hybrid was 2180 cpm.

increase correlated with an increasing level of *E. coli* RNA synthesis and presumably represented synthesis of rRNA from *E. coli* rRNA cistrons.

Addition of small quantities of T7 DNA to the T2 DNA/$\phi 80\ d_3$ $rrnB^+$ ilv^+ su^+7 DNA template mixture increased the extent of rRNA synthesis while strongly competing T2 RNA synthesis. Thus 2.2 μg T7 DNA, the greatest quantity added, competed T2 RNA synthesis by 88% while enhancing rRNA synthesis to 185%. This increase in rRNA synthesis was also apparent when T7 DNA was added to a reaction mixture containing only $\phi 80\ d_3$ $rrnB^+$ ilv^+ su^+7 DNA and lacking T2 DNA (fig.2).

In the presence of 0.2 mM ppGpp, a nucleotide which alters the initiation specificity of RNA polymerase [3] a different pattern of competition was observed. Both T7 DNA and *E. coli* DNA at low concentrations competed rRNA and T2 RNA synthesis to similar extents (fig.3), and thus the character of the competition observed was relatively independent of the nature of the competing template. Further ppGpp reduced the effectiveness of *E. coli* DNA as a competitor of rRNA synthesis. A comparison of the data from figs.1 and 2 tabulated in table 1 shows that this reflects the degree to which

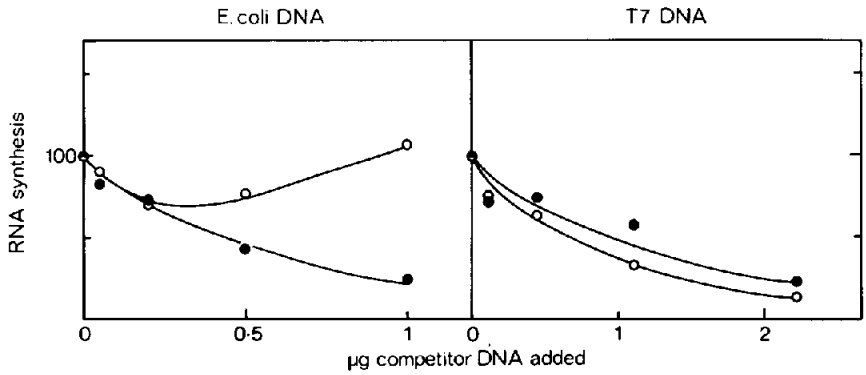

Fig.3. Competition of rRNA (o-o-o) and T2 RNA (•-•-•) synthesis by *E. coli* DNA and T7 DNA in the presence of 0.2 mM ppGpp.

Table 1
Effect of addition of *E. coli* DNA on the sensitivity of rRNA synthesis to ppGpp.

μg *E. coli* DNA added	Total RNA synthesis		rRNA in hybrid		T2 RNA in hybrid	
	Control	+0.2 mM ppGpp	Control	+0.2 mM ppGpp	Control	+0.2 mM ppGpp
	cpm/50 μl aliquot extracted RNA					
0	71 466	55 137	919	427	14 208	14 010
0.05	60 392	50 052	488	384	12 234	11 420
0.2	58 474	46 255	377	301	11 301	9821
1	53 783	41 189	573	445	4696	3538

the nucleotide inhibits rRNA synthesis. In the absence of any competing DNA ppGpp inhibits rRNA synthesis by 45% while T2 RNA synthesis is only slightly inhibited. However with sufficient *E. coli* DNA present to reduce rRNA synthesis by ∿45% ppGpp no longer inhibits rRNA synthesis preferentially, the reduction in rRNA and T2 RNA synthesis being respectively 8% and 20%.

4. Discussion

The data show that when purified RNA polymerase transcribes T2 DNA and rDNA in a mixed template system T2 RNA synthesis is preferentially reduced by addition of T7 DNA while rRNA synthesis is preferentially reduced by addition of *E. coli* DNA. Thus the character of the competition observed depends on the nature of the competing template. This observation suggests that RNA polymerase molecules form a mixed population in which the enzyme can assume two, or more, states each of which has a preferred affinity for a particular type of binding site.

The pattern of competition is changed by the nucleotide ppGpp. In this case the character of the competition is not dependent on the nature of the competing templates tested. Thus under these conditions there is no evidence for the existence of multiple forms of RNA polymerase. The nucleotide binds to RNA polymerase [7] and alters the initiation specificity of the enzyme, reducing its affinity for rRNA promoters while having little effect on its affinity for ϕ80 promoters [3]. Thus ppGpp strongly inhibits rRNA synthesis only when RNA polymerase has a high affinity for rRNA promoters. Table 1 shows that such inhibition is observed here in the absence of any competing template but that in the presence of *E. coli* DNA rRNA synthesis becomes relatively insensitive to ppGpp. This suggests that under the latter conditions rRNA synthesis is initiated by polymerase with a low affinity for rRNA promoters. Consequently *E. coli* DNA must have many binding sites for the form of RNA polymerase with a high affinity for RNA promoters. This observation would explain why rRNA synthesis from *E. coli* DNA itself with template excess is not preferentially sensitive to ppGpp and has a high transition temperature for promoter opening [2,8]. The nature of these binding sites is uncertain. They could be bacterial promoters or some other structural feature of *E. coli* DNA preparations. By contrast T7 DNA increases the sensitivity of rRNA synthesis to ppGpp while concomitantly reducing T2 RNA synthesis. This result suggests that T7 DNA has binding sites for a form of RNA polymerase with a high affinity for T2 promoters and a low affinity for rRNA promoters. Thus T7 DNA reduces the interference in the initiation of rRNA synthesis resulting from the binding of this form of the enzyme to rRNA promoters and hence stimulates the overall extent of rRNA synthesis.

The experiments in this paper support the hypothesis that RNA polymerase can exist in functionally distinct forms each of which preferentially initiates at a particular class of promoter sites. The possible existence of such isomers of the enzyme is made more credible by the identification of two forms of polymerase holoenzyme which differ in the rate at which they bind rifampicin [9].

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