EFFECT OF H_1 PROTEIN ON IN VITRO RIBOSOMAL RNA SYNTHESIS

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1. Introduction

The in vitro synthesis of *Escherichia coli* ribosomal RNA (rRNA) is strongly dependent on the conformation of the DNA template [1]. With naked DNA two forms of the rRNA promoter can be operationally distinguished at 0.1 M KCl; below 35°C the promotor is closed, above 36°C it is open. Yet in vivo the DNA is probably covered with tightly bound protein [2]. Three *E. coli* DNA binding proteins H_1 [3,4], H_2 [4] and D [5] have been shown to stimulate the initiation of phage RNA synthesis in vitro. We show here that H_1 protein also increases the transition temperature between the open and closed forms of the rRNA promotor, this increase being about 6°C when H_1 and DNA are present in equal weights.

2. Materials and methods

2.1. *RNA polymerase and E. coli DNA*

RNA polymerase containing sigma factor was prepared from *E. coli* CA 285 by the method of Burgess and Travers [6]. *E. coli* DNA was prepared according to the method of Marmur [7]. H_1 protein was prepared according to the method of Cukier-Kahn et al. [4].

2.2. *Preparation of in vitro RNA*

The reaction mixtures for in vitro RNA synthesis contained in a volume of 0.2 ml 0.04 M Tris–HCl (pH 7.9 at 20°C), 0.1 M KCl, 0.01 M $MgCl_2$, 0.002 M dithiothreitol, 0.0001 M EDTA, 0.25 mM ATP, 0.25 mM CTP, 0.25 mM GTP, 0.0045 mM [^{3}H]UTP (specific activity 13 000 Ci mol^{-1}), *E. coli* DNA 20 μg ml^{-1} RNA polymerase holoenzyme 20 μg ml^{-1} and H_1 protein as specified. Before the addition of RNA polymerase the reaction mixture was incubated for 10′ at the indicated temperature. RNA synthesis was then started by the addition of RNA polymerase and allowed to proceed for 15′ at the same temperature. The reaction was terminated by the addition of pancreatic DNase to a final concentration of 50 μg ml^{-1}. The incubation was continued for a further 10′ after which the reaction mixture was diluted with an equal volume of 4 X SSC and the resulting mixture was shaken with an equal volume of water saturated phenol. The aqueous phase resulting from this extraction was used directly as a source of RNA for hybridisation. The amount of total RNA synthesis was determined by trichloracetic acid precipitation of a 20 μl aliquot of the aqueous phase.

2.3. *Determination of rRNA in the in vitro transcript*

The amount of rRNA in labelled *E. coli* RNA was determined by hybridisation in triplicate of 50 μl aliquots of RNA to denatured *E. coli* DNA in the presence and absence of 6 μg ml^{-1} unlabelled rRNA [8]. The hybridisation efficiency of in vivo ^{32}P-labelled rRNA to *E. coli* DNA varied between 18–22% in the experiments described. Where appropriate total rRNA synthesis is calculated assuming that the

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efficiency of hybridisation of in vitro synthesised RNA is the same as that of in vivo labelled rRNA.

3. Results

The effect of H_1 protein on in vitro transcription is dependent upon the weight ratio of H_1 to DNA [4]. To test the effect of H_1 on transcription of rRNA from the open form of the rRNA promoter *E. coli* DNA was first incubated in the presence of increasing amounts of H_1 at 38°C and 0.1 M KCl. RNA synthesis was then started by the addition of RNA polymerase and the resulting transcript analysed for total and rRNA synthesis. Fig. 1 shows that as the H_1 concentration increases total *E. coli* RNA synthesis is stimulated to about 50% when equal weights of DNA and H_1 are present. Further increase in H_1 then inhibits transcription. The effect of H_1 on *E. coli* DNA transcription is thus very similar to its effect on T4 DNA transcription [4]. In contrast rRNA synthesis initially parallels total RNA synthesis but at higher concentrations is strongly inhibited, both absolutely and relatively to total RNA synthesis. Indeed the proportion of rRNA in the transcript changes from ~11% at a H_1/DNA weight ratio ~0.5 to ~2% at

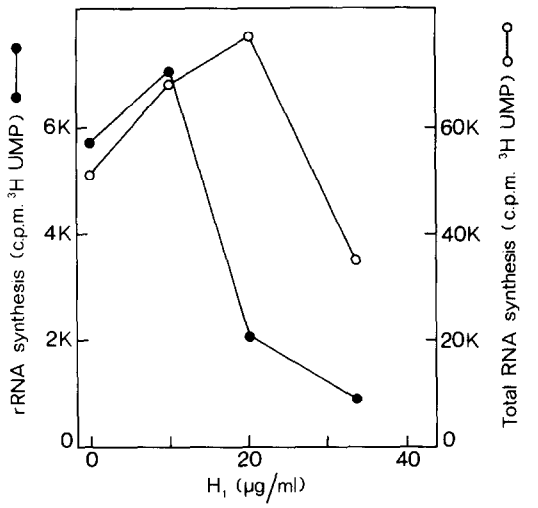

Fig. 1. Effect of H_1 concentration on total RNA and rRNA synthesis at 38°C.

H_1/DNA ~1. Thus H_1 clearly can alter the quality of an in vitro transcript in a specific manner.

Since the rate of rRNA synthesis is strongly temperature dependent [1] the effect of H_1 on rRNA synthesis was tested at temperatures between 28° and 45°C. Table 1 shows that with equal weights of

Table 1
Effect of H_1 protein on in vitro rRNA synthesis

Temperature (°C)	No H_1			+ H_1 (20 μg/ml)		
	RNA synthesis (cpm ^{3}H UMP) Total	rRNA	% RNA	RNA synthesis (cpm ^{3}H UMP) Total	rRNA	% rRNA
28	17 100	880	5.2	21 470	1470	6.8
30	17 050	990	5.8	15 300	1030	6.7
33	41 020	1030	2.5	n.d.	n.d.	n.d.
34	56 830	1420	2.5	56 900	1020	1.8
38	45 410	5090	11.2	68 610	1850	2.7
39	51 320	5030	9.8	69 230	970	1.4
40	62 000	6700	10.8	91 000	2640	2.9
42	62 170	6020	9.7	73 810	9580	13.0
45	44 000	6210	14.1	43 600	5570	12.8

The data shown for each temperature is the result of a single experiment. Each temperature point, with the exception of 30°C and 45°C was repeated at least three times. In no case did the % of rRNA in the transcript differ by > 2% from the values shown; n.d. = not determined.

H_1 and DNA H_1 markedly reduced the proportion of rRNA in the transcript only over the temperature range of 38°–40°C. At 42°C and above H_1 had no effect on rRNA synthesis from the open form of the rRNA promoter. Similarly at 34°C and below H_1 did not significantly affect the low rate of rRNA synthesis from the closed form of the rRNA promoter. Thus we conclude that one effect of H_1 is to increase the transition temperature between the open and closed forms of the rRNA promoter.

4. Discussion

We have shown that H_1 protein can preferentially affect in vitro rRNA synthesis relative to total *E. coli* RNA synthesis by increasing the transition temperature between the open and closed forms of the rRNA promoter. Two lines of evidence suggest that this effect on rRNA synthesis is probably an effect on initiation. First H_1 clearly stimulates transcription of T4 DNA by stimulating the initiation of RNA chains [3]. Second, agents such as glycerol which affect rRNA synthesis in vitro by depressing the transition temperature between two forms of the promoter, alter the level of initiation by acting principally on the DNA template and not RNA polymerase [9].

During the initiation of RNA synthesis by RNA polymerase holoenzyme the initial recognition of a promoter site is followed by localised strand separation [10–12]. On this model a DNA binding protein could affect the rate of RNA chain initiation either by altering the recognition properties of the DNA or by increasing or decreasing the energy required for local melting. H_1 preferentially increases the T_m of G–C rich DNA while destabilising A–T rich DNA [13]. This property is clearly compatible with the latter mechanism. Nevertheless it remains possible that H_1 may act by altering the secondary structure of the promotor, possibly by inducing changes in the diameter of the double helix.

Although there is no direct indication of the in vivo role of H_1, a possible function for this protein would be to stabilise the conformation of the DNA genome, particularly in promoter and other control regions. This would imply that the rRNA promoters would normally be closed in vivo and so would be fully consistent with the observation that the in vivo characteristics of the control of rRNA synthesis are only paralleled in vitro by synthesis from the closed form of the promoter [14].

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