ppApp ALTERS TRANSCRIPTIONAL SELECTIVITY OF *ESCHERICHIA COLI* RNA POLYMERASE

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1. Introduction

In bacteria the nucleotide ppGpp, guanosine 3'-diphosphate 5'-diphosphate, has been implicated as a principal effector of the stringent response [1–3]. In vivo its accumulation is, in general, strongly correlated with a cessation of stable RNA synthesis, an effect which is mimicked in both crude and highly purified in vitro systems [4–7]. One target of ppGpp is RNA polymerase [7]. Physiological concentrations of the nucleotide selectively reduce the ability of the enzyme to initiate at the ribosomal RNA (rRNA) and sub1 tRNA promoters relative to phage φ80 and λ promoters [6–8], an effect which is reflected in a decreased salt optimum for rRNA synthesis.

Recently ppApp, the adenine analogue of ppGpp, has been reported to accumulate during the initiation of sporulation of *Bacillus subtilis* [9]. Like ppGpp [10], ppApp is synthesised by ribosome-associated factors [11]. In this paper I show that ppApp can also affect transcriptional selectivity in vitro. However, by contrast to ppGpp, ppApp increases the optimal salt concentration for the initiation of rRNA synthesis. Thus in this respect ppApp and ppGpp act as functionally opposing effectors.

2. Materials and methods

2.1. Materials

RNA polymerase was prepared by the method in [12] from *Escherichia coli* MRE 600. Enzyme so prepared was >95% pure as judged by polyacrylamide gel electrophoresis and contained at least 0.75 mol σ subunit/2 mol α subunit. λ ds ilv DNA [13] was prepared by gentle phenol extraction of purified phage particles [7]. Adenosine 3'-diphosphate 5'-diphosphate and guanosine 3'-diphosphate 5'-diphosphate were obtained from ICN Pharmaceuticals.

2.2. In vitro transcription

The reaction mixtures for rRNA synthesis contained 0.04 M Tris–HCl, pH 7.9, 10 mM MgCl₂, 6 mM 2-mercaptoethanol, 250 µM each of ATP, CTP and GTP, 0.004 mM [³H]UTP (spec. act. 23 Ci/mmol), KCl and DNA as indicated. The reaction mixture was preincubated for 5 min at 30°C and RNA synthesis was started by the addition of RNA polymerase holoenzyme to a final 20–40 µg/ml and allowed to proceed for 15 min at 30°C. RNA synthesis was terminated by the addition of 200 µl 0.06 M NaCl, 0.06 M Na citrate and 400 µl water-saturated phenol. rRNA synthesis was analysed as in [7].

To measure preinitiation complex formation between RNA polymerase and rDNA, reaction mixtures lacking nucleoside triphosphates and containing 19 µg/ml λ ds ilv DNA, but otherwise as above, were preincubated for 5 min at 30°C. RNA polymerase was then added to 200 µg/ml and the incubation continued for 10 min at 30°C. Heparin was then added to a final 400 µg/ml, together with the nucleoside triphosphates including 0.018 mM UTP (spec. act. 4.5 Ci/mmol). The incubation was continued for 20 min at 30°C and rRNA synthesis determined.
3. Results

To investigate possible effects of the purine nucleoside tetraphosphates on transcriptional selectivity, rRNA synthesis from \( \lambda d_\delta ilv \) DNA [13] was measured as a function of KCl concentration, a parameter known to affect both the non specific binding of RNA polymerase to DNA [14] and its capacity to transcribe different DNA templates [15]. In vitro the functional interaction of ppGpp with RNA polymerase is apparently at least biphasic, for whereas the \( K_i \) for the selective inhibition of rRNA synthesis is \( \sim 10^{-4} \) M [6,7] that for the inhibition of su\(_{\text{III}}\) tRNA transcription [8] and of poly d(I-C) directed GpCpG formation [16] is \( \sim 5 \times 10^{-6} \) M. Figure 1 shows that, in agreement with [6,7], addition of \( 2 \times 10^{-4} \) M ppGpp greatly increased the inhibition of RNA transcription by increasing salt concentration. A lower nucleotide concentration, \( 10^{-5} \) M, although again strongly inhibiting rRNA synthesis at high ionic strength substantially stimulated the production of this RNA at low ionic strength. By contrast at \( 10^{-5} \) M ppApp elicited the opposite response to that observed with ppGpp, inhibiting rRNA synthesis at low ionic strength and stimulating it at high, the optimal KCl concentration being increased from \( \sim 0.06 \) to \( 0.11 \) M. Raising ppApp to \( 2 \times 10^{-4} \) M further increased this optimum to \( \sim 0.16 \) M KCl. Thus ppApp and ppGpp alter the in vitro characteristics of rRNA synthesis in opposing ways respectively increasing and decreasing the optimal salt concentration.

It is clear that the response of rRNA synthesis to increasing concentration of either purine nucleoside tetraphosphate is strongly dependent on KCl concentration. Accordingly, the detailed dependence of rRNA transcription on ppApp concentration was determined at 0.06 M, 0.11 M and 0.16 M KCl. At all salt concentrations a bimodal response of rRNA synthesis was apparent with maxima at \( 5\sim 20 \times 10^{-6} \) M and \( 1\sim 2.5 \times 10^{-4} \) M ppApp (fig.2). The precise
Table 1

<table>
<thead>
<tr>
<th>ppApp</th>
<th>ppApp present during</th>
<th>Total RNA</th>
<th>rRNA in hybrid</th>
</tr>
</thead>
<tbody>
<tr>
<td>(M)</td>
<td>preincubation</td>
<td>synthesis</td>
<td>(cpm [³H]UMP/50 µl reaction mixture)</td>
</tr>
<tr>
<td>0</td>
<td>–</td>
<td>–</td>
<td>64 212</td>
</tr>
<tr>
<td>10⁻⁵</td>
<td>+</td>
<td>–</td>
<td>59 445</td>
</tr>
<tr>
<td>–</td>
<td>+</td>
<td></td>
<td>68 830</td>
</tr>
<tr>
<td>2 × 10⁻⁴</td>
<td>+</td>
<td>+</td>
<td>70 382</td>
</tr>
<tr>
<td>–</td>
<td>+</td>
<td></td>
<td>61 178</td>
</tr>
</tbody>
</table>

KCl was 0.06 M

Effect of ppApp on the formation of preinitiation complexes of λ d₅ ilv DNA

position of these maxima was KCl dependent, such that a higher KCl concentration raised the ppApp concentrations at which both maxima were observed.

ppGpp directly interacts with free RNA polymerase and alters the capacity of the enzyme to form preinitiation complexes at different promoters [7]. To test whether ppApp acts at a similar stage in the transcription process RNA polymerase was first preincubated with λ d₅ ilv DNA in the absence of nucleoside triphosphates to allow the formation of polymerase-promoter preinitiation complexes. These complexes were then assayed by the simultaneous addition of nucleoside triphosphates and heparin, a polyanion that sequesters polymerase molecules which are free or weakly bound to DNA [17,18]. When ppApp was present during the preincubation and subsequent synthesis rRNA production was stimulated by both low and high concentrations of the nucleotide (table 1). By contrast ppApp did not significantly affect either total or rRNA synthesis when the nucleotide was added immediately after heparin. Thus to influence transcription ppApp, like ppGpp, must be present prior to the initiation of RNA synthesis.

4. Discussion

The experiments described here show that adenosine 3’-diphosphate 5’-diphosphate can alter transcriptional selectivity in an in vitro system containing RNA polymerase holoenzyme and a DNA template as the sole macromolecular components. In particular ppApp affects rRNA transcription in an opposite manner to another transcriptional effector, ppGpp. The latter nucleotide alters the pattern of in vitro transcription by changing the promoter preference of RNA polymerase [7]. The necessity for ppApp to be present prior to the initiation of an RNA chain suggests that this nucleotide may also act in this way. The opposing effects of ppApp and ppGpp on transcriptional selectivity are paralleled by opposing effects on the structure of RNA polymerase holoenzyme. We show elsewhere (Debenham, Buckland, Butler and A.A.T., in preparation) that ppApp increases and ppGpp decreases the apparent sedimentation coefficient of the enzyme by ~0.5 S. ppApp and ppGpp thus provide another example of functionally opposing effectors, a regulatory mode also invoked for the purine nucleotides cAMP and cGMP [19].

An unusual feature of the response of rRNA synthesis to ppApp is its bimodal nature. A possible explanation for this phenomenon may be differential effects of the nucleotide on initiation from each of the two tandem promoters of an rRNA cistron [20].

To what extent is regulation of transcriptional selectivity by the purine nucleoside tetraphosphates applicable to in vivo transcription pattern. While the occurrence of ppGpp in E. coli is well documented, ppApp has not yet been detected in this organism. By analogy with the regulation of ppGpp levels [3] if ppApp does occur in E. coli its maximum level should be attained during nutritional upshift. However, the reported occurrence of the latter nucleotide in B. subtilis [8] suggests that ppApp may have a direct role in the control of sporulation. Thus its ability to act as a transcriptional effector in vitro implies that the changes in transcription occurring at the onset of the sporulation process may be initiated by the direct interaction of nucleoside polyphosphates with RNA polymerase.
Acknowledgements

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References