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Protein synthesis inhibitors and catalytic RNA

Effect of puromycin on tRNA precursor processing by the RNA component of *Escherichia coli* RNase P

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RNase P and ribosomes must interact with similar substrate molecules, tRNA precursors in the case of RNase P and aminoacyl-, peptidyl- or free tRNAs in the case of ribosomes. In order to compare the substrate recognition mechanisms between ribosomes and RNase P, protein synthesis inhibitors have been assayed for their effect on the catalytic activity of the RNA component of *Escherichia coli* RNase P (M1 RNA). Puromycin has an inhibitory effect that could be related to similar substrate recognition mechanisms by rRNA in the ribosome and by M1 RNA in RNase P.

RNase P; RNA, catalytic; RNA, M1; Puromycin; Protein synthesis

1. INTRODUCTION

RNase P is an endoribonuclease which cleaves tRNA precursors to generate the 5′-end of mature tRNAs [1] leaving 3′-OH and 5′-phosphate at the cleavage site [2]. In prokaryotes, this enzyme consists of a catalytic RNA (M1 RNA in *Escherichia coli*) and a protein cofactor [3,4]. Both components are essential in vivo [5], but in vitro the RNA component can process tRNA precursors in the absence of the protein. This has been demonstrated in *E. coli* [6], *Bacillus subtilis* [7] and *Salmonella typhimurium* [8]. It is remarkable that the *E. coli* enzyme can cleave not only all the different tRNA precursors in *E. coli*, but also those from other species [9] and unusual substrate molecules [10]. The mechanism by which RNase P or its catalytic RNA subunit is able to recognize all these different substrates and cleave them at the correct site remains unknown.

Ribosomes must also recognize many different tRNA molecules either as aminoacyl-, peptidyl- or free tRNA in a precise way at the A-, P- or E-sites. There is now firm evidence that ribosomal RNA plays an essential role in tRNA binding during protein synthesis [11] and is itself directly and crucially involved in the process of translation [12]. Recently, a direct and functionally relevant interaction between tRNAs and 16 S RNA has been shown [13].

It has been proposed that a similar mechanism is used by rRNA and RNase P RNA for recognition of tRNAs [14]. This suggestion is based on similarities between the secondary structure of 16 S rRNA and a proposed secondary structure for M1 RNA [14]. However, these similarities are not supported by other models of the secondary structure of M1 RNA [15,16], although no extensive comparison of the primary and secondary structure between RNase P RNA and rRNAs has been made.

Many antibiotics that inhibit protein synthesis interfere with functions related to tRNA binding or movement along the ribosome cycle [17]. It has been shown that some of these antibiotics bind to rRNA at positions involved in tRNA interactions [18,19].

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In order to investigate whether RNase P RNA and rRNA recognize tRNAs in an analogous way, I have studied the inhibition of M1 RNA catalytic activity by a number of protein synthesis inhibitors. Only puromycin showed a significant inhibitory effect. The implications of this result are discussed.

2. EXPERIMENTAL

M1 RNA was prepared by in vitro transcription with T_7 RNA polymerase using as template plasmid pJA2 digested with *Fok*I, as described [20]. The protein cofactor (C5 protein) was purified from an overproducing *E. coli* strain as in [20].

^{32}P-labelled substrate was prepared by in vitro transcription with T_7 RNA polymerase, in the presence of [^{32}P]GTP, of a plasmid that contains a synthetic gene for the precursor of $tRNA^{Tyr}$ (Baer, M. and Guerrier-Takada, C., unpublished). Run-off digestion of this plasmid after digestion with *Fok*I produces an RNA 131 nucleotides long corresponding to the precursor of $tRNA^{Tyr}$ found in vivo.

RNase P activity of M1 RNA was assayed in 10 μl of a reaction mixture containing 50 mM Tris-HCl (pH 7.5), 100 mM NH_4Cl, 100 mM $MgCl_2$, 2.4 ng/μl of M1 RNA and 1000–2000 cpm of ^{32}P-labelled substrate. RNase P activity of the holoenzyme was assayed in 10 μl of a reaction mixture containing 50 mM Tris-HCl (pH 7.5), 400 mM NH_4Cl, 10 mM $MgCl_2$, 0.3 ng/μl of M1 RNA, 0.6 ng/μl of C5 protein and 1000–2000 cpm of ^{32}P-labelled substrate. Reaction mixtures were incubated with the indicated concentrations of antibiotic for 5 min at 37°C prior to the addition of substrate. After addition of the substrate, reaction mixtures were incubated for 5–10 min at 37°C and the degree of processing measured by separating the substrate from the products on 8% acrylamide, 7 M urea gels and densitometric analysis of the corresponding autoradiograms, as described [20].

3. RESULTS AND DISCUSSION

Various protein synthesis inhibitors were assayed for their effect on the RNase P activity of M1 RNA. These inhibitors were selected because their inhibition is related to interference with some tRNA function [17] or because their interaction with ribosomal RNA is well documented [18,19]. Streptomycin, neomycin, kanamycin, kasugamycin, sparsomycin, spectinomycin and paramomycin had no effect on RNase P activity at concentrations as high as 20 mM. Puromycin showed an inhibitory effect (fig.1).

Puromycin produced 50% inhibition at a concentration of ~3 mM, with an M1 RNA concentration of 20 nM in the assay mixture. This result suggests that, if inhibition is due to the binding of

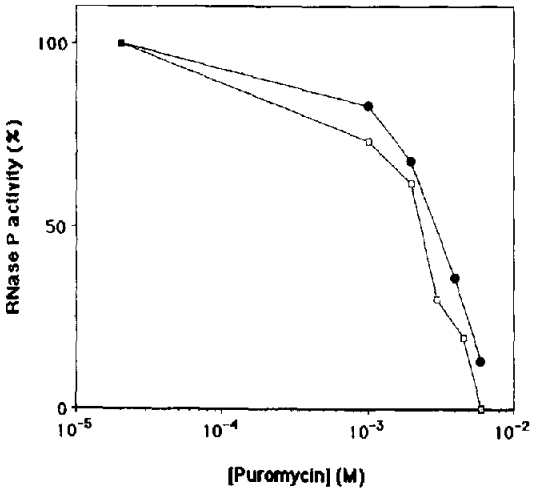

Fig.1. Effect of puromycin on precursor tRNA processing by M2 RNA (●) and the RNase P holoenzyme (□). Assays were performed as described in section 2 with the indicated concentrations of puromycin. Activity is expressed as percentage of that obtained in the absence of puromycin.

one molecule of puromycin to M1 RNA, the binding constant is of the order of 10^3. This is only one order of magnitude lower than the binding constant of puromycin to ribosomes (~10^4) [21]. The RNase P holoenzyme is also inhibited at the same or slightly lower puromycin concentrations (fig.1). This inhibition is reduced when lower NH_4Cl concentrations are used (Guerrier-Takada, C. and Altman, S., personal communication).

Puromycin is an analog of the 3′-terminal end of aminoacyl-tRNA and can function as a substrate for the peptidyltransferase activity of the ribosome. Through its -NH_2 group, puromycin can be linked to the C-terminal end of a peptidyl group [17]. These data indicate that the peptidyltransferase center recognizes aminoacyl-tRNAs by the 3′-end of the tRNA part, and does not require the whole tRNA molecule for recognition. Similarly, RNase P does not require the whole tRNA molecule and can process truncated tRNA precursors that contain only the aminoacyl and T stems of the tRNA moiety and the T loop [23].

The 3′ CCA end of tRNAs has been implicated in the interaction of tRNA precursors with M1 RNA [22,23]. However, *B. subtilis* RNase P appears to be unaffected by the presence or absence of CCA in their substrates [24]. It would be in-

teresting to determine whether puromycin has any effect on *B. subtilis* RNase P RNA activity.

In any case, inhibition by puromycin of tRNA precursor processing by M1 RNA or the RNase P holoenzyme suggests that M1 RNA and 23 S RNA recognize their substrates in a similar manner. A specific region in 23 S RNA that interacts with puromycin has not been detected, probably due to its low binding constant [19], therefore no direct comparison between M1 RNA and a relevant region of 23 S RNA can be made at this point. However, it is noteworthy that there is a strong similarity in primary and secondary structure between the exit site in *E. coli* 23 S rRNA (positions 2109–2179) and a region in M1 RNA encompassing positions 76–96 and 230–242 (Altman, S., personal communication). Specific recognition of the 3′-terminal adenosine of a tRNA in the exit site of *E. coli* ribosomes has been described recently [25].

In addition, this work suggests that protein synthesis inhibitors could become useful tools in the analysis of the catalytic mechanism of RNase P.

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