EFFECTS OF PURINE AND PYRIMIDINE NUCLEOSIDE 5′-DI(TRI)PHOSPHATE-3′-DIPHOSPHATES ON THE *ESCHERICHIA COLI* CELL-FREE TRANSCRIPTION AND TRANSLATION ACTIVITY

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1. Introduction

The pleiotropic roles of (p)ppGpp as a unique effector or a signal molecule in stringent control of bacteria have been well studied [1–3]. Occurrence and special function of its adenine analogues (p)ppApp were suspected in possible relation to the bacterial growth and sporulation [3–7]. We have studied the effects of the 8, namely cytidine and uridine as well as the above 2 purine nucleoside 5′-di(tri)phosphate-3′-diphosphates on the wheat germ cell-free eukaryote mRNA translation system and found that each of them exerts various and specific effects, either positive or negative, depending on the mRNAs employed [8]. Here, we have tested the effects of these 5′,3′-polyphosphonucleotides (p)ppNpp on the bulk RNA and protein synthesis by *Escherichia coli* cell-free coupled transcription–translation and uncoupled poly(U)-directed translation systems.

2. Methods and materials

(p)ppGpp and (p)ppApp were generous gifts from Sanraku Ocean Co. (Tokyo). (p)ppCpp and (p)ppUpp were synthesized utilizing a broad acceptor specificity of *Streptomyces adephospholyticus* [9] or *morookaensis* [10] ATP nucleotide pyrophosphokinase (EC 2.7.6.4) reaction, for example CDP + dATP → ppCpp + dAMP as in [11]. A cell-free coupled transcription–translation lysate S-30 was prepared from *E. coli* CP-78, a stringent strain kindly donated by Dr Kaziro [12], and used according to [13]. DNA and unfractionated tRNA were prepared from the same strain as in [14] and [15], respectively. [5,6-^{3}H]UTP (42 Ci/mmol), [U-^{14}C]leucine (339 mCi/mmol) and [U-^{14}C]phenylalanine (521 mCi/mmol) were purchased from the Radiochemical Centre (Amersham).

3. Results and discussion

The above lysate incorporated both UMP and leucine to the extents of 0 or only 10% of that of the complete mixture when DNA was omitted or 10 μM rifampicin was added. Puromycin at 10 μM caused 90% inhibition of leucine incorporation. The experiments showed that only (p)ppGpp distinctly inhibited both the transcription (UMP) and translation (leucine incorporation) steps while all the other (p)ppNpp were without effects in the coupled system (fig.1). None of these, including (p)ppGpp, exerted any significant effects when added to the uncoupled, poly(U) translation system. Apparent slight stimulation of the phenylalanine incorporation by ppApp and ppCpp might be regarded, in view of their inertness in the coupled expression system above, as accidental or unspecific effects which may possibly happen in this kind of unstable lysate system even if or even all the the more when they are not natural effectors (fig.2). It can thus be said that the inhibition of leucine incorporation by ppGpp in the coupled system is the effect of specific ppGpp inhibition of relevant mRNA- as well as rRNA- [14] and some tRNA-gene transcriptions as characterized in [1,2], excluding its direct effect on the translational step. Since rRNA synthesis is usually ~25% of the total RNA synthesis in [14], the above 50% inhibition of UMP incorporation by ppGpp at 1 mM [1] must represent almost complete inhibition of rRNA synthesis, indicating fully preserved

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response to stringent control of the lysate preparation used and so validity of the experimental results. Each pppNpp showed essentially the same effects as the corresponding ppNpp; for example, pppGpp to ppGpp and pppCpp to ppCpp, respectively, in this incorporation (not shown). The same observations were true also of the effects of all the (p)ppNpp in the eukaryotic mRNA translation reaction reported in [8]. Common nucleoside 5′-di- and triphosphates, when tested separately under the same conditions, caused ⩽20% deviation from the control (not shown).

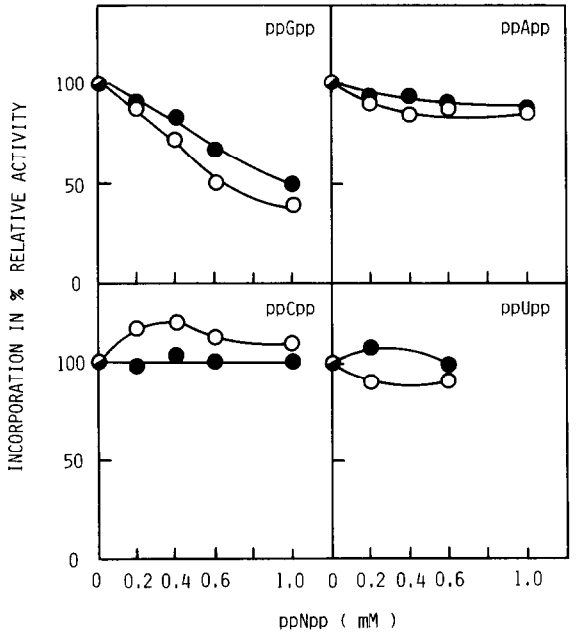

Fig.1. Effects of ppNpp on DNA-dependent bulk RNA and protein synthesis in *E. coli* cell-free, coupled transcription–translation system. Each mixture (50 μl) consisted of 44 mM Tris-acetate (pH 8.2), 55 mM potassium acetate, 27 mM ammonium acetate, 15 mM magnesium acetate, 7.5 mM calcium acetate, 1.4 mM dithiothreitol, 21 mM trisodium phosphoenolpyruvate, 1.4 μg each of pyridoxine–HCl, NADP, FAD and folic acid, 0.6 μg *p*-amino benzoic acid, 0.5 mM cAMP, 2.2 mM ATP, 0.55 mM each of GTP and CTP, 0.1 μCi [^{3}H]UTP, 0.22 mM each of 20 amino acids minus leucine, 0.05 μCi [^{14}C]leucine, 4.5 μg DNA, 5 μg tRNA, one of (p)ppNpp at an indicated concentration and 20 μl S-30 lysate (0.7 mg Folin's protein). The reaction lasted 60 min at 30°C with shaking and was terminated by diluting the mixture with 1 ml 5% casamino acid and cooling it on ice: A 0.5 ml aliquot was mixed with 0.5 ml cold 10% trichloroacetic acid and, after 10 min standing in the cold, filtered through Whatman GF/C filter, washed and dried. The remaining 0.55 ml aliquot was acidified with 0.55 ml 20% trichloroacetic acid, put in a boiling water bath for 10 min, cooled and filtered as above. The radioactivity on the filters was counted in 5 ml Omnifluor toluene (4 g/l) mixture using Beckman LS-3150 scintillation spectrometer. Actual cpm values ranged from 2000–3000: (–○–) [^{3}H]UMP; (–●–) [^{14}C]leucine.

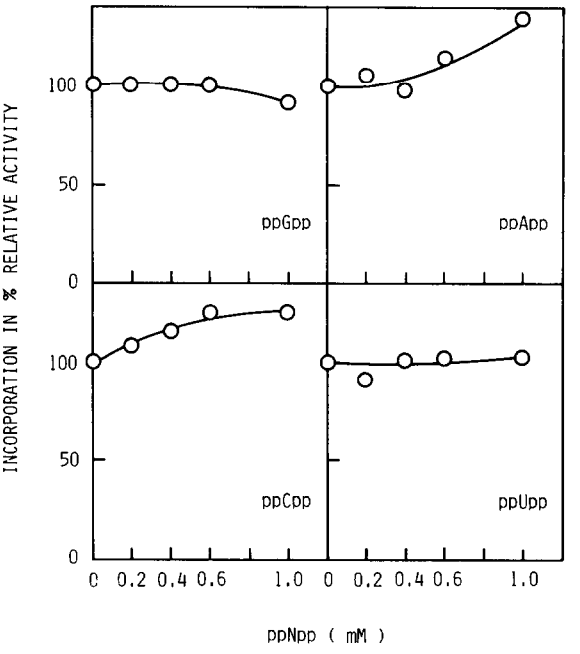

Fig.2. Effects of ppNpp on poly(U)-directed phenylalanine incorporation in *E. coli* cell-free uncoupled translation system. The same reaction conditions were used as in the coupled system (fig.1) except that DNA was omitted, 10 μg poly(U) was added as a mRNA, [Mg^{2+}] and [Ca^{2+}] were 10 mM and 5 mM respectively; 0.04 μCi [^{14}C]phenylalanine was added to each tube and the reaction proceeded for 40 min at 35°C. Termination and incorporation assay were done as for translation in fig.1.

These results confirm that (p)ppGpp is a unique effector or a signal molecule in stringent control and that all the other (p)ppNpp are probably not natural effectors in the bacterial gene expression as far as bulk RNA and protein synthesis are concerned. This situation contradicts our data on the effects of (p)ppNpp on eukaryotic mRNA translation in vitro where each of them exerted unique and mRNA species-specific positive or negative effects. This may reflect the fundamental and qualitative difference of gene expression machinery between the prokaryotes and the eukaryotes discussed in [8]. Natural occurrence and functions of these and other 3′-pyrophosphoryl nucleotides are under study [11,16–19].

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