

Table 1 Radioactivity from the alkaline hydrolysates of rRNAs isolated from *E. coli* CP78 spheroplast

³² P-labelled purine nucleotides	Radioactivity (c.p.m.) obtained from TLC spots with rRNA samples*	
	p16S†	p23S†
³² pppAp	367	0
³² pppGp	0	0
AMP ³²	187	386
GMP ³²	146	337

* γ -³²P-labelled ATP or GTP was used to label RNA isolated from *E. coli* CP78 spheroplasts. The RNA extract (for method see legend under Fig. 1) was subjected to a linear 5–20% sucrose gradient centrifugation at pH 5.1, using SW 40 rotor and Beckman ultracentrifuge L5-40 at 4 °C and centrifuging for 12 h at 25,000 r.p.m. The fractions containing p23S and p16S RNAs were collected and their specific activities determined by A_{260} (410 and 487 c.p.m. μg^{-1} , respectively). These two species of rRNA were first dialysed in distilled water for 6 h to eliminate sucrose, and then hydrolysed separately by treating with 0.3 M KOH at 37 °C for 16 h. The hydrolysate contains nucleotide tetraphosphates and monophosphate⁶. The hydrolysates were then neutralised by adding appropriate volumes of 1 M perchloric acid. The precipitate, potassium perchlorate, was pelleted by centrifugation at 12,000 r.p.m. for 20 min in a Sorvall RC2B centrifuge at 4 °C. Fractions of the supernatant (600 c.p.m. each or approximately 1.5 μg) were spotted on and analysed by thin-layer chromatography using plastic backed sheet obtained from Macherey-Nagel, Polygram cel 300 PEI. Non-radioactive ATP AMP, GTP and GMP were also spotted on the same sheet as reference markers. The chromatographic sheet was run in 1 M LiCl, 1 M acetic acid for 2.5 h at 4 °C. The various spots could be identified by exposing the sheet under a mineral ultraviolet lamp. The tetraphosphates and monophosphates spots were cut out carefully, dried at room temperature for 2 h and then counted on a Packard scintillation counter (Model 3375, Tri-Carb) in toluene-based Omnifluor.

†p16S and p23S refer to precursors of 16S and 23S rRNA species.

and separated. The results are tabulated in Table 1. There is a conspicuous tetraphosphate spot for p16S RNA hydrolysate that is not detectable in the case of p23S rRNA hydrolysate. Therefore, for the p23S rRNA hydrolysate, the region corresponding to the tetraphosphate spot was located by adjusting with the 16S rRNA spot by eye estimation, and a considerable area of the TLC around the region was cut. Lines 1 and 3 of column 4 (Table 1) represent the radioactivity (c.p.m.) of the tetraphosphate spots for p16S (precursor of 16S rRNA) and p23S (precursor of 23S rRNA) rRNA hydrolysates, which are 367 c.p.m. and 0 c.p.m. respectively. The first and third line in Table 1, column 5 are the radioactivity due to XM³²P spots of p16S and p23S hydrolysates.

Table 1 indicates that there is a breakdown of the γ -³²P-triphosphate label, but the extent is not very high. Considering the specific activity of γ -³²P-ATP (2.5 $\mu\text{Ci ml}^{-1}$) used for labelling rRNAs and the fact that only one 5'-³²P-labelled ATP would appear on the 5' end of each p16S RNA molecule the amount of ³²P-monophosphate, incorporated along the length of p16S or p23S rRNA is calculated as less than 0.0001% of input radioactivity. Incorporation of the labelled phosphorus into the entire length of the rRNA molecules is presumably the reason for the presence of approximately double the number of c.p.m. in the monophosphate spots of p23S compared with that of p16S RNA. γ -³²P-nucleotide triphosphates were incorporated into the p16S rRNA but not into the p23S rRNA. Because the first nucleotide incorporated always retains its triphosphate ends, we can minimise the possibility of pyrophosphate exchange into the triphosphate position. Therefore any radioactivity present in the tetraphosphate spots would be the γ -labelled ³²P-nucleotide triphosphates.

To verify which of the two purines (γ -³²P-ATP or γ -³²P-GTP) was incorporated as the first nucleotide, similar experimental procedures were repeated using only γ -³²P-GTP. Results are tabulated in Table 1, lines 2 and 4. Tetraphosphate spots of both p16S and p23S RNA hydrolysates contained no detectable radioactivity. This suggests that GTP was not the

initiating nucleotide triphosphate and therefore ATP must be the first nucleotide incorporated.

We therefore conclude, since the p16S rRNA hydrolysate contained ³²P-radioactivity in its tetraphosphate spot and p23S rRNA did not, that: (1) initiation of rRNA transcription can only take place in the 16S rRNA gene, (2) ATP is the initiating nucleotide, and (3) transcription initiating at 16S rRNA proceeds towards 23S rRNA without further reinitiation.

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- Doolittle, W. F., and Pace, N. R., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1786 (1971).
- Doolittle, W. F., and Pace, N. R., *Nature*, **228**, 125 (1970).
- Bleyman, M., Kondo, M., Hecht, N., and Woese, C. R. *J. Bact.*, **99**, 535 (1969).
- Nikolavev, N., Silengo, L., and Schlessinger, D., *J. biol. Chem.*, **248**, 7967 (1973).
- Takanami, M., *J. molec. Biol.*, **23**, 135 (1967).
- Maitra, V., and Hurwitz, J., *Proc. natn. Acad. Sci. U.S.A.*, **54**, 815 (1975).
- Bea-Hamida, F., and Gros, F., *Biochimie*, **53**, 71 (1971).
- Guha, A., Saturen, Y., and Szybalski, W., *J. molec. Biol.*, **56**, 53 (1971).
- Jorgensen, S. E., Buch, L. B., and Nierlich, D. P., *Science*, **164**, 1067 (1969).

Corrigenda

In the article "Collimation of auroral particles of time-varying acceleration" by D. S. Hall and D. A. Bryant (*Nature*, **251**, 402; 1974) there is an error in equation (10). The denominator should read $\sqrt{2}(\sigma/E_0)$ and not as printed. Also the value of A in the legend to Fig. 1 should read $9.1 \times 10^8 \text{ cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1} \text{ keV}^{-1}$ and not as printed.

In the article "Oxidation of nickel by nitric oxide as a new strong oxidant" by Y. Takasu, Y. Matsuda, S.-I. Maru and N. Hayashi (*Nature*, **255**, 544; 1975) there are several errors in the figures and legends. On the ordinate of Figs 2 and 3, for K_1 read K_1' . The legends to Figs 2 and 3 should read:

Fig. 2 Relationship between K_1' ($\text{g cm}^{-2} \text{ s}^{-1}$) and temperature ($P_{\text{NO}} = 10 \text{ mmHg}$). $E = 17.8 \text{ kcalorie}$.

Fig. 3 Relationship between K_1' ($\text{g cm}^{-2} \text{ s}^{-1}$) and the pressure of nitric oxide (700 °C). $n = 0.74$.

Errata

In the article "Excision of thymine dimers from specifically incised DNA by extracts of xeroderma pigmentosum cells" by K. Cook, E. C. Friedberg and J. E. Cleaver (*Nature*, **256**, 235; 1975) the following corrections should be made. In line 3 of the legend to Table 1, for 50–100 erg mm^{-2} read 5–10 J m^{-2} . The list of authors should include as the last author H. Slor, Department of Human Genetics, University of Tel Aviv, Israel. The asterisk after J. E. Cleaver should indicate that his address is the Laboratory of Radiobiology, University of California School of Medicine, San Francisco, California.

In the article "Pathogenicity and cerato-ulmin production in *Ceratocystis ulmi*" by S. Takai (*Nature*, **252**, 124; 1974) there is an error in the second sentence of paragraph 6. For (120 mg ml^{-1}) read (120 $\mu\text{g ml}^{-1}$).