A THERMOSENSITIVE MUTANT DEFECTIVE IN RIBOSOMAL 30 S SUBUNIT ASSEMBLY

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1. Introduction

A number of *Escherichia coli* mutant strains, defective in ribosome assembly have already been described [1–4]. Several of them are conditional cold-sensitive mutants, which grow normally at 42°, but do not produce active ribosomes at 22°. We have now succeeded in isolating a thermosensitive strain the growth of which stops at 42°. In this preliminary communication, it is shown that this strain fails to assemble normal 30 S subunits at the non-permissive temperature, while it is still able to accumulate 50 S particles which can form 70 S ribosomes with normal 30 S subunits *in vitro*.

2. Experimental

The mutant strain 219 is a K 12 derivative, the phenotype of which is dependent on at least two mutational events. Its isolation and genetics will be described in details elsewhere.

To follow the biosynthesis of proteins or RNA, 3 μCi of L-^{14}C-arginine (3 μCi/mM) or 6 μCi or L-^{14}C-uracile (160 μCi/mM) were added to 10 ml aliquots of cultures grown at 30° on minimal 68 medium [5] containing 0.2% glucose. At the appropriate time, the culture flasks were transfered to a shaking incubator at 42°. Growth was followed by measurement of A_{420} absorbance. ^{14}C-arginine incorporation into hot-acid insoluble precipitate and ^{14}C-uracile incorporation into cold-acid insoluble precipitate were measured on 0.1 ml aliquots.

To prepare doubly labelled cells for sucrose gradient analysis of ribosomes and ribosomal precursors, a 25 ml culture on 1.7% Difco Bacto Tryptone – 0.3% sodium chloride (BT medium) was grown up to 3×10^7 cells/ml in the presence of 30 μCi of ^{14}C-uracile (48 mCi/mM). Cells were harvested and washed once with BT medium supplemented with 200 μg/ml uracile by centrifugation at 0°. Cells were resuspended in 25 ml of BT uracile medium at 30° for 15 min. Growth resumed almost immediately in these conditions. 25 ml of BT uracile medium, prewarmed at 42°, were then added and the culture transfered at 42°. After 20 min, 2 mCi of carrier-free $^{32}PO_4H_3$ were added and the incubation was prolonged for 90 min. Cells were harvested on ice in the presence of 50 mg (wet weight) of carrier exponential *Escherichia coli* RNase I^-_{10} cells and 500 mg of KH_2PO_4. The pelleted cells were washed once in 10 ml of low Mg^{2+} buffer (10 mM Tris-HCl, pH 7.8, 30 mM NH_4Cl, 0.3 mM $MgCl_2$, 6 mM β-mercaptoethanol). The final pellet was resuspended in 1.5 ml of the same buffer containing 30 μg of DNase and homogenized in a French pressure cell at 10,000 psi. Unbroken cells and debris were eliminated at 30,000 *g* for 20 min. 1 ml of the supernatant was layered on a 5–20% sucrose gradient in low Mg^{2+} buffer and spun at 24,000 rpm for 15 hr in a Spinco SW 27 rotor. Gradients were collected in 1 ml fractions on an ISCO D gradient fractionator. 0.1 ml aliquots were diluted in 10 ml of Bray's scintillation fluid [6] and counted in a Packard Tri-Carb 3380.

To measure the association of doubly labelled 50 S particles with normal 30 S subunits, 300 μl of the top fraction of the 50 S peak in the previous gradient were mixed with A_{260} units of 30 S subunits and 2 A_{260} units of 50 S subunits prepared from *Escherichia coli* RNase I^-_{10} [7] in a total volume of 600 μl of buffer adjusted to 10 mM Mg^{2+}. After incubation at

37° for 60 min the sample was layered on a 5–20% sucrose gradient prepared in the presence of 10 mM Mg^{2+} and centrifuged at 20,000 rpm for 15 hr in a Spinco SW 27 rotor. The gradient was fractionated as before and the total fraction was used for measuring radioactivities.

RNA extractions were performed by phenol-m-cresol deproteinization as previously described [8]. Agarose-polyacrylamide gel electrophoresis was done according to Dahlberg et al. [9]. 1 mm slices of gel were cut, incubated overnight in concentrated ammonia and counted in 10 ml Bray's fluid.

3. Results and conclusions

3.1. *Kinetics of incorporation of ^{14}C-uracile and ^{14}C-arginine after transfer to 42°*

As judged by ^{14}C-uracile incorporation in cold-acid insoluble products, the total RNA content of *Escherichia coli* 219 cells increased continuously, although at a progressively diminishing rate, during the first three hours which followed the shift to the non-permissive temperature. The incorporation of ^{14}C-arginine in hot-acid insoluble molecules decreased much more rapidly and almost completely stopped after about 2 hr at 42° (fig. 1). These results suggest

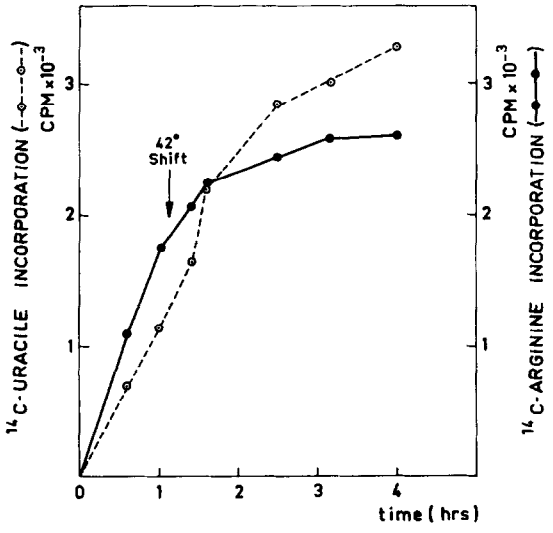

Fig. 1. RNA and protein biosyntheses by mutant 219 during growth at 30° and after a shift at 42°.

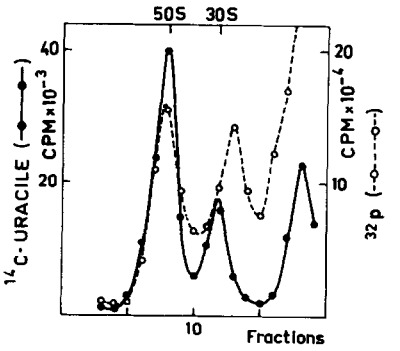

Fig. 2. Sucrose gradient analysis of a crude extract from mutant 219. ^{14}C-uracile was incorporated during growth at 30°. $^{32}PO_4H_3$ was added after shifting to 42°. For details see the experimental part.

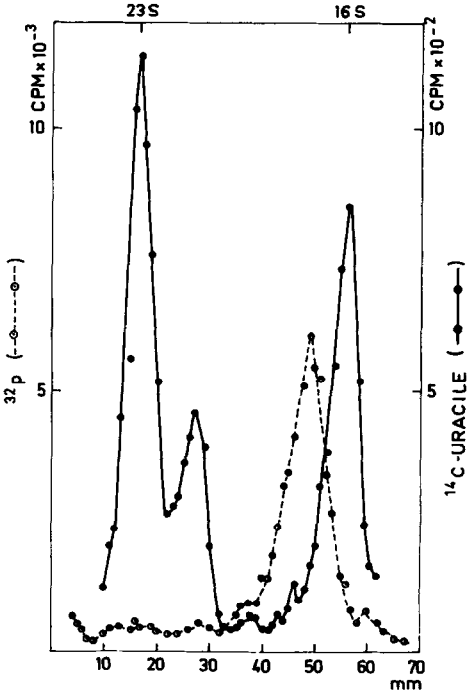

Fig. 3. Gel electrophoresis of ^{32}P-labelled RNA extracted from the 26 S particle accumulated by mutant 219 at 42°. A ^{14}C-labelled sample of ribosomal RNA from mutant 219 grown at 30° was added as a marker. The small ^{14}C-peak on the right of the 23 S peak denotes a slight accidental degradation of the 23 S marker.

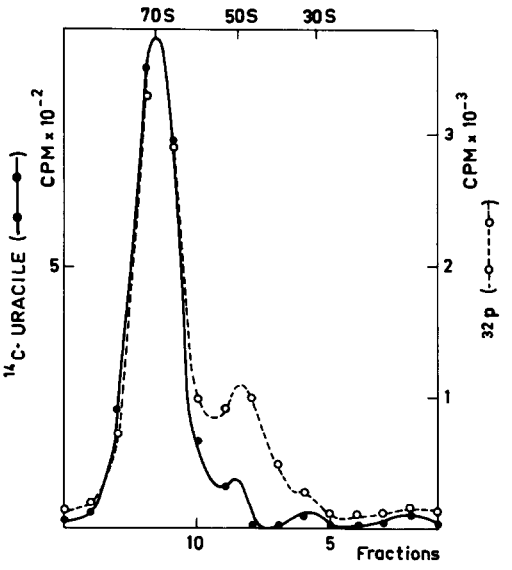

Fig. 4. 70 S ribosome formation from ^{32}P-50 S particles accumulated by mutant 219 at 42° analyzed by sucrose gradient sedimentation.

that the protein-synthesizing machinery of cells which were grown at the permissive temperature was progressively inactivated after the temperature shift.

3.2. *Accumulation of ribosomal subunits and precursors at the non-permissive temperature*

Extracts of cells labelled with ^{14}C-uracile at 30° and with ^{32}P-orthophosphate after transfer to 42° were analyzed on sucrose density gradients prepared in a 0.3 mM Mg^{2+} containing buffer (fig. 2). While a large fraction of the ^{14}C-radioactivity sedimented at 30 S and 50 S, two ^{32}P-labelled peaks sedimenting at 26 S and 50 S were detected.

3.3. *Electrophoretic properties of the ^{32}P-labelled RNA present in the 26 S particle*

The electrophoretic mobility on agarose-polyacrylamide gels of the ^{32}P-labelled RNA extracted from the top fraction of the 26 S peak in fig. 2 was compared with those of ^{14}C-labelled rRNAs extracted from *Escherichia coli* 219 grown at 30° (fig. 3). The ^{32}P-RNA from the 26 S peak, which migrated slightly more slowly than mature ^{14}C-16 S RNA, was similar in this respect to precursor-16 S RNA [10]. It can therefore be concluded that, in the thermosensitive strain 219, the normal assembly of 30 S subunit is prevented at 42°. A 26 S particle, which contains precursor-16 S RNA, accumulates instead.

3.4. *In vitro formation of 70 S ribosomes from the 50 S subunit made at 42°*

An aliquot of the top fraction of the 50 S peak (fig. 2) was incubated for 60 min at 37° in a 10 mM Mg^{2+} containing buffer in the presence of unlabelled normal 30 S and 50 S subunits. Sucrose gradient analysis of the mixture demonstrated that the ^{32}P-labelled 50 S subunits made at the non-permissive temperature were able to associate with 30 S subunits into 70 S ribosomes (fig. 4).

The distribution of ^{14}C- and ^{32}P-radioactivities showed that the affinity for normal 30 S ribosomes of the 50 S subunits formed at 42° was almost the same as that of the ^{14}C-labelled 50 S subunits assembled at 30°. Since no real biological activity test has yet been made on the non-permissive 50 S particles, it would be permature to conclude that in *Escherichia coli* 219, a block in the assembly of the 30 S subunit does not prevent the normal assembly of biologically active 50 S subunit. At least this block does not prevent the formation of particles which sediment at 50 S and which can form 70 S ribosomes.

Acknowledgements

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References

[1] L.J. Lewandowski and B.L. Brownstein, Biochem. Biophys. Res. Commun. 25 (1966) 554.
[2] R.E. McDonald, G. Turnock and J. Forschammer, Proc. Natl. Acad. Sci. U.S. 57 (1967) 141.
[3] C.H. Guthrie, H. Nashimoto and M. Nomura, Proc. Natl. Acad. Sci. U.S. 63 (1969) 384.
[4] H. Nashimoto and M. Nomura, Proc. Natl. Acad. Sci. U.S. 67 (1970) 1440.
[5] G.N. Cohen and H.V. Rickenberg, Ann. Inst. Past. 91 (1956) 673.
[6] G.A. Bray, Anal. Biochem. 1 (1960) 279.
[7] A. Kikuchi and R. Monier, FEBS Letters 11 (1970) 157.
[8] R.A. Gomes, C. Jeantet and R. Monier, European J. Biochem. 8 (1969) 55.
[9] A.E. Dahlberg, C.W. Dingman and A.C. Peacock, J. Mol. Biol. 41 (1969) 139.
[10] G. Attardi and F. Amaldi, Ann. Rev. Biochem. 39 (1970) 183.