Variations in the Average Lifetime of Bacterial Messenger Ribonucleic Acid with Growth Medium and Temperature

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The average lifetime of the messenger RNA of *Escherichia coli* M.R.E. 600 was measured by the kinetic method of Bolton & McCarthy (1962), in which the time-course of labelling unstable messenger RNA and stable RNA is followed during the incorporation of [5-³H]uracil into steadily growing cultures. The labelled RNA in any sample was assayed by DNA-RNA hybridization by the method of Gillespie & Spiegelman (1965), as described for *E. coli* M.R.E. 600 by Gray & Midgley (1968).

Experiments were carried out in lactate-salts, glucose-salts and the enriched-broth media of Roberts, Cowie, Abelson, Bolton & Britten (1957) over the temperature range 25–45°, in increments of 5°. The division times of the cultures were constant to within ± 5 min. at any given temperature and in any given medium. However, cultures grew in lactate-salts, glucose-salts and broth in the rate ratio 1:2:4 at a given temperature.

In lactate-salts and glucose-salts media, the ratio (average lifetime of messenger RNA)/(division time of the cultures) was a constant (0.054 ± 0.003) over the whole range of temperatures. The cells also contained a constant 2.6% of their total RNA as messenger. Similar results were obtained with enriched-broth cultures. In this case the corresponding ratio was 0.095 ± 0.005 , and 3.9% of the total RNA was messenger RNA. At all temperatures, in any growth medium, the rates of entry of labelled nucleic acid precursors (as measured by exogenous uracil) into the messenger RNA and the stable RNA fractions had a constant 1:2 ratio. This indicated a fairly close control between the two biosynthetic processes. Aspects of the cellular control of messenger RNA lifetime in *E. coli* were discussed.

Bolton, E. T. & McCarthy, B. J. (1962). *Proc. nat. Acad. Sci., Wash.*, **48**, 1390.

Gillespie, D. & Spiegelman, S. (1965). *J. molec. Biol.* **12**, 829.

Gray, W. J. H. & Midgley, J. E. M. (1968). *Biochem. J.* **108**, 34P.

Roberts, R. B., Cowie, D. B., Abelson, P. H., Bolton, E. T. & Britten, R. J. (1957). *Publ. Carnegie Instn. no. 607: Studies in Biosynthesis in Escherichia coli*, p. 5.

Growth Hormone and the Binding of Aminoacyl-Transfer-Ribonucleic Acid to Liver Ribosomes

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Hypophysectomy of rats reduces and treatment of rats with growth hormone increases the ability of ribosomes from liver to incorporate amino acids into proteins *in vitro* (Korner, 1968). This communication reports the effect of hypophysectomy and of growth-hormone treatment on the binding of aminoacyl-t-RNA* to liver ribosomes.

Rat liver ribosomes were prepared as described by Munro, Jackson & Korner (1964). The ¹⁴C-labelled aminoacyl-t-RNA was prepared from the pH 5.2 fraction of 104000g supernatant of a homogenate of normal rat liver. It was resuspended in 20 ml. of medium M [20 mM-tris (pH 7.6)–10 mM-magnesium acetate–100 mM-KCl–40 mM-NaCl–6 mM-mercapto-ethanol], charged with 30 μ C of ¹⁴C-labelled amino acid mixture or with [¹⁴C]phenylalanine in the presence of 10 mM-ATP at 37° for 20 min. and extracted with water-saturated phenol at 2° as described by Moldave (1968). The binding of ¹⁴C-labelled aminoacyl-t-RNA to ribosomes was carried out at 37° for 20 min. the presence of 0.05 mM-GTP in medium M with 5 mM-magnesium acetate (Leder, 1968). The ¹⁴C-labelled aminoacyl-t-RNA-ribosomal complex was collected on Millipore filters, which were dried, and the radioactivity was assessed in a Packard scintillation counter.

It was noted that the binding of aminoacyl-t-RNA increased with temperature between 0° and 37°. Omission of GTP decreased binding, and binding was further decreased by addition of the GTP analogue guanylyl methylenediphosphonate (Hershey & Monro, 1966).

The binding of ¹⁴C-labelled aminoacyl-t-RNA to normal ribosomes was greater than to ribosomes from livers of hypophysectomized rats. Binding varied with the concentration of Mg²⁺, but the difference in binding to ribosomes from normal and hypophysectomized rats persisted at all concentrations of Mg²⁺ tested (5–30 mM). Treatment of hypophysectomized rats with ox growth hormone (250 μ g. injected into 150g. rats) either 1 hr. or 12 hr. before death resulted in increased binding of aminoacyl-t-RNA to ribosomes compared with untreated hypophysectomized rats.

Similar differences were noted when binding of [¹⁴C]phenylalanyl-t-RNA to ribosomes in the presence of polyuridylic acid was examined.

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* Abbreviation: t-RNA, transfer RNA.