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.

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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.

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Synthesis of Ribonuclease-Resistant Ribonucleic Acid by Fowl-Plague-Virus-Induced Ribonucleic Acid Polymerase

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An RNA-dependent RNA polymerase is induced in chick-embryo fibroblast cells infected with fowl-plague virus (Skehel & Burke, 1968; Scholtissek & Rott, 1969). The RNA synthesized by this enzyme *in vitro* has now been investigated and compared with RNA formed in infected cells *in vivo*.

Chick-embryo fibroblast cells grown in roller bottles were infected at an input multiplicity of 5–10 plaque-forming units/cell (Borland & Mahy, 1968). The microsomal fraction prepared from cells at 6 hr. after infection was incubated at 28° with a reaction mixture containing (μ moles/ml.): tris-HCl, pH 7.8, 250; 2-mercaptoethanol, 12; $MgCl_2$, 2.5; ATP, CTP and GTP, 0.15 each; [3H]-UTP, 0.005 (5 μ C). Incorporation into acid-insoluble radioactivity increased linearly with time of incubation up to 60 min. When samples of the reaction mixture taken at any time were treated with ribonuclease (1 μ g./ml. for 10 min. in 0.15M-NaCl–0.015M-sodium citrate buffer), only about 50% of the radioactivity was rendered acid-soluble. Labelled RNA extracted from the reaction mixture after 30 min. incubation with phenol–sodium dodecyl sulphate or phenol–cresol (Kirby, 1965) was 37% resistant to ribonuclease treatment. The ribonuclease-resistant fraction showed a sharp thermal transition at about 90° in 0.15M-NaCl–0.015M-sodium citrate buffer.

The polymerase product RNA sedimented in the 7–18s region on sucrose density gradients, and ribonuclease-resistant fractions were found at 12–14s. If the product RNA was treated with ribonuclease before sedimentation, all the acid-insoluble radioactivity banded sharply at 12s. To compare the RNA product obtained *in vitro* with RNA formed *in vivo*, cells were treated with actinomycin D (2 μ g./ml.) at 5.5 hr. after infection then pulse-labelled with [3H]uridine (10 μ C/ml.)

for 15 min. before extraction of RNA from the microsomal fraction. The bulk of the RNA labelled *in vivo* sedimented in the 18s region, i.e. in a similar position to RNA from purified fowl-plague virus (Barry & Davies, 1968), but 5–10% was resistant to ribonuclease and sedimented at 12s.

Hybridization experiments were carried out between 3H -labelled polymerase product RNA and ^{32}P -labelled fowl-plague-virus RNA. The virus RNA was completely susceptible to ribonuclease treatment, but became 37% resistant after mixing with polymerase product RNA under annealing conditions. The results of further annealing studies will be presented.

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The Use of Gradient Centrifugation to Study Events in the Life Cycle of Cultured Cells

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Exponentially growing P815Y neoplastic mast cells (Schindler, Day & Fischer, 1959) were briefly exposed to radioactive precursors, collected by centrifugation, washed and layered on to 40 ml. of a gradient (5–10%, w/v) of Ficoll in growth medium (Morris, Cramer & Reno, 1967). After centrifugation at 80g_{av} for 7 min. the tube was punctured and samples were collected. The number of cells in each sample, and their mean volume, was measured with a Coulter counter. Precursor incorporated into cells was determined by filtering a portion of each sample, washing with trichloroacetic acid and measuring the radioactivity.

The distribution of cells through the gradient showed a broad peak near the centre. The mean volume of cells near the top was about half that at the bottom. When thymidine was used as a precursor, the incorporated radioactivity/cell showed a broad maximum just below the cell peak, in agreement with the results of Morris *et al.* (1967). When proline, uridine or choline was used, the incorporated radioactivity/cell increased from the top to the bottom of the gradient.

When cells that had been separated by gradient centrifugation were resuspended in medium, they grew with a certain degree of synchrony; those from the top of the gradient divided after 10–15 hr. (mean generation time 15 hr.) whereas those from the bottom began division within 5 hr. Hence cells near the top of the gradient appear to be