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# The Metabolic Stability of Ribosomal Protein

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*Summary*. Measurements of the metabolic stability of ribosomal proteins of exponentially growing *Escherichia coli* B/r showed that their rate of degradation to free amino acids is zero to within limits of the experimental accuracy of $\pm 0.7\%$ per hour or $\pm 0.5\%$ per doubling period.

The known instability of ribosomes in *E. coli* upon magnesium starvation (MCCARTHY, 1962) raises the possibility that they may also be unstable during normal growth, though there exist no cogent reasons to suspect that ribosomes *are* degraded in normally growing cells. Previous measurements of ribosome stability in growing *E. coli* have shown that ribosomal RNA is not degraded (DAVERN and MESELSON, 1960), and that the rate of exchange or renewal of the proteins on the "CsCl cores" is less than 5% per hr (MESELSON *et al.*, 1964). (The "CsCl cores" are the subribosomal particles having lost about 40% of the normal ribosomal proteins which are obtained by equilibrium centrifugation of ribosomes in CsCl.) The measurements to be presented here are directed to the, as yet, unanswered question of the stability of *ribosomal proteins* during growth. For this purpose, the rate of degradation of ribosomal protein was measured by following the rate of disappearance of radioactively labeled ribosomal protein from ribosomes.

The ratio of the mass of ribosomal protein to total cellular protein in growing cells closely obeys the relationship

$$\frac{R}{P} = \frac{C}{T} \tag{1}$$

(MAALØE and KJELDGAARD, 1966), where $R$ and $P$ are masses of ribosomal and total protein per unit volume of culture, $C$ is a constant ($C$ equals approximately 8 min) and $T$ is the doubling time of the culture. In the experiment to be reported here the cellular protein was labeled with a radioactive amino acid while the bacteria were growing in a "rich" medium supporting rapid growth (small $T$). Labeling under these conditions therefore placed a high fraction of the radioactivity in ribosomal protein, according to equation (1). Subsequent to the labeling, the bacteria were transferred to a "poor" medium supporting slow growth (large $T$). If the labeled ribosomal proteins are degraded to free amino acids during the slow growth, only a small fraction of the released labeled amino acids would be reincorporated into newly synthesized ribosomal proteins the majority of such amino acids being directed towards nonribosomal proteins,

according to equation (1). The rate of such transfer of radioactivity from ribosomes to nonribosomal protein is directly related to the rate of ribosomal protein degradation. This rate can be calculated from the decrease with time of the ratio of radioactivity in ribosomes to the total radioactivity in all cellular protein, from its initial value shortly after radioactive labeling to its final value after a long period of growth in the "poor" medium. The ratio of labeled amino acids present in ribosomes to those present in total protein can be measured by centrifuging cell extracts through a $D_2O$ sucrose-gradient with density $\varrho$ varying linearly from $\varrho = 1.20$ to $\varrho = 1.30$ and measuring the radioactivity in drops collected. Use of this high density sucrose gradient has been found necessary to prevent nonribosomal proteins from sedimenting with ribosomes (SCHLEIF, 1967).

## Materials and Methods

*E. coli* B/r was grown with a doubling time of 100 min on a mineral salts medium with succinate as the carbon source present at 0.2% ("poor" medium). The "rich" medium, giving a doubling time of 35 min, represents the minimal medium supplemented with glucose at 0.2%, leucine at 20 $\gamma$/ml, 18 other amino acids in the ratio of their occurrence in *E. coli* protein (ROBERTS *et al.*, 1963), and the four bases adenine, guanine, cytosine, and uracil at 40 $\gamma$/ml. Proline was not added to the medium so as to allow its use as a radioactive label.

Bacteria were grown exponentially in the succinate-containing "poor" medium for many generations before the measurement. The supplements necessary to make the "rich" medium were added when the culture had reached an optical density at $\lambda = 450$ m$\mu$ ($OD_{450}$) of 0.1, and 5 min later 30 $\mu$C $^3$H proline was added. The added $^3$H proline was completely incorporated into cellular protein within the next 5 min, after which excess nonradioactive proline was added. After a further 20 min growth, the medium was changed by filtering the culture with a preboiled (to remove plasticizers) 0.45 $\mu$ Millipore filter, and the bacteria collected on the filter were resuspended in the "poor" medium. After 60 min growth, a volume of the culture was withdrawn and added to 50 times its volume of nonradioactive carrier bacteria before washing in $5 \times 10^{-3}$ *M* Tris·HCl pH 7.2, $10^{-4}$ *M* Mg acetate and cell disruption by sonication. The remainder of the culture was diluted 100-fold in "poor" medium and grown exponentially with a doubling time of 100 min. After 14.6 hr of growth, when the $OD_{450}$ had reached 1.2, the bacteria were washed and sonicated. The unfractionated cell extracts were centrifuged 21 hrs at 107,000 g through the $D_2O$ sucrose gradients. Drops from the bottoms of the tubes were dripped directly into scintillation vials and counted. For additional experimental details see SCHLEIF (1967).

## Results

Cells were first grown in the "poor" medium, then shifted to the "rich" medium and pulse labeled with radioactive proline, then shifted back to the "poor" medium. The ratio of radioactivity in ribosomes to total radioactivity in all cellular protein was 0.249 in the extract taken 1 hr after the shift back to the "poor" medium, in agreement with the value of $8/35 = 0.23$ predicted from equation (1). After an additional 14.6 hrs of growth in the "poor" medium the measured ratio was 0.253. The fact that the ratio of radioactivity in ribosomes to the total radioactivity in all cellular proteins did not significantly change during the 14.6 hrs of growth, when, according to equation (1), the fraction of total cellular protein present as ribosomal protein should have fallen to $8/100 = 0.08$ shows that ribosomal protein is degraded at a very low rate, if at all, in exponentially growing *E. coli* B/r.

## Discussion

The measurements presented here show that the rate of loss of radioactively labeled ribosomal protein from the ribosomes of exponentially growing *E. coli* is very low. The rate and error limits on the rate of degradation of ribosomal protein will now be calculated in terms of the measured ratios of radioactivity in ribosomes to total radioactivity.

$$\text{Let } \omega(t) = \frac{\text{Radioactivity in ribosomal protein at time } t}{\text{Radioactivity in total cell protein at time } t},$$

let $x$ be the rate of degradation of ribosomal protein to free amino acids in mass units degraded per mass unit present per unit time, and let $\varepsilon$ be the rate of synthesis of ribosomal protein divided by the rate of synthesis of total protein during the 14.6 hr period of slow growth in the "poor" medium. As ribosomal protein is degraded, a fraction $\varepsilon$ of the resulting freed amino acids is reincorporated into ribosomal protein, and a fraction $1-\varepsilon$ is incorporated into nonribosomal protein. The total radioactivity in ribosomes, $R(t)$, will fall with increasing time as $R(t) = R(0)e^{-(1-\varepsilon)xt}$. The total radioactivity in cells remains unchanged, however, and therefore the fraction of radioactivity in ribosomes $\omega(t)$, falls as $\omega(t) = w(0)e^{-(1-\varepsilon)xt}$. Experimentally it was found that $(1-\varepsilon)xt < 1$, hence the Taylor expansion of the exponential is justified, yielding

$$x = \frac{\omega(0)-\omega(t)}{\omega(0)\,(1-\varepsilon)t} \tag{2}$$

The experimental data are $\omega(0) = 0.249$, $\omega(14.6) = 0.253$, $t = 14.6$, $\varepsilon = 0.08$, and substituting into equation (2) yields $x = -0.12\%/\text{hr}$. Fifteen independent measurements of the value of $\omega$ for *E. coli* B/r cultures growing on glucose minimal medium carried out over the past year have yielded values for $\omega$ ranging from 0.14 to 0.17 with an average of 0.155. From this we can estimate the error in the measurement of $\omega$ is approximately $\Delta\,\omega = 0.015$. From standard methods of error analysis it follows that for an uncertainty $\Delta\,\omega$ in the measurement of $\omega$ the corresponding uncertainty in $x$ is $\Delta\,x = \frac{\sqrt{2}\,\Delta\omega}{\omega(0)\,(1-\varepsilon)\,t}$ and substituting the data from the measurement gives $\Delta\,\omega = 0.7\%/\text{hr}$. The rate of degradation of ribosomal protein is therefore zero to within experimental error.

The discussion has considered the possibility that ribosomal protein is degraded to free amino acids which are then reincorporated into newly synthesized protein. Ribosomal protein could also be lost from ribosomes, but not degraded to free amino acids. In this case, the "degraded" ribosomal protein, freed from the ribosomes would appear in these measurements as nonribosomal protein. Equation (2) giving the rate of degradation of ribosomal protein $x$ is modified in this case by setting $\varepsilon$ equal to zero, and it can be seen that this alters the values of $x$ or $\Delta\,x$ by very small amounts.

Very little interference in these measurements is expected from the degradation of nonribosomal protein, since this rate is low, 0.6% per hr, (WILLETTS, 1967), (we estimate the uncertainty of this measurement is less than 0.1% per hr) and since only 8% of the freed amino acids could enter ribosomal protein.

The rate of degradation of nonribosomal protein was also estimated by similar methods. The cells were labeled when they were growing in "poor" medium so that only a small fraction of incorporated radioactivity entered ribosomes, then grown on the "rich" medium so that the relatively greater rate of ribosomal protein synthesis would incorporate a large fraction (0.25) of freed amino acids into ribosomal protein. In this case the rate of transfer of label *to* ribosomes is related to the rate of degradation of nonribosomal protein. This value was found to be $-0.7\% \pm 2\%$ per hr. It should be remarked that this measurement does not depend on the validity of the assumption used by WILLETTS (1967) that amino acid molecules resulting from protein breakdown are quantitatively released to a medium containing an excess of the amino acid in question.

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