RNA COMPOSITION OF ESCHERICHIA COLI AS A FUNCTION OF GROWTH RATE

by

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The total RNA content per cell of various bacteria is known to vary as a function of the rate of growth. The faster the growth, the higher is the amount of total RNA. For all growth rates higher than a minimal value, linear relationships between the ratios RNA/DNA or RNA/protein and the growth rate have been found and the rate of protein synthesis per unit weight of RNA is approximately constant (1,2,3). Since transfer RNA (t RNA) and ribosomal RNA (r RNA), which together presumably represent 95-97 % of total cellular RNA, are both involved in the constitution of the protein synthesizing machinery, one would a priori expect that the ratio between t RNA and r RNA should remain constant irrespective of the growth rate, in all cases where the relationships mentionned above are observed. Neidhardt and Magasanik (2) have actually concluded that the ratio t RNA/r RNA is constant in Aerobacter aerogenes, but they have examined a limited number of cases only. Their conclusion is not supported by recent findings by Kjeldgaard and Kurland (4) with Salmonella typhimurium. In the latter case, it was found that the ratio t RNA/r RNA continuously increases, when the growth rate decreases, while the ratio t RNA/DNA remains constant.

In the course of studies on the behaviour of t RNA in vivo, it became necessary for us to examine the situation in Escherichia Coli. E. Coli, strain ML308, was grown at 30°C with aeration on a basal mineral medium containing in 1 liter : $K H_2PO_4$ 13.6g, $Mg SO_4,9H_2O$ 0.2g, $Fe SO_4,7H_2O$ 0.0005g (pH adjusted to 7.0 with KOH). To this medium various carbon and nitrogen sources were added as indicated in the first two columns of the Table, in order to obtain the growth rates, μ, reported in the third column. RNA, DNA and protein were measured by conventional techniques (5,6) on aliquots of exponential cultures, growing under conditions of balanced growth. Both the ratios RNA/DNA and RNA/protein vary linearly as a function of the growth rate for all growth rates examined (Table).

Table

Nitrogen Source (2g/1)	Carbon-Energy Source (4g/1)	μ +)	RNA/DNA	RNA/protein	t RNA	
					% of total RNA	moles p. ++ 70 S ribosome
Casamino acids	Glucose	1.09	5.9	0.36	12.5	9.4
$(NH_4)_2 SO_4$	Glucose	0.95	5.42	0.33	11.6	8.6
$(NH_4)_2 SO_4$	Succinate	0.60	4.43	0.24	12.4	9.3
L-alanine	Glucose	0.51	4.33	0.21	11.4	8.5
$(NH_4)_2 SO_4$	Rhamnose	0.31	3.70	0.16	15.3	11.7
L-glutamate	Succinate	0.25	-	-	17.2	13.5

+) μ = number of generations per hour.
++) The M.W. of t RNA, 16 S RNA and 23 S RNA have been assumed to be 26 000, 550 000 and 1 100 000, respectively (11).

From other aliquots of the same cultures, cells were harvested and washed by centrifugation. These operations and all the following ones were made at or near 0°C, unless otherwise stated. The cells were homogenized in 0.05 M Tris-HCl buffer, pH 7.5, 0.01 M $MgCl_2$ by shaking with glass beads for 10 min. Unbroken cells and debris were eliminated by centrifuging 10 min. at 15,000 g. RNA was extracted from the supernatant in the presence of one volume of 90 % phenol and of 0.25 % bentonite (7). After centrifugation and decantation of the aqueous phase, the phenol and protein layers were washed 3 times with buffer. RNA was recovered and freed of phenol by 3 precipitations with 2 volumes of 95 % ethanol at -10°C. Contaminating glycogen was eliminated by a 30 min. centrifugation at 30 000 g. The resulting supernatant was then analyzed by chromatography at room temperature on methylated serum albumin columns (8), t RNA and r RNA being separately eluted with the help of a sodium chloride concentration gradient (9) and estimated by measuring the optical density of the effluents at 260 mμ. The total yield of the extraction and analysis procedures was determined in control experiments and found to be 97 $\pm$ 2 % of the amount of acid insoluble RNA determined by orcinol colorimetry in the homogenate freed of cells and debris. It has also been checked that the chromatographic technique does not entail a systematic loss either of t RNA or of r RNA. Under the present conditions t RNA is eluted along with a low molecular weight RNA normally present in ribosomes (10). The values for t RNA, which are reported in the Table, have been corrected for this product. For all values of μ higher than 0.5, the percentage of t RNA in total RNA is approximately constant and it increases only for the two lowest values of μ. Similar but less reproducible

results were obtained when the extracted RNA was analyzed by sedimentation in a sucrose concentration gradient (12).

Contrary to the observations of Kjeldgaard and Kurland (4) on S. typhimurium, it appears that, in E. Coli ML 308, grown and analyzed under our conditions, the ratio t RNA/DNA does not remain constant and the ratio t RNA/r RNA does not vary continuously,when the growth rate varies. When the cells are growing with a doubling time shorter than 2 hours, the rates of synthesis of t RNA and r RNA in E. Coli are so adjusted that the number of molecules of t RNA per 70 S ribosome remains constant. But this adjustment is no longer effective, when the cells grow very slowly. Provided that the RNA identified as t RNA by chromatography and sedimentation in the present experiments really be t RNA and not another unrelated low molecular weight polyribonucleotide, our results therefore do not invalidate the conclusion of Kjeldgaard and Kurland (4) that "the bacterial cell is able independently to regulate the synthesis of t RNA and r RNA", but, in our case, this becomes apparent only when the growth rate falls below a certain minimum value.

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