A MUTATION AFFECTING DEGRADATION OF STABLE RNA IN *ESCHERICHIA COLI*

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1. Introduction

Turning over processes are very prominent in bacteria during starvation periods. Degradation of stable RNA was shown to take place when bacteria were starved for various essential metabolites [1–3]. However, little is known about the enzymes that participate in these reactions.

Here we report on a mutant strain of *Escherichia coli* altered in its ribonuclease II activity [4–6], that upon starvation degrades its stable RNA to a larger extent than does its parental strain. Therefore the enzyme ribonuclease II [7, 8] is most likely an enzyme that participates in degradation of stable RNA during starvation.

2. Results

Strain N4752 was isolated as a mutant unable to recover from a 72 hr starvation at 42° on an agar plate [9]. Since the strain is also unable to grow at 42° [4] and exhibits a remarkable increase in RNase activity when grown at the nonpermissive temperature [4–6], we studied certain of its characteristics with respect to starvation at 42°. When this strain is starved in a medium devoid of a carbon source, its viability drops continuously and exponentially, while the viability of the parental strain (112–130) remains almost unaffected (fig. 1). (Here we show only data for the first 12 hr of starvation, since most of the biochemical analysis was carried out only during this period.)

When in an experiment similar to that shown in fig. 1, the stable RNA was first labelled during exponential growth and then its degradation was followed during starvation (fig. 2), it became evident that at 42° stable RNA was degraded to a larger extent in the mutant strain than stable RNA of the parental strain. To ensure that these results were not due to disintegration of cells, cultures were monitored during the starvation period and not more than 5% cell losses could be detected. To find out the fate of the ribosomes, rRNA and tRNA in these cultures, extracts and RNA were prepared from the starved cells and analyzed on either low Mg^{2+} (10^{-4} M) sucrose gradients (extracts) or polyacrylamide gels (RNA). The results of some of these experiments (sucrose gradients) are summarized in table 1. Analyses were carried out 0, 8 and 12 hr after starvation, since in both strains the total insoluble material remained constant between 12 and 24 hr after starvation and the tendency to level off in the parental strain (112–130) was already evident after 8 hr (see fig. 2).

The analysis on sucrose gradients revealed that at least the 50 S peak became virtually nonexistent in cell extracts of strain N4752 starved for 8 hr, while about 10% of the 50 S peak remained intact in the parental strain after 8 hr of starvation. (Since sedimentation of 50 S and 30 S degradation product occurs in a variety of positions across the gradient, only estimates of the 50 S region are reliable in such experiments.)

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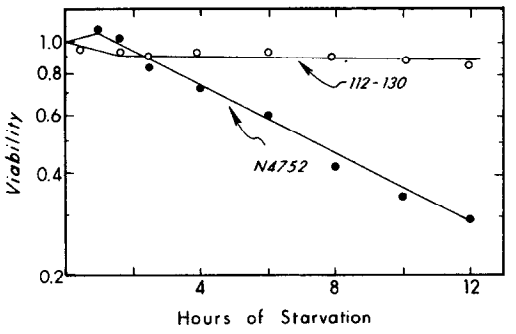

Fig. 1. Viability after starvation. Cultures of strains 112–130 and N4752 (both require L-cysteine and L-leucine) were grown in 0.2% glycerol minimal medium [4], to an A_{560} of about 0.3, centrifuged at 9,000 *g* at room temp, and resuspended in basal medium (the same medium devoid of any carbon source or amino acids). The cells were recentrifuged and resuspended in basal medium, in a similar volume to that they were growing in. Samples were withdrawn diluted and plated on nutrient broth agar plates [4] that were incubated at 30° and inspected after 48 hr. The 1.0 value represents $2-3 \times 10^8$ colony forming units per ml.

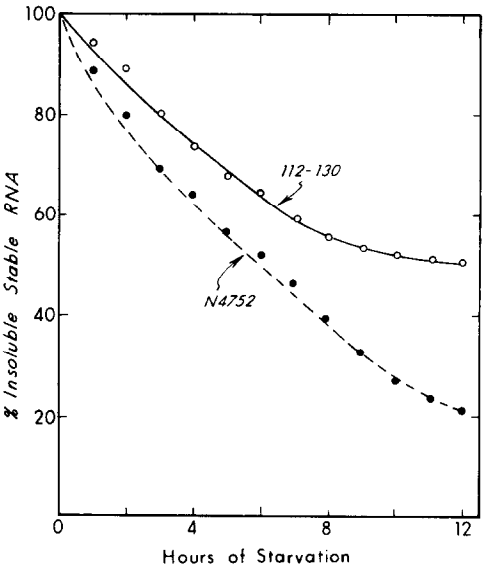

Fig. 2. Degradation of stable RNA during starvation. Cultures were grown as described in legend to fig. 1. 5 μCi of 5-^{3}H-uracil (20.5 Ci/mmole) were added to each ml of the culture. The label was present at least during 3 doublings of the culture. After the cells were washed and resuspended in starvation medium (see legend to fig. 1) 0.2 ml samples were withdrawn at the indicated time intervals and the RNA was precipitated by addition of 3–4 ml of cold 5% trichloroacetic acid. Samples were filtered on glass fiber filters, and the filters were dried and their radioactivity counted in a tricarb scintillation counter. 100% corresponds to 130,000 DPM for 112–130, and 90,000 DPM for N4752. The values obtained in 2 different experiments are presented.

The analysis of RNA on polyacrylamide gels revealed clearly 2 features of starvation. The first is that there are very little intermediate degradation products. Less than 4% of the total RNA was found to migrate between 23 S to 16 S and 16 S to 4 S. This was true for both strains throughout the starvation. The second feature was the difference in the ratios of the various RNA molecules in both strains. In strain 112–130 the ratio of 23 S to 16 to 4 S was almost constant throughout the starvation, while in the mutant strain (N4752) the ratio of 23 S and 16 S to 4 S decreased progressively to about one tenth of the original value by the end of a 12 hr starvation period. No quantitation of the analysis of the RNA on polyacrylamide gels was attempted, since the opening of the cells and the extractions resulted in too many losses.

3. Discussion

The experiments presented in this paper show clearly that in the mutant N4752 degradation of stable RNA during starvation is much more extensive than that in the parental strain 112–130. Since the difference between the 2 strains with regard to degradation of stable RNA during starvation is related to the *sts* 4752 mutation [4] and since this mutation is most likely responsible for a structural modification in the ribonuclease II enzyme [4–6, and Gorelic and Apirion, in preparation], we conclude that ribonuclease II participates in degradation of stable RNA during starvation.

The participation of RNase II in degradation of stable RNA was already indicated by the studies of Maruyama and Mizuno [10], who found that in an *E. coli* strain the level of 5′-mononucleotides in the nucleotide pool increased during starvation; 5′-mononucleotides are the products of degradation of this enzyme. That an enzyme like RNase II participates in degradation of stable RNA during starvation is further supported by the fact that relatively little intermediate RNA molecules were found in the starved cells, since this enzyme degrades RNA molecules progressively [11].

Table 1
Distribution of RNA during starvation.

Strain	Time of starvation (hr)	Total RNA in cells (%)	50 S (%)	30 S (%)	4 S (%)
	0	100	44	23	19
112–130	8	56	4.5	9	13
	12	48	2.2	6	11
	0	100	45	22	18
N4752	8	34	0.4	8	8
	12	19	0.2	3	4

Cells from both strains were grown and labelled with ^{3}H-uracil as described in fig. 2. Samples were withdrawn at the indicated times. Cells were opened by alumina grinding and the supernatants from a 10,000 *g* centrifugation were dialyzed against a Tris-HCl buffer that contained 0.1 mM magnesium acetate and was centrifuged in a sucrose gradient in the same buffer. Total RNA in cells was determined in aliquots taken from the samples that were analyzed in the sucrose gradients as described in fig. 2. The amount of material in 50 S, 30 S and 4 S represent the fraction of radioactivity sedimenting in these regions as compared to all of the gradient, multiplied by the percentage of the total RNA in the sample as compared to zero starvation time. 36 to 37 fractions were collected from each gradient and only the values from 4 fractions were totaled for the calculations of each of the peaks.

The participation of a pre-existing ribonuclease in a starvation degradation process is in accord with the finding of Goldberg [12] that pre-existing protease(s) participates in protein degradation during starvation of *E. coli*. The teleological arguments for having pre-existing enzymes participate in essential processes during starvation when metabolic activities should be kept to a minimum are obvious, and clearly degradation of one molecule to completion before starting degradation of the other could be of great selective advantage to a starving cell.

The experiments reported here with strain N4752 together with those presented earlier [4, 5] show that the same lesion changed mRNA as well as stable RNA degradation, and therefore they imply that a similar mechanism is responsible for degradation of all RNA species in *E. coli*. If degradation of stable RNA during starvation is an essential function for survival (a generally accepted, even though unproven assumption), these experiments raise the possibility that perhaps the instability of mRNA is a by-product for the necessity of providing the cell with a mechanism to degrade its stable RNA. Obviously both processes could be beneficial and could be evolved simultaneously.

Some of the results and ideas presented here were presented during the 62nd Annual Meeting of the American Society of Biological Chemists [13].

Acknowledgement

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