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Control of ribosome turnover during growth of the haloalkaliphilic archaeon *Natronococcus occultus*

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Abstract

The metabolism of ribosomes during growth of the haloalkaliphilic archaeon *Natronococcus occultus* was examined. The ribosome content was higher during exponential growth and diminished to 35% of the maximum in the stationary stage. The incorporation of ^{3}H-orotic acid and ^{14}C-uracil into rRNA was higher during exponential growth. After that, it decreased to 39% of the maximum in the stationary stage. The labeling of non-ribosomal RNA took place almost exclusively in the exponential stage. From loss of radioactivity, the half-life of rRNA was 11.43, 14.85, 5.28 and 7.14 h during the initial, exponential, late exponential and stationary growth stages, respectively. These results suggested that increased synthesis combined with diminished degradation were responsible for the high ribosome content displayed by *Ncc. occultus* during exponential growth. In contrast, diminished synthesis together with increased degradation provoked its posterior loss.

Keywords: Halophilic archaea; *Natronococcus occultus*; Ribosome synthesis; Ribosome degradation

1. Introduction

Protein synthesis depends to some extent on the cell ribosome content. Thus, the cell sets up the environment and responds to the necessity of proteins by increasing its ribosome content. Several studies have established that ribosome content increases in direct relationship with the cell growth rate [13,17]. That content may be controlled throughout degradation, biogenesis, or both. For instance, bacteria under stationary growth and starvation exhibit the following features: decreased biogenesis of ribosomes; decreased synthesis of tRNA; and enhanced protein breakdown [12]. Conversely, the increased availability of nutrients enhances ribosome biogenesis [14]. Based on their ribosome size, the archaea can be separated into two groups: one with characteristics similar to those of the bacterial ribosome (halophiles and methanogens, except for *Methanococcus* sp.); the other with similar size as the eukaryotic ribosome (sulfur-dependent archaea and *Methanococcus* sp.). Granted that the sizes of archaeal rRNAs are identical, the higher mass of the second group depends on protein composition [3]. In addition, a substantial number of proteins are reasonably preserved within archaea and eukarya [11,21], including those of ribosomes [20].

In archaea, data regarding ribosome turnover are scarce. Consequently, this work considers the effect of biogenesis and degradation on the ribosome content of growing *Natronococcus occultus*.

2. Materials and methods

2.1. Bacterial strains and growth conditions

Ncc. occultus NCMB 2192 cells were aerobically grown without light in a rotating shaker at 150 rpm and 37 °C for 8 days in a liquid medium described by Tindall et al. [28], except that casamino acids were replaced by yeast extract [25].

2.2. Metabolic labeling

Cells (100–400 μg DNA) were incubated in the growth medium containing 0.75 μCi/ml of either ^{3}H-orotic acid

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[21 Ci/mmol] or ^{14}C-uracil [58 Ci/mol] for 2 h. Then, they were harvested and analyzed for radioactivity incorporated into RNA. This value also represented the zero time labels for analysis of breakdown. For this, additional sets of cells were labeled for 2 h, washed with 3 M NaCl, incubated in fresh medium for 4 h, and analyzed for radioactivity retained by RNA. The maximum label at exponential phase was 19 700 and 400 cpm per 100 µg DNA for ^{14}C and ^{3}H, respectively.

2.3. Preparation of fractions containing ribosomes

Cells (100–400 µg DNA) were collected by centrifugation, washed once with 3 M NaCl and suspended in 4 ml of 100 mM acetic acid–Na acetate buffer, pH 5. Then, they were sonicated six times for 30 s with 30 s intervals, mixed with 4 ml of the same buffer and stored for 1 h at 4 °C. The precipitate was collected by centrifugation at 12 000 *g* for 1 h. This material containing the total cell ribosomes (80 ± 12% of total RNA) was defined as the ribosomal fraction [4]. Its RNA composition was analyzed by extraction with TriZOL reagent (Invitrogen) as specified by the manufacturer. Then, 10 µg of RNA were analyzed by gel electrophoresis in 1% agarose–formaldehyde gels as indicated by Sambrook et al. [27]. The pH 5 supernatant was mixed with 100% (w/v) TCA to obtain a 10% (w/v) concentration and stored for 1 h at 4 °C. The resulting precipitate was collected by centrifugation for 20 min at 4000 *g* and was identified as "non-particulated RNA".

Cytosolic ribosomes were also isolated. For that, the cells were washed with 3 M NaCl, mixed with 5 ml of 100 mM Tris–HCl buffer (pH 7.5) containing 2 mM Mg^{2+} acetate at 4 °C and sonicated 6 times for 30 s with 30 s intervals. After centrifugation at 7500 *g* for 15 min, the supernatant was made up to 2 mM Mg^{2+} and pH 5 by addition of Mg^{2+} acetate and glacial acetic acid, respectively. After 30 min at 4 °C, the precipitate was collected by centrifugation at 12 000 *g* for 30 min. Tested for both RNA and protein composition, this fraction was essentially composed of ribosomes [4,19].

2.4. Estimation of radioactivity

Both the ribosomal fraction and the non-particulated RNA were exposed to 0.3 N KOH at 37–40 °C for 16 h. Then they were neutralized, mixed with 1 N PCA, and stored for 1 h at 4 °C. The supernatant containing the hydrolyzed RNA was collected after centrifugation at 4000 *g* for 20 min and analyzed for radioactivity as described by Iappalucci-Espinoza et al. [16].

2.5. Estimation of RNA and DNA content

The contents of DNA and RNA of cells as well as that of RNA of ribosomal fraction were analyzed by the method of Fleck and Munro [10]. The content of rRNA was estimated as 80% of total RNA [2]. The ribosome amount was obtained from the content of rRNA multiplied by the ratio between the Avogadro's number and 3×10^6 kDa, which is the halophilic ribosome size [1]. The result was related to total cellular DNA.

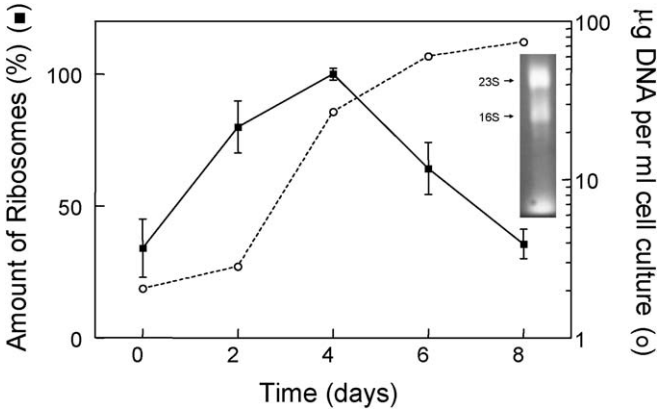

Fig. 1. Changes in rRNA content during growth of *Ncc. occultus*. Average data ± SEM of 5 independent tests are expressed as percentage of the highest value. This corresponds to 7.16×10^{11} ribosomes per µg DNA. The growth curve in terms of µg DNA per ml of culture is also indicated. The inset shows a typical RNA profile of the ribosomal fractions analyzed during growth.

3. Results and discussion

Most of the assessments in this work were made after 2, 4, 6 and 8 days of growth, reflecting changes at the initial, exponential, late exponential and stationary phase of growth, respectively (Fig. 1). The changes found in the ribosome content of *Ncc. occultus* during growth are compatible with those observed in *Escherichia coli* [17]. The number of ribosomes per µg of DNA reached its highest value (100%) during exponential growth (Fig. 1). Then it declined, at an average rate of 16% per day, until reaching 35% of the maximum in the stationary phase. Indeed, the RNA composition of all the studied fractions was that of the prokaryotic ribosome (Fig. 1, inset). The analyzed cellular fraction is mainly composed by rRNA [4]. Therefore, it is suitable for the evaluation of both the synthesis and breakdown of rRNA as indicators of ribosome turnover.

The changes in the number of ribosomes during growth may have been due to changes in the rate of biogenesis, the rate of degradation, or both. The incorporation of radioactive precursors (^{3}H-orotic acid or ^{14}C-uracil) into rRNA indicated that cells in the exponential phase of growth displayed the highest rate of synthesis (100%). Then, this decreased gradually until reaching 39% of the maximum in the stationary phase. In bulk, such behavior is directly related to the capacity for protein synthesis displayed by *Ncc. occultus* during growth [25]. They also are in harmony with those displayed by bacteria [2,7–9,12,13,26].

The rate of degradation of rRNA was evaluated from the percent of radioactivity lost per hour (Fig. 2b). This was 3.44, 2.45, 11.33 and 9.14 for the initial, early exponential, late exponential and stationary phase of growth, respectively. Assuming first-order kinetics, the respective averages half-lives were: 11.43, 14.85, 5.28 and 7.14 h. The data shown in Figs. 1 and 2 indicate that increased synthesis combined with diminished degradation caused accretion of ribosomes in the exponential phase. On the other hand, diminished synthesis together with increased degradation caused subsequent loss of ribosomes. In the studied periods of growth, the half-lives of *Ncc. occultus* ribosomes (11.43,

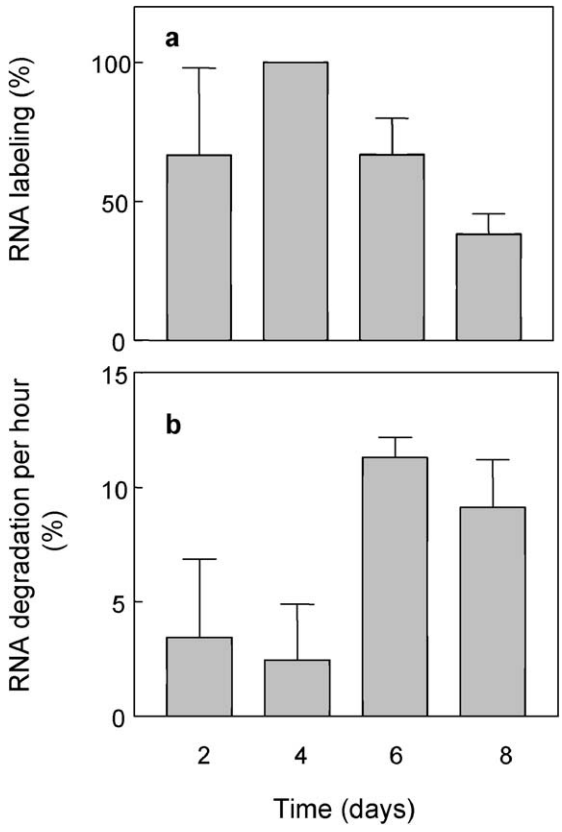

Fig. 2. (a) Estimation of rRNA synthesis during growth of *Ncc. occultus*. Cells were pulse-labeled with either ^{3}H-orotic acid (3 assays) or ^{14}C-uracil (2 assays) for 2 h. The value of 100% was assigned to the highest incorporation observed in each assay. Data are means ± SEM of 5 tests. (b) Estimation of rRNA degradation during growth of *Ncc. occultus*. Cells were pulse-labeled with ^{3}H-orotic acid for 2 h. Percent radioactivity lost by rRNA per h was determined during the subsequent 4 h. Data are average ± SEM of 3 independent tests.

14.85, 5.28 and 7.14 h) were lower than those of 82, 10 and 20 h described for *E. coli* in exponential and stationary growth and starvation, respectively [22,23]. It should be noted that the average half-lives of *Ncc. occultus* proteins are also lower than those of *E. coli* proteins [25]. These two features are possibly due to the extremely acidic nature of the proteins of halophilic archaea, including those of ribosomes [1,15,18,25].

The rRNA was gradually degraded with culture development. However, cells in the stationary phase conserved a low amount of ribosomes (35%). They are probably retained for use in fast recovery processes, like those occurring during transfer to a new culture medium [9]. On the other hand, degraded rRNA may be used for production of both amino acids and nucleotides [5,22,24].

The incorporation of radioactivity into RNA soluble at pH 5 was almost exclusively during the exponential growth phase (Fig. 3), which correlates with the required high rate of protein synthesis during this stage of growth [6,12,25].

In conclusion, turnover of ribosomes was measured for the first time in a haloalkaliphilic archaeon, *Ncc. occultus*. It was found that the number of ribosomes reached its highest value during exponential growth due to a combination of high synthesis and low degradation. Conversely, it decreased in stationary growth due to both high degradation and low synthesis. The half-lives displayed by the archaeal ribosomes were remarkably lower than those reported for bacteria.

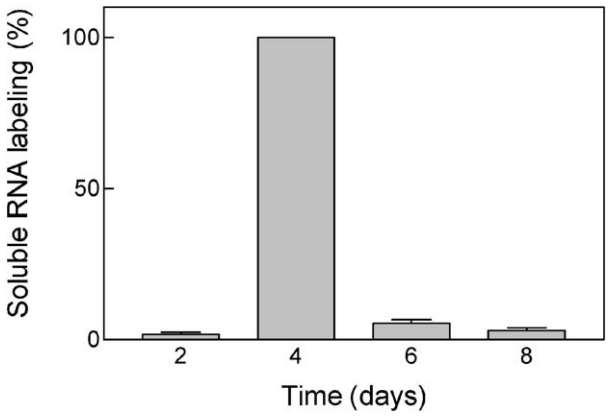

Fig. 3. Estimation of non-particulated RNA synthesis during growth of *Ncc. occultus*. Cells were pulse-labeled with either ^{3}H-orotic acid (3 assays) or ^{14}C-uracil (2 assays) for 2 h. The value of 100% was assigned to the highest label measured in each assay. Data are means ± SEM of 5 assays.

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