KINETIC EVIDENCE FOR THE PROCESSING OF RIBOSOMAL PROTEINS S6 AND S8

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Received 19 August 1976

1. Introduction

It is clear that the 30 S ribosomal subunit of *Escherichia coli* can be reconstituted in vitro from its purified components in a well-defined sequence of events [1,2]. Nonetheless, the actual pathway of subunit assembly which occurs in vivo is unknown. Since a frequent feature of the assembly of bacteriophages and viruses is proteolytic cleavage of capsid protein precursors [3,4], I am investigating the possible occurrence of similar reactions during the biogenesis of the 30 S subunit in *E. coli*. In this communication, I report the results of measurements of the kinetics of appearance of proteins S6 and S8 which lend credence to the hypothesis that these ribosomal proteins are indeed derived from precursors by some form of post-translational modification.

2. Methods

The technique employed for the assay of ribosomal proteins in crude extracts relies on the ability of some 30 S subunit proteins to bind specifically to 16 S RNA with high affinity [5]. The details of this procedure are described fully elsewhere (manuscript submitted to Biochemistry). In brief, cultures of strain H882 *groE*$_{44}$ [6] are pulse-labelled with carrier-free [^{35}S]H_2SO_4 (New England Nuclear), then chased as necessary with excess unlabelled Na_2SO_4. Samples are removed at various times, the cells collected quickly on nitrocellulose filters, and extracts prepared by treating the cells on the filters with 66% acetic acid in the presence of 33 mM $MgCl_2$ [7,8]. These acid extracts are dialyzed into reconstitution buffer (50 mM Tris–Cl, 350 mM KCl, 20 mM $MgCl_2$, 5 mM β-mercaptoethanol, 0.25 mM phenylmethylsulphonyl fluoride, pH 7.8), and mixed on ice with a slight excess of 16 S RNA. Incubation at higher temperatures, as is normally the case for ribosomal reconstitution, leads to the degradation of the added RNA by contaminating ribonucleases. Proteins which bind to 16 S RNA under these conditions are separated from the unbound proteins by sucrose gradient centrifugation of the protein–16 S RNA complex. The bound proteins are extracted from the 16 S RNA by the acetic acid method [7,10], and resolved from each other by column chromatography [9,10]. Only S4, S6, S8, S15, S16/17, S18, and S20 bind to the 16 S RNA under the conditions used, along with small quantities of S7, S9, S11 and S19. This is in general agreement with other authors [2,10]. The recovery of the eight major proteins is entirely reproducible under defined conditions of labelling. The purity of S6 and S8 has been assessed by tryptic fingerprinting and by SDS–polyacrylamide gel electrophoresis. S8 is essentially pure, whereas S6 can be contaminated with S8 to a maximum extent of 25%. This may result in an overestimate of the amount of S6 recovered in some of the experiments discussed below. The reference protein throughout these experiments, S4, is clearly resolved from all other 30 S subunit proteins by chromatography on phosphocellulose [9].

3. Results

The recovery of protein S4 from pulse-labelled cells using the assay described has been quite repeatable under a variety of conditions, and is typically 0.17–0.19% of the total acid-extractable radioactivity. In contrast, the yields of several other proteins,

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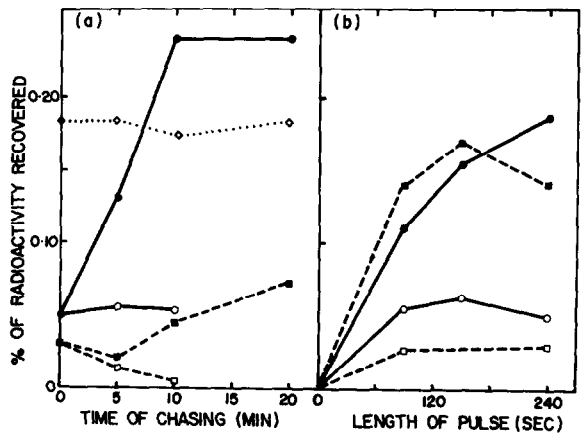

Fig.1. (a) Kinetics of chasing of S6 and S8. A culture of *E. coli* K12 H882 *groE*$_{44}$ was grown to sulphate exhaustion at 28°C, shifted to 44°C, and pulse-labelled 25 min later for 4.0 min with 200 μCi/ml carrier-free [^{35}S]H_2SO_4. Portions of the culture were chased at 44 or 28°C in the presence of 5 mM Na_2SO_4. Samples of 5 ml were taken at the indicated times, and the acid-extractable proteins which bound to 16 S RNA were separated by column chromatography [9] and quantitated by liquid scintillation counting. The radioactivity recovered in a particular protein peak is expressed as a percentage of the total input in the reconstitution assay. Fig.1 (b) Kinetics of labelling of S6 and S8. A culture was labelled as above except that 5 ml samples were taken after 90, 150 and 240 s. The processing of these samples and the analysis of the ribosomal proteins was as before. The results are expressed as the percentage of the input radioactivity recovered as S6 or S8, normalized to a recovery of S4 of 0.19%, the average in the six samples. (■– – – – –■) S6 (28°C); (□– – – – –□) S6 (44°C); (●————●) S8 (28°C); (○————○) S8 (44°C); (◇. . . . . .◇) S4 (44°C).

particularly S6, S8, S18, and S20, have been variable. Accordingly, the behaviour of S6 and S8 has been examined in more detail. After a pulse of 4.0 min at 44°C, only very small quantities of either S6 or S8 can be detected (fig.1a). Figure 1a also shows that these amounts are not increased by chasing the labelled cells for up to 10 min at 44°C. However, the recoveries of both these proteins rise when the cells are chased at 28°C prior to their extraction. The amount of S8 capable of binding to 16 S RNA reaches a plateau after 10 min of chasing, while that of S6 increases more slowly throughout the chase period. Thus it would appear that both S6 and S8 are synthesized in cells exposed to 44°C, but remain in forms that are either unextractable or incapable of binding to ribosomal RNA under the conditions used for reconstitution in these experiments. On the other hand, the recovery of S4 which was monitored simultaneously, was consistently between 0.17 and 0.19% in seven determinations after different times of labelling and chasing. For clarity, only data for S4 derived from a chase at 44°C are included in fig.1a; essentially identical results were obtained at 28°C. The effects of higher temperature appear to be confined to only some of the ribosomal proteins, and do not exert a general inhibition on the biosynthesis of all ribosomal proteins.

I have also examined the kinetics of labelling of several 30 S subunit proteins at either 28 or 44°C. S4 and S16/17 become maximally labelled after 90 s exposure of the culture [^{35}S]H_2SO_4 at either temperature (data not shown). Figure 1b shows that the rate of labelling of S8 is comparatively slow relative to that of S4, even at 28°C, as the fraction of S8 in the acid extract reaches only two-thirds its maximum value after 4.0 min of labelling. In contrast, S6 is labelled quite rapidly at 28°C. At 44°C, neither S6 nor S8 is labelled substantially after any period of time.

4. Discussion

The most straightforward interpretation of the results presented above is that S6 and S8 are initially synthesized in the form of precursors. Conversion of these putative precursors to the corresponding mature ribosomal proteins is apparently blocked at 44°C, and, in the case of S8, relatively slow even at 28°C. The nature of the temperature-sensitive event in the processing of these two proteins remains to be identified. The successful recovery of both S6 and S8 from cells initially labelled at 44°C and subsequently chased at 28°C argues against the activation of a protease and the concomitant destruction of these proteins in cells exposed to the higher temperature. Two other controls have also been performed to eliminate this possibility. First, cells were labelled at 28°C for 4.0 min and then chased for 10 min at 44°C before their extraction. The recoveries of S6 and S8 were practically identical to those found after a 4.0 min pulse at 28°C alone (cf. fig.1b). Secondly, mixing experiments indicate that there is no diffusible substance in or released from cells exposed to 44°C which can degrade proteins S6 or S8 labelled at 28°C. Attempts to isolated and

characterize the putative precursors to these two proteins are underway.

While the results of these and other experiments (my unpublished results) support the existence of precursors to some proteins of the 30 S subunit, further work is necessary to clarify the nature of the processing steps and to establish the significance of such reactions to the process of ribosomal assembly in vivo.

Acknowledgement

This work was supported by a grant from the MRC of Canada.

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