SYNTHESIS AND ASSEMBLY OF RIBOSOMAL PROTEINS AT THE ONSET OF PYRIMIDINE STARVATION

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Summary Ribosomal proteins from a pyrimidine auxotroph of Escherichia coli were pulse labelled with ^{14}C-leucine prior to and following depletion of uridine. The incorporation of label into different 70S ribosomal protein bands decreased differentially at the onset of starvation. Readdition of uridine following the ^{14}C-leucine pulse did not alter the labelling pattern. A sequential turn-off in the synthesis of different ribosomal proteins and a tight coupling between synthesis and assembly were indicated.

In vivo maturation of ribosomes proceeds through distinct intermediate stages (1-3), each differing by the amount of ribosomal proteins present with the ribosomal RNA. In vitro removal of specific ribosomal proteins with salt treatment and their reassembly into functional ribosomes (4,5), also strongly suggests a sequential attachment of the different proteins during ribosome biogenesis. Recent experiments by Davis and Sells (6,7) and Cozzone et al (8) on exponentially growing cells and on cells recovering from chloramphenicol treatment have traced the differential incorporation of certain proteins into ribosomes. The relationship between synthesis and assembly however, remains unclear. We have used a pyrimidine auxotroph in order to investigate this relationship during the turn-off of ribosome synthesis. The incorporation of labelled amino acid into different ribosomal proteins as the cells entered into pyrimidine starvation was examined. Only completion of growing ribosomes would be expected under these

conditions since it has been shown by Nakada (9) that no new ribosomes are synthesized during pyrimidine starvation. The results indicated a sequential turn-off in the synthesis of different 70S proteins and a tight coupling between the regulations of synthesis and assembly.

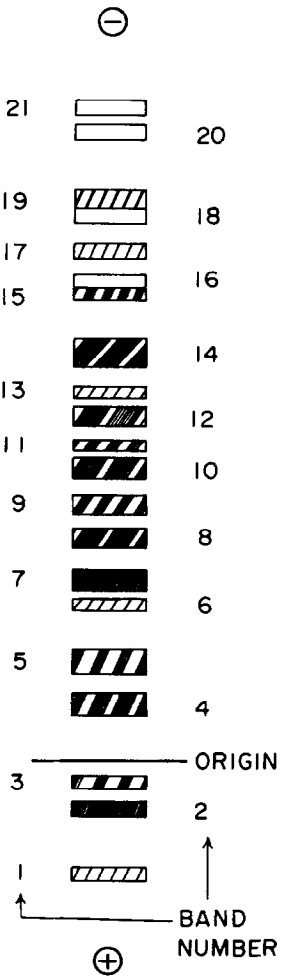

Figure 1. Starch Gel Electrophoresis of Ribosomal Proteins. Lyophylized 70S ribosomes were extracted in 6M urea 0.05M sodium acetate buffer pH 5.6 and run on a urea acetate gel prepared according to Waller (13). Each slot contained 0.1 to 0.2 mg labelled ribosomes and 1 mg unlabelled ribosomes. The bands were cut out and dehydrated overnight in absolute alcohol followed by one hour in anhydrous ether. Each band was ground to a fine powder, air dried, and counted in 10 ml of toluene PPO-POPOP mixture in a Nuclear Chicago Mark II scintillation counter. At optimized settings the efficiency was 55 $\pm$ 3.5%. In the tested range of 0 - 45 mg the amount of dried gel powder had no quenching effects. The bands chosen for study were 5, 7, 8, 9 and 11. The total number of bands seen on the gel were 21. The relative specific activity of each band was calculated as cpm/mg total labelled r-protein in the gel slot.

Materials and Methods

Escherichia coli strain ATCC 12632, a pyrimidine auxotroph, was grown in medium 63 (10) with 0.5% glycerol, plus either 30 μg/ml uridine for non-limiting, or 7 μg/ml for exhaustion experiments. Each 200 ml sample, containing 4.8×10^{10} cells, was pulse-labelled with 18 nmoles of ^{14}C-leucine (273 mC/mM) which sufficed for less than one minute of exponential growth. The cells were harvested by adding NaN_3 to 20 mM and pouring over frozen medium. After centrifugation, the cells were resuspended in 0.01 M tris-HCl, 0.01 M Mg-acetate pH7.4 and broken in a French pressure cell. The ribosomes were purified according to Tissieres (11). Electrophoresis and radioactive counting of ribosomal proteins are detailed in Figure 1. Protein determinations were carried out according to Lowry et al (12).

Results and Discussion

Depletion of uridine from the medium was marked by a sharp break in the growth of strain 12632 cells as measured by absorbancy at 450 mμ. The rate of amino acid incorporation into the total cellular proteins also decreased on uridine starvation. Moreover, when the cells were exposed to a short pulse of ^{14}C-leucine followed by a further twenty minutes of incubation, the fraction of the label entering the different ribosomal proteins decreased with the onset of uridine depletion. As shown in Fig. 2a, the rate of decrease differed for the different bands studied. Bands 7, 9, and 11 showed a steeper drop relative to bands 5 and 8. The entry of the labelled amino acid into the structural proteins of the ribosome (r-proteins) can be discussed in two stages:

amino acid $\xrightarrow[(I)]{\text{synthesis}}$ precursor r-protein unattached to r-RNA $\xrightarrow[(II)]{\text{assembly}}$ r-protein attached to r-RNA

If an r-protein is synthesized with the r-RNA as a template (14-16), stages I and II would become coincidental and the soluble pool of precursor r-protein would become non-existent. In terms of the above scheme, the drop in label distribution into different r-proteins seen in Figure 2a could result from

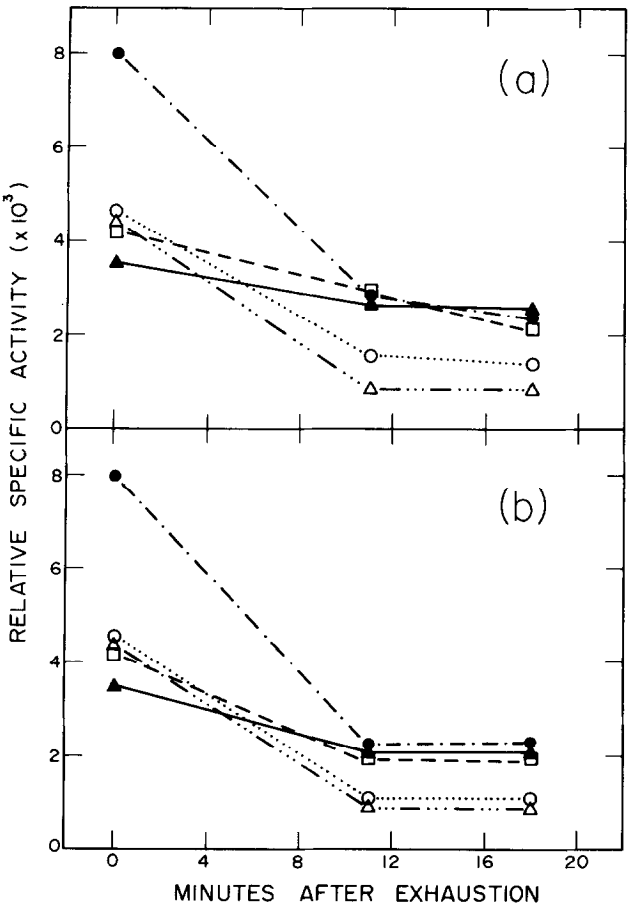

Figure 2. Cells were grown in a limiting amount of uridine (7μg per ml). Duplicate samples were taken during exponential growth (zero time sample) and at different times after uridine exhaustion. Each sample was pulsed with .09 nmoles/ml of ^{14}C-leucine. In (a) the samples were incubated for twenty minutes in a 37° shaker bath before harvesting (see methods). In (b) the cells were treated as in (a) but uridine (5μg per ml) was added to each sample after the twenty minute incubation and the cells were incubated for a further fifteen minutes before harvesting. The ribosomes were collected in each case and the relative specific activities of the r-protein bands determined as described in Fig. 1.

(_▲_ protein bands no. 5; —•—●—•— band no. 7; _ _□_ _ band no. 8; ....○.... band no 9; ..._△_... band no. 11).

regulation at the level of synthesis or assembly. To distinguish between these two alternatives, in Fig. 2b uridine was readded to the cells following the twenty minute incubation. The cells were further incubated for fifteen minutes to allow reinitiation of ribosome synthesis. If the r-proteins were synthesized

with normal efficiency but failed to become assembled upon uridine exhaustion, the labelled proteins in the soluble precursor pool would be chased into ribosomes on readdition of the uridine. From the striking similarity between the results of Fig. 2a and 2b, it is clear that the readdition of uridine after the pulse labelling failed to chase additional label into the r-protein bands examined. This indicates that the drop in incorporation of the labelled amino acid resulted from a regulation at the synthesis level. However, it could be argued that the r-proteins were synthesized and then broken down in the absence of ribosomal RNA (r-RNA). In order to examine this possibility, an experiment similar to that described in Fig. 2b was performed. Instead of adding the uridine to the starved cells twenty minutes after the addition of the ^{14}C-leucine, it was added at intervals of three, five, ten, and twenty minutes after the addition of the ^{14}C-leucine in order to detect the possible breakdown of the precursor r-proteins with time. As seen in Figure 3, varying the interval between labelling with ^{14}C-leucine and the readdition of uridine

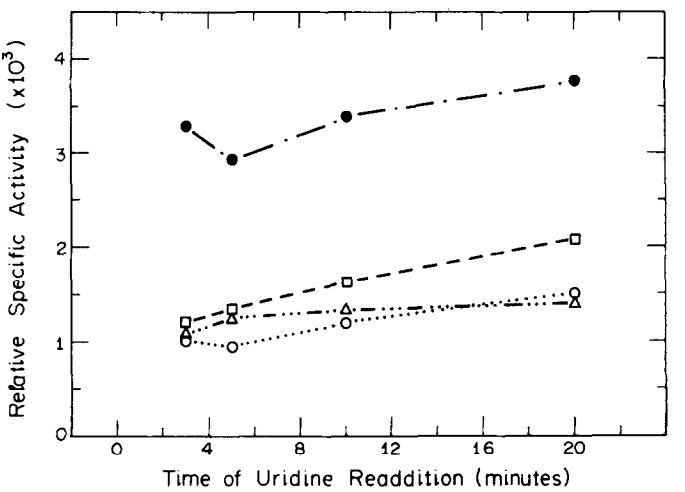

Figure 3. Cells were grown in limited uridine as in Fig. 2. Ten minutes after uridine exhaustion the cells were pulsed with ^{14}C-leucine at a concentration of .09 nmoles/ml. Uridine (5µg/ml) was added to 200 ml aliquots of cells at 3, 5, 10, and 20 minutes after the addition of the ^{14}C-leucine pulse. The cells were harvested after a further incubation of 15 minutes and the r-proteins were processed as outlined in Fig. 1. The curves are designated as in Fig. 2.

from three to twenty minutes did not alter the levels of labelled r-proteins in the completed ribosomes. A similar experiment with cells labelled five minutes instead of ten minutes after uridine exhaustion gave the same results. These experiments therefore do not support the idea that a significant continued breakdown of precursor r-proteins occurs in the absence of r-RNA synthesis. It is concluded that the r-proteins were synthesized only in quantities which could become assembled immediately into the 70S ribosomes, pointing to a highly co-ordinated regulation of synthesis and assembly in these cells. Since the declines in label incorporation seen in Fig. 2a represent regulation at the level of synthesis, the much sharper declines of bands 7, 9 and 11 relative to bands 5 and 8 also implicate a sequential mechanism in turning off the synthesis of the former group prior to the latter.

Two relevant control experiments are shown in Figures 4 and 5. In Fig. 4 preformed ribosomes are shown to be largely stable under pyrimidine starvation.

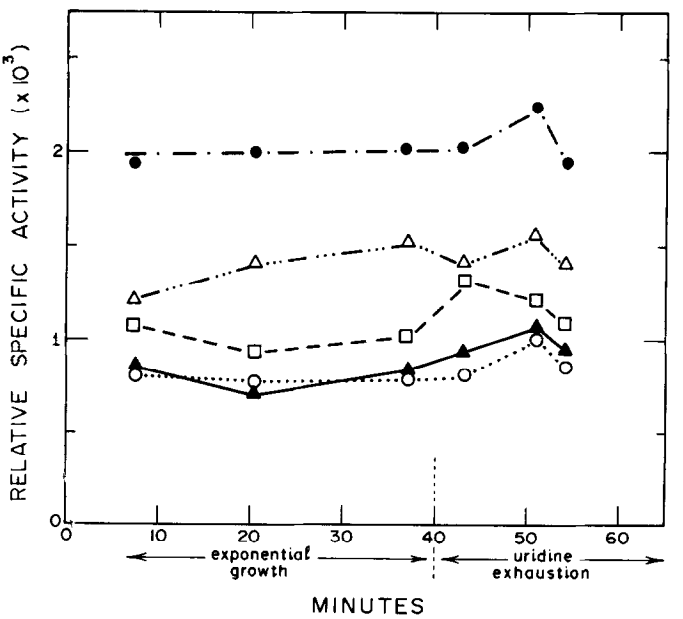

Fig. 4. Stability of preformed ribosomal proteins during uridine exhaustion. The cells were pulsed with ^{14}C-leucine during exponential growth at about -50 minutes, and samples were taken during exponential growth and after exhaustion. The specific activities of the ribosomal proteins were normalized for changes in cell mass (absorbancy at 450 mu); decreases would provide a measure for any breakdown of preformed ribosomes during uridine exhaustion. The curves are designated as in Figure 2.

Thus the drop in radioactivity of the r-proteins bands in Figure 2a was not due to breakdown of completed ribosomes causing a flooding of precursor r-protein pools. The possible flooding of precursor pools was even more clearly ruled out by the findings in Fig. 5. The rapid entry of labelled amino acid into the r-proteins is not consistent with the existence of large pools either before (Figure 5a) or after (Figure 5b) uridine exhaustion. Various estimates of r-protein pools have been reported (17-20). Our experiments favour the small pools found by Schleif (17). It should be noted that small precursor pools alone do not provide a sufficient basis for

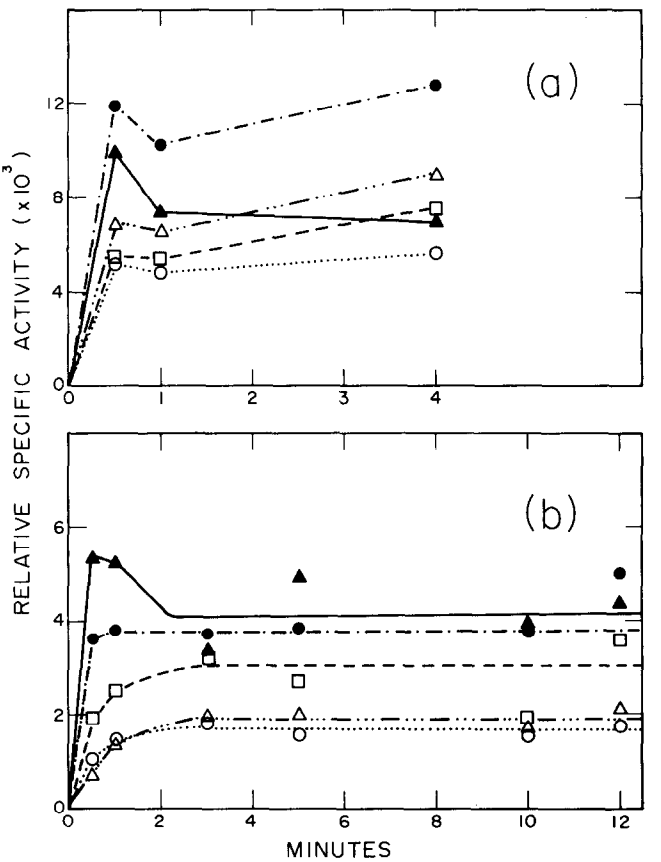

Fig. 5. Rate of labelling of ribosomal proteins during exponential growth and uridine starvation. Cell samples were pulsed with ^{14}C-leucine during (a) exponential growth and (b) 8 minutes after uridine exhaustion. The samples were harvested at different times as indicated and the ribosomes prepared as in Methods. The relative specific activities of the r-protein bands were calculated and the curves designated as in Fig. 2.

distinguishing between a coordinated regulation of synthesis and assembly, as suggested in this study, and a rate-limiting synthesis stage relative to assembly.

There is substantial evidence for sequential attachment of r-proteins during ribosome biosynthesis including the identification of intermediate precursor particles (1-3), and the different rates of appearance of labelled r-proteins into 50S (7) and 70S (8) particles during exponential growth. Our results agree with these data and further demonstrate the existence of a sequential regulation of r-protein synthesis which is tightly coordinated to assembly. Whether this involves a sequential inactivation of different m-RNA activities or their feed-back repression by unassembled precursor r-proteins, the rapid turn-off in synthesis appears to require m-RNA activities with only very short functional half-lives. This is entirely consistent with the possibility that r-RNA itself carries the m-RNA activities for some r-proteins (14-16). However, alternative explanations are by no means excluded and deserve equal consideration.

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