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TIGHT AND LOOSE COUPLE RIBOSOMES INTERCONVERT DURING *ESCHERICHIA COLI* GROWTH

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ABSTRACT: Ribosome is a cellular organelle which synthesizes protein in all living organisms. The translocation of tRNAs during the elongation phase of protein synthesis coupled with mRNA has several intermediates with pre and post translocation discrete states significantly altered in conformation. It is well known that there are two types of ribosomal populations in *E. coli*, namely Tight couple (TC) and Loose couple (LC) ribosomes. LC ribosomes are generally believed to be inactive particles. In this report we demonstrate the *in vivo* interconversion of TC and LC ribosomes during exponential and stationary phases of E. coli growth. This novel demonstration proves that LC ribosomes represents a physiological functional and discrete conformational state of *E. coli* ribosomes.

KEYWORDS: Protein Synthesis, Ribosome, Translocation, TC and LC Ribosomes.

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1.INTRODUCTION

During the elongation phase of protein synthesis, sequential addition of amino acids to the growing polypeptide chain takes place. After peptide bond formation, elongation factor-G (EF-G) binds to ribosome and catalyzes the translocation of peptidyl- tRNA from its aminoacyl site to peptidyl site along with movement of mRNA by one codon [1]. This process of translocation is accompanied by significant conformational change, defined as pre- and post-translocation conformational state of the ribosome (Fig.1).

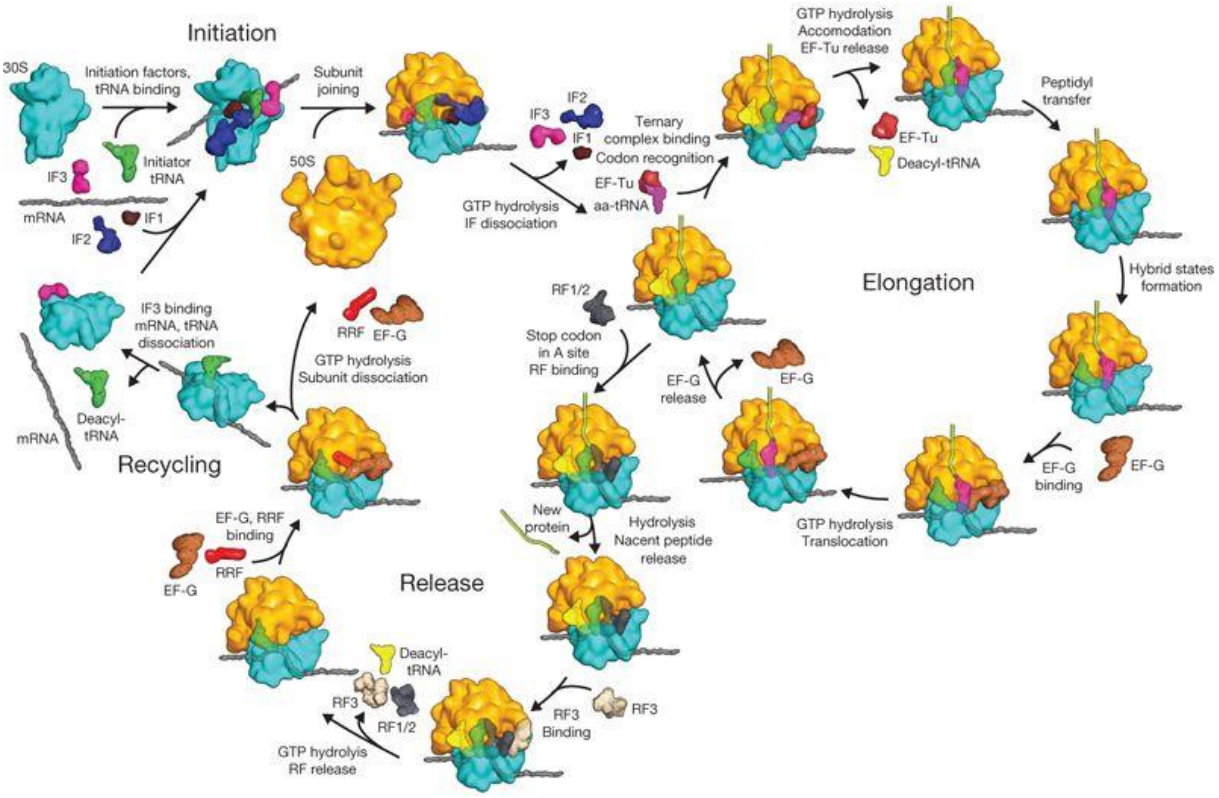

Figure 1: A overview of translation in bacteria. (Adapted from Nature. 2009; 29:1234-42)

It is well established that there are two types of 70S ribosomal populations in *E.coli* called TC and LC ribosomes. TC ribosomes remain associated at low Mg^{++} concentration (4mM or so) while LC ribosomes requires higher Mg^{++} concentration (10mM or so) for association [2]. It has been suggested that LC ribosomes may be inactive whereas TC ribosomes are active particles [3], although no difference in RNA and protein composition was found in LC ribosome as compared to TC ribosome [4]. Studies carried out with TC and LC ribosomes have suggested that LC ribosomes might not be inactive ones as believed, but may represent conformationally altered form of ribosome [4-5]. Interconversion of these two populations *in vitro* with the help of EF-G, GTP analogue and fusidic acid, an inhibitor of translocation has been demonstrated [6,7]. Ribosome function in protein synthesis requires dynamic flexibility of the ribosomal structure. During the elongation cycle, the ribosome reciprocates between a pre- and post- translocation conformation [8-14]. In this report, we demonstrate the interconversion of the two populations during exponential and stationary phase of the growth in *E. coli*.

2. MATERIALS AND METHODS

E. coli MRE600 was used in these experiments. Carrier free radioactive inorganic phosphate was procured from Bhabha Atomic Research Center, Bombay, India. All other chemicals used were of analytical grade.

2.1 Incorporation of radioactive inorganic phosphate at different phases of growth of *E. coli*: *E. coli* MRE600 (RNase I^-) was grown at 37□ from a single colony with aeration in minimal medium

containing 1.5 mM phosphate (specific activity 1.33 x 10^7 counts/min/μmole), 22 mM glucose, 0.2 mM magnesium sulphate, 1.1 mM sodium citrate, 7.5 mM ammonium sulphate and 100 mM Tris-HCl (pH 7.5). An aliquot, sample I (50 ml), was sampled at 3 hr within exponential phase and cooled to 4^0 immediately. The remaining cell culture was allowed to grow upto the stationary phase and a second aliquot, sample II (50 ml), was collected at 6 hr. The remaining cell suspension was diluted with equal volume of minimal medium and excess cold phosphate was added to make the final concentration of phosphate 100 mM so as to dilute out the radioactive phosphate. The cells were allowed to grow again and a third sample III (50 ml), was collected within the exponential phase at 7 hr. The growth was continued till the stationary phase was attained again and a fourth and final sample IV (50 ml), was collected at 10 hr. To determine the amount of radioactivity incorporated in the cells at each stage of the sample collection, trichloroacetic acid insoluble counts at each stage was determined by passing the acid - treated aliquots of each of the sample (0.05 ml) through Whatman glass fibre filters. The filters were dried and counted in scintillation fluid in Beckman liquid scintillation counter LS1701.

2.2 Preparation of radiolabeled ribosomes at different phases of growth of *E. coli*: All operations were carried out at 4^0 unless otherwise stated. Ribosomes were prepared from all the four samples collected as above at different stages. The cells were immediately harvested by centrifugation at 13,000 x g and were washed twice with 20 mM Tris-HCl (pH 7.5). The cell extract was prepared by passing the cells suspended in 5 ml of TMA-10 (20 mM Tris-HCl pH 7.5, 30 mM ammonium chloride, 10 mM magnesium acetate and 5 mM β-mercaptoethanol) twice through French pressure cell press at 7000 psi. After high speed centrifugation of the extract at 23,000 x g for 30 min, the ribosomes were pelleted by centrifugation at 1,50,000 x g for 2 hr 30 min. The pellet was suspended in TMA-10 and the suspension was treated with 15 units/ml of DNase I (RNase free) for 1 hr. The suspension was recentrifuged at 1,50,000 x g for 2 hr 30 min and the pellet obtained was suspended in TMA-10. The final preparation of ribosomes was dialyzed for 18 hrs against TMA-4 (same as TMA-10 but containing 4 mM magnesium acetate).

2.3 Analysis of radiolabeled ribosomal populations by sucrose density gradient centrifugation: An aliquot (total counts 3 x 10^5/min) of the dialyzed radiolabeled preparation of ribosomes from the samples collected at different phases of growth, was subjected to centrifugation at 1,15,000 x g for 2 hr 30 min at 4^0 through 5 ml of 10-35% sucrose density gradient containing TMA-4. Two- drop fractions were collected on Whatman 3MM filter paper and the dried papers were counted in scintillation fluid in Beckman liquid Scintillation counter.

3. RESULTS AND DISCUSSION

E. coli cells were grown in low phosphate (of high radioactivity) containing minimal medium. The incorporation of radioactivity at various phases of cell growth shows about 50% more radioactivity associated with the cells isolated at the first stationary phase as compared to those isolated at the

first exponential phase as shown in figure1. Due to dilution with cold phosphate and thus subsequent lowering of specific activity, no further incorporation of radioactivity into the cells was observed during the second stage of cell growth either at exponential or stationary phase (Fig.2).

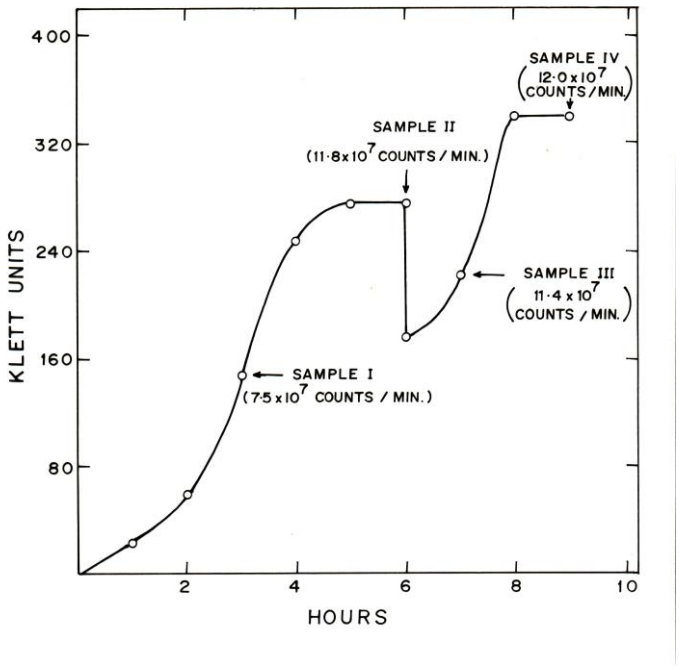

Figure 2: Incorporation of ^{32}PO4-3 at different phases of growth of *E. coli*. *E. coli* was grown in minimal medium containing radioactive phosphate and samples were collected and trichloroacetic acid insoluble counts determined at different phases of growth (as discussed in detail under materials and methods). Counts of the samples (taking into account the dilution at the second phase) are mentioned in the figure. The remaining portions of the samples were processed as described in legend to Fig. 3. for analysis of radioactivity of 70S, 50S and 30S ribosomes.

A fraction of incorporated radioactivity is carried by ribosomes of the cells due to their radiolabeled ribosomal RNA. Analysis of ribosomal populations at different phases of the cell growth by sucrose density gradient centrifugation (10-35%) in the presence of 4 mM Mg^{++} was carried out as described under materials and methods to allow the separation of TC and LC ribosomes (fig. 3).

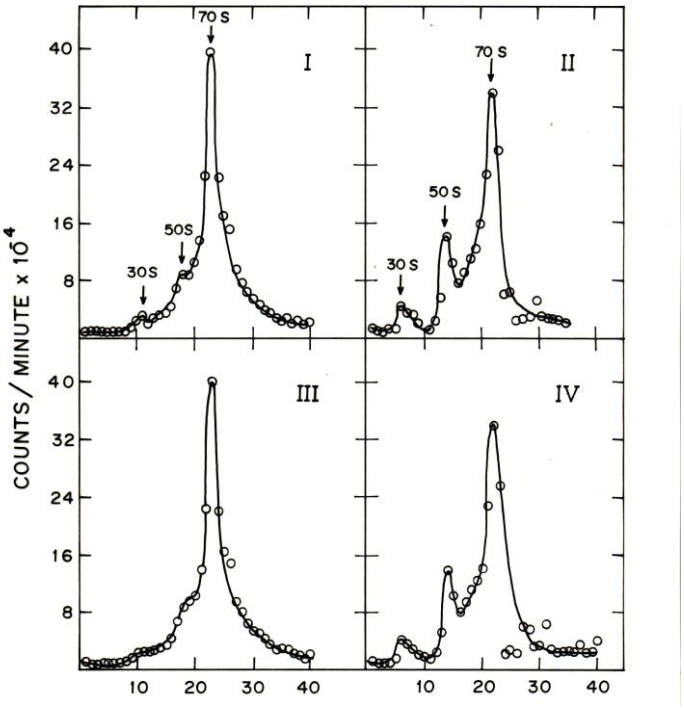

Figure 3: Analysis of radiolabeled populations at different phases of growth of *E. coli* by sucrose density gradient centrifugation. The samples collected at different stages (Fig.2) were processed as described under materials and methods and ribosomal preparations were subjected to sucrose density gradient analysis. Marking of samples at different stages of growth are as in Fig. 2. Fraction Numbers are plotted on x-axis.

70S ribosomes in the gradients represents the TC population as they remain undissociated at 4 mM Mg^{++} whereas 30S and 50S ribosomes represent LC population as they get completely dissociated. At the exponential phase in both the stages, (sample I and III, fig. 2) major amount (80-90%) of ribosomes were TC and approximately 10-20% LC ribosomes were present as determined by the counts associated. Whereas in the case of ribosomes isolated from stationary phase cells in both the stages, (sample II and IV, fig. 2) about 30-35% population were LC and remaining 65-70% of TC type. The increased amount of radioactivity associated with LC ribosomes from 10% to 30% in the stationary phase I (II, fig. 2) as compared to exponential phase I (I, fig. 2) must have been derived from the conversion of TC ribosomes of the exponential phase I (I, fig. 2). Surprisingly, again there is an increase in the total radioactivity associated with TC ribosomes at exponential phase II (III, fig. 2) as compared to that of stationary phase I (II, fig. 2). This points towards reconversion of LC ribosomes of stationary phase I to TC ribosomes during exponential phase II as evident with the increased amount of radioactivity from 70% to 90% associated with TC ribosomes. This demonstrates the novel interconversion of TC and LC ribosomes during different phases of cell growth in *E.coli*. The highly reduced radioactivity of phosphate in the medium in the second stage of cell growth rules out the breakdown and new synthesis of radioactive TC ribosomes. The only

possible way is the conversion of LC to TC ribosomes. The same is true for the conversion of TC to LC ribosomes at both the stages, from exponential to stationary phase. It should be noted carefully that the total radioactivity associated with the cells remained constant after addition of excess of cold phosphate (lll, lV fig.1). The specific activity of the ribosomes was also same in all the cases. These results indicate the interconversion of TC and LC ribosomes during the exponential and stationary phases of *E.coli* growth. This remarkably demonstrates the physiological relevance of loose couple ribosomes as against the belief that they are inactive particles. Ribosome is a highly flexible structure with dynamic properties being a prerequisite for its function. It has been known for a long time that ribosome oscillates between a contracted and extended conformation during protein synthesis [15]. Translocation is one of the crucial steps in the elongation cycle during protein synthesis promoted by EF-G. During this, A and P-site tRNAs move to the P and E sites respectively and mRNA is advanced by one codon. There are several lines of evidence suggesting that the translating ribosome undergoes reversible conformational transitions between pre- and post-translocational states promoted by elongation factor-G [8,9,13].

4. CONCLUSION

This novel demonstration of interconversion of TC and LC ribosomes during exponential and stationary phase of *E. coli* growth provides clear evidence of the physiological and functional relevance of loose couple ribosomes.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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