Protein folding by ribosome and its RNA

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We show that the protein-folding activity of ribosome evolved probably prior to evolution of its translational activity since the total ribosomal RNA could also assist refolding of denatured proteins. This also points at some general protein-folding activity of ribosomal RNA, a kind of activity of RNA that has not been reported earlier.

We have shown earlier that ribosomes from a variety of organisms, like *E. coli*, wheat germ, rat liver and archaebacterium *M. barkeri* could fold a number of enzymes, viz. bacterial alkaline phosphatase, glucose 6-phosphate dehydrogenase, lactate dehydrogenase and horse radish peroxidase to their active forms when the enzymes, denatured with guanidine–HCl, were subsequently incubated with ribosomes[1,2]. No energy currency like ATP, GTP, UTP etc. was needed for these reactions, unlike most of the chaperone-mediated refolding of denatured proteins where ATP or its analogue was required[3].

Folding of polypeptides to their biologically active forms after synthesis on the ribosome has been shown to be not spontaneous as was initially thought[4] from genetic and biochemical experiments. A large number of proteins called molecular chaperones have been implicated in the folding of newly synthesized polypeptides in the cell[5,6]. We have earlier shown that many ribosomes from different sources could refold a wide variety of denatured proteins to their active forms. Although these reactions were shown to be different from chaperone-mediated protein folding in being independent of the requirement of ATP or any other energy supplying cofactor, the question remained that the ribosome, although washed with high salt and ethanol, could still have some bound chaperones as contaminants. In order to find the answer to that suspicion and locate the activity on the ribosome, we started deproteinizing it and found that in course of time the total ribosomal RNA, when thoroughly deproteinized with phenol, could still assist the folding of denatured enzymes. In this report we show that both the ribosome and total ribosomal RNA could assist the folding of a number of proteins from their denatured states to active forms.

E. coli 70S ribosome used in these experiments was prepared following the method described elsewhere[1]. *M. barkeri* ribosome was prepared by slightly modifying the procedure of Elhardt and Böck[7]. These ribosomes were free from GroEL-like proteins which belong to the Hsp60 family and are sometimes reported to be associated with 70S ribosome preparations[8]. No immunoprecipitation was detected between polyclonal antibody against Hsp60 and our ribosome preparations. Total rRNA from *E. coli* and *M. barkeri* was extracted following slightly modified protocol of Traub & Nomura[9] by phenol extracting 70S particle 8 times in the buffer containing 10 mM Tris–HCl pH 7.6, 4 mM Mg-acetate and 0.2 mM EDTA. It was then precipitated with chilled ethanol and dialyzed with a few changes against the same buffer. The rRNA preparations were found intact. They showed about 40% hyperchromicity when treated with RNAase I.

Figure 1 shows the reactivation of denatured pig muscle lactate dehydrogenase in the presence of *E. coli* 70S ribosome and its RNA. The enzyme (0.10 µM with respect to monomer) was denatured for 90 min. at 25° C with 1.0 M guanidine–HCl in buffer containing 20 mM Tris–HCl pH 7.5 and 5 mM 2-mercaptoethanol. It was diluted 40-fold in the renaturation buffer (20 mM Tris–HCl pH 7.5, 10 mM Mg-acetate, 5 mM 2-mercaptoethanol) in the presence of different concentrations of ribosome or ribosomal RNA. Incubation was carried out

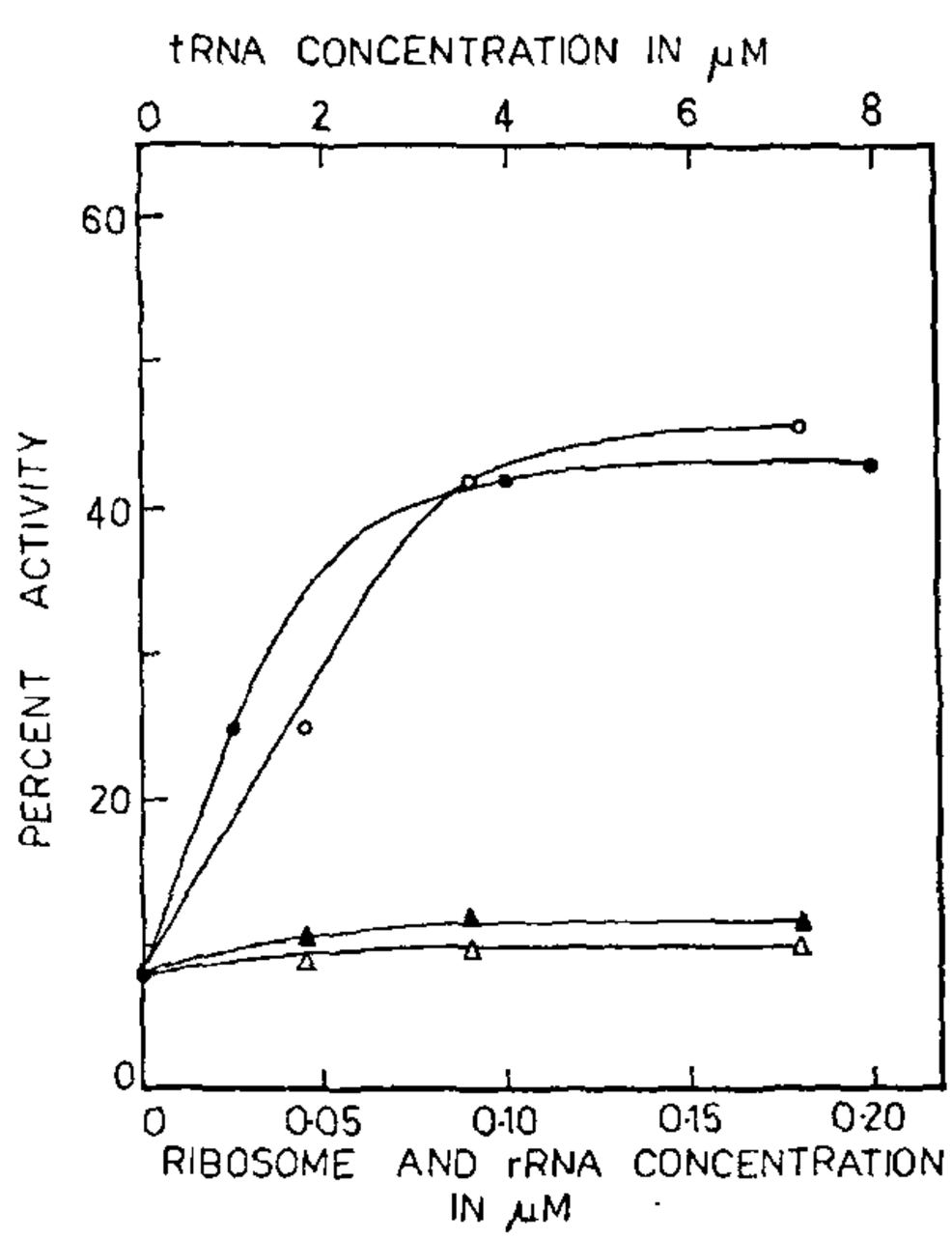

Figure 1. Effect of *E. coli* 70S ribosome (–●—●–), total RNA (–O—O–), total RNA treated with RNAase (–Δ—Δ–) and tRNA (–▲—▲–) on the reactivation of denatured pig muscle lactate dehydrogenase.

for 30 min at 37° C and the enzyme activity assayed for 5 min at 37° C. The control sample had no ribosome or rRNA added to it. In the other set of samples, ribosomal RNA was first digested with RNAase (final conc. 100 µg/ml) at 37° C for 45 min followed by 30 min at 65° C and then added to the denatured enzyme. As shown in Figure 1, both 70S ribosome and its RNA could reactivate the denatured enzyme, whereas there was no recovery of activity when the denatured enzyme was left to renature by itself or by RNAase-treated rRNA. *E. coli* tRNA (about 40 times higher molar concentration than rRNA) also had no effect on the recovery of enzyme activity. The time course of ribosome and rRNA– mediated recovery of enzyme activity showed linear increment upto 20 min in case of LDH (data not shown).

The recovery of activity of denatured horse radish peroxidase is shown in Figure 2. The enzyme (1.1 µM with respect to monomer) in 20 mM Tris–HCl pH 7.5 was denatured by treatment with 6 M guanidine–HCl at 25° C for 45 min. The denatured enzyme was then diluted 40-fold in the buffer containing 20 mM Tris–HCl pH 7.5, 10 mM Mg-acetate and 25 mM KCl and allowed to renature by itself, in presence of 70S ribosome, in presence of total ribosomal RNA and in presence of RNAase (100 µg/ml) treated ribosomal RNA. Renaturation time was 30 min at 20° C. The enzyme activity was assayed for 3 min at 20° C. As shown in the case of lactate dehydrogenase, horse radish peroxidase was also reactivated by ribosome and ribosomal RNA whereas RNAase-treated ribosomal RNA, could not refold the enzyme. Transfer RNA also had no effect on the recovery of enzyme activity.

The recovery of enzyme activity was not only due to *E. coli* ribosome[1] or ribosomal RNA from *E. coli* as reported earlier, but ribosome from other organisms also presented this protein-folding activity. In Figure 3, we show the reactivation of denatured horse radish peroxidase with ribosome and ribosomal RNA from the archaebacteria *M. barkeri*. The enzyme and ribosome denaturation and rRNA-mediated recovery were carried out as described in the case of *E. coli* ribosome and rRNA. Ribosomal RNA lost this activity when treated with RNAase as in *E. coli* rRNA. In the case of both *E. coli* and *M. barkeri* ribosome and rRNA, the time course of refolding the denatured HRP was linear for about 20 min at 20° C and slowly attained a saturation level in about 30 min (data not shown).

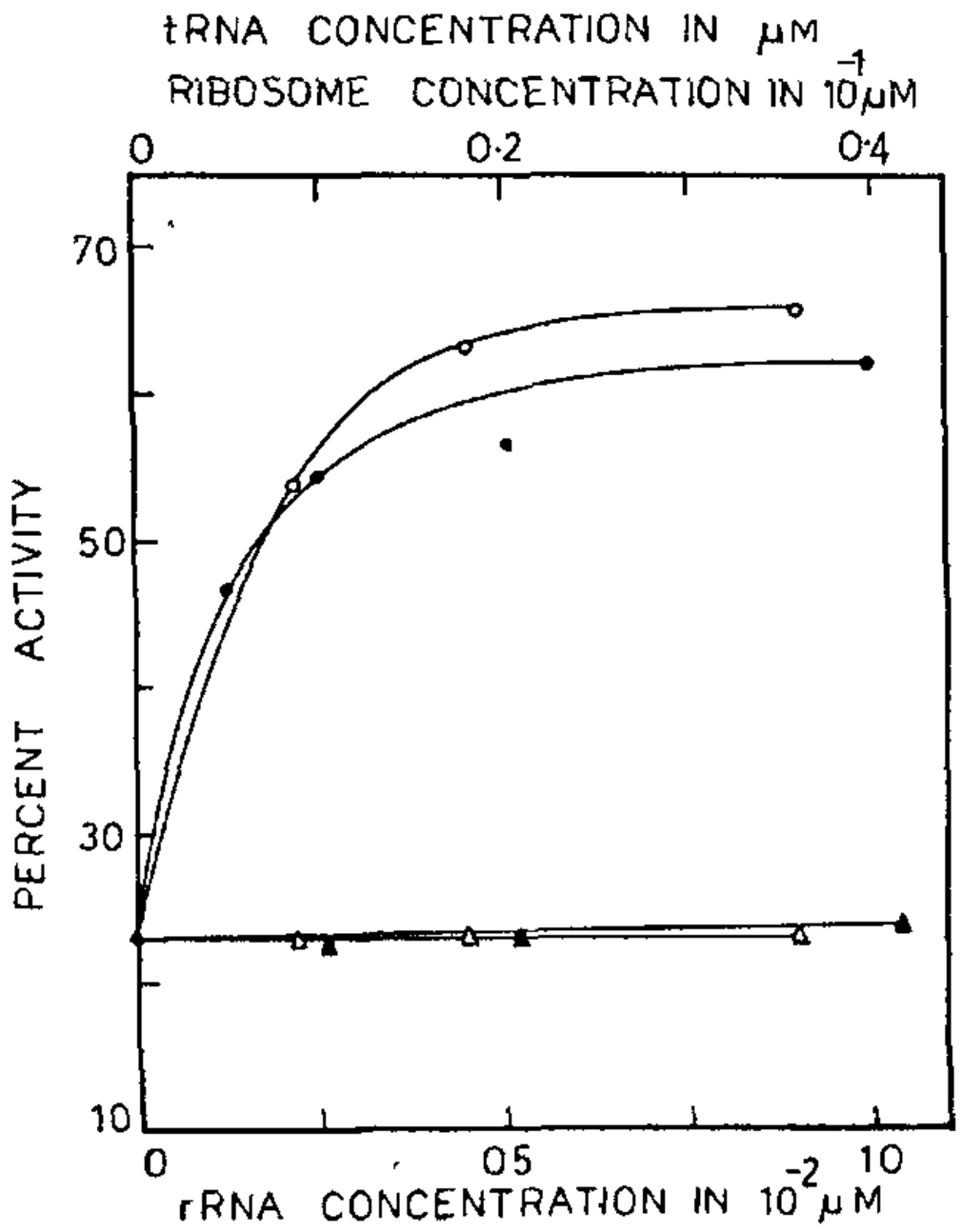

Figure 2. Effect of *E. coli* 70S ribosome (-●—●), total RNA (-O—O-), total RNA treated with RNAase (-Δ—Δ) and tRNA (-▲—▲-) on the reactivation of denatured horse radish peroxidase.

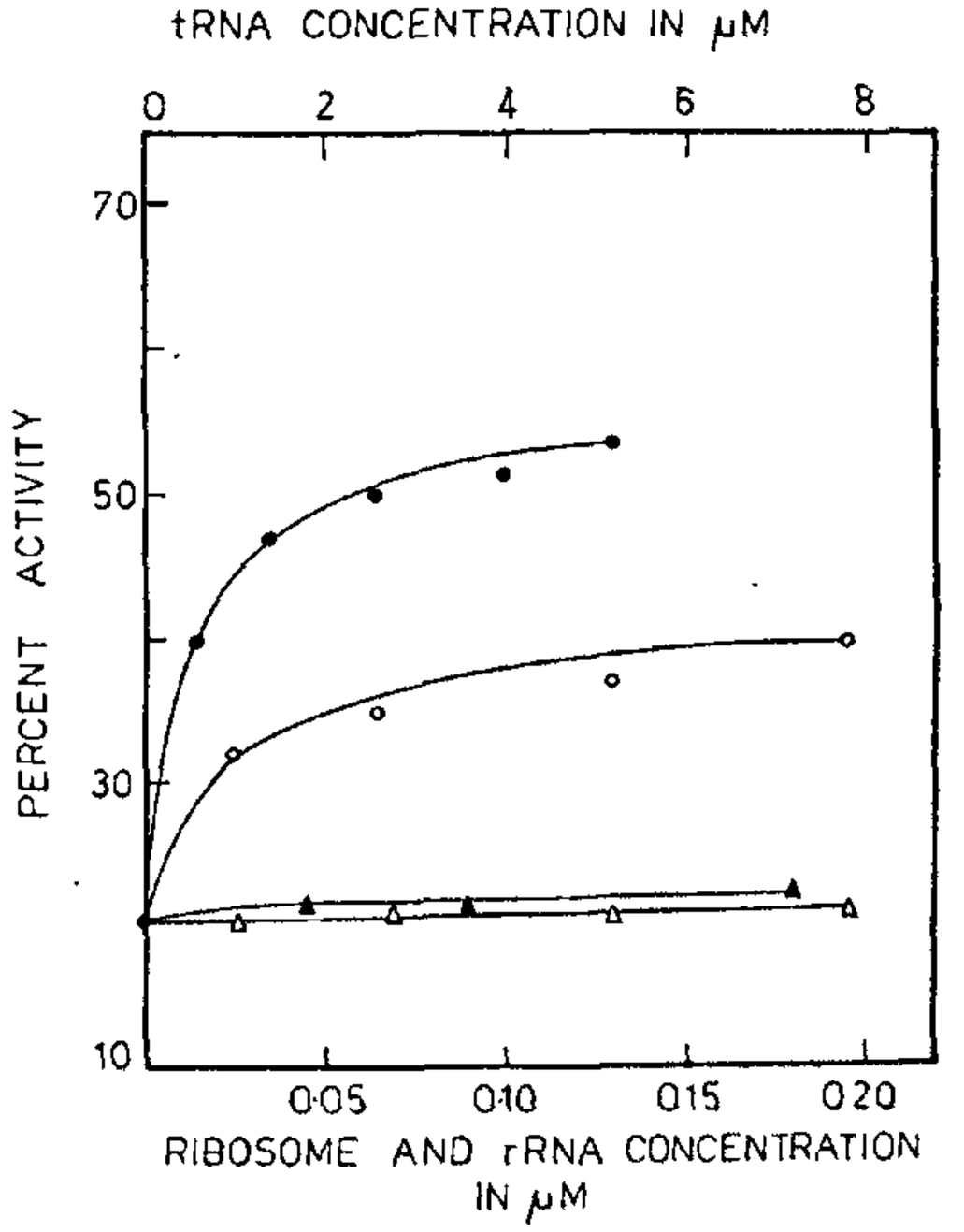

Figure 3. Effect of *M. barkeri* 70S ribosome (-●—●), total RNA (-O—O), total RNA treated with RNAase (-Δ—Δ) and *E. coli* tRNA (-▲—▲-) on the reactivation of denatured horse radish peroxidase.

The A_{260} of the rRNA preparations from both *E. coli* and *M. barkeri* ribosomes did not change during the recovery of activity of the enzymes, indicating that rRNA did not denature or degrade within that time.

As seen earlier, the recovery of enzyme activity depended on the state of protein denaturation. Even when samples were picked up at definite times from denaturation reaction the residual activities varied to some extent in different experiments. The ribosome-mediated recovery of enzyme activity also varied accordingly. However, denatured enzyme samples having the same residual activity would always exhibit the same level of recovery of activity. The data presented in Figures 1–3 are from one set of experiments. Although the experiments were repeated many times, the graph could not be plotted with average of a number of data for the reason just mentioned. As we can see from Figure 3, the rRNA-mediated recovery of activity was poor compared with the recovery in presence of 70S ribosome. We do not have an explanation for this at this moment. It was not due to degradation of that rRNA. The reason hopefully would come when the individual rRNA molecule is checked for protein-folding activity.

In this report, therefore, we have shown some generalized protein-folding activity in the ribosome and total ribosomal RNA. The protein-folding activity of RNA seems to be specific for ribosomal RNA only and is not dependent on any nonspecific secondary structure containing extensive hairpins and loops since transfer RNA from *E. coli* having double-stranded and looped regions failed to reactivate the denatured enzymes at about 40 times higher molar concentration. This activity on rRNA deserves special attention, since these findings point towards the evolution of the protein-folding activity of ribosome even before its translational activity evolved, as the latter activity also needed the ribosomal proteins. It should be noted that RNAs exhibit not only enzymatic activity on nucleic acids, but also could take part in refolding the polypeptides after their synthesis on ribosome surface. Recently, folding of nascent polypeptide on ribosome surface has been demonstrated in *in vitro* translation system in crude cell extracts from wheat germ and *E. coli*[10].

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Myoinositol trisphosphate sensitive calcium stores in *Entamoeba histolytica*

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Calcium mobilization from internal stores of the parasitic protozoan *Entamoeba histolytica* was studied by fluorescence measurements of the calcium indicator quin 2 in saponin permeabilized amoeba. Both Ins (1,4,5)P_3 and Ins(2,4,5)P_3 could release calcium equally well from permeabilized *E. histolytica*. On an average, about 40% of the A23187 releasable calcium pool could be mobilized by 2 μM $InsP_3$. Neither GTP nor cAMP could influence $InsP_3$-mediated Ca^{2+} mobilization. $InsP_3$-mediated Ca^{2+} release from internal stores of *E. histolytica* occurred in an $InsP_3$ receptor-dependent manner. Differential interference contrast microscopy revealed that increased motility and pseudopod formation could be produced in *E. histolytica* through $InsP_3$ treatment.

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INTRACELLULAR calcium and protein kinase C play important roles in the cytolytic activities of *Entamoeba histolytica*, a parastic protozoan[1,2]. TMB-8, an intracellular calcium antagonist causes significant reduction in vesicle exocytosis in this enteric parasite[1]. However, very little else is known about calcium homeostasis in this organism.

Trypanosoma brucei, another parasitic protozoan, has very recently been shown[3,4] to possess a large extramitochondrial calcium pool and contain inositol phosphates, especially Ins(1,4,5)P_3. Both the circulating and