Splitting of ribosome into its subunits by unfolded polypeptide chains

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***E. coli* ribosomes can fold a number of unfolded proteins *in vitro* and the newly synthesized proteins *in vivo*. During the process of folding, the 70S ribosome is found to be dissociated into the 50S and 30S subunits. The separation of 30S subunit is a consequence of interaction of the 50S subunit with the unfolded protein. This finding suggests that completed polypeptide chains may play a role in releasing the 50S and 30S subunits so that they are available for the next round of protein synthesis.**

THE different steps in protein synthesis on ribosome – the initiation, elongation and termination of synthesis of the polypeptide chain – have been worked out in great detail over the last several decades. The fourth step, the disassembly of the post-termination complex of ribosome, mRNA and tRNA have also been deciphered in detail *in vitro* and *in vivo*[1] and more information is coming up on this step[2,3]. The dissociation of ribosome from the mRNA and release of tRNA are prerequisites for the next round of protein synthesis. The next round starts with positioning small ribosomal subunit on mRNA followed by association of the large subunit. But the factors that govern the dissociation of the ribosome into the small and large subunits prior to the next round of protein synthesis have not been clearly identified. We describe below some *in vitro* experiments that suggest a role of the newly synthesized protein in carrying out the process of dissociating the ribosome into its subunits in the course of its folding into its active state.

We reported earlier that the ribosome has a role in protein folding both *in vitro* and *in vivo*[4–6]. The protein-folding activity comes from the large subunit. The ribosome and its large subunit can fold unfolded proteins whereas the small subunit fails to do so (Figure 1). For protein folding studies, BCA was unfolded in 6 M guanidium hydrochloride for 3 h at 25°C. The loss of secondary structure of the protein was confirmed by CD spectral analysis (not shown). The unfolded BCA was diluted hundredfold in the folding buffer (50 mM Tris HCl, pH 7.5, 100 mM NaCl and 10 mM Mg-acetate) containing the 70S ribosome or its 50S subunit. As shown in Figure 1 *a*, the activity, however, appears to be more in the large subunit than in the intact ribosome, although the time course of the event appears identical in the two cases (Figure 1 *b*). We have shown that the active site of protein folding is the domain V of 23S rRNA[7–10] in *E. coli* and other eubacterial ribosomes. In the crystal structure of ribosome[11–13], this site is exposed in the 50S subunit[14]. But when the 30S subunit joins the 50S counterpart, the domain V region may remain partly inaccessible for the protein folding purpose. This suggests that for more efficient folding the ribosomal subunits should preferably dissociate after protein synthesis. This is also supported by *in vivo* observations from our and other laboratories[6,15,16] that the newly synthesized full length polypeptide remains associated with 50S particle for a considerable length of time (about 5 min in case of β-galactosidase)[6] during which the protein folding process remains sensitive to some antibiotics that bind to domain V of 23S rRNA. The dissociation of 70S to 50S and 30S particles is completed within this time. Although the crystal structure of ribosome suggests that the complete polypeptide chain could fold as it exits from the tunnel in the 50S subunit, the antibiotic sensitivity pattern of the process confirms that the folding polypeptide subsequently associates with domain V of 23S rRNA in the 50S subunit to complete the process of folding and attain an active state[6]. When the polypeptide moves through the 'non-stick' exit tunnel, it does not find the appropriate surface for hydrophobic collapse. But the exposure of domain V RNA on splitting the ribosome provides the correct hydrophobic surface[8] for protein folding. We also found that when deacylated tRNA molecules bind to the P and E site of the 70S particle at 25 mM Mg^{++} (ref. 17), its protein folding activity goes down from 65% to 28% at a molar stoichiometry of ribosome : tRNA of 1 : 3, when the 31 kD monomeric enzyme bovine carbonic anhydrase (BCA) was used as a substrate. This is true in case of folding of a number of other proteins. Thus, tRNA molecules bound to 70S particles during protein synthesis may inhibit the folding activity and the ribosome may have to wait for complete release of tRNA which only happens at the end of the fourth step of protein synthesis and is followed by the dissociation of 50S and 30S particles[18].

The above discussion suggests that the ideal time for protein folding by ribosome would be when it dissociates into the subunits after completion of protein synthesis so that the newly synthesized protein gets full access to the large subunit. The question is, what facilitates the dissociation of the ribosome into its subunits at this stage? The elongation factor G, release factors, the ribosome recycling factor, IF3, etc. are known to be involved in releasing the full length newly synthesized polypeptide, and the deacylated tRNA and hence dissociate the ribosomal subunits[18–22]. Such experiments included cell extract required for translation and it is difficult to say which protein(s) directly cause dissociation of ribosomal subunits. We attempted to see whether the polypeptide

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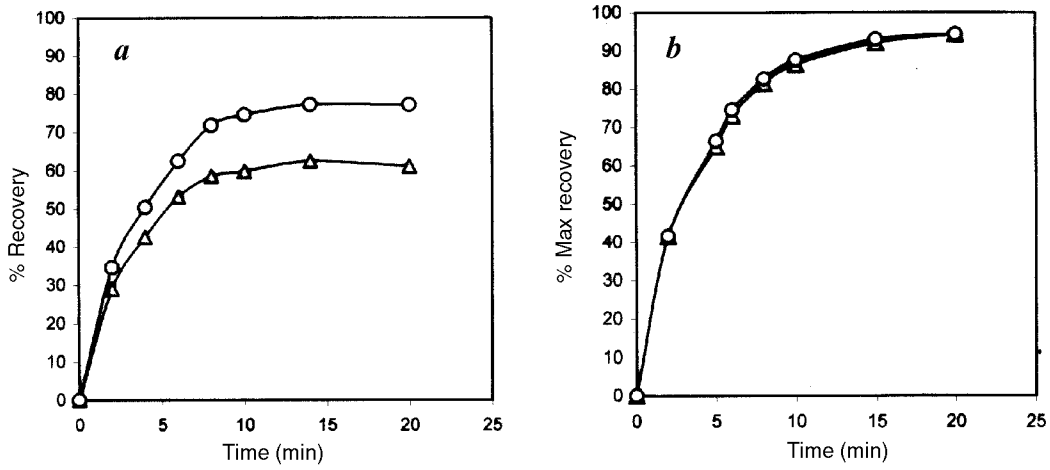

Figure 1. Time course of folding of unfolded BCA. BCA was unfolded at a concentration of 20 μM in 6 M guanidium hydrochloride at 25°C for 3 h and diluted 100-fold in folding buffer (50 mM Tris.HCl pH 7.5, 100 mM NaCl and 10 mM Mg-acetate) containing 150 nM each of 70S or 50S particles. Refolding was done at 25°C. Samples were withdrawn at various times and assayed for recovery of activity of BCA. Although the extent of refolding differed in case of 50S and 70S particles, as shown in (*a*), the rate of attainment of maximum folding was the same for both, as shown in (*b*).

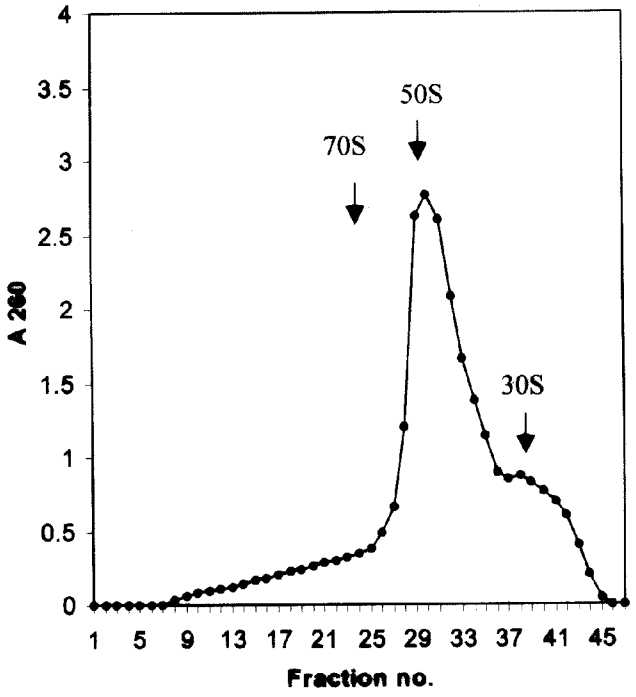

Figure 2. 5–20% sucrose density gradient profile of the refolding mixture containing unfolded BCA and 70S ribosome in 5 : 1 molar ratio. BCA which unfolded at a concentration of 150 μM in 6 M guanidium hydrochloride, at 25°C for 3 h, was diluted 100-fold in protein folding buffer (containing 300 nM 70S so that 70S : unfolded protein molar ratio was 1 : 5). The high concentration was required to get measurable concentration of ribosomes in gradient fractions. 0.2 ml of reaction mixture was loaded on 4.8 ml gradient and run at 28000 rpm for 1.5 h. The 70S, 50S and 30S particles were run in three separate tubes in the same experiment and their peak positions are marked here. The splitting of 70S to 50S and 30S is clear from the profile.

chain itself could bring about the dissociation in the process of folding when it accesses the domain V of the 23S rRNA.

BCA was unfolded as mentioned earlier and diluted in folding buffer containing 70S ribosome so that the 70S : unfolded protein ratio was 1 : 5. This should allow all the 70S ribosomes to interact with the unfolded protein. The splitting of 70S ribosome to 50S and 30S subunits was verified by running the folding mixture of 70S ribosome and BCA in a 5–20% sucrose gradient. The 70S particle resolved into 50S and 30S subunits as shown in the sucrose gradient profile (Figure 2). The 50S and 30S particles showed identical sedimentation profile with control 50S and 30S particles. These particles as well as complete 70S ribosome were run separately in different centrifuge tubes.

In another experiment, unfolded BCA was added to the 70S ribosome in the same ratio as mentioned above and the fall in light scattering intensity consequent to separation of ribosomal subunits was measured. The scattering intensity of light at 350 nm dropped from an arbitrary value of 140 to 104. When the same concentration of 70S was allowed to dissociate into the subunits in absence of magnesium in the buffer, the light intensity dropped to 100. There was no fall of intensity when the same amount of native BCA was added to 70S. The light scattered by BCA itself was negligible.

These *in vitro* experiments corroborate the assumption based on experiments described in Figure 1 that the 50S subunit must be free to ensure optimum folding of the freshly synthesized polypeptide on the 70S ribosome. This is achieved if the folding protein dissociates the 70S particle in the process of accessing the domain V of 23S rRNA of the 50S subunit at a time when all the tRNAs dissociated from the ribosome on completion of polypeptide synthesis. We repeated the experiments with malate dehydrogenase and lysozyme, and found similar splitting of 70S ribosome in course of folding these pro-

teins. It is interesting to assume that this should also be true *in vivo* during protein synthesis because, after synthesis, the folding of the polypeptide remains sensitive to antibiotics for several minutes due to its association with the domain V of 50S subunit[6]. Our aim is to do similar experiments in *in vitro* translation system in the presence of release factors, elongation factor EFG and the ribosome recycling factor.

1. Nakamura, Y., Ito, K. and Isakson, L. A., *Cell*, 1996, **87**, 147–150.
2. Janosi, L. *et al.*, *EMBO J.*, 1998, **17**, 1141–1151.
3. Atarashi, K. and Kaji, A., *J. Bacteriol.*, 2000, **182**, 6154–6160.
4. Das, B., Chattopadhyay, S. and DasGupta, C., *Biochem. Biophys. Res. Commun.*, 1992, **183**, 774–780.
5. Das, B., Chattopadhyay, S., Bera, A. K. and DasGupta, C., *Eur. J. Biochem.*, 1996, **235**, 613–621.
6. Chattopadhyay, S., Pal, S., Pal, D., Sarkar, D., Chandra, S. and DasGupta, C., *Biochim. Biophys. Acta*, 1998, **1429**, 293–298.
7. Chattopadhyay, S., Das, B. and DasGupta, C., *Proc. Natl. Acad. Sci. USA*, 1996, **93**, 8284–8287.
8. Pal, S., Chandra, S., Chowdhury, S., Sarkar, D., Ghosh, A. N. and DasGupta, C., *J. Biol. Chem.*, 1999, **274**, 32771–32777.
9. Chowdhury, S., Pal, S., Ghosh, J. and DasGupta, C., *Nucleic Acids Res.*, 2002, **30**, 1278–1285.
10. Sanyal, S. C., Pal, S., Chaudhuri, S. and DasGupta, C., *Nucleic Acids Res.*, 2002, **30**, 2390–2397.
11. Cata, J. H., Yusupov, M. M., Yusupova, G. Z., Earnest, T. N. and Noller, H. F., *Science*, 1999, **285**, 2095–2104.
12. Nissen, P., Hansen, J., Ban, N., Moore, P. B. and Steitz, T. A., *Science*, 2000, **289**, 920–930.
13. Ban, N., Nissen, P., Hansen, J., Moore, P. B. and Steitz, T. A., *Science*, 2000, **289**, 905–919.
14. Culver, G. M., Cate, J. H., Yusupova, G. Z., Yusupova, M. M. and Noller, H. F., *Science*, 1999, **285**, 2133–2136.
15. Teter, S. A. *et al.*, *Cell*, 1999, **97**, 755–765.
16. Choi, K. M. and Brimacombe, R., *Nucleic Acids Res.*, 1998, **26**, 887–895.
17. Joseph, S. and Noller, H. F., *EMBO J.*, 1996, **15**, 910–916.
18. Karimi, R., Pavlov, M. Y., Buckingham, R. H. and Ehrenberg, M., *Mol. Cell*, 1999, **3**, 601–609.
19. Hirashima, A. and Kaji, A., *J. Mol. Biol.*, 1972, **65**, 43–48.
20. Subramaniun, A. R. and Davis, B. D., *J. Mol. Biol.*, 1973, **74**, 45–56.
21. Pavlov, M. Y., Fresitroffer, D. V., Heurgue-Hamard, V., Buckingham, R. H. and Ehrenberg, M., *J. Mol. Biol.*, 1997, **273**, 389–401.
22. Selmer, M., Al-Karadaghi, S., Hirokawa, G., Kaji, A. and Liljas, A., *Science*, 1999, **286**, 2349–2352.

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STMS-based DNA fingerprints of the new plant type wheat lines

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New plant type wheat lines have been developed at the Indian Agricultural Research Institute, New Delhi, by combining some of the negatively correlated yield contributing traits such as more grains per panicle, larger grain size and optimum tillers per plant through inter-varietal hybridization followed by a modified-bulk pedigree method of selection. Some of these lines have been found to give 15–22% higher yield in experimental plots than the most popular national check varieties being cultivated in the north-western part of India and thus constitute a valuable source of genetic variation for breaking yield barriers in wheat. With the objective of precise differentiation and identification of new plant type wheat lines, 33 sequence tagged microsatellite site markers, which are genetically located on different chromosomes of wheat genome, were employed. These markers in combination differentiated the new lines from 137 wheat varieties released for commercial cultivation in India. Fourteen markers detected polymorphism among the thirteen genotypes included in this study, of which eight markers in combination differentiated the seven new wheat lines from each other as well as from their parents. Inheritance of parent-specific alleles revealed the direction of selection during breeding. Grouping of the lines based on cluster analysis reflected the nature of their relationship with the parent varieties. A graphic presentation of the genetic constitution of the new plant type lines was developed, which can be used as bar-coded molecular tags for identification of the respective seed samples.

THE Green Revolution in India in the 1960s was primarily due to the introduction and large-scale cultivation of semi-dwarf wheat varieties developed at CIMMYT, Mexico. The new plant architecture replacing the traditional tall phenotypes was responsible for a quantum jump in wheat yield. To bring further significant improvement in the yield potential, attempts are being made to modify the wheat plant by breaking some of the negative correlations among the yield contributing traits, namely number of productive tillers per plant, number of grains per panicle and grain size. At the Indian Agricultural Research Institute (IARI), New Delhi, strategic research during last seven years has led to the development of a new plant type by combining intermediate to high tiller

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