



Recycling of eucaryotic ribosomes

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Summary

Until recently protein biosynthesis has been viewed as a process involving only three steps: initiation of translation, elongation of the nascent polypeptide chain and release of the completed unfolded polypeptide. In recent years fourth step in translation has been distinguished – it is the recycling of the ribosome. In this process posttermination complexes termed post-TC consisting of ribosomes with deacylated tRNA(s) and mRNA are dissociated with the help of ribosome recycling factor (RRF), elongation factor G (EF-G) and initiation factor 3 (IF3) in Prokaryotes. The mechanism of this final step in Eucaryotes was unknown for a long time, but the work of Pisarev et al. sheds a light on splitting 80S ribosomes and preparing them for the next cycle of translation.

Key words:

recycling of ribosomes, release factor, initiation factor.

1. Introduction

The ribosome recycling process is now considered as the fourth essential step in protein biosynthesis. After the releasing of the newly synthesized unfolded polypeptide the ribosome is still bound with mRNA and deacylated tRNA in the P site. Since the ribosomes are used periodically in protein synthesis, after one round of synthesis they must split into small and large subunits and release tRNA and mRNA before another cycle. At first ribosomal decomposition has been thought to occur spontaneously after the termination step (1,2). Successively it has been shown that this process is more complicated, catalyzed by newly identi-

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fied in Prokaryotes so called ribosome release factor (RRF) (3,4). To perform its function this factor (further re-named to ribosome recycling factor) cooperates with elongation (EF-G) and initiation (IF3) factors (5,6).

During procaryotic termination mRNA stop codon in the ribosomal A-site is recognized by class-1 release factors RF1 and RF2 that trigger the release of nascent polypeptide chain from the P-site bound peptidyl-tRNA (7,8). RF3, class-2 release factor, possessing GTPase activity catalyses the recycling of RF1 and RF2 (9,10). The ribosome and tRNA bound to mRNA form now the post-termination complex (post-TC). In 2005 it was shown that in the first step the combined action of RRF and EF-G splits ribosomes into subunits for a new round of translation (11-13). Successively IF3 releases deacylated tRNA from the P-site of 30S subunit, but mRNA is thought to dissociate spontaneously with the rate comparable to that of initiation and termination. It is also possible that mRNA is actively released from post-TC with the help of RRF and EF-G (14). It has been proposed that as a result of a major conformational change in the Switch I domain of EF-G and GTP hydrolysis, a large-scale movement of EF-G domain IV induces domain rotation of RRF (15). Furthermore, the interaction between 23S rRNA and RRF can disrupt the intersubunit bridges B2a and B3, and thus cause a separation of the small and large subunits (16).

2. Ribosome recycling in Eucaryotes

Basing on the fact that eIF3 with eIF1 can dissociate 80S ribosome in the presence of RNA (17) and that eIF1 can dissociate 48S complexes containing mutated initiator tRNA, (18) it was presumed that eucaryotic ribosomal recycling process does not require a special factor similar to procaryotic RRF. Moreover, it has been shown that Eucaryotes do not encode RRF (although homologs were identified in mitochondria and chloroplasts), thus the mechanisms leading to recycling of the ribosomes must be different than procaryotic one.

In 2007 it has been described the disassemblance of the posttermination 80S ribosomes in work of Pisarev et al. (19). Previously it has been shown that eukaryotic release factor 3 (eRF3) assure rapid and effective hydrolysis of peptidyl-tRNA performed by eRF1 (20). Binding of eRF1 and eRF3-GTP causes a conformational change in the ribosome but it is not sufficient for releasing the free subunits. Further rearrangement, as a result of GTP hydrolysis, is needed to properly place eRF1 in the peptidyl transferase center (PTC) (21).

Using sucrose gradient analyses, 80S ribosomal posttermination complexes were isolated (19). Those complexes were further subjected to incubation with eRF1, eRF3, GTP, a variety of other ribosomal factors (eIF2, eIF3 (lacking its 3j subunit), eIF3j, eIF1, eIF1A, eIF4A, eIF4B, eIF4F, eIF5, eIF5B; eEF1H, eEF2) and analysed again on sucrose gradient. It was shown that the only factor that alone possessed dissociation activity was eIF3. The highest dissociation potency showed eIF3s, together

with eIF1, eIF1A and eIF3j, as they promoted almost complete splitting of post-TC. The addition of these initiation factors disassembled ribosomes into subunits, even when termination factors and the GTP were replaced by antibiotic puromycin. Factors interacting with the small ribosomal subunit, such as eIF2, eIF1, eIF1A and eIF3j in the absence of eIF3 could not promote the dissociation. eIF2/GTP/Met-tRNA^{Met} did not affect in any way the dissociation by eIF3 or upon addition of eIF1, eIF1A and eIF3j. eIF1 and eIF1A only slightly stimulated eIF3's dissociating activity, while eIF3j strongly enhanced splitting of pre-TC (up to 70-75%). eEF2 did not dissociate post-TC, moreover it did not influence the dissociation by eIF3 and eIF1, eIF1A, eIF3j.

Data presented by Pisarev et al. (19) show that the principal factor involved in the eucaryotic ribosomal recycling is eIF3, promoting splitting of post-TC into subunits. It has been also shown that after the disassemblance this factor remains bound to the small subunit, preventing its nonfunctional reassociation with the 60S subunit. Other factors, such as eIF3j, eIF1 and eIF1A are needed to enhance eIF3's activity. Altogether they mediate near complete dissociation of posttermination ribosomes. eIF1 is the factor that promotes the release of deacylated tRNA from recycled 40S subunit (19). These findings are in agreement with previous results, when it was shown that eIF1 has the ability to dissociate 48S complexes, containing initiator tRNA mutated in an anticodon stem (22). To investigate which factors are necessary for mRNA release, different combinations of eRFs, eIFs and puromycin with post-TC were analysed. eRF1/eRF3 stimulated the release of 35-40% of mRNA from posttermination ribosomes. Incubation of eRF1/eRF3 and eIF3 with pre-TC revealed the association of 25-30% of mRNA with 80S ribosomes and 20% with the small subunit. Moreover, these data indicated that eIF3j can dissociate mRNA from 40S subunit only if it is not stabilized by deacylated tRNA, after eIF1-induced release of tRNA.

Mammalian eIF3 is a large (~ 750 kDa) multiprotein particle that binds to the solvent side of the small ribosomal subunit and recruits mRNAs bearing a methylated guanosine cap at the 5'-end through direct interaction with eIF4F (23,24). Moreover, it was shown that eIF3 interacts with other initiation factors that detect the start codon, and helps to assemble active ribosomes and prevents premature association of the 40S and 60S. Binding of eIF3 most probably causes conformational changes in the small subunit that leads to ribosome dissociation. eIF3 is involved in interaction with helix 34 of 60S subunit, below the 40S platform, and contributes to the intersubunit B4 bridge (25,26). eIF1 and eIF1A both bind to the intersubunit surface of 40S. It has been proposed that eIF1 blocks the access of the large ribosomal subunit to 18S rRNA fragments involved in forming B2b and B2d bridges (25,26).

The sequence of events that makes posttermination 80S ribosomes disintegrated has been proposed. It starts after the peptide release, when eRFs (one or both) remain bound to 80S ribosomes. In the next step, several factors (eIF3, eIF1, eIF1A and eIF3j) cooperatively disassemble these posttranslational complexes releasing the large (60S) subunit. The small one, still remains bound to mRNA and deacylated

tRNA in the P site. Then, eIF1 ejects deacylated tRNA from the P site, which results in weakening of the interactions between 40S/eIF3 and mRNA. The complete release of 40S requires the presence of eIF3j. It is now well established that eIF3 is the factor that initiates recycling in Eucaryotes, but the order in which other factors join the process still remains unknown. This sequence of events, despite some obvious differences, is similar at some steps to those present in prokaryotic ribosomes. It seems that the dissociation of eukaryotic posttranslational complex involves the interaction with eIF3 from the solvent side of small ribosomal subunit, which is in contrast to prokaryotic (27,24). Furthermore, major factors that are involved in releasing the deacylated tRNA from 40S subunit, differ between Eucaryotes (eIF1) and Prokaryotes (IF3), but perform similar functions – they bind to identical regions in the small subunit and play equivalent roles during the initiation of protein biosynthesis.

Although the work performed by Pisarev et al. (19) constitutes the foundation of eucaryotic recycling, several questions still must be answered. For instance, it is of key importance to determine whether this process requires energy. For the disassemblance of prokaryotic ribosomes energy input in form of GTP hydrolysis is necessary (28) while in Eucaryotes the process does not seem to need energy. Perhaps one or more not yet identified factors that hydrolyze high-energy compounds are involved in eucaryotic ribosome recycling process. It is also possible that the energy is supplied during the termination reaction and thus it is not needed to hydrolyze GTP or ATP during recycling, but this possibility seems not very likely.

3. Conclusions

For years through an oversimplified linear view of protein biosynthesis we were ignoring the fact that the translation has a cyclic nature. After releasing of newly synthesized polypeptide the ribosomes are not intended for degradation, but undergo a process in which they are disassembled. Thus, the ribosome recycling process in which posttermination complexes consisting of mRNA-bound ribosomes and deacylated tRNA are dissociated, is now considered as the last essential step in protein biosynthesis. This process is necessary to initiate the preparation of ribosomes for the next round of protein biosynthesis.

Although the process of recycling of prokaryotic ribosomes is relatively well known, the mechanism in Eucaryotes is only becoming to be resolved. The work of Pisarev et al (19) brought us closer to the big mystery of eukaryotic recycling, but still several issues remain to be solved. Hopefully, the advances in biochemical and genetic studies, macromolecular crystallography that have been made during last decades will help in elucidating all molecules and steps necessary for eucaryotic ribosome recycling.

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