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Hotspots in ribosomal proteins – determinants of the translation machinery and beyond

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Motivation and Aim: Ribosome is a giant ribonucleoprotein machinery, which synthesizes proteins in all organisms from bacteria to human according to the program incoming as messenger RNA (mRNA) copied from definite parts of the genomic DNA. It is composed of two subunits; each consists of ribosomal RNAs and several dozens of ribosomal proteins. The structures of ribosomes from all kingdoms of life to date are deciphered at near-atomic level thanks to achievements in X-ray crystallography and cryo-electron microscopy. This structural information gives the rationale for the obtained earlier data on the arrangements of ribosomal ligands (mRNAs and tRNAs) in the respective functional sites of the mammalian ribosome obtained by site-directed cross-linking with the use of chemically or photochemically reactive tRNA and mRNA derivatives [1]. Specific amino acid residues of ribosomal proteins (RPs) located very close (within 3–5 angstroms) to mRNAs and tRNAs at these sites have been determined. It is assumed that these residues make contacts with the respective ligand, but structural information itself does not provide an idea on contributions of these contacts to the translation process and its regulation. In particular, it remained unclear which consequences would take place upon changes at amino acid residues contacting mRNAs and tRNAs. This information has been obtained recently in our studies described below.
Methods and Algorithms: To study contribution of specific amino acid residues of RPs to the translation process, we carried out investigations with human HEK293T cells, in which target RPs contained substitutions or deletions of amino acid residues that had been found earlier in close proximity to ribosomal ligands. Cells were transfected with plasmid constructions encoding FLAG-tagged wild type or mutant RP under investigation, and lysate of these cells were centrifuged in sucrose density gradients to analyze the content of the labeled RP in fractions of ribosomal subunits, 80S ribosomes and polysomes. If the mutation interferes the subunits assembly, the level of mutant protein is lower than that of the wild type RP in all fractions. If it stops translation at the initiation step, mutation does not affect the content of the RP in fractions of the subunits but it decreases in fractions of 80S ribosomes and polysomes. Finally, if the mutation blocks elongation, labeled RP content decreases only in fractions of polysomes. Then experiments *in vitro* enables identification the particular step of initiation or elongation affected by the mutation.
Results: We studied functional roles in translation of those amino acid residues of RPs, which had been identified earlier to cross-link to tRNA and mRNA derivatives [1]. In particular, the C-terminal portion of the RP uS19 (RPS15) (amino acid residues in positions 130–145) was found in the decoding site of the mammalian 40S ribosomal subunit; it has no homology in bacterial counterparts. The application of HEK293T cells

producing mutant RP uS19 lacking the C-tail and analysis of the ribosomal material from these cells have revealed that the tail is not important for the assembly of the post-initiation 80S ribosome but is essential for the correct accommodation of incoming aminoacyl-tRNA (aa-tRNA) at the A site [2]. This step of the elongation cycle is an essential prerequisite enabling the participation of the cognate aa-tRNA in the peptide bond formation. RP uS3 (RPS3) is exposed at the solvent side of the 40S subunit near the mRNA entry pore and its tetrapeptide 60-GEKG-63 possesses an ability, unique among RPs, to selectively cross-link to single-stranded model RNAs containing aldehyde groups, e. g., an abasic site [3]. We found that this tetrapeptide is critically important for maintaining the correct structure of the 48S preinitiation complex competent for the eIF5A-dependent binding of the 60S subunit, the way by which 80S ribosome is formed upon the completion of initiation of translation. Conserved motif YxxPKxYxK of the RP eS26 (RPS26) neighbors mRNA upstream of the E site bound codon. Replacement of the conserved residues of this motif for alanines did not significantly affect the bulk translation but led to accumulation of the light polysomes containing initiation factor eIF3 that did not leave the ribosome at elongation. Detailed analysis enabled the conclusion that this conserved RP eS26 motif is implicated in fine tune regulation of translation of selected mRNAs to maintain balance of proteins currently required for the cell life [4]. Finally, we determined functional role of the conserved motif 45-QSGYGGQTK-53 in the RP eL42 (L36AL) located close to the 3'-terminus of deacylated tRNA at the P site after peptide bond formation (in this state, the acceptor stem of the tRNA is shifted to the E site, which is known as "hybrid" P/E state). It turned out that the eL42 motif plays a critical role in the ability of the human ribosome to perform properly elongation cycle at the step of deacylated tRNA dissociation from the E site in the human cell [5].

Conclusion: The application of the approach based on the use of human cells producing labeled target mutant RP with alterations in the region of interest and the subsequent analysis of the incorporation of the mutant RP into ribosomal fractions allowed us to reveal for the first time translational assignment of specific RPs amino acid residues contacting tRNA and mRNA. Some of these assignments turned out to be not those that were expected from the structural data.

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