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ADP-ribosylation of human ribosomal proteins by enzymes PARP1 and PARP2 *in vitro*

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Motivation and Aim: ADP-ribosylation is a posttranslational modification of proteins that plays a key role in many regulatory mechanisms and cellular processes. The modification is performed by enzymes of the PARP family (ARTD) and results in the attachment of one (mono-ADP-ribosylation) or several (poly(ADP-ribosyl)ation) of ADP-ribose units to proteins [1]. Previously, it was believed that ADP-ribosylation occurs mainly during DNA repair, but recently a lot of data has been obtained on the participation of PARP enzymes in a variety of cellular processes, in particular, in the regulation of ribosome biogenesis and gene expression at the translation level [2]. Proteomic analysis revealed many ADP-ribosylated proteins, including several dozen ribosomal proteins (RPs). However, it is still unknown which enzymes of the PARP family under what conditions modify certain RPs and what consequences their ADP ribosylation leads to in the cell. The study of this type of modification is an urgent task, since violations of the assembly or operation of ribosomes, as well as many of the non-ribosomal functions performed by RPs, are associated with the development of a number of neurodegenerative diseases and are associated with carcinogenesis.
Methods and Algorithms: Modification of human ribosomal proteins with PARP enzymes was carried out *in vitro* by incubation of reaction mixtures containing the enzyme PARP1/2, total ribosomal protein from 40S or 60S ribosomal subunits from human placenta, DNase I-treated DNA as an enzyme activator, co-factor HPF1, and [^{32}P]-NAD^{+}. The labeled protein products of the ADP-ribosylation reaction were separated in an SDS-containing polyacrylamide gel followed by radioautography.
Results: We show that ribosomal proteins of the human large (60S) ribosomal subunit of are able to effectively undergo ADP-ribosylation by enzymes PARP1 and PARP2. The dependence of the modification reaction on the presence of the HPF1 cofactor, DNA activator, as well as on the amount of substrate (total 60S subunit ribosomal protein) was studied. It was found that modification occurs only in the simultaneous presence of the DNA activator and the HPF1 cofactor. Almost all the same patterns with 60S RPs are observed with PARP2. An unexpectable result was that when the concentration of the substrate (RPs) increases to a certain threshold, the modification reaction is completely inhibited (both modification of RPs and automodification of the PARP). Pre-incubation of RPs with proteinase K restores automodification of PARP, which indicates that the effect of complete inhibition of the reaction is caused by the intact ribosomal proteins. The identification of ribosomal proteins modified by each of the PARPs is the task of further research. Remarkably, under conditions used for modification of ribosome-free RPs, the mature ribosomal subunits were not modified by the enzyme PARP1.
Conclusion: For the first time, we found out which enzymes and under what conditions ADP-ribosylate human ribosomal proteins. It was shown for the first time that the

reaction depends on the simultaneous presence of a DNA activator and a HPF1 cofactor, and is completely inhibited when the concentration of ribosomal proteins reaches a certain threshold value.

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