The influence of the catalytic ricin A subunit on the translational machinery

ABSTRACT

Ricin is one of the most toxic substances synthesised in the plant kingdom. It represents type II ribosome-inactivating proteins (RIPs) and consists of two subunits: chains A and B linked via a disulfide bridge. Ricin A chain (RTA) is a catalytically active subunit of ricin, and ricin B chain (RTB) facilitates endocytosis of the toxin into the eukaryotic cell by binding to its surface. The target of the RTA activity is the sarcin-ricin loop (SRL), which is the most conserved part of the rRNA of the large ribosomal subunit. RTA hydrolyses the N-glycosidic bond in the adenine nucleotide located on the SRL, i.e. it depurinates rRNA. The sarcin-ricin loop is an essential element of the GTP-ase associated centre (GAC) and stimulates GTP hydrolysis by translational factors, i.e. proteins with GTPase activity supporting each step of the translation process. Adenine removed by ricin plays a key role in the formation and stabilisation of the interaction network formed close to the catalytic centre of each translational factor after binding to SRL; hence, this base is indirectly involved in the induction of GTP hydrolysis. Investigations conducted with the use of *in vitro* translation systems have shown that ricin-induced depurination of the sarcin-ricin loop inhibits the binding of the eEF2 elongation factor to the ribosome, GTP hydrolysis by eEF1 and eEF2, and the translocation step in the elongation cycle of translation. *In vitro* analyses have shown that ricin-modified ribosomes are inactivated and incapable of translation. *In vivo* studies in mammalian cells have demonstrated that the exposure of these cells to ricin induces apoptotic cell death. Yet, there is no information about the mechanism of the induction of apoptotic cell death by deactivation of ribosomes via SRL depurination.

To elucidate the effect of SRL depurination on the translation process in a eukaryotic cell, we characterised the function of the translational machinery in yeast cells in which controlled RTA expression was carried out. A plasmid containing the RTA-coding gene (pRTA) was introduced into W303 strain yeast cells. Gene expression was controlled via the GAL1 promoter - the culture of cells in glucose medium was aimed at repression of gene expression, whereas the cell growth in galactose medium ensured induction of gene expression. Preliminary analyses showed that cells with the pRTA plasmid did not grow on the galactose-supplemented medium, which indicated high toxicity of RTA and concurrently confirmed the functionality of the experimental system. A method for evaluation of the SRL depurination level based on the RT-qPCR technique was adopted and developed. It was found that 1.6% of the ribosomes in the pRTA plasmid-containing yeast cells grown in the repression conditions were depurinated, which

was probably a reflection of the so-called promoter leakage phenomenon. Additionally, 3-, 6-, and 9-h induction of RTA-coding gene expression was employed. The RT-qPCR analysis demonstrated that the level of depurination increased during the induction and reached a maximum after 6 hours (10.2%), which was followed by a decline to 6% after 9 hours. The SRL depurination was shown to include a translationally active pool of ribosomes in the cell. The *western blotting* technique confirmed the presence of RTA in the protein fraction obtained from cells in which RTA synthesis was induced for 6 and 9 hours, with a higher amount of the protein noted after the 9-h induction. The translation efficiency was nearly two-fold lower than in the control as a result of the RTA synthesis in repression conditions, and the RTA synthesis induction did not enhance the inhibitory effect of SRL depurination on translation. The level of translation inhibition did not correlate with the level of SRL depurination. Such experiments as polysome profiling and determination of parameter $T_{1/2}$ (ribosomal half-transit time) did not reveal any significant changes in the efficiency of any of the translation steps at repression of the expression. Cells that were subjected to the 6-h induction of RTA synthesis exhibited low but significant inhibition of translation at the elongation or termination stage. Additionally, cells with RTA were characterised by an increased frequency of errors of the ribosome, i.e. amino acid misincorporation and -1 frameshifting in the 5' direction. However, the level of the changes in both types of errors did not correlate with the depurination level. Particularly noteworthy is the fact that the RTA activity in the yeast cells did not cause their death but inhibited their capability of mitotic division and generation of daughter cells. Yeast cells carrying out the RTA synthesis were reproductively active for one-tenth of their lifespan and thus generated several times lower numbers of daughter cells than the control. These cells remained in the post-reproduction stage for a greater part of their lifespan, which was manifested as lack of growth.

The results of the analyses indicate that the mechanism of RTA toxicity does not result directly from blockage of the ribosome function via SRL depurination. The modification of SRL is not a direct cause of inhibition of translation in RTA-containing yeast cells. Depurination of the sarcin-ricin loop in the ribosome pool estimated at merely 1.6% is probably a signal inducing metabolic pathways of reduction of the translation efficiency. The RTA toxicity observed in the yeast cells is probably relayed to the induction of metabolic pathways involved in the regulation of the cell cycle.

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