Abstract

This doctoral dissertation concerns the functional characteristics of the phosphorylation modification of ribosomal P-stalk proteins. These proteins are the basic protein element of the eukaryotic GTPase-associated center (GAC). The GAC is located on the large subunit of the 60S ribosome and its best-defined function is binding and stimulating the GTPase activity of translational GTPases. The ribosomal P-stalk proteins form the uL10-$(P1/P2)_2$ pentameric complex, which binds to the rRNA of the ribosome via the uL10 protein. All P proteins: uL10, P1, and P2 have a conserved C-terminal fragment in their amino acid sequence (EESEESDDDMGFGLFD), within which there are serine residues (S304 and S307 for uL10, S101, and S104 for P1 and S102 and S105 for P2) undergoing dependent phosphorylation by CK2 kinase. Despite the description of the possibility of phosphorylation of these proteins *in vitro*, their phosphorylation status *in vivo*, as well as the role of this post-translational modification, have never been defined.

This study aimed to define *in cellulo* the phosphorylation status of ribosomal P proteins in the steady-state and stress conditions, as well as to determine whether CK2 kinase is responsible for the phosphorylation of P proteins *in vivo*. The other aim is to characterize the role of changes in the phosphorylation status of these proteins in the process of cell response to changing environmental conditions.

Non-cancer mammalian cell lines, i.e. MEF, and cancer cell lines, i.e. HeLa and HCT116, were used as the research model. The implementation of the goal began with the analysis of the phosphorylation status of the uL10 protein, which is the 'structural backbone' of the entire P-protein complex. Biochemical studies using the 2D electrophoresis technique indicated that the uL10 protein may occur in five isoforms that differ in charge, which corresponds to different degrees of its phosphorylation. *In silico* studies showed the possibility of phosphorylation of the uL10 protein within its C-terminal as well as N-terminal domain. Further confocal microscopic and biochemical studies based on mimetic variants of the uL10 protein showed that its phosphorylation within the N-terminal domain, at tyrosine 24 and threonine 59, disturbs its binding to the ribosome molecule. In the subsequent stages of this work, the state of phosphorylation of P-stalk proteins *in cellulo* was characterized. The use of the Pro-Q staining method and the Phos-tag/SDS-PAGE method showed that under basic conditions, i.e. in the absence of stress, ribosomal P-stalk proteins are present on ribosomes only in the phosphorylated form. Proteomic analyses and phosphoproteomic databases were consistent

with biochemical analyses and confirmed that P-proteins are phosphorylated in steady-state conditions. Monitoring of the phosphorylation status of P-stalk proteins under stress conditions, i.e. ER stress, oxidative stress, mitochondrial stress, or amino acid starvation conditions, showed no changes in their status compared to the steady-state conditions. Using a specific CK2 kinase inhibitor, CX-4945, it was shown that *in vivo* CK2 is responsible for the phosphorylation of P-stalk proteins, and its inhibition results in their accumulation in a non-phosphorylated form. Polysome profile analysis indicated that both phosphorylated and non-phosphorylated P-stalk proteins bind to translationally active ribosomes. Studies on the effect of CX-4945 on cell metabolism have shown the activation of the integrated stress response (ISR) pathway dependent on GCN2 kinase. Activation of GCN2 kinase under CX-4945 stress was not caused by a decrease in the concentration of amino acids in the cell, which indicates a different than canonical mechanism of its activation under these conditions. Describing the functioning of the translational machinery in the quantitative aspect, GCN2-dependent inhibition of translation was additionally demonstrated by the reduction of puromycin incorporation into peptides and the decrease in the number of polysomes. Further examination of the polysomal profile showed that GCN2 kinase is associated with ribosomes and is increased on ribosomes under CX-4945 stress. In addition, stress induced by CX-4945 has been shown to activate the autophagy and proteasome-mediated degradation of P-stalk proteins and other ribosomal components, which is associated with GCN2-dependent phosphorylation of eIF2α.

In summary, this doctoral dissertation shows that under basic conditions P-stalk proteins are phosphorylated by CK2 kinase and are present in a phosphorylated form on translationally active ribosomes. In addition, it was shown that the use of the CX-4945 inhibitor leads to a change in the phosphorylation state of P-stalk proteins and GCN2-dependent activation of the ISR pathway. This observation indicates a correlation between the emerging pool of non-phosphorylated P-stalk proteins and the activation of the ISR pathway, in which GCN2 kinase may act as both a receptor (detection of changes in the phosphorylation state of P-proteins) and a stress signal transmitter (activation of the ISR pathway).

Key words: ribosome, P-stalk proteins, CK2 kinase, GCN2 kinase, ISR pathway

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