ABSTRACT

The PhD dissertation is focused on the role of ribosomal P proteins, which are the main protein elements in the eukaryotic GTPase Associated Centre. This centre is located on a large ribosomal subunit and its function is to bind and stimulate the GTPase activity of translational factors, i.e. trGTPases. GAC is involved in the release of energy accumulated in GTP. This energy is the main driving force in the ribosome function and ensures the unidirectional nature of the translation process. Eukaryotic P proteins form two hetero-dimers P1-P2, which do not interact directly with ribosomal RNA but bind to the ribosome via the uL10 protein and thus form a pentameric uL10-$(P1\text{-}P2)_2$ complex. This complex in yeast cells has a modified configuration uL10-(P1A-P2B)(P1B-P2A). A unique feature of the proteins included in the complex is the multiplication of P proteins containing a conserved C-terminus responsible for the direct interaction with trGTPases. There are five identical C-terminal fragments in the eukaryotic complex: four originating from two P1-P2 dimers and the fifth from the P domain in the uL10 protein. The presence of only one such element on the ribosome is sufficient for stimulation of translational factor-dependent *in vitro* GTP hydrolysis, which ensures cell viability.

The aim of the research is to determine the function of eukaryotic P proteins in the respective stages of the translation process and, primarily, the role of multiplication of these proteins in the function of the translational machinery.

Saccharomyces cerevisiae yeast cells were used as a research model, i.e. a set of mutants based on the BY4741 strain, which were characterised by a modified configuration of the P protein complex. Three yeast strains were used: BY4741 with an unchanged uL10-(P1A-P2B)(P1B-P2A) complex and mutants $uL10_{\Delta h2}$ and $uL10_{\Delta h1h2}$ with a uL10-(P1A-P2B) and uL10 complex configuration, respectively. The implementation of the research tasks was commenced with phenotypic characterisation of the yeast mutants. It was found that yeast strain $uL10_{\Delta h1h2}$ with ribosomes devoid of all P proteins exhibited a slow growth rate. It was revealed with the use of a flow cytometer that the mutant exhibited disturbances in the G1 phase of the cell cycle, which resulted in a significantly prolonged time of yeast cell generation. Additionally, the phenotypic analyses revealed that mutant $uL10_{\Delta h1h2}$ had a metabolic defect, i.e. it did not utilise the so-called non-fermentable carbon sources, e.g. ethanol or glycerol. The next steps consisted in description of the translational machinery function in quantitative and qualitative aspects. Quantitative analyses involved determination of the translation efficiency at its individual stages using such approaches as “polysome

profiling", including "*runoff*" and "*half transit time*" analyses. The investigations demonstrated that the absence of P proteins on the ribosome did not impair translation efficiency

at the respective stages of the translational cycle. However, the most puzzling result was provided by the qualitative analysis, which showed that ribosomes with a defective P-protein complex exhibited impaired accuracy of genetic information decoding. The analyses were carried out based on the *Dual Luciferase Assay Reporter System*. It was demonstrated that the disturbances in the complex architecture had an impact primarily on the decoding process, i.e. amino acid *misincorporation*, "read-through" stop codon suppression, or shifting the reading frame by 1 nucleotide towards 3', i.e. the so-called "*frameshifting*". The results of the analyses based on the luciferase reporter system are supported by the observations indicating that the yeast mutant devoid of the P protein is hypersensitive to antibiotics from the aminoglycoside group, which impair the decoding process in translation. This demonstrates a synergistic effect between the decoding defect associated with the P protein deficiency and the aminoglycoside applied. Importantly, these analyses reveal a correlation between the number of copies of P proteins on the ribosome and the accuracy of the genetic information translation by the ribosome. Depletion of a single dimer P1-P2 on the ribosome is only a moderate decoding defect, whereas the absence of two dimers increases the frequency of ribosome errors. Moreover, the analyses of the operation of the translational machinery demonstrated that the yeast mutant with disorders in the architecture of the P protein complex acquired resistance to sordarin, i.e. a specific antifungal antibiotic.

The doctoral dissertation research showed that the multiplication of P proteins, and mainly the presence of the five characteristic C-terminal fragments of P1/P2 proteins and the uL10 protein, plays an important role in the process of genetic information decoding. Therefore, it can be concluded that the main role of P protein multiplication is the functional coupling with the qualitative aspect of the ribosome action, i.e. genetic information decoding. The level of these proteins does not influence the ribosome biogenesis process or the efficiency of initiation or elongation in the translational cycle. Additionally, these studies indicate that the P protein complex may play an important role in the interaction of sordarin with the ribosome. This result paves the way for elucidation of the molecular basis of the effect of sordarin on the translational machinery and, hence, development of effective anti-fungal antibiotics.