

ABSTRACT

The Shelterin protein complex and telomere notion are closely intertwined. The initial part of this thesis focuses on their comprehensive characteristics, structure, and role in living organisms, including humans. Next, it provides answers to fundamental questions about the molecular aspects of telomere function that have effect on telomere length homeostasis. Furthermore, how telomeres solve the problem of terminal replication, and how they counteract the myriad of cellular pathways that can sense and act on the ends of DNA? The answer to posed questions is presented by analysis of the activity of the telomerase enzyme, belonging to the group of reverse transcriptase, and the Shelterin complex, consisting of six distinct proteins (TRF1, TRF2, TIN2, POT1, TPP1 and Rap1). The importance of these telomeric components in tumor diseases is demonstrated, and targeted strategies for therapies against them are indicated. The collected information accents the importance of achieving the main aim of this thesis.

The second part of the theoretical considerations describes selected properties of proteins that are currently used in their purification processes. It presents the advantages of modern approaches in the preparation of fusion protein with different labels. It accents the advantage of bioproduction fusion proteins in bacterial expression systems and the simplicity of their purification using dedicated chromatographic techniques. The presented information helps understand the selected strategies for obtaining target proteins from the Shelterin complex.

The experimental part shows the results that confirm obtaining the full variants of hTRF1 and hTRF2 (human TRF1/2) and their Myb1 and Myb2 binding domains in the *Escherichia coli* bacterial expression system. The results from individual stages of their preparation processes support the previously described advantages of biosynthesis and purification of recombinant fusion proteins with selected labels. The performed studies on the specificity of interactions the Shelterin proteins, using the EMSA method and BLItz technology, confirm their native properties. The interaction of hTRF1 and hTRF2 recombinant proteins and their binding domains with telomeric dsDNA is demonstrated, as well as the interaction of hTRF1 and hTRF2 with TIN and Apollo peptides. To sum up, the detailed aims were met, which consequently allowed achieving the main aim. The usefulness of the obtained proteins of the Shelterin complex in *in vitro* interaction models is confirmed.