Abstract

Precise DNA replication is crucial to ensuring accurate inheritance of genetic information. The mechanism of DNA replication is highly evolutionarily conserved: from simple single-cell organisms such as yeast to human cells. Many individual proteins and protein complexes are responsible for the progress and regulation of subsequent stages of the DNA replication process. DNA replication in eukaryotes begins with the binding of the ORC complex to replication origins on the DNA strand. The next step is to formation a replisome - molecular machine which is able to starting and continuing DNA replication. The key component of the replisome is the CMG helicase complex (**C**dc45-**M**CM-**G**INS), which unwinds double-stranded DNA and coordinates the work of other components of the replisome, including DNA polymerases. A group of replication proteins such as Cdc6, Dbf4, Sld3, Sld7, Sld2 and Mcm10 are involved in the regulation of replisome assembly steps. Almost all cells do a limited number of replication rounds during their lifetimes, which means that their division potential is also limited. Upon it's depletion, cells typically do not die immediately but enter the aging phase, which lasts until death. Aging is defined as a progressive decline in physiological integrity, leading to impaired biological functions, including fertility and increasing susceptibility to death. DNA replication disorders often lead to replication stress and are identified as one of the potential factors determining the rate of aging. The aim of this doctoral dissertation was to examine how the lack of one copy of genes involved in the initiation of DNA replication in heterozygous *Saccharomyces cerevisiae* cells affects cell physiology and aging. The research material used was yeast strains lacking one copy of the *ORC* complex genes, referred to as *ORC/orc*Δ, strains lacking one copy of the *CMG* complex genes, referred to as *CMG/cmg*Δ, and strains having only one functional copy of genes encoding essential replication proteins, such as Cdc6, Dbf4, Sld3, Sld7, Sld2 and Mcm10, referred to as lowPICC. Based on the conducted research, it was shown that cells with only one copy of the genes involved in the initiation of DNA replication, *ORC/orc*Δ, *CMG/cmg*Δ, and lowPICC strains mostly showed a significant decrease in the level of their mRNA transcripts, cell cycle disorders and prolonged doubling time, and were characterized by changes in the biochemical profile. It has also been shown that reducing the expression of genes involved in the initiation of replication significantly affects the reproductive potential of cells, with the exception of *ORC6/orc6*Δ and *MCM2/mcm2*Δ. Interestingly, in relation to the *ORC/orc*Δ and *CMG/cmg*Δ mutants, no differences were observed in the overall survival of mitotically active cells (replicative aging model). In contrast, lowPICC heterozygotes were characterized

by a shortened overall lifespan in this model, i.e. until the appearance of an accelerated aging phenotype. It has also been shown that disturbances in the initiation of DNA replication also influence the rate of aging of post-mitotic cells (chronological lifespan) in each of the tested strains. Additionally, we observed a correlation between RNA level and polysaccharides in yeast and their reproductive potential, as well as a correlation between the level of fatty acids and the cell doubling time in *CMG/cmg*Δ heterozygotes. Moreover, reduced expression of lowPICC genes led to an abnormal response to DNA damage and affected cellular and mitochondrial DNA content. Importantly, a strong negative correlation was also demonstrated between the content of cellular macromolecules (RNA, proteins, lipids, polysaccharides) and the aging process in this group. The data presented as part of the doctoral dissertation also shed new light on the potential usefulness of yeast in the study of potential therapeutic targets in the treatment of cancer.