

## Summary

Yeast *Saccharomyces cerevisiae* is one of model organisms for research connected with ageing processes. Despite many features typical for this group e.g. the presence of cell wall, closed mitosis, budding as a mechanism of cytokinesis, they are models for research on universal methods of this process. One of the models used in yeast gerontology is co-called *replicative life span*. It has been assumed that by researching the influence of various factors on RLS value, it will be possible to explain the basic ageing mechanisms, as well as longevity of humans and animals. The study is connected with physical separation of daughter cells from mother cells and counting these newly produced daughter cells with the usage of micromanipulation technique. The measure of age and longevity of yeast cells is a number of daughter cells produced by "mother cell" during lifetime. This way of expressing age does not include cell lifetime. Because of that reason the term replicative lifespan was replaced by the term reproductive potential. This term better describes the essence of research, as well as it also allows a different cause of reduced reproductive capacity of yeast cells than ageing process.

The aim of this research was analysis of changes in cells size during the reproductive phase of life, analysis of reproductive potential and cells lifetime of selected mutants, belonging to the group of "long-lived". Taking into consideration the fact that phenotypic effect of the mutations is often dependent on genetic background. Yeast cells representing three different genetic backgrounds were used in that analysis: SP-4, BY4741 and BMA64-1A. Wild strains representing particular genetic backgrounds differ from each other as far as the average and maximum value of reproductive potential is concerned, what can influence phenotypic expression of studied mutations. Three genes *FOBI*, *SCH9*, *RPL20B* were chosen for that analysis and their deletion, according to data in literature, causes increase of reproductive potential. Deletion mutant *sfp1Δ* which is considered to be short-lived, was also used.

The results concerning reproductive potential indicate that phenotypic effect for most mutants is not identical and dependent on genetic background. The only gene which knock-out showed similar effects in each genetic background was gene *FOBI*. The remaining strains showed variation within the genetic background in varying degrees. The role of cell volume increase rate in regulation of reproductive potential was determined. The obtained results indicate hypertrophy as one of possible factors regulating cell reproductive potential. In most cases the maximum size which mutants with increased reproductive potential obtain

equals the size of wild strains. It is the evidence of slower rate cell volume increase per one generation. Similar volume can be reached by mutants with decreased reproductive potential or those who do not have an influence on number of generations. According to results of the study some of analysed mutations lead to higher volume obtained by a cell than wild strains, but it is always associated with increase of reproductive potential.

According to generally accepted interpretation of study results of yeast replicative lifespan, the measure of cell age is a number of produced daughter cells. In reality, RLS does not reflect the real lifespan. Yeast cell lifetime is not only time, when it produces next daughters (reproductive lifespan), but also time after reproduction (postreproductive lifespan). The duration of cells reproductive lifespan is determined by two parameters: number of cycles i.e. reproductive potential and duration of one single cycle. Obtained data show that both parameters can work together or separately, leading to the same result, e.g. deletion mutants *fohlΔ* and *sfp1Δ* in genetic background of BMA64-1A strain, despite significant differences in reproductive potential, have identical reproductive phase of life. When the last cell cycle is over, the cell does not die, but lives for some time. The duration of this period of time depends among other things on doubling time and a number of cell cycles of "mother cell".

Increase of a number of generations, produced by a cell contributes to a shorter life after the process of reproduction. It can be connected with significant energy load of a "mother cell", resulting from effort incurred to increase its volume during next cycles on one hand and efforts connected with reproduction (with production of new daughter cells) on the other hand. Taking reproductive and post reproductive lifetime into account we receive the total lifespan, which can be a basis to state whether we deal with longevity or not.

The analysis of total lifespan has provided us with crucial data concerning longevity, which totally change conclusions of previous research on ageing with the usage of yeast *S. cerevisiae*. None of the analysed strains defined as "long-lived" according to literature, in reality lives no longer than reference strain. Only deletion mutant *sfp1Δ*, despite the fact that it is defined as 'short-lived' according to literature (on the basis of generation number), after the analysis of its total lifespan it turned out to be the only long-lived strain among those analysed.

An attempt to explain the mechanism leading to longevity of mutant *sfp1Δ* was an analysis of parameters concerning cell bioenergetics: profile of polysomes, *in vivo*

incorporation of [<sup>35</sup>S]-labeled methionine, isothermal microcalorimetry, indication of content of ATP and metabolic activity with the usage of fluorescent probe FUN-1. Taking into consideration Pearl's theory of pace of life, conclusions concerning reproductive potential of analysed strains, doubling time, duration of reproductive and post reproductive phase, we can conclude that decreased rate of cell metabolism can be responsible for longevity of mutant *sfp1Δ*. Reproductive potential was the basic measure of age and longevity during research with yeast as a model organism in gerontology. It was responsible for criterion of dividing mutants into long-lived and short-lived ones. Results of analyses presented in this thesis make us change our mind about determining the longevity of yeast, including particularly lifetime and not only numerical value of cell reproductive potential.