Tracing the aging phenotype at the cellular level

Aging is a process from which there is no escape or exception. It applies to all organisms, regardless of whether they are single-celled or composed of many differentiated cells. Interestingly, at the cellular level, aging is very similar for all of them. Aging cells accumulate DNA, protein, and lipid damage because they can no longer repair or remove them. They produce energy less efficiently. They respond more slowly or incorrectly to signals from the environment. They stop dividing, and the only thing they can do is to live, although it must be admitted that individual cells deal with it differently - some live longer, others shorter. The data collected so far indicate high inter-species similarity of the mechanisms that determine aging. However, in this case, we should rather think of the mechanisms that begin to fail, resulting in aging. Since these mechanisms are similar, why not study them in a system that is convenient for the researcher? Therefore, baker's yeast cells are a frequently used model in aging studies. Two experimental approaches are used in research using yeast: analysis of replicative lifespan, i.e., testing how many divisions a given cell is able to carry out, and analysis of chronological lifespan, i.e., checking how long a given cell survives in unfavorable conditions (limited availability of nutrients) while maintaining the ability to resume the growth and division as soon as nutritional conditions improve.

Recently, while examining chronological lifespan, we discovered something quite unexpected. By measuring the DNA content of aging cells, we found that initially diploid cells gradually lose their DNA during aging, down to the level typical of haploid cells. The limiting amount of DNA for growth was the DNA content - *1c* - i.e., exactly one copy of the parental DNA, which was surprising because diploid cells usually have at least two such copies, one from each parent, i.e., *2c*. However, before the next division and after the round of DNA synthesis is finished, they have four such copies, *4c*. We also found that if they remained viable, aging cells with a DNA content of *1c* regenerated a population of diploid cells containing DNA from *2c* to *4c* when provided access to an abundance of nutrients**. The proposed project aims to explain how the described changes in DNA content occur, i.e., its reduction during aging and its restoration of the appropriate amount after improving growth conditions.**

At the respective time points, we want to isolate viable cells from the chronologically aging yeast population by high-efficiency sorting (for this purpose, a fluorescence-activated cell sorter, FACS, is required). Then, we will characterize their phenotypic features using various biochemical and cytometric tests, fluorescence microscopy, whole genome sequencing, etc. Our preliminary data suggest that DNA loss involves various autophagy pathways, which help remove damaged molecules and even non-functional organelles from cells. We will check the impact of nucleophagy, microautophagy of the nucleus (PMN), and mitophagy pathways on cell survival during aging. We suspect that the activation of nucleophagy may be responsible for the rapid decline in cell viability in the initial phase of aging, and the activation of PMN enables the removal of damaged DNA repair products from the cell nucleus, along with the enzymes involved in this process, which allows cells to survive and helps avoid genomic rearrangements. We believe that the process of recovering lost copies of genomic DNA during cell growth on a rich medium involves auto-duplication (endoreduplication). The experiments planned in the project will allow for the verification of this hypothesis.

The proposed research will expand our knowledge of cell aging and lead to the discovery of previously unknown processes and rules. Since, as we mentioned at the beginning, aging mechanisms are universal among living organisms, the discovered rules may also apply to human cells. Especially since aging human cells also show signs of malnutrition.