The impact of metabolism on aging and cell size in single yeast cells
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Summary of thesis

In this thesis, we used a newly developed microfluidic dissection platform to investigate how metabolism affects yeast replicative lifespan (RLS) and cell size. In Chapter 2, we hypothesized that cells may sense and regulate their metabolism based on the rate of metabolism (Chapter 1). We also proposed that the intracellular level of the metabolite fructose-bis-phosphate (FBP) is a component of a flux-dependent regulation network in yeast. In Chapter 3, we described the development and use of the microfluidic dissection platform, which makes it possible to measure RLS under environmentally stable conditions using continuous high-resolution microscopy. Using this novel tool, we analysed how a reduction in the extracellular glucose concentration, referred to as calorie restriction (CR), affects yeast RLS (Chapter 4). Surprisingly, we did not find any lifespan extension under CR conditions in our aging experiments with the microfluidic dissection platform. CR did however extend RLS when we measured RLS using another method called the classical microdissection technique suggesting that the effect of CR is method-specific. Next, we used the microfluidic dissection platform to study cell size as a function of replicative age in Chapter 5. We observed that cell size increased linearly with replicative age and that the rate of this increase was strongly dependent on carbon source. Cells grow in size with each round of cell division due to temporary protein overproduction after mitosis. We speculate that this growth is a requirement for cell cycle progression in mother cells. Overall, our findings illustrate that the microfluidic dissection platform is a valuable tool for studying different aspects of yeast replicative aging. Using this tool, we found that a reduction in extracellular glucose concentration did not necessarily alter yeast RLS, while different carbon sources clearly did alter the rate of cell size increase with age. Apparently, metabolism has a variable impact on different aspects of yeast aging.
Future outlook

In this thesis, we made several key findings concerning metabolism and yeast aging. Below, we will discuss what future research could be performed to continue the work presented in this thesis and how to further improve our ability to study yeast aging at the single cell level.

Towards understanding metabolic regulation in yeast

In Chapter 2, we show that cells may sense their carbon uptake rate via their intracellular levels of fructose-bis-phosphate (FBP) and use this information to regulate their metabolism. The next steps will be to identify how the levels of FBP (the ‘flux signal’) is mechanistically established and to identify the targets of this flux signal. Proteins interacting with FBP could be identified using screening methods, like pull-down essays (1). Flux sensing may play a role in aging, since metabolic changes were observed in aging yeast cells under constant environmental conditions. These metabolic changes could be caused by the increase in cell size with age, which may reduce the carbon uptake rate per cell volume.

The microfluidic dissection platform greatly facilitates yeast aging research

In Chapter 3, we described the development and use of the microfluidic dissection platform, which is an important methodological improvement for yeast aging research. With the introduction of the microfluidic dissection platform, the bottleneck of lifespan determination is no longer the number of cells that can be tracked during a single experiment, but the amount of time that is needed to extract the desired information from the experiment. For example, in our hands it requires only five experimental days to perform an aging experiment, but to determine the RLS of 100 cells requires at least another three days of image analysis. Although these time scales represent an enormous improvement over the microdissection technique, where several weeks are required to complete an aging experiment (2), application of automated image analysis tools would further enhance the throughput of aging experiments.

Improving the reproducibility of RLS measurements with the microdissection technique

In Chapter 4, we performed a systematic analysis of RLS data of *S. cerevisiae* under normal and CR conditions. To verify the literature data, we performed RLS measurements with the microdissection technique. Based on our experiences and observations using this method, we recommend that the following measures are taken to improve reproducibility: First, aging experiments should always be performed with a minimum of 100 cells to have sufficient statistical power (3). Second, we found that it was crucial to keep the amount of time that cells spend inside the microdissection needle to an absolute minimum to retain
cell viability and RLS. Third, cells must grow exponentially prior to the start of the experiment. If cells are not growing exponentially, their lifespan will be reduced. In addition, we determined from our meta-analysis that the average RLS of BY4741 and BY4742 yeast cells under standard experimental conditions (YPD medium with 2% (wt/vol) glucose) was 25.9 buds, which could be used as a reference value in future RLS measurements with the classical microdissection technique.

**Calorie restriction does not robustly extend RLS**

In Chapter 4, we also studied the effect of CR on RLS using the microfluidic dissection platform and found that there was no effect of calorie restriction on RLS in contrast to the classical microdissection technique. This discrepancy was difficult to explain until Mei & Brenner (2015) (4) found that calorie restriction extends RLS via a dialyzable extracellular factor that is secreted by cells growing under CR conditions. If cells under CR conditions are moved to fresh CR medium during a classical microdissection experiment, the RLS of these cells is no longer extended. In the microfluidic dissection platform, a secreted factor would be flushed away by the flow of medium explaining why CR is no longer able to extend RLS inside the microfluidic dissection platform. Addition of nicotinic acid or nicotinamide riboside to the medium restored the longevity benefit of CR in cells moved to a new environment during microdissection experiments (4). We would therefore expect that addition of nicotinic acid or nicotinamide riboside to the medium should extend the RLS of cells under CR conditions in the microfluidic dissection platform. If so, the next step should be to identify the yeast longevity factor, *e.g.* by comparing the secretome of cells growing under normal and CR conditions. It would be interesting to understand how this longevity factor is able to extend RLS in yeast under CR conditions and how it could contribute to longevity in cells growing under standard conditions.

**Dissecting the role of cell size in yeast aging**

The role of cell size in yeast aging is currently debated due to conflicting findings made by various research groups. It was shown by some that cell size strongly correlated with RLS and that cells died at a particular cell size (5). In support of these findings, cell size and RLS are regulated by similar pathways, such as the Target of Rapamycin (TOR) pathway (6-8) suggestively linking cell size to RLS. In contrast, others found that there was not always a correlation between cell size and RLS (9) and that there was significant variation in cell size at cell death (10). In addition, diploid cells are twice as large as haploid cells, but have an extended RLS (11). Combined we believe that these latter findings suggest that cell size is not necessarily a cause of cell death. However, the increase in cell size with age may still affect cellular physiology (12). An increase in cell volume lowers the ratio between cell surface and cell volume, which may reduce the import rate of nutrients per cell volume. A reduction in metabolic flux can cause metabolism to shift from fermentation towards respiration (Chapter 2). Interestingly, a
Conclusion and Outlook

A metabolic shift from fermentation towards gluconeogenesis and respiration has been observed in aging cells (13, 14). To explore the potential ability of cell size to alter metabolism in aging yeast cells, we could compare the metabolism of cells that are artificially enlarged by temporary treatment with mating pheromone with that of untreated cells. These studies would decouple cell size increase from other aging phenotypes and make it possible to understand the role of cell size in aging.

Conclusion

In this thesis, we show that the newly developed microfluidic dissection platform can be used to study several aspects of yeast aging. Using this tool, we observed how metabolic interventions, e.g. different carbon sources or extracellular glucose concentrations, affect yeast aging and cell size. Future research should focus on how cells sense their rate of metabolism, since this does not only represent a potentially novel manner in which cells regulate their metabolism, but may also be an important regulator of aging.

References


