Biological variability and the aging process at the single-cell level in yeast

Supervisor's name: Jean-Pascal Capp (capp@insa-toulouse.fr)

Laboratory: Toulouse Biotechnology Institute (TBI) at INSA Toulouse

Expected goals:

Understanding the mechanisms of aging has great implication such as promoting the development of therapeutics towards aging-related diseases and improving the performance of microorganisms in the industrial use. Yeast offers an attractive eukaryotic model that has dramatically accelerated aging research [1,2]. Yeast aging is, as human aging, characterized by the hallmarks of genomic instability, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, and mitochondrial dysfunction. Especially, trehalose metabolism on the one hand, and epigenetic regulation on the other hand, have been linked to longevity in yeast. Nevertheless, this process remains largely uncomprehended, especially because of these many different biological processes involved. One way to tackle this question is to start from the observation that cells grown in the same environment exhibit heterogeneous lifespans that could rely on metabolic, epigenetic and gene expression variability (noise) from cell-to-cell. Indeed, single-cell variability of lifespan is observed in yeast populations. Recent studies exploring the dynamics of the expression noise of a reporter gene during yeast replicative aging showed a progressive increase in intercellular variability (a measure of the population-level heterogeneity) [3].

To better understand the process of aging, it thus appears of great significance to investigate the role of gene expression noise, and its interplay with variability at the metabolic and epigenetic in the production of cell-to-cell differences in lifespan. Especially, we will study whether variability in trehalose metabolism on the one hand and in epigenetic regulation on the other hand (both well-known to modulate longevity in yeast) could impact the aging process at the single-cell level in yeast.

We will study for the first time expression noise during chronological aging (and not replicative aging), and noise in genes whose fusions with fluorescent reporters are functional (some fusions are already constructed in the lab and functional). More specifically, we will ask whether:

(1) gene expression noise of key genes in trehalose metabolism *(TPS1* and *TSL1*) or epigenetic regulation (*SIR2*) (previously studied in the lab in the context of cell-to-cell heterogeneity, see [4] and [5] respectively) changes during normal chronological aging, and under conditions known to modulate longevity (for instance caloric restriction, inhibition of cell signaling or genetic mutants).
(2) cells sorted depending on their level of Tps1, Tsl1 or Sir2 levels harbor differences in lifespan, together with other differences in other phenotypes linked to aging measured using flow cytometry (listed above).

For these two first points, whether these differences of gene expression from cell-to-cell are correlated with differences of other phenotypes linked to aging measured using flow cytometry (cell viability, measurement of ROS accumulation, mitochondrial membrane potential, membrane modification…) will be studied.

(3) targeted modulation of gene expression mean and/or noise of these genes allow tuning the aging process (libraries of promoter variants integrated at the native locus will are already constructed in the lab (*TPS1* and *TSL1*), others will be constructed (*SIR2*)).
(4) the transfer in industrial yeast strains of the best promoter variants modulating the expression mean and/or noise of these genes and consequently the aging process allows improving their performance.

These studies would help elucidating the possible central role of gene expression noise in aging, its possible relationships with other types of biological variability and if its modulation could promote longevity and better yeast performance. This project is in line with previous works in the lab aiming at studying different types of cell-to-cell phenotypic heterogeneity in yeast so as to find strategies of improvements for industrial use (see among others [4-7]).

Scientific methods:

Classical yeast growth cultures will be performed, and media composition will be modified with several kinds of limitations (caloric restriction) or presence of specific inhibitors. Genetic tools will include CRISPR-Cas9 to create fusions of our genes of interest with fluorescent proteins at the chromosomal level at their native locus. Genetic mutants known to modulate lifespan will be also generated by this technology. Also, to modify the mean expression and/or noise levels of *TSL1*, *TPS1* or SIR2, we will produce variants of their own promoters by applying error-prone PCR applied on wild-type promoters, followed by rapid and error-free replacement of the native promoter by the variants in the tagged strains, using CRISPR-Cas9. This approach has been successfully used in the lab on another yeast promoter and allowed construction of a library of 20 000 promoter variants [7]. Individual clones distributed in 96-well plates will be analyzed by high-throughput flow cytometry. The best candidates with changed mean and/or noise will be selected for further analysis of different aspects of the aging process at both the single-cell and the population levels, and finally for transfer in strains of industrial interest (again with CRISPR-Cas9). This project also mainly relies on flow cytometry technologies for expression analysis and cell sorting, but also for many aspects of cellular aging: cell viability, measurement of ROS accumulation, mitochondrial membrane potential...

Time arrangement:

2022-09 -> 2023-03: molecular biology: creation of the lacking genes tagged with fluorescent markers, and creation of genetic mutants in the tagged strains.

2023-04 -> 2023-10: analysis of the changes in mean and/or noise in the expression of our genes of interest during normal chronological aging, and in various environmental or genetic contexts known to modulate lifespan. Complementary methods using flow cytometry will be used to study phenotypes related to aging at the single-cell level.

2023-11 -> 2024-05: experiments of sorting of cells depending on their levels of expression of our genes of interest, followed by their analysis in terms of expression dynamics, chronological aging and various phenotypes related to aging at the single-cell level.

2024-06 -> 2024-12: creation of the libraries of promoter variants of our genes of interest and analysis of the libraries by high-throughput cytometry.

2025-01 -> 2025-05: analysis of promoter variants of interest (with modified mean and/or noise levels) in terms of aging of whole populations or sorted subpopulations. Effects of this modulation of expression of our genes of interest on other phenotypes related to aging at the single-cell level.

2025-06 -> 2025-10: transfer of the most interesting variants that slow down the aging process at the population level in industrial yeast to measure the benefit because yeast cell aging is known to impact fermentation performance. (Yeast cells undergo constant modifications in terms of physiology, morphology and gene expression and such characteristics play an important role in the performance of yeast during alcoholic beverage production, influencing sugar uptake, alcohol and flavour production and also the flocculation properties of the yeast strain.).

2025-11 -> 2026-02: Writing of PhD manuscript and PhD defense.

References:

1. Longo, V.D.; Shadel, G.S.; Kaeberlein, M.; Kennedy, B. Replicative and chronological aging in Saccharomyces cerevisiae. *Cell Metab* **2012**, *16*, 18-31, doi:10.1016/j.cmet.2012.06.002.
2. Denoth Lippuner, A.; Julou, T.; Barral, Y. Budding yeast as a model organism to study the effects of age. *FEMS Microbiol Rev* **2014**, *38*, 300-325, doi:10.1111/1574-6976.12060.
3. Sarnoski, E.A.; Song, R.; Ertekin, E.; Koonce, N.; Acar, M. Fundamental Characteristics of Single-Cell Aging in Diploid Yeast. *iScience* **2018**, *7*, 96-109, doi:10.1016/j.isci.2018.08.011.
4. Arabaciyan, S.; Saint-Antoine, M.; Maugis-Rabusseau, C.; Francois, J.M.; Singh, A.; Parrou, J.L.; Capp, J.P. Insights on the Control of Yeast Single-Cell Growth Variability by Members of the Trehalose Phosphate Synthase (TPS) Complex. *Front Cell Dev Biol* **2021**, *9*, 607628, doi:10.3389/fcell.2021.607628.
5. Liu, J.; Mosser, L.; Botanch, C.; Francois, J.M.; Capp, J.P. SIR2 Expression Noise Can Generate Heterogeneity in Viability but Does Not Affect Cell-to-Cell Epigenetic Silencing of Subtelomeric URA3 in Yeast. *G3 (Bethesda)* **2020**, *10*, 3435-3443, doi:10.1534/g3.120.401589.
6. Liu, J.; Francois, J.M.; Capp, J.P. Gene Expression Noise Produces Cell-to-Cell Heterogeneity in Eukaryotic Homologous Recombination Rate. *Front Genet* **2019**, *10*, 475, doi:10.3389/fgene.2019.00475.
7. Liu, J.; Lestrade, D.; Arabaciyan, S.; Cescut, J.; Francois, J.M.; Capp, J.P. A GRX1 Promoter Variant Confers Constitutive Noisy Bimodal Expression That Increases Oxidative Stress Resistance in Yeast. *Front Microbiol* **2018**, *9*, 2158, doi:10.3389/fmicb.2018.02158.