Identifying longevity factors that mediate mono unsaturated fatty acid (MUFA) dependent lifespan extension

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Introduction

- **Chronological aging** is a primary risk factor for **chronic diseases** and **mortality**[1].
- **Biological aging** is characterized by advancing **decay of tissue structure** and **function**, regarded as **multifactorial process** (Fig.1).
- Focus on the **role of lipids**, especially **MUFA Oleic Acid** (**OA**), in **aging-related metabolic changes** (Fig.2) [2].

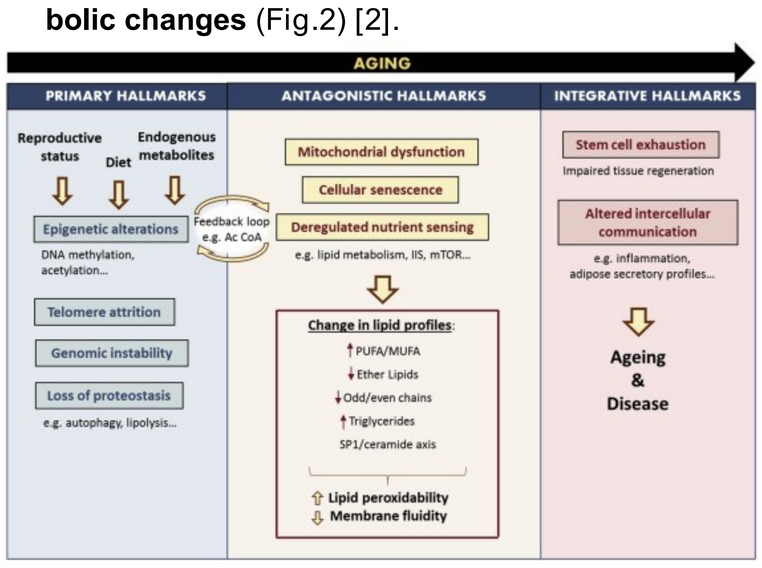


Fig. 2 Key aspects of lipids in aging and their connection to the hallmarks of aging (figure adapted from [2]).

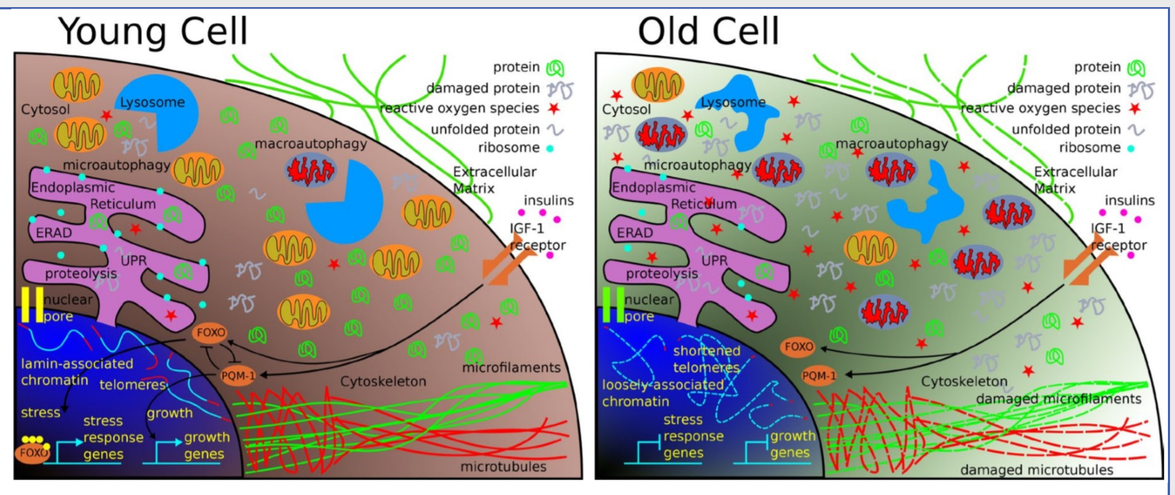


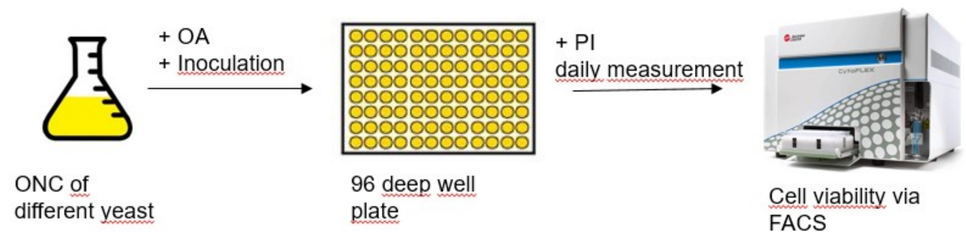
Fig. 1 Major features of cellular aging. As cells age translational defects and entropy progressively increase the amount of cellular damage (figure adapted from [1]).

Aims

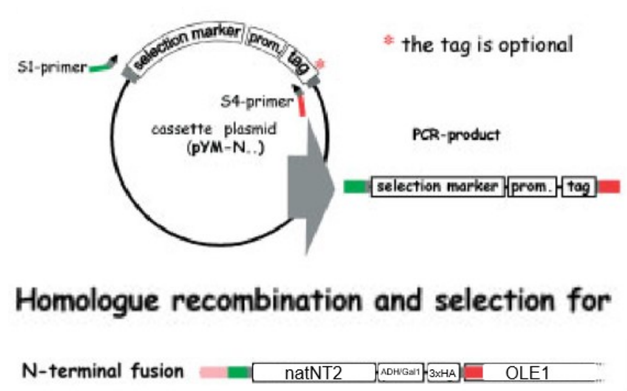
- Identification of **downstream factors** in **MUFA OA** mediated lifespan extension
- Understanding complex **relationship** between **lipids and aging**

Methods

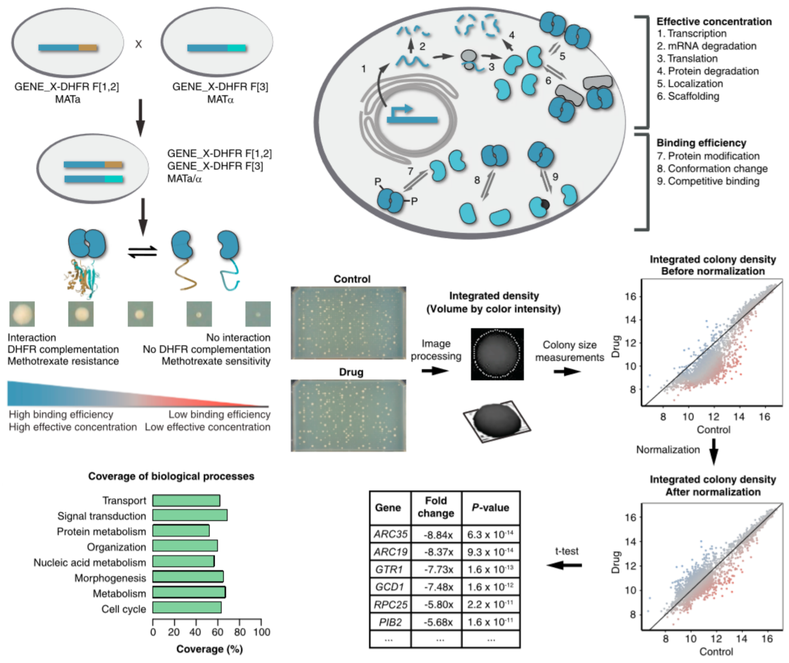
A Workflow yeast aging experiments



B Genetic approach to increase endogenous OA level



C Homomer dynamics DHFR protein complementation assay



Gene	Fold change	P-value
ARC35	-8.84x	6.3×10^{-14}
ARC19	-8.37x	9.3×10^{-14}
GTR1	-7.73x	1.6×10^{-13}
GCD1	-7.48x	1.6×10^{-12}
RPC25	-5.80x	2.2×10^{-11}
PIB2	-5.68x	1.6×10^{-11}
...	...	...

Fig. 3 Different approaches for analysing aging yeast and identify possible targets of OA

A) Schematic workflow and analysis of aging experiments.

B) Chromosomal promotor exchange upstream of OLE1 gene to increase endogenous amount of OA

C) Homomer dynamics DHFR protein-fragment complementation assay for detection of the condition-dependent states of proteins. (figure adapted from [3]).

Results

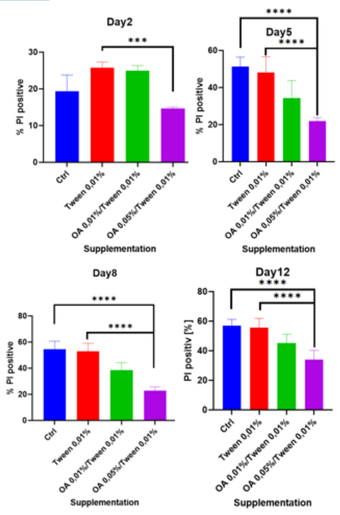


Fig. 4 Pharmacological approach of OA supplementation increases longevity in yeast. Measurement via PI staining and FACS. Normal distribution was shown by Shapiro-Wilk. Significance was assessed by one-way ANOVA with Tukey post hoc test. Asterisks indicate p-values: *** p<0.001, **** p<0.0001

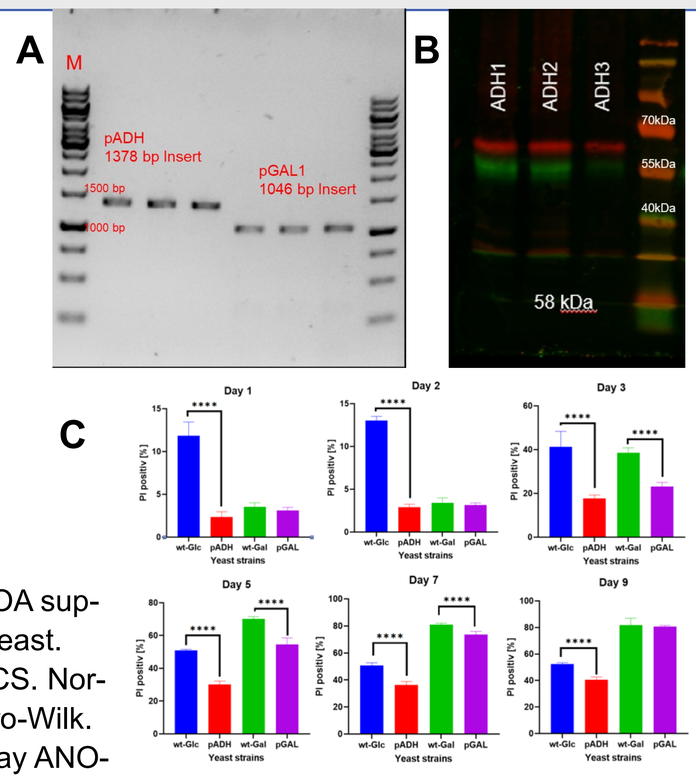


Fig. 5 Genetic approach to increase endogenous amount of OA.

A) Agarose gel confirms correct chromosomal promotor exchange, M = marker, pADH = ADH promotor, pGAL1 = GAL promotor

B) Immunoblotting against HA tag proofs proper chromosomal promotor exchange of constitutive ADH promotor, red = anti-HA (58kDa), green = anti-Tubulin (55kDa)

C) Ageing experiment with exchanged promotors. Measurement was performed by FACS via PI staining. Normal distribution was shown by Shapiro-Wilk. Significance was assessed by one-way ANOVA with Tukey post hoc test. Asterisks indicate p-values: **** p<0.0001

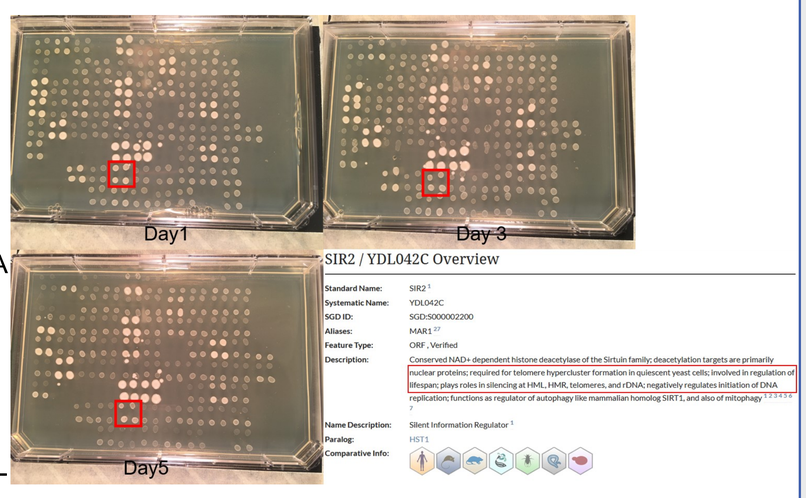


Fig. 6 Exemplary hdPCA plates at different points of time with possible hit (here: Sirtuin 2, displayed in the red rectangle. Upper row = without OA, bottom row with OA treatment). Sirtuin 2 is a conserved NAD+ dependent histone deacetylase of the Sirtuin family, involved in regulation of lifespan. Quantification and image analysis was performed via Fiji ImageJ software

Conclusions

- Pharmacological and genetic approach led to **increased lifespan** in yeast
- **Identification of pathways** which are **altered** as a specific response to **OA treatment**

References

[1] Diloreto, R. and Murphy, C. T. (2017) *Molecular Biology of the Cell.* **26**, 4524-4531

[2] Fuchs, B et al. (2019) *Chemistry and physics of lipids.* **222**, 59-69

[3] Stynen et al. (2018) *Cell.* **175**, 1418-1429