Yeast Lifespan Controlled by the Lipid Regulator Gemfibrozil via the UBR1-CUP9 Dependent Pathway

Sebastian Troncoso[1], Chong He[2]

[1]Santa Rosa Junior College, [2]The Buck Institute for Research on Aging

Abstract

Multiple pathways link the expression of PTR2, the transporter of di-peptides and tripeptides in the yeast *Saccharomyces cerevisiae.* PTR2 degradation is induced by both amino acids and dipeptides with destabilizing N-terminals. These dipeptides bind to UBR1, the E3 ubiquitin ligase of the N-end rule pathway, and accelerate the UBR1-dependent degradation of CUP9, a transcriptional repressor of *PTR2*. Previous studies have found that UBR1 deletion allows for the proliferation of CUP9 in the cytosol, resulting in high transcriptional repression of PTR2, and lifespan reduction in yeast *S. cerevisiae*. On the contrary, it has also been found that CUP9 deletion allows for the proliferation of PTR2 in the cytosol, indicating a high uptake of di-peptide and tripeptides through the plasma membrane and thus stimulating lifespan extension. A previous study found that defects in the E3 ubiquitin ligase UBR1 lead to the Johnson-Blizzard Syndrome (JBS), a rare autosomal recessive disorder characterized by exocrine pancreatic insufficiency, resulting in facial anomalies. In this study, we explore the N-end rule pathway and the effects of modifying the UBR1-CUP9 dependent pathway by introducing gemfibrozil, a pharmaceutical drug belonging to a group known as fibrates used to lower lipid levels in humans. We found that exposure of gemfibrozil prolonged the lifespan of wild type yeast by 37.5% in comparison to the control. Our studies lay the foundation for further investigation in the Johanson-Blizzard syndrome and the UBR1-CUP9 dependent pathway.

Introduction

Membrane Transport System

Figure 1

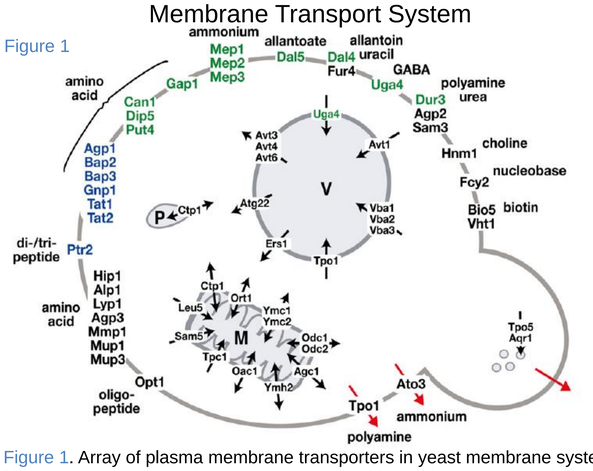

Figure 1. Array of plasma membrane transporters in yeast membrane system. Mitochondria and vacuole are represented by M and V, respectively (1).

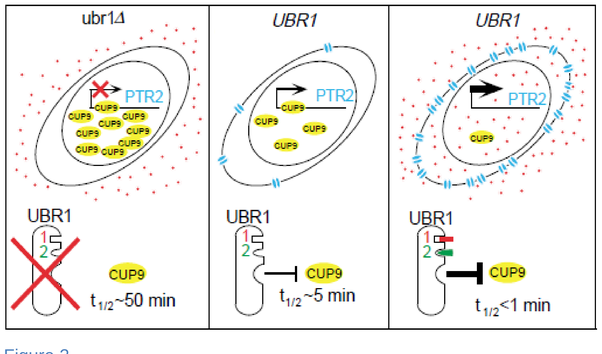

Figure 2

Deletion of UBR1 allows the over proliferation of CUP9 in the cell. This action decreases the propagation of PTR2, resulting in shortening yeast lifespan (2).

UBR1 controls the proliferation of CUP9, and CUP9 controls the proliferation of PTR2. No mutation in wild type yeast (2).

UBR1 is not deleted, but CUP9 is deleted and therefore proliferation of PTR2 becomes unregulated. There is more dipeptide uptake. As a result, yeast lifespan increases (2).

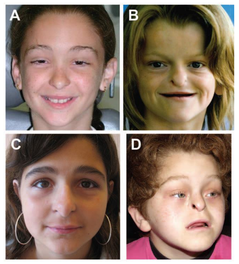

Figure 3. Johanson-Blizzard Syndrome is caused by mutations in the gene UBR1. Patients display facial anomalies, loss of weight, growth development inhibition, and mental retardation (3).

From previous literature, it was found that suppression of the UBR1 gene inhibited the side effects of the syndrome, but also affected the organism lifespan.

Methods

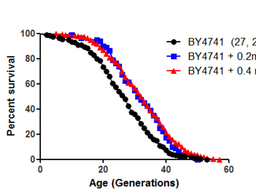

Figure 4. shows the percent survival vs age (generations) of wild type yeast on 0.2 mmol and 0.4 mmol gemfibrozil.

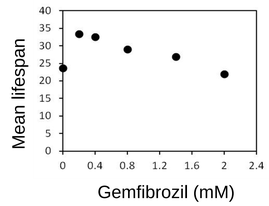

Figure 5 shows the mean lifespan of wild type yeast in different gemfibrozil concentrations.

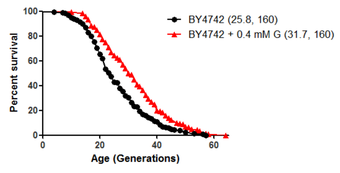

Figure 6 shows the gemfibrozil concentration required for optimal yeast lifespan extension.

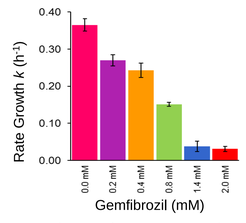

Figure 7 shows the rate growth of wild type yeast in different gemfibrozil concentrations.

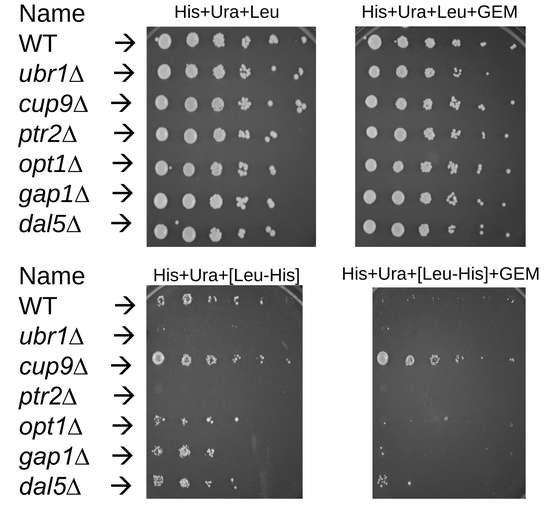

Figure 8. All strains in the BY4743 background, Gemfibrozil (GEM) at 0.4 mM. Media were YNB (1.7 g/l) w/o amino acids and nitrogen, allantoin (1 g/l) –for nitrogen source, dextrose (20 g/l). Photographs were taken after 5 days.

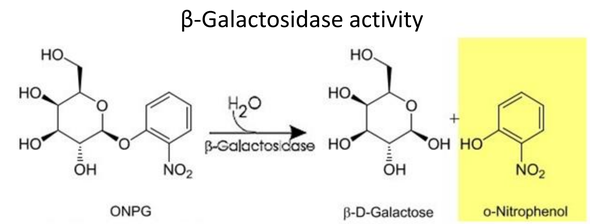

Figure 9. UBR1 allows the degradation of ortho-nitrophenyl-β-galactoside (ONPG). Deletion of UBR1 promotes the hydrolysis of ONPG by β-galactosidase, proliferating ortho-nitrophenol, a substrate that emits a yellow color in situ (4).

Results

Lifespan extension by Gemfibrozil is dependent on dipeptide transporter

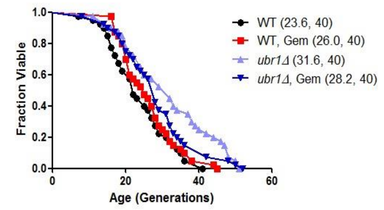

Figure 10 shows a graph between the percent survival (fraction viable) versus the lifespan (Age). The labels are wild type (W), UBR1 deleted in the genome (*ubr1Δ),* and Gemfibrozil (Gem).

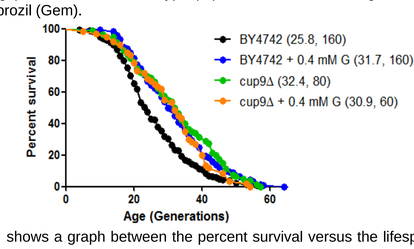

Figure 11 shows a graph between the percent survival versus the lifespan (Age). BY4742 is wild types yeast (W), CUP9 is the deletion (*cup9Δ)*, and concentration is 0.4mM Gemfibrozil (G).

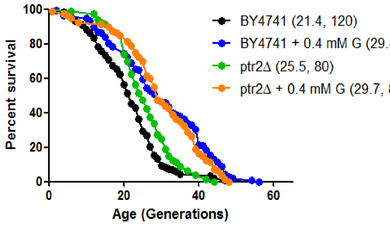

Figure 12 shows a graph between the percent survival versus the lifespan (Age). BY4741 is wild type yeast (W), PTR2 is the deletion (*ptr2Δ),* and concentration is 0.4mM Gemfibrozil (G).

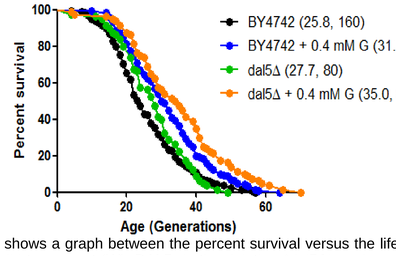

Figure 13 shows a graph between the percent survival versus the lifespan (Age). BY4742 is wild type yeast (W), DAL5 is the deletion (*dal5Δ),* and concentration is 0.4mM Gemfibrozil (G).

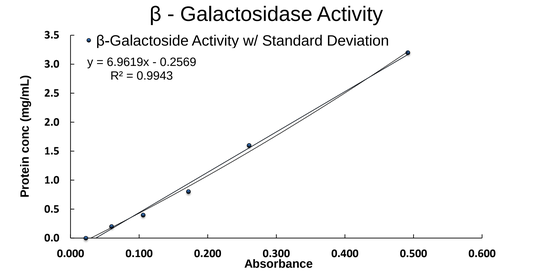

Figure 14 a graph between the UBR1 protein concentration versus the absorbance (nm). The dots next to the function are the standard deviation from the theoretical plot. The ratio between the protein concentration and the absorbance is the β-galactoside concentration.

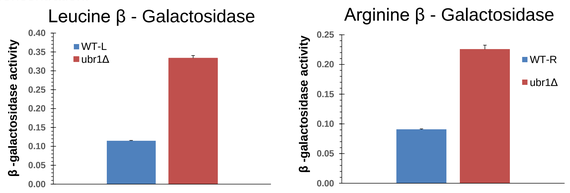

Figure 13 shows two bar graphs between the control (WT) and knockout yeast (*ubr1Δ*). Knockout yeast display a high concentration of β-galactoside as opposed to the control yeast. Arginine and Leucine are substrate-binding sites in Ubr1p that recognize β-galactoside.

Conclusion & Future Direction

The purpose of this experiment was to find how the lipid regulator gemfibrozil extended yeast lifespan. From previous literature, it is known that *cup9Δ* deletion allows PTR2p proliferation in cytosol, which leads toward extending yeast lifespan. It is also known that *ubr1Δ* allows CUP9p proliferation in cytosol, thus reducing yeast lifespan. In this experiment, it was found that *cup9Δ* in the yeast genome under a 0.4 mmol gemfibrozil, the knockout yeast lifespan remained long-lived, shown in Figure 10. This means that the lipid regulator gemfibrozil somehow controls the degradation of PTR2 in the cytosol. We also found that *ptr2Δ* in the genome under 0.4 mmol gemfibrozil concentration caused a long-lived lifespan in knockout yeast. This means that the lipid regulator gemfibrozil not only performed similar roles as CUP9, it also resembled the roles PTR2 has in the yeast organism to further extend lifespan. In addition, the results may potentially lead to treatment discovery for the Johansen-Blizzard Syndrome (JBS) caused by UBR1 mutations to the N-end rule pathways. It is known that UBR1p degrades β-galoctoside in the vacuole. Therefore, a DC Assay was performed to measure the concentration of β-galoctoside found in mutant and untreated wild type yeast. This research has paved the way to further understand yeast lifespan induced by Gemfibrozil, but it also has ignited a new research toward investigating the possible role that Gemfibrozil can have in the Johansen-Blizzard syndrome.

Acknowledgements

This research was supported by the Buck Institute for Research on Aging and the Mathematics Engineering Science Achievement program (MESA) of SRJC. Special thanks to Dr. Chong He for her guidance in understanding the N-end rule pathway and the overall research.

References

1. Ljungdahl, Per O., and Bertrand Daignan-Fornier. "Regulation of Amino Acid, Nucleotide, and Phosphate Metabolism in *Saccharomyces Cerevisiae*." *Genetics* 190.3 (2012): 885–929. *PMC*. Web. 25 Apr. 2016.
2. Hershko, Avram, Aaron Ciechanover, and Alexander Varshavsky. "The ubiquitin system." *Nature medicine* 6.10 (2000): 1073-1081.
3. Hwang, Cheol-Sang, et al. "Ubiquitin ligases of the N-end rule pathway: assessment of mutations in UBR1 that cause the Johanson-Blizzard syndrome." *PLoS One* 6.9 (2011): e24925.
4. Hwang, Cheol-Sang, et al. "Ubiquitin ligases of the N-end rule pathway: assessment of mutations in UBR1 that cause the Johanson-Blizzard syndrome." *PLoS One* 6.9 (2011): e24925.
5. Tasaki, Takafumi, et al. "The N-end rule pathway." *Annual review of biochemistry* 81 (2012): 261.
6. Hwang, Cheol-Sang, and Alexander Varshavsky. "Regulation of peptide import through phosphorylation of Ubr1, the ubiquitin ligase of the N-end rule pathway." *Proceedings of the National Academy of Sciences* 105.49 (2008): 19188-19193.
7. Lee, Kang-Eun et al. "N-Terminal Acetylation-Targeted N-End Rule Proteolytic System: The Ac/N-End Rule Pathway." *Molecules and Cells* 39.3 (2016): 169–178. *PMC*. Web. 25 Apr. 2016.