Δ*tor1* and Yeast Longevity via Chronological Lifespan Assay

Amelia Keyes and Megan Bestwick
Department of Chemistry, Linfield College, McMinnville, OR

Introduction

Overview and Importance. TOR, Target of Rapamycin, is a highly conserved signaling pathway in eukaryotic organisms that was discovered in the 1990s through its inhibition by rapamycin. There are two complexes formed, TORC1 and TORC2, with TORC1 having either Tor1p or Tor2p and TORC2 having Tor2p (Teixeira *et al.*). TOR pathway impacts metabolism, growth, cell cycle progression, macromolecule biosynthesis, and autophagy through nutrient sensing (Laplante and Sabatini). Inhibition of Tor1p results in limited anabolism and halted normal cell cycle progression through the translation of defensive proteins involved in cell adaption and tolerance to environmental stress (Laplante and Sabatini). Stress signaling through creation of reactive oxidative species (ROS) causes similar cellular response to Tor1p inhibition. ROS damage is implicated in neurodegenerative disorders because of reduced cellular viability through cellular damage (Tellone *et al.*). Bonawitz *et al.* have demonstrated that reduced TOR pathway signaling extends chronological lifespan by increased mitochondrial respiration, which increases the translation of defensive proteins that help reduce cellular damage by ROS.
Xie *et al.* demonstrated a number of proteins in the TOR1 pathway that were also inhibited by rapamycin, most notably SOD1, SOD2, CTR1, and LYS7. Double gene deletion strains of Δ*lys7*/Δ*tor1*, Δ*sod1*/Δ*tor1*, Δ*sod2*/Δ*tor1*, and Δ*ctr1*/Δ*tor1* are not well-characterized for their effects on *S. cerevisiae* longevity.

Goal. In this work, partial inhibition of TOR by deletion of *TOR1* can be studied through *S. cerevisiae* longevity via a chronological lifespan assay, with intention of studying the double gene deletion strains of *Δlys7/Δtor1*, *Δsod1/Δtor1*, *Δsod2/Δtor1*, and *Δctr1/Δtor1*.

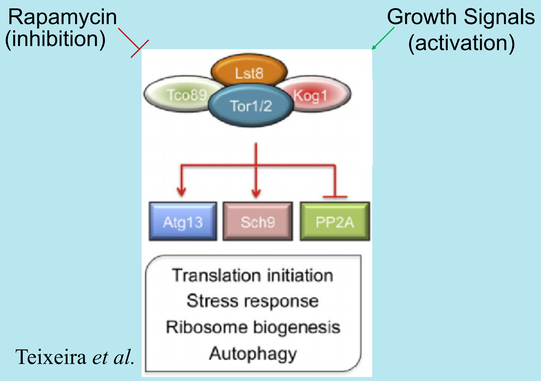
Methods

- BY4741 *S. cerevisiae* were transformed using the PCR product generated from the Forward and Reverse KanMX primers from Table 1.
- Growth on standard YPD-G418 plates and in standard YPD-G418 liquid media was used to confirm uptake of KanMX cassette.
- *TOR1* check primers (Table 1) were developed and PCR and gel electrophoresis performed to confirm *TOR1* deletion and insertion of the disruption gene. (Figure 1)
- An oxidative stress test was performed to further confirm *TOR1* deletion and insertion of the disruption cassette (Figure 2).
- Chronological lifespan (CLS) assay was done over a period of twenty days using hemocytometer counting with Trypan Blue staining to compare percentage of live cells of wild type BY4741 to *Δtor1* BY4741 (Figure 3).

Table 1. Primer Sequences used

Primer name	Sequence 5' -> 3'
Forward primer KanMX	CAT ACA TCA ACC GGC TAG CAG GTT TGC ATT GAT CGT ACG CTG CAG GTC GAC
Reverse primer KanMX	GCTC GAG CTT AAG TAG CTA AAC AAA GCA CGA AAT GAA AAA TGA CAC CGC
Forward primer Tor1 check	GGG TCT TCT TGA GCT TAC
Reverse primer Tor1 check	GTA TAC GTG TGC AAG TAT TG

TOR Pathway



Teixeira *et al.*

Results

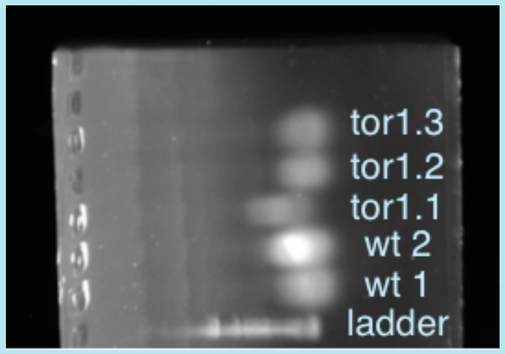


Figure 1: PCR agarose gel to confirm Δ*tor1*. Candidate tor1.1 was confirmed positive for the deletion of the *TOR1* gene.

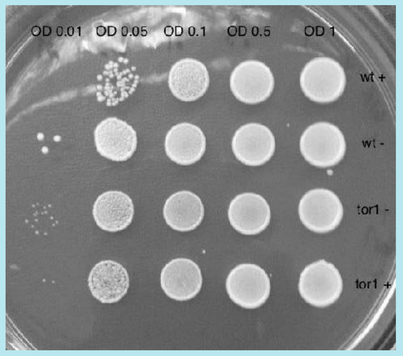


Figure 2: Environmental Oxidative Stress test. The Δ*tor1* and WT strains were incubated for 2 hours in the presence (+) or absence (-) of 2mM H_2O_2. A dilution series growth test on YPD media shows Δ*tor1* sensitivity to H_2O_2 (a ROS molecule).

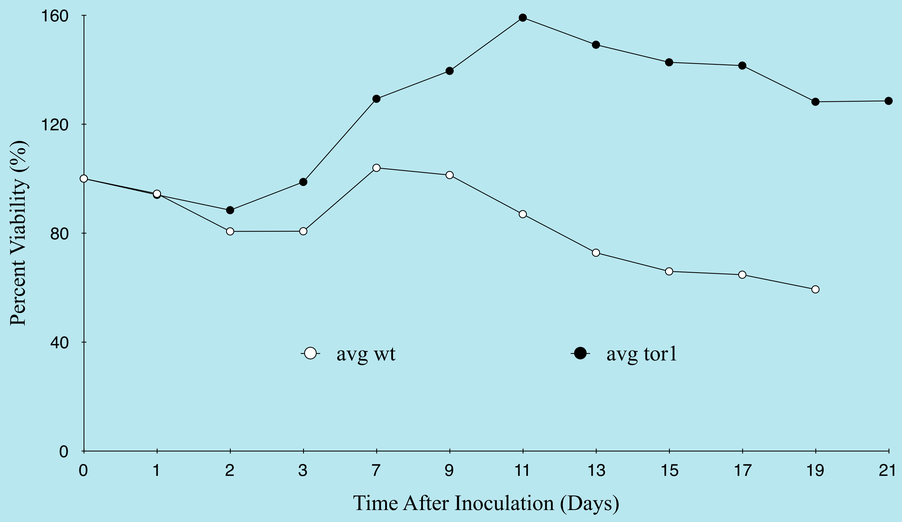


Figure 3: CLS Assay of wild type and *TOR1* mutant yeast. The CLS assay of wild type and *TOR1* mutants shows increased longevity in the *TOR1* mutant. From other research, this is what is to be expected from *TOR1* mutants.

Discussion

- Growth in YPD-G418 media, sequencing primers, and oxidative stress test all confirmed deletion of *TOR1* gene.
- The CLS assay of wild type and Tor1p mutants shows increased longevity in the Tor1p mutant (Figure 3), and also demonstrate the phenotype expected of *Δtor1* BY4741 compared to wild type BY4741.
- The oxidative stress test (Figure 2) also shows the expected phenotype, with *Δtor1* colonies that grow in the presence of environmental oxidative stress, but grow much smaller colonies that wild type BY4741.
- Further research includes attempting to disrupt other genes of interest involved in the TOR1 signaling pathway to examine the effects on chronological lifespan of those gene double-deletions.

Conclusions

This research is related to lifespan of many species, as TOR is a highly conserved eukaryotic pathway (Teixeira *et al.*). In addition, the TOR pathway has implications in human health, as mutations to this pathway result in neurodegenerative diseases like ALS and Huntington's (Tellone *et al.*) due to reactive oxidative species damage.

Future Directions

- Using the *Δtor1* strain, generate double deletion strains *Δlys7/Δtor1*, *Δsod1/Δtor1*, *Δsod2/Δtor1*, and *Δctr1/Δtor1*.
- For each deletion strain, conduct a CLS Assay.
- Test each deletion strain with oxidative stress, such as the H_2O_2 test.

References

1. Bonawitz, N. D.; Chatenay-Lapointe, M.; Pan, Y.; and Shadel, G. S. (2007) *Cell Metab. 4*, 265-277.
2. Laplante, M.; and Sabatini, D. M. (2012) *Cell. 149*, 274-293.
3. Teixeira, V.; Medeiros, T. C.; Vilaça R.; Moradas-Ferreira, P.; and Costa, V. (2014) Microbial Cell. 1, 21-36.
4. Tellone, E.; Galtieri, A.; Russo, A.; and Ficarra, S. (2016) *Neural Regen Res 11,* 86-87.
5. Xie, M. W.; Jin, F.; Hwang, H.; Hwang, S.; and Anand, V., Duncan, M. C., Huang, J. (2005) *PNAS Genetics. 102*, 7215-7720.

Acknowledgements

Murdock Charitable Trust Natural Sciences Grant

National Science Foundation MRI-Grant

Linfield College Student Faculty Collaborative Research Grant

Linfield Chemistry and Biology Departments

The Wendell L. Foote Fund