**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 Δsod1Δtor1, Δsod2Δtor1, and Δctr1Δtor1 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 Δlys7Δtor1, Δsod1Δtor1, Δsod2Δtor1, and Δctr1Δtor1.

**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).

**Results**

- **Rapamycin (inhibition)**
  - Growth Signals (activation)

**Discussion**

- **Growth** in YPD-G418 media, sequencing primers, and oxidative stress test 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 Δlys7Δtor1, Δsod1Δtor1, Δsod2Δtor1, and Δctr1Δtor1.
- For each deletion strain, conduct a CLS assay.
- Test each deletion strain with oxidative stress, such as the H2O2 test.

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**References**