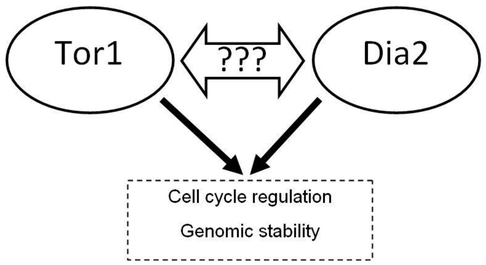
Effects of Rapamycin on Dia2 in *S. cerevisiae*

Peter W. Newhouse IX and Deanna Koepp

University of Minnesota, College of Biological Sciences

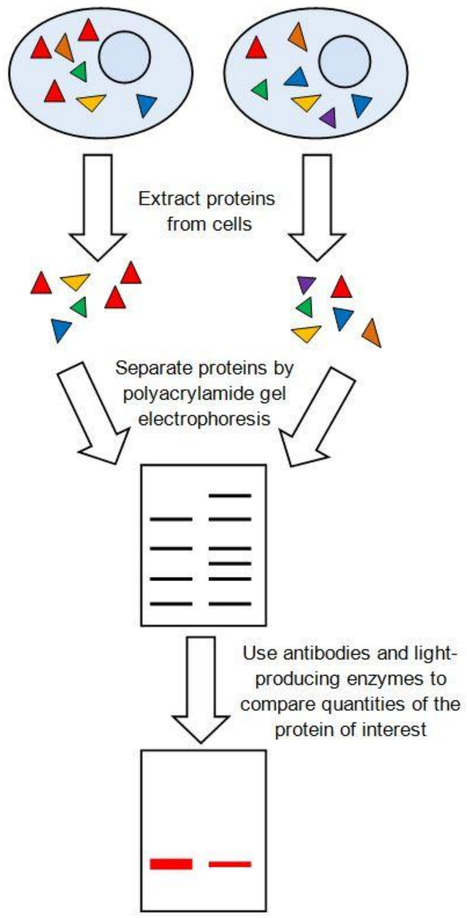
Background

- **Dia2**
  - F-box protein with role in S-phase checkpoint regulation.
  - Loss leads to chromosomal damage.
  - Found at rDNA loci
- **Tor1**
  - Protein kinase with role in regulating cell growth & division.
  - Inhibited by rapamycin
  - Loss leads to chromosomal damage.
  - Found at rDNA loci
- **Dia2Δ – Tor1Δ Mutant**
  - Lacks function of Dia2 and Tor1.
  - Synthetic phenotype: more severe chromosomal damage.
- **Hypothesis**
  - Dia2 and Tor1 are functionally related, and act in similar pathways to control cell division and genomic stability.



Methods

- **Western Blotting**



- **Stability Assays**
  - Determine Dia2 protein turnover rate
  - Treatment with cyclohexamide to inhibit protein synthesis
  - Timepoints every 30 minutes for 3 hours

Results

- **Western Blotting**
  - Decrease in Dia2 abundance was associated with rapamycin treatment.
  - PGK loading control confirmed that the same quantity of protein was loaded from each sample.
  - Performed with G1-arrested cells to control for variation in Dia2 levels, since rapamycin inhibits S phase progression.

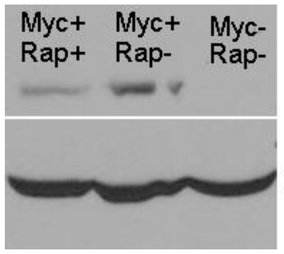


Figure 1. Dia2 abundance is decreased in the presence of rapamycin. Top: Dia2 protein is less abundant in rapamycin-treated cells compared to untreated cells. As a negative control, cells with untagged Dia2 do not show any 9Myc-Dia2 protein. Bottom: Pgk1 as a loading control. All samples contained similar levels of Pgk1.

- **Stability Assays**
  - Following cyclohexamide treatment, Dia2 levels decreased more rapidly in rapamycin-treated cells.
  - Performed with G1-arrested cells.

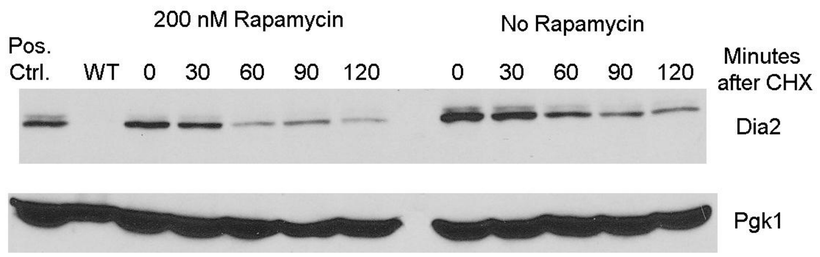
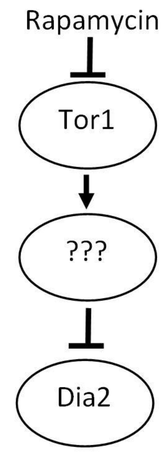


Figure 2. Dia2 degradation observed to be accelerated in rapamycin-treated cells. Following cyclohexamide exposure, Dia2 levels decrease in both samples. This loss appears to occur more rapidly in the rapamycin-treated cells. PGK served as a loading control.

Discussion

- **Conclusions**
  - The Tor1 inhibitor rapamycin reduces Dia2 abundance and increases the rate of Dia2 degradation.
  - This investigation provides further evidence of a functional relationship between Dia2 and Tor1.



- **Future Directions**
  - Further studies of Dia2's pathway will be necessary, both to better understand Dia2 itself and to investigate its relationship with Tor1.
  - Investigate whether Tor1 influences transcriptional regulation of Dia2 by use of reverse transcription PCR.

Bibliography

Blake, D. et al. The F-box protein Dia2 overcomes replication impedance to promote genome stability in Saccharomyces cerevisiae. Genetics 174, 1709–1727 (2006).

Ha, C. W. & Huh, W.-K. Rapamycin increases rDNA stability by enhancing association of Sir2 with rDNA in Saccharomyces cerevisiae. Nucleic Acids Res. 39, 1336–1350 (2011).

Kile, A. C. & Koepp, D. M. Activation of the S-phase checkpoint inhibits degradation of the F-box protein Dia2. Mol. Cell. Biol. 30, 160–171 (2010).

Acknowledgments

The author is grateful to the members of the Koepp lab, especially Dong-Hwan Kim and Chi Meng Fong, for their advice and assistance in this project. This work was funded by RO1 grant #GM076663 from the National Institutes of Health.