Effects of Rapamycin on Dia2 in S. cerevisiae

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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
  - 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.

Results
- Western Blotting
  - Following cyclohexamide treatment, Dia2 levels decreased more rapidly in rapamycin-treated cells.
  - Performed with G1-arrested cells.

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.

Background Diagram:
- Tor1
- Dia2
- Cell cycle regulation
- Genomic stability

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.

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.

Bibliography

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