Effects of Environmental Heat Stress on Changes in RAD51 in Yeast

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Objective

DNA damage must be repaired to maintain genomic stability and cell survival. RAD51 is a key protein responsible for repairing double-stranded DNA breaks. Environmental stressors such as elevated temperature may disrupt protein expression and DNA repair pathways.

Research Question: How does environmental heat stress affect cell growth and RAD51 protein abundance in Saccharomyces cerevisiae?

Hypothesis: RAD51 knockout yeast will have a slower rate of cell growth and demonstrate less protein expression when under heat stress compared to wild-type RAD51 yeast in the same conditions.

Methodology

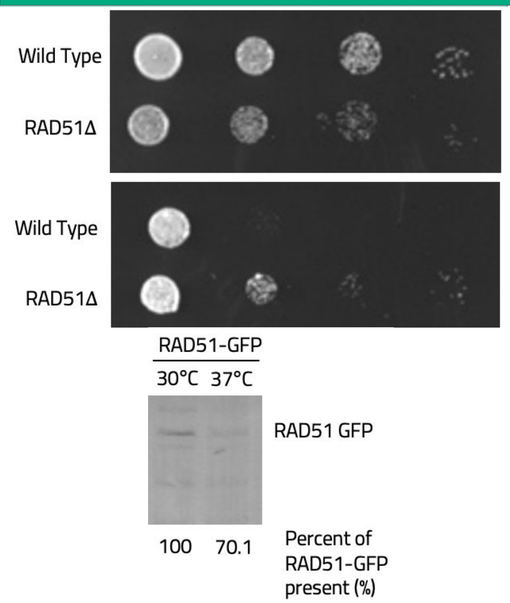
Growth Assays

- Wild type and RAD51 knockout yeast strains cultured in YPD media
- Serial dilutions plated on agar plates
- Incubated at 30°C (normal condition) and 37°C (heat stress condition)
- Colony growth compared qualitatively and quantitatively

Western Blot

- GFP-tagged RAD51 strains grown at 30°C and 37°C
- Protein extracted and separated
- Transferred to membrane and probed with anti-GFP antibody
- PGK1 used as loading control
- RAD51 abundance compared between temperatures

Results



30°C: RAD51 knockout exhibited slower growth compared to wild type.

37°C: Knockout strain demonstrated improved growth compared to wild type, even under heat stress.

Western blot analysis: RAD51-GFP protein abundance decreased by 30% under heat stress.

Conclusions

- RAD51 contributes to normal cellular growth.
- Heat stress reduces RAD51 protein abundance.
- Slower growth in knockout strain under normal conditions supports RAD51's role in DNA repair.
- Unexpected improved growth at 37°C suggests possible secondary mutations.

Future Directions

- Repeat experiments for statistical validation
- Sequence RAD51 knockout strain to assess secondary mutations.