Effects of Environmental Heat Stress on Changes in RAD51 in Yeast

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Introduction

DNA damage occurs naturally in all living organisms and must be repaired to maintain genomic stability and cell survival. RAD51 is a DNA repair protein involved in repairing double-stranded DNA breaks. As DNA is damaged a lot, experimenting with the presence of the RAD51 observes its importance in how it affects cell growth. Environmental stressors, such as elevated temperature, can disrupt normal cellular processes, including protein expression and stability. Studying RAD51 in S. cerevisiae, or yeast, allows controlled experimentation on stress responses that would not be feasible in more complex organisms. Understanding how heat stress affects RAD51 expression in a model organism may provide introductory insight into how environmental conditions might affect DNA stability and survival in more complex organisms and how cells cope with damage under stressful conditions. It is hypothesized that yeast with RAD51 knockout lacking the RAD51 protein will experience a slower rate of cell growth and demonstrate less protein expression when under heat stress compared to wildtype RAD51 in the same conditions.

RAD51 DNA Repair Enzyme

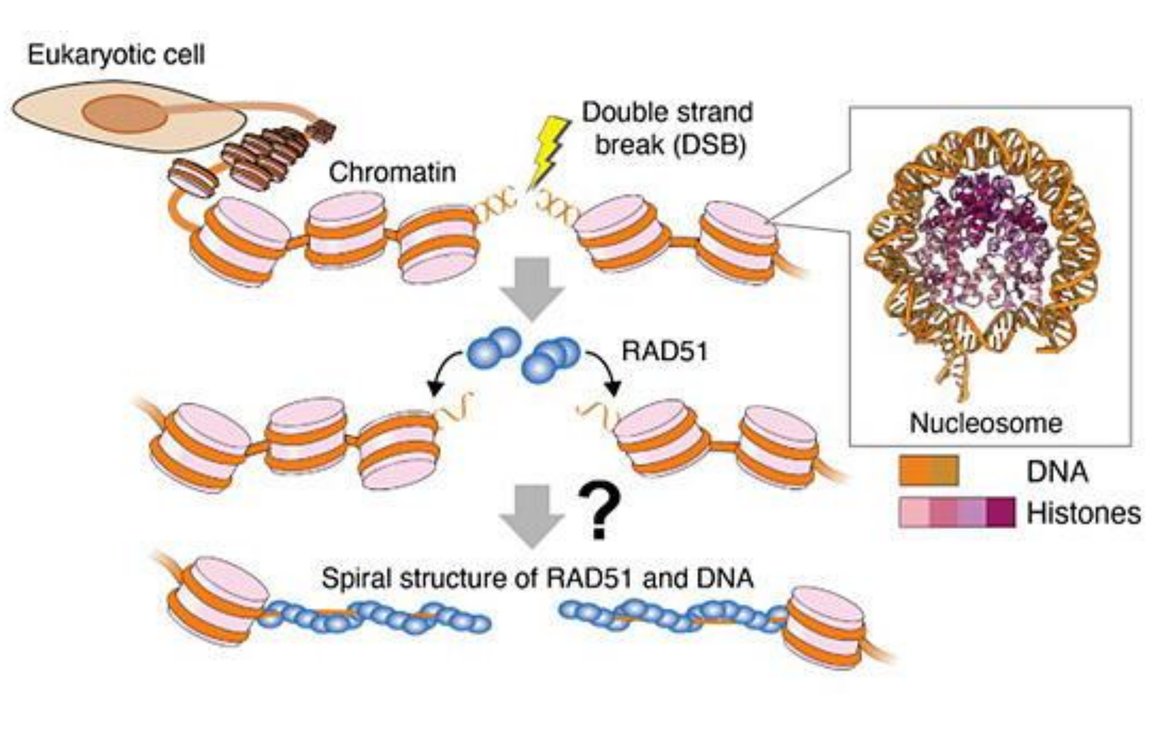


Figure 1 DNA double strand break on a chromatin template repaired by RAD51

Japan Science and Technology Agency. "Function of RAD51 Protein in Repairing Double-Strand DNA Breaks on Chromosomes Clarified by the University of Tokyo — Opening a Path to Understanding Cancer Development." Japan Science and Technology Agency, 17 May 2024, https://sj.jst.go.jp/news/202405/n0517-01k.html.

Methodology

Growth Assays

VJ4476 wild-type and RAD51 knockout yeast strains were streaked onto YPD (nutrient-rich growth medium) agar media. Streaked yeast were incubated overnight at 30°C until single colonies were present. The VJ4476 wild-type and RAD51 knockout yeast strains were each inoculated in 5mL of liquid YPD media and incubated at 30°C, rotating, overnight. The OD600 (optical density at 600 nm light) spectrophotometer was used to determine the OD of each strain using a 1:25 dilution factor (48 µL YPD with 2 µL yeast strain). The spectrophotometer was blanked using 50 µL YPD (without yeast). 0.2 OD units were used on the growth assays. The wildtype and knockout strains from the wells were then plated onto four YPD agar media plates. 4 µL of each dilution was pipetted onto plates containing agar growth medium. Once plates were dry, they were transferred to the incubators. Plates #1 and #2 were incubated at 30°C, and plates #3 and #4 were incubated at 37°C. Plates were imaged after sufficient growth was shown.

Western Blots

Two tubes of GFP-tagged RAD51 yeast strain were inoculated in liquid YPD media. One tube was incubated overnight at 30°C and the other at 37°C. Two tubes of wild-type yeast strain was inoculated in liquid YPD media. One tube was incubated overnight at 30°C and the other at 37°C to serve as the control. 5 OD units were harvested, and the cells were lysed by NaOH and SDS detergent to release the proteins. The samples were loaded on a 10% polyacrylamide gel and ran at 200 volts for one hour in the Bio-RAD Mini-PROTEAN blotting system. After gel electrophoresis, an electrophoretic transfer was performed onto a cellulose membrane. The membrane, now with proteins, was washed with a skim milk-based blocking buffer solution with TBS/T to prevent nonspecific binding. The membrane was probed with a 1:20,000 dilution of GFP probe and imaged, then probed with a 1:20,000 dilution of anti-PGK1 and imaged. 1:40,000 dilution of the alexa fluor antibody was used as the secondary probe to tag the antibodies. The membrane was imaged on a Licor Odyssey DLX Membrane Imager.

Results

Figure 1: Growth assay of plate #2 incubated at 30°C overnight.

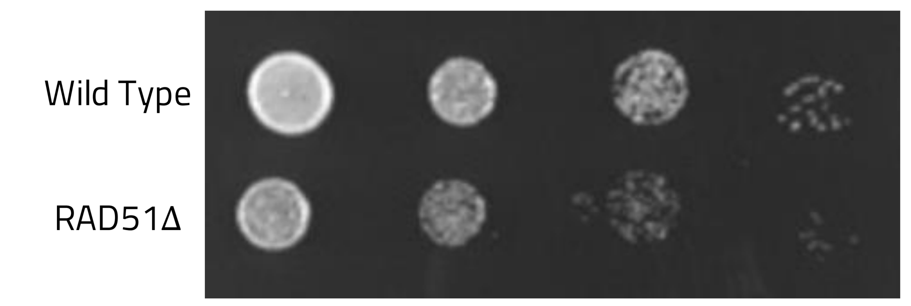


Figure 2: Growth assay of plate #3 incubated at 37°C for five days.

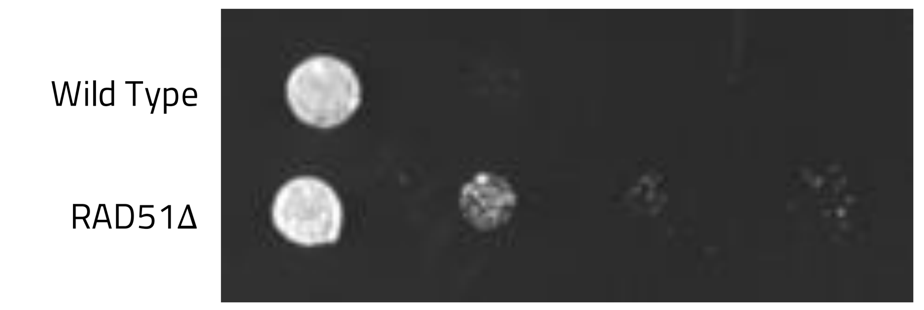


Figure 3: Western blot for RAD51-GFP

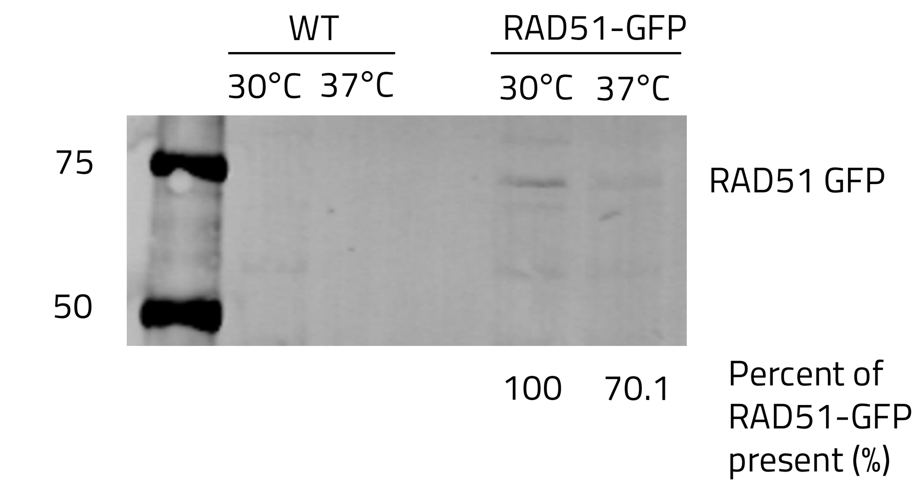
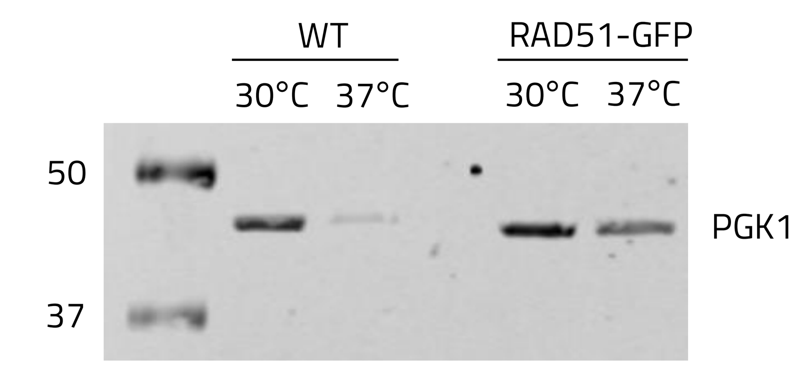


Figure 4: Western blot for PGK1.



Conclusion

The results from the growth assays demonstrated slower growth in the RAD51 knockout in 30°C but quicker growth in 37°C. The results from the Western blot demonstrated that RAD51 abundance was reduced at high temperatures.

Therefore, the hypothesis that an organism lacking RAD51, the protein that repairs damaged DNA, will have less protein abundance when exposed to heat stress was supported. However, the hypothesis that RAD51 knockout would grow slower in heat stress was not supported. This suggests that the RAD51 protein is necessary for cellular growth in normal conditions but may cause other mutations under heat stress.

Future Directions

- Repeat experiments multiple times
- Investigate whether secondary mutations in the RAD51 knockout strain, arising from impaired DNA repair, may have accumulated and contributed to the enhanced high-temperature growth observed in Figure 2

Acknowledgements

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