4-25-2019

Analysis of Cross Complementation of Archaeal Bax1 Protein in Saccharomyces cerevisiae

Kelly Holmin
College of Saint Benedict/Saint John's University, kholmin001@csbsju.edu

Analysis of Cross Complementation of Archaeal Bax1 Protein in *Saccharomyces cerevisiae*

Kelly Holmin, advisor Dr. Michael Reagan
College of Saint Benedict/Saint John's University
Biology Department

Introduction

- Ultraviolent (UV) light exposure can cause damage to DNA bases and result in distortion of it's native conformation.
- When DNA damage occurs, a cellular process called nucleotide excision repair (NER) occurs in which DNA lesions are repaired.
- NER in eukaryotes is well understood, however, very little is known about Archaeal NER.
- In eukaryotic NER, Rad1 and Rad2 proteins are part of the 9-1-1 cell checkpoint complex that is called to the area of DNA damage. Rad1 protein functions as a checkpoint exonuclease and Rad2 protein functions as a checkpoint endonuclease.
- Recent studies suggest the archaeal Bax1 gene could function as a DNA endonuclease, serving the same role in DNA repair in archaea as the Rad1 protein does in eukaryotes.

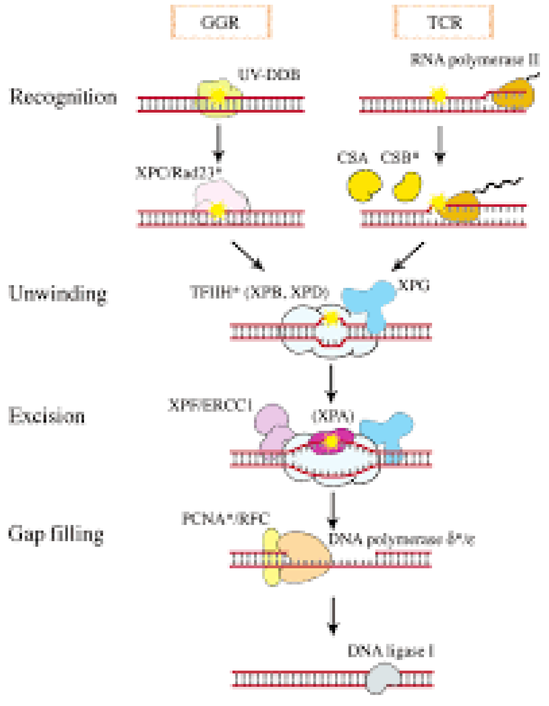

Figure 1: The NER process in eukaryotes. NER has two direct pathways; global genome repair (GGR) and transcription coupled repair (TCR). GCR, the steps of which are shown right side of the figure, repairs transcriptionally silent regions, whereas TCR, the steps of which are shown on the left side of the figure, repairs transcriptionally active regions of the genome. The main distinction between the two pathways is their mechanism of DNA damage, as is shown in the figure above. *Image source:* https://core.ac.uk/download/pdf/132564416.pdf

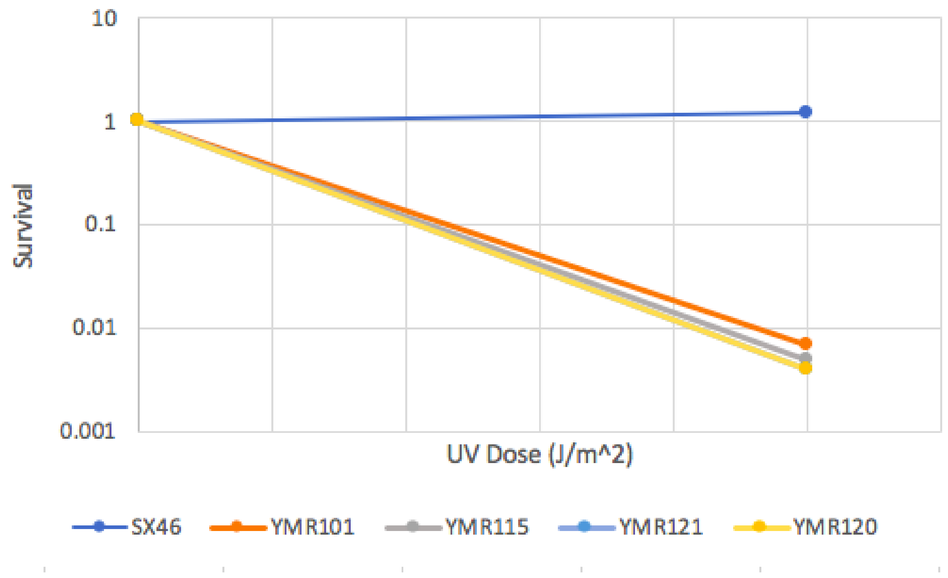

Figure 2: Survival of SX46, YMR101, YYMR115, YMR120 and YMR121 *S. cerevisae* when exposed to UV light.

Methods

- Five strains of the yeast *Saccharomyces cerevisiae* were obtained; *S. cerevisiae* Δrad1 with a Bax1 gene plasmid (YMR120) or a vector plasmid (YMR101), *S. cerevisiae* Δrad2 with a Bax1 plasmid (YMR121) or vector plasmid (YMR115), and a wild type *S.cerevisiae* and no plasmid (SX46).
- Each of the five strains were grown in dextrose broth culture for 72 hours at 30℃. the yeast strains were grown in the absence of uracil to ensure plasmid retention, with the exception of the wild type *S.cerevisiae* which held no plasmid and was grown in uracil.
- After 72 hours, the strains were transferred to galactose broth culture for another 24 hours at 30℃ to induce expression of the archaeal gene.
- After 24 hours in the galactose broth, cells were plated on YPR/G media and exposed to UV light for two seconds.
- After exposure, plates were incubated at 30℃ for 72 hours and removed. Cell counts for each plate where then taken to determine percent survival after UV light exposure.

Results

- Wild type SX46 *S. cerevisiae* had high survival rates despite UV light exposure, suggesting functional NER.
- YMR101 and YMR115, both strains with no genes coding for DNA repair mechanisms, had virtually no survival after UV light exposure, suggesting high UV sensitivity and no functional NER.
- YMR120 and YMR121, both strains no native Rad protein and a Bax1 plasmid, also had virtually no survival after UV light exposure, suggesting high UV sensitivity and no functional NER.

Discussion

- Low survival suggests that *S. cerevisiae* containing Bax1 genes (YMR121 and YMR120) did not have functional NER.
- Low survival in Δrad2+Bax1 (YMR121) and Δrad1+Bax1 (YMR120) suggests that Bax1 cannot substitute for Rad1 and Rad2 proteins in *S. cerevisiae.*
- Future research should be conducted to test for cross-complementation of other DNA repair archaeal genes in *S. cerevisiae.*

References

- Guo, Caixia, Tang, Tie-Shan, Friedberg, Errol C. (2010) Snapshot: Nucleotide Exicision Repair. *Bejjing Institute of Genomics*. pg. 754 DOI 10.1016/j.cell.2010.02.033
- Habraken, Y, Sung, P, Prakash, L, Prakash, S. (1993) Yeast excision repair gene RAD2 encodes a sigle-stranded DNA endonuclease. *Nature,* pg. 365-368. DOI: 10.1038/366365a0
- Kelman, Z. and White, M. (2005) Archaeal DNA Replication and Repair. *Current Opinion in Microbiology. pg. 669-679,* DOI 10.1016/j.mib.2005.10.001

Acknowledgements:
Thank you to Dr. Reagan for sharing his time, knowledge, lab space, and materials to this project.