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Analysis of potential archaeal NER endonuclease homologs using Saccharomyces cervisiae

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Analysis of potential archaeal NER endonuclease homologs using *Saccharomyces cerevisiae*

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Introduction

- Environmental agents can create distorting lesions in DNA and disrupt cell function[1]
- Nucleotide Excision Repair (NER) involves complex cooperation of proteins to remove DNA lesions[2]
- NER process and proteins involved are well understood for eukaryotes, but not for archaea[3]
- rad1 (known as XPF in eukarya) is 5' endonuclease in NER and is found in all eukaryotes[2]
- Based on amino acid sequencing and biochemical function, archaeal proteins Bax1 and Hef1 could perform rad1 role[4,5]

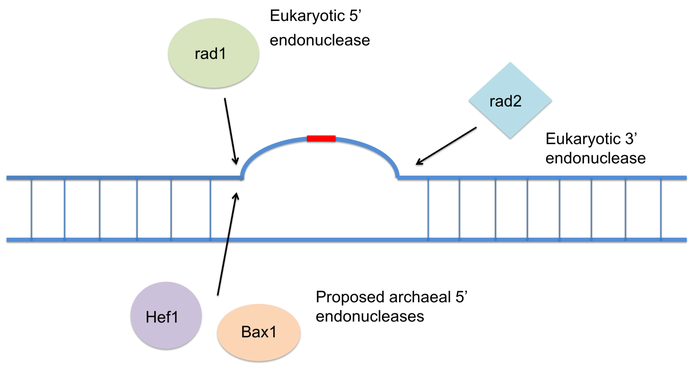

Figure 1. Eukaryotic nuclease function and proposed archaeal homologs in NER. rad1 functions as a 5' endonuclease in eukaryotic NER; Bax1 and Hef1 are proposed to perform the same function based on structure and nuclease activity.

Methods

- rad1 deletion (Δrad1) *S. cerevisiae* strain was obtained and archaeal genes were inserted using pYES2/NT vector[6]
- Growth in Uracil-deficient media ensures that plasmid will be maintained in cells
- *S. cerevisiae* strains were grown with dextrose for 72 hours at 30°C
- Transferred to galactose to induce expression of archaeal gene and grown at 30°C
- After 24 hours, cells were plated on agar and exposed to Ultraviolet (UV) light
- Cells were grown for 72-120 hours at 30°C and survival rates were calculated

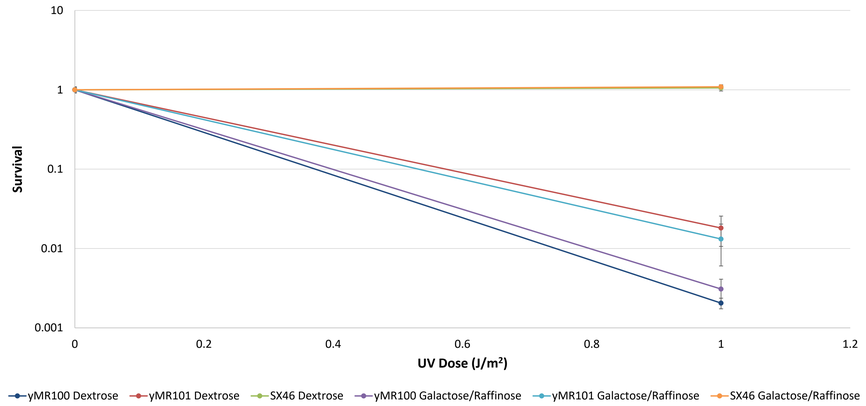

Figure 2. Survival of Δrad1 *S. cerevisiae*+*M. voltae* Hef1 (yMR100) when utilizing different carbohydrate sources. yMR100, wild type (WT), and Δrad1+pYES2/NT vector (yMR101) were plated with dextrose or galactose+raffinose and exposed to UV. Error bars represent standard error of the mean.

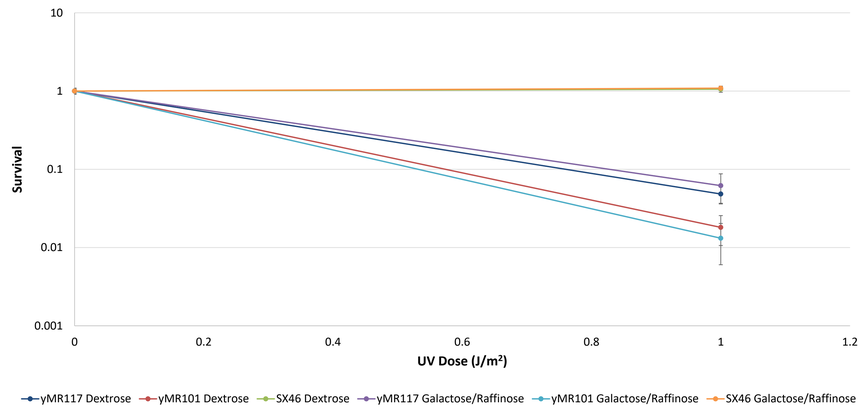

Figure 3. Survival of Δrad1 *S. cerevisiae*+*M. acetivorans* Hef1 (yMR117) when utilizing different carbohydrate sources. yMR117, WT, and Δrad1+pYES2/NT vector (yMR101) were plated with dextrose or galactose+raffinose and exposed to UV. Error bars represent standard error of the mean.

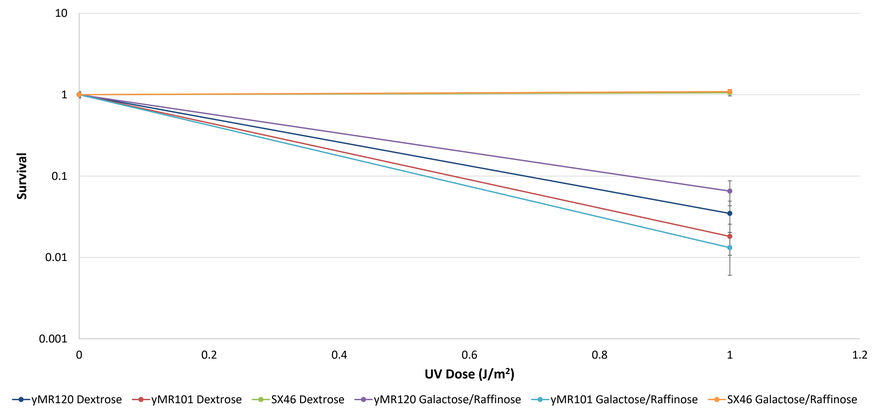

Figure 4. Survival of Δrad1 *S. cerevisiae*+*M. acetivorans* Bax1 (yMR120) when utilizing different carbohydrate sources. yMR120, WT, and Δrad1+pYES2/NT vector (yMR101) were plated with dextrose or galactose+raffinose and exposed to UV. Error bars represent standard error of the mean.

Results

- Δrad1 + pYES2/NT vector shows high UV sensitivity consistent with nonfunctional NER
- Δrad1 + *M. voltae* Hef1 did not have a higher rate of survival than Δrad1 + pYES2/NT vector with either carbohydrate source (Fig. 2)
- Δrad1 + *M. acetivorans* Hef1 had significantly higher survival rates than Δrad1 + pYES2/NT vector with both carbohydrate sources (Fig. 3)
- Δrad1 + *M. acetivorans* Bax1 had significantly higher rates of survival than Δrad1 + pYES2/NT vector on galactose/raffinose agar (Fig.4)
- Δrad1 + *M. acetivorans* Bax1 plated on galactose/raffinose agar had higher rates of survival than when plated on dextrose agar (Fig. 4)

Discussion

- Increased survival of Δrad1 + *M. acetivorans* Bax1 and Hef1 strains indicates that Bax1 and Hef1 have potential to perform rad1 function in archaea
- Increased survival of Δrad1 + *M. acetivorans* Bax1 on galactose+raffinose agar suggests that plasmid maintenance and expression is critical for repair in initial hours after UV exposure
- *M. acetivorans* Hef1 was synthesized to correct for the codon bias in *S. cerevisiae* and can account for higher survival than uncorrected *M. voltae* Hef1

References

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