Analysis of double-stranded DNA break repair in haploid *Saccharomyces cerevisiae* under spaceflight conditions.

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Abstract

Due to the advancement of space age, all spaceflight-associated health risks should be studied in detail. Mistakes in double-stranded DNA break (DSBs) repair can lead to carcinogenesis. Previous studies reported contradictory results regarding the effect of microgravity on DSB repair. Cells have two major mechanisms that repair DSBs: homologous recombination (HR) and non-homologous end joining (NHEJ). HR requires a second, good copy of the damaged DNA for repair, while NHEJ adheres two DNA ends together. The goal of this experiment is to determine how spaceflight impacts NHEJ. To accomplish this goal, we will use the budding yeast *Saccharomyces cerevisiae* as an experimental model. Yeast have several advantages for this line of investigation: 1) Their NHEJ repair mechanisms are well conserved with humans; 2) Their fast proliferation rate generates sufficient material for cellular and molecular analyses; 3) Yeast can live as either diploids or haploids; haploids must exclusively use NHEJ to repair DSBs; 4) DNA damaging agents have been well-studied in this model. Using the confines of a NanoRacks MiniLab, we have engineered an experimental system which will expose proliferating haploid yeast to bleomycin, a DNA damaging agent. Preliminary experiments have identified an optimal bleomycin concentration (1.5 µg/mL) and a sufficient cell density (2.5 x 10^6 cells/mL). Using these parameters,we will measure NHEJ in both an experimental sample sent to the International Space Station and an Earth control. The results of these analyses will be crucial in forwarding our understanding of NHEJ in space.

DSB Repair Mechanisms

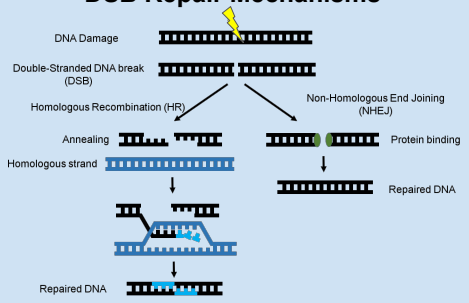

Figure 1: The two major types of DSB repair are HR and NHEJ. HR relies on a homologous strand of DNA that is usually present in diploid but not haploid organisms. On the other hand, NHEJ simply rejoins the two strands. Although HR is more precise, NHEJ is the preferred repair pathway in mammalian cells.

Experimental Design

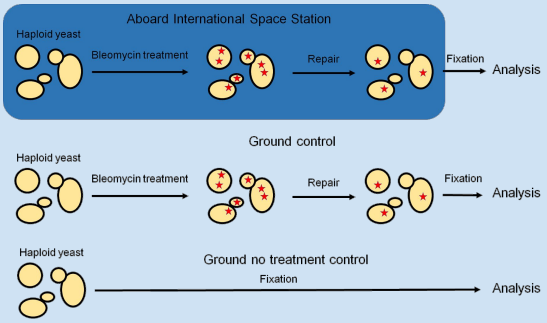

Figure 2: The goal of the experiment is to expose haploid *Saccharomyces cerevisiae* to bleomycin (BLM), a DSB inducing agent, allow time for sufficient repair, fixate and finally analyze the extent of remaining damage in a laboratory setting. Differences in damage between the experimental sample and ground control will be indicative of spaceflight conditions influencing NHEJ.

Bleomycin Delivery

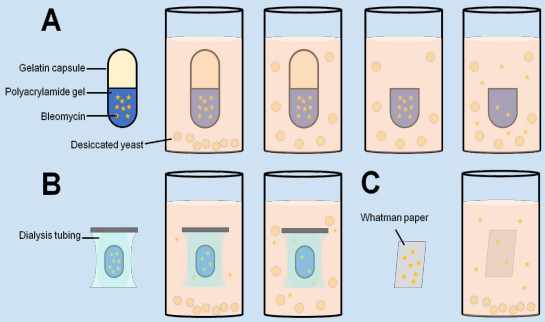

Figure 3: Due to the long transit time to the ISS, yeast will need to be desiccated for survival. Our data showed that desiccated yeast are very susceptible to high concentrations of BLM. Multiple delivery systems were engineered to ensure yeast survival. A: polyacrylamide hydrogel containing BLM was kept inside a water soluble gelatin capsule. After the yeast recovered, the capsule would dissolve and slowly diffuse BLM from the hydrogel. B: BLM containing hydrogel was kept inside a dialysis tubing. This delivery method would slow the diffusion rate enough to allow yeast recovery. C: a carefully measured BLM dose was administered at the start of experiment from a whatman paper. Such a dose will influence recovery time but not kill the yeast. After heavy experimentation, approach C was chosen as the final delivery method. Approaches A and B raised concerns over exposure of yeast to compounds in capsule/hydrogel and BLM stability in a hydrogel.

Bleomycin Dose

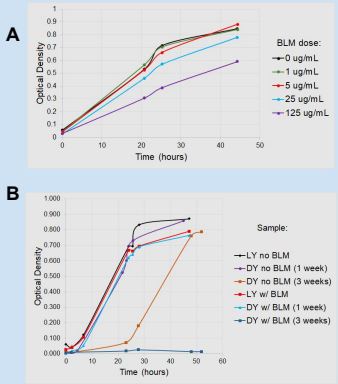

Figure 4: Bleomycin toxicity analysis. Due to its DNA damaging properties, BLM is highly toxic to yeast cells. It is therefore crucial that the BLM dose is not high enough to kill the yeast, yet high enough to elicit a response. A: response of fresh, liquid yeast to BLM treatment. B: response of yeast that have been desiccated for 21 days. It was determined that yeast age is a major factor for determining BLM toxicity. BLM dose of 5 ug/mL was chosen.

Dessicated Yeast Stability

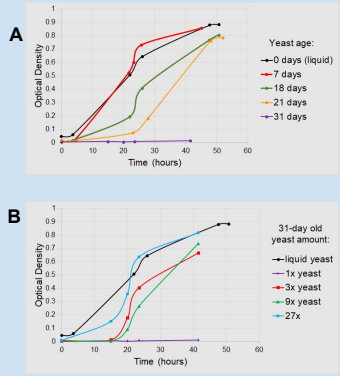

Figure 5: Yeast viability analysis. Prolonged time periods spent in a desiccated state will decrease viability of yeast. This decrease of viability results in longer recovery time and possibly death. A: recovery of yeast desiccated for different periods of time. B: recovery of yeast dessicated for 31 days with different starting cell counts. The problem shown in A was solved by increasing starting yeast cell count 27-fold.

Final Design

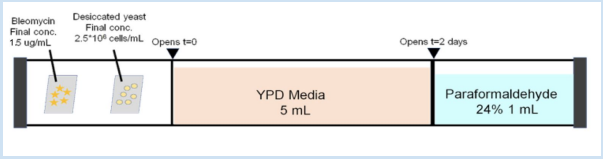

Figure 6: The NanoRacks MiniLab tube will be split into three chambers by two clamps. First chamber will contain BLM and desiccated yeast spotted on whatman paper. Second chamber will contain 5 mL of YPD media. The experiment will be initiated when the clamp separating these two chambers is released. The third chamber contains 1 mL of 24% paraformaldehyde solution. The clamp separating this chamber will be released after two days since the start of the experiment. After mixing, paraformaldehyde will fixate the experiment at a concentration of 4%. The experiment will then be sent back to Earth for analysis.

Methods of Analysis

- Cell count measurement: Cell count will be obtained using a hemocytometer. This will indirectly offer insight on DSB repair efficiency. In addition it will provide information necessary for other analyses.
- γ-H2AX assay: When a DSB occurs, nearby H2AX histones are phosphorylated to become γ-H2AX histones. These are used by cells to direct repair machinery towards the DSB. Amount of γ-H2AX will be quantified using antibody based method. This quantity will be representative of DSB prevalence, and therefore NHEJ effectiveness.
- Southern Blot: DNA will be seperated on a gel by fragment size. Next, a probe detecting single-stranded DNA present at DSB sites will be applied. This will indirectly quantify amount of DSBs.
- RT-qPCR: Expression of 19 genes involved in DNA damage repair pathways will be analyzed. If spaceflight conditions influence DSB repair through epigenetic changes, this will be detected.