ABSTRACT

The objective of this experiment was to determine the effect of a microgravity environment on an ARCUS nuclease's ability to locate a specific sequence and create a double-stranded break in DNA. The experiment served as a first step to determine whether this technology could be used in an extraterrestrial habitat, possibly someday repairing DNA damage caused by exposure to space radiation.

Introduction

Immaculata Catholic School collaborated with Space Center Houston and Precision BioSciences, to conduct a MixStix experiment aboard the International Space Station.

Precision Biosciences has developed a molecular tool called ARCUS, an enzyme that can be engineered to recognize any 22 base pair DNA sequence and generate a double-stranded break allowing removal, addition, or replacement of DNA at that site. Precision BioSciences has shown that ARCUS nucleases can function in plants and animals. ARCUS is currently being used to improve life by developing therapies to fight cancer, viral infections, genetic diseases, and make better foods.

Methods

Generation of Reagents:

Under the leadership of scientists at Precision BioSciences, students purified an ARCUS nuclease by standard chromatography methods and extracted plasmid DNA containing the 22 base pair recognition sequence specific for that ARCUS nuclease from E. coli. For increased stability, the nuclease was dried to a powder form.

The MixStix was prepared as illustrated in Figure 1. Clamps separate the three compartments containing the dried ARCUS nuclease (volume 1) a solution of plasmid DNA containing the recognition sequence (volume 2) and a stop solution containing SDS detergent (volume 3). Two identical experiments were packaged; one for deployment to the ISS and one as an Earth control.

The MixStix was transported to the International Space Station aboard SpaceX CRS-16. Once on station, an ISS crewmember released the first clamp mixing the ARCUS nuclease and plasmid DNA. After an incubation period, the second clamp was removed, mixing the detergent with the DNA and ARCUS mix and stopping the reaction.

Results

The experiment was designed to determine if the ARCUS nuclease can make a double stranded break in a circular plasmid containing the ARCUS recognition sequence resulting in a linear fragment of DNA (Figure 1).

On return to Earth it was evaluated by gel analysis and compared to the Earth control to determine if the DNA was cut and linearized by the ARCUS nuclease under microgravity conditions (Figure 2).

The plasmid DNA that was incubated with nuclease on Earth appeared completely linearized. A portion (~50%) of the plasmid DNA that was incubated with nuclease on the ISS was linearized. When BglII was added to the DNA sample that was previously incubated with nuclease either on Earth or the ISS, the DNA that was linearized by the nuclease was cut into 2 fragments as expected (~1380 & 4600 bp). In the ISS DNA sample that was digested with BglII, a band corresponding to linear DNA remains due to BglII-linearization of the uncut plasmid DNA by BglII.

Conclusion:

The ARCUS nuclease digested target DNA in a microgravity environment on the ISS.