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THE FIDELITY OF DNA REPLICATION IN MICROGRAVITY

Abstract

Current space missions involving low earth orbit, in addition to prospective interplanetary exploration missions, will expose participants and their cells to extended periods of microgravity, an environmental stressor for which no earth-based organism has evolved to survive. As well, ionizing radiation present in space is known to have mutagenic effects on DNA. Despite these potential health risks, the impact of exposure of microgravity and ionizing radiation on DNA replication and repair machinery has not been fully explored. DNA polymerases play a major role in both these processes, and thus can be considered central to the maintenance of genomic integrity, especially in the presence of radiation-induced DNA lesions. The "Polymerase Error-Rate in Space (PolERIS) experiment" was designed to assess whether a differential in DNA polymerase fidelity and replication rate exists under conditions of microgravity generated by parabolic flight when compared to earth gravity (1G). A flexible platform for conducting genetics and enzymological experiments during parabolic flight was developed for this experiment. The payload was designed to automatically inject two fluid samples into reactions housed in a temperature-controlled thermal cycler in triplicate and mechanically mix them. During flight, DNA polymerization reactions on a primed synthetic single-stranded DNA template were initiated by introduction of an enzyme mixture (Klenow Fragment +/-; with and without proofreading exonuclease activity) upon commencement of a parabolic arc. Reactions were quenched after 20 sec using a divalent cation chelator (EDTA) and heat inactivated following the completion of a parabola. While initial results do not indicate a significant change in DNA replication product length in flight, assessment of replication fidelity is still underway. Sequencing to determine replication fidelity utilizes the PacBio Single Molecule Real Time circular consensus read platform (Pacific Biosciences). When coupled with the novel single-molecule combinatorial DNA tag mechanism developed for this experiment, we posit that an extremely accurate determination of any differential in DNA polymerase error rate will be achieved. The PolERIS experiment represents a new approach to enzymology research in microgravity, and introduces a flexible automatic payload suitable for testing other life-science related experiments. Furthermore, it is our hope that the engineering, genetics, and bioinformatics techniques developed here may prove invaluable to future space health research.