CALIFORNIA STATE SCIENCE FAIR
2004 PROJECT SUMMARY

Name(s)
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Project Title
Characterization of Mus81/Mms4's Role in Homologous DNA Repair during S-Phase of the Mitotic Cell Cycle

Abstract

Objectives/Goals
The Mus81/Mms4 heterodimer is a highly conserved member of the XPF endonuclease superfamily and part of the Rad52 epistasis group, which is known to serve a large role in recombinational DNA repair. Previous extraction of Mus81/Mms4 for in vitro experimentation has varied based on the proteins' host and has differed in conclusions about the proteins' function. The objective of my research was to directly isolate and characterize the Mus81/Mms4 heterodimer, along with its respective mutant, from a native eukaryotic host, Saccharomyces cerevisiae; ultimately providing a tool necessary for comprehending the Rad52 epistasis group of proteins as well as the mechanisms of DNA repair synthesis.

Methods/Materials
An overexpression plasmid was created and propagated within E. coli cells prior to being transformed into S. cerevisiae cells for protein overexpression. Following Comassie staining and Western Analysis to verify overexpression, the endonuclease was purified through affinity chromatography. The applicability of the protein was verified through DNA sequencing and complementation testing, prior to conducting nuclease assays using P-32 labeled DNA substrates.

Results
DNA sequencing of the purified protein revealed the endonucleases was identical to the accepted sequence. Complementation tests verified that the addition of protein tags in no way inhibited or altered the in vivo functions of Mus81/Mms4. Mus81/Mms4 nuclease assays reveal the structure specific endonuclease displays a penchant for cleaving the 3' Flap of ssDNA-dsDNA junctions. The findings suggest Mus81/Mms4 serves to maintain stability in stressed replication fork elongation, and not to resolve the four-way Holliday Junction resulting from homologous DNA fork repair, as is suggested by the current model of DNA repair synthesis.

Conclusions/Discussion
Comprehending Mus81/Mms4's in vivo substrate preference assists in developing a strong understanding of the endonuclease's function during S-Phase cellular division. The fact that cells lacking the Mus81/Mms4 heterodimer are highly sensitive to carcinogens, suggests the possibility of a homologous understanding of failures in cancer averting mechanisms. By better comprehending the components and processes of DNA repair synthesis during cellular division, we may be in a better position to pinpoint the failed mechanisms attributed to tumor formation and cancer development.

Summary Statement
Purification and biochemical characterization the Mus81/Mms4 protein to determine its role in DNA repair synthesis during the S-Phase of mitotic cellular division.

Help Received
Use of Equipment at the University of California Davis Microbiology Department under the supervision of Kirk Ehmsen.