Aug 6th, 9:30 AM - 12:00 PM

The Role of recN in stationary phase mutagenesis in bacillus subtilis

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Repository Citation
Here, we examine mutagenic programs that are independent of growth, such aspects of the evolutionary process are novel and have been implicated in the formation of cancers in animal cells and the acquisition of antibiotic resistance in animal pathogens. Adaptive or stationary phase mutagenesis is a genetic program to increase diversity in cells under conditions of stress whereby cells escape non-dividing conditions. Previous research has shown that recombination functions are required to generate mutations that promote growth in *Escherichia coli* cells starved for carbon. This project tests the hypothesis that recombination functions are required for the generation of mutations that promote growth in response to amino acid starvation stresses in *Bacillus subtilis* cells. In *B. subtilis* cells, *recN*, in addition to *recA*, mediates recombination events and may influence the formation of adaptive mutations. A RecN“ strain will be generated by standard molecular techniques and compared to a RecN‘ strain for its ability to accumulate mutations that affect amino acid biosynthesis. We speculate that *recN* does not affect stationary phase mutagenesis in *B. subtilis* and discussed other novel mechanisms mediating the generation of mutations in non-dividing cells.
The Role of recN in Stationary Phase Mutagenesis in Bacillus subtilis

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Abstract
Here mutagenic programs that are independent of growth were examined. Such aspects of the evolutionary process are novel and have been implicated in the formation of cancers in animal cells and the acquisition of antibiotic resistance in animal pathogens. Adaptive or stationary phase mutagenesis is a genetic program to increase diversity in cells under conditions of stress whereby cells escape non-dividing conditions. Previous research has shown that recombination functions are required to generate mutations that promote growth in E. coli cells. This project tests the hypothesis that recombination functions are required for the generation of mutations that promote growth in stationary phase Bacillus subtilis cells. In B. subtilis cells, recN, in addition to recA, mediates legitimate and illegitimate recombination events and may influence the formation of adaptive mutations. A recN strain was generated by standard molecular techniques and compared to a wild-type B. subtilis for its ability to accumulate mutations that affect amino acid biosynthesis. We report that recN affects stationary phase mutagenesis in B. subtilis and discussed other novel mechanisms mediating the generation of mutations in non-dividing cells.

Methods
- Genomic DNA was extracted from Bacillus subtilis strain B3281 (recN::cat).
- An isogenic recN knockout was prepared by transforming the parental strain 168SS with DNA from B3281.
- Chloramphenicol (300 μg/ml) cassettes within recN gene produced a fragment that is 2.2 kb larger than wild type recN and also rendered the strain resistant to chloramphenicol.
- Colonies were then isolated on TBA medium containing chloramphenicol (5 μg/ml).
- Knockout was verified by PCR and gel electrophoresis.
- Sample cultures of wild type and mutant were grown to stationary phase.
- The cultures were then plated on minimal media containing trace histidine, methionine, and leucine.
- Number of revertants were then scored daily for 5 days while the survival of background cells were monitored every other day.

Results

Conclusions/Future Directions
The preliminary data is indicative that recN plays a role in the mutagenesis in B. subtilis. However, the influence of this gene is dependent on the genetic event required for cells to escape non-dividing conditions in B. subtilis. Further analysis is required to elucidate how recN influences stationary phase mutagenesis.

These experiments are being repeated and a fluctuation test will also be conducted to determine if recN plays an active role in exponential growth.

Acknowledgments
Great gratitude and appreciation is extended to the entire Yasbin/Robleto lab for demonstrating patience, allowing me to gain research experience and permitting me to grow as a scientist. To Dr. Willis Derby, Mary Giac, Holly Martin, Caitlin Murphy, Marlan Schmidt, and Carmen Valin for their constant encouragement and support. This project was funded by the National Science Foundation.