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 *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 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 RecN strain 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 BG281 (recN:cat)*

*• An isogenic recN knockout was prepared by transforming the parental strain YB955 with the DNA from BG281.*

*• Chloramphenicol (cm⁺) 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 TBAB medium containing chloramphenicol (5μg/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 9 days while the survival of background cells were monitored every other day*

Conclusions/Future Directions

The preliminary data is indicative that recN is involved in the stationary phase mutagenesis in *B. subtilis*. However, the influence of this gene is dependent on the genetic event required for cells to escape non-growing 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. The following individuals provided constant encouragement and support: Mary Gizard, Holy Martin, Gordon Murphy, Maran Schmidt, and Carmen Valin. I also thank Dr. Willie Darby, Hampton University for his advice and encouragement. This project was funded by the U.S. National Science Foundation REU Program (NSF 0649267).

Figure 1 - Revertant Plating

Figure 2 - Survivals

(Left): Graphs show the accumulation of revertants on minimal media over a period of nine days. (Right): Graphs show viability of the non-revertant background.