DNA secondary structures and their contribution to mutagenesis in B. subtilis stationary phase cells.

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Abstract

It is widely known and accepted that the cause of many mutations in cells are generated during the replication process of actively dividing cells, however more recent research has shown that mutations also arise in non-growing conditions, a phenomenon known as stationary phase mutagenesis. Much of what is known comes from studies in eukaryotic and bacterial models. It has been proposed that in non-growing cells, the process of transcription plays an important role in mutagenesis. We test the hypothesis that DNA secondary structures, formed during transcription, promote mutagenesis. The transcription-generated structures are speculated to be prone to by blocking the RNA polymerase which has potential to trigger a gratuitous response from transcription coupled repair proteins like mfd. Genes up-regulated in response to stress with secondary structures can accumulate mutations due to this gratuitous repair. To test this hypothesis, I am using two Bacillus subtilis genes, argF and thiF, predicted by in silico, to form secondary structures. By altering the base sequence of these genes, the stability of their stem-loop structures are affected, thereby allowing us to test whether transcription of the altered sequence influences the accumulation of mutations in argF and thiF by impeding the RNA polymerase. Our assay for detecting mutations is based on phenotypic reversion back to prototrophy in cells under conditions of starvation. Ultimately, these experiments will increase our understanding of how mutations occur in cells of all domains of life.

Hypothesis

Transcription associated mutations in stationary phase are dependent on the formation of SLS. SLS stability, as measured by free energy of formation, influences the accumulation of mutations.

Methods

Find a marker gene that forms SLS in Bacillus subtilis

Construct alleles that differ in ability to form SLS using PCR

Results

• Defective thiF and argF alleles differing in their ability to form SLS have been constructed (see figure 3).

Future Plans

• Conduct a stationary phase assay and score mutant reversion to thiamine and arginine prototrophy.
• Conduct stationary phase assay in the presence of sub-inhibitory concentrations of gyrase inhibitors.
• Conduct stationary phase assay without transcription strand specific repair pathways such as knocking out mfd gene.
• Sequence analysis of Thi+ and Arg+ reversion to see if they map to stem loop.

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References