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 mutagenic 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 have used 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 in Bacillus subtilis FC40 system has been proposed. One pathway of bacterial differentiation in Bacillus subtilis stations phase irreversible growth arrest and stops DNA replication and division. This is accompanied by a shift of the metabolic program that results in increased levels of stationary phase specific transcripts. Our research project is aimed at elucidating the molecular mechanisms that generate stress-induced or adaptive mutations.

Background

• Stationary phase mutagenesis was first evidenced in the 1950s by Francis J. Ryan in a paper in Genetics: “In the meanwhile, the fact that mutagenesis can disappear in populations of bacteria whose numbers are not increasing must be accepted.”

• Later on, Cairns and coworkers revisited the concept of mutagenesis in conditions of carbon starvation and showed that cells under stress accumulated LecA mutations (1990). Research for the last 30 years has elucidated molecular mechanisms that generate stress-induced or adaptive mutations.

• Two pathways in the E. coli FC40 lac system has been proposed. One that generates point mutations and another that generates amplifications.

• Recent evidence in Bacillus subtilis suggests that aspects of transcription mediate the formation stationary phase mutations. Further, it has been speculated that secondary structures formed during the process of transcription have been found to contribute to mutations in E. coli. This concept has been extended to explain the high frequency mutability in certain cancer genes, such as p53 tumor suppressor gene.

• Stem loop structures (SLS) form as a result of transcription driven negative super-coiling (see figure 1). DNA residues located to single stranded regions within a SLS have been shown to be prone to mutagenic events.

• The likelihood of forming SLS is sequence-dependent and may be estimated by calculating Gibbs free energy value, which suggests that transcription-associated mutations occur at hotspots in the genome.

• Here, we report the construction of thiF alleles that differ in their ability to form SLS. These alleles will be used to test whether stationary phase mutations are dependent on transcription and take place at hot spots.

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

Construct alleles that differ in ability to form SLS using PCR

Figure 1

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 proteotrophy.

• 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.

Acknowledgements

A special thanks to everyone in the Robleto lab for sharing their knowledge with me. This project was supported by the following grants MCB0843606, and 2P20 RR016463 Nevada INBRE. I would also like to acknowledge Katherine Ona and Holly Martin for her involvement in the research project.

References


