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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 mutations by exposing regions of single-stranded DNA to lesions. We examined the *Bacillus subtilis* gene thiF, predicted by in silico analysis to be prone to mutations at particular locations during transcription. By altering the base sequence of this gene, the stability of its stem-loop structures is affected, thereby allowing us to test whether transcription of the altered sequence influences accumulation of mutations in thiF. Our assay for detection of mutations is based on reversion to thiamine 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.

**Background**

- Stationary phase mutagenesis was first evidenced in the 1950s by Francis J. Ryan in a paper in Genetics.
- Later on, Cairns and coworkers revisited the concept of mutagenesis in conditions of carbon starvation and showed that cells under stress accumulated Lac' mutations (1990).
- 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.
- Transcription and its transient changes in DNA topology have been linked to formation and stabilization of secondary structures such as hairpins and Z-DNA G4 structures in both the transcribed and non-transcribed strand. These structures are stabilized with increased transcription and estimated negative supercoiling.
- Recent work has shown how G4 DNA, when located in the non-transcribed strand, can block DNA repair and Mammalian RNNAPII. This arrest has the potential to initiate a "gratuitous" TCR response that can lead to mutagenesis.
- Work in *E. coli* has also evidenced a role for stem loop structures or hairpins in mutagenesis in association with transcription and replication.
- 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**

Find a marker gene that forms SLS in *Bacillus subtilis*

- Conduct a stationary phase assay and score mutant alleles differing in their ability to form SLS using PCR
- Knockout thiF gene eliminating stem loop sequence to prevent recombination.
- Conduct a stationary phase assay to score mutant alleles differing in their ability to form SLS.
- Conduct a stationary phase assay in the presence of subinhibitory concentrations of gyrase inhibitors.
- Conduct a stationary phase assay without transcription strand specific repair pathways such as knocking out mfd gene.
- Sequence analysis of Thi' reversions.

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**References**

- Ryan, Francis J. (1953). Spontaneous Mutations in Non-Dividing Bacteria. Genetics

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