2012

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

Carmen Vallin
University of Nevada, Las Vegas

Holly Anne Martin
University of Nevada Las Vegas

Christian Ross
University of Nevada, Las Vegas, rosscg@unlv.nevada.edu

Ronald Yasbin
University of Nevada, Las Vegas

Eduardo Robleto
University of Nevada, Las Vegas, eduardo.robleto@unlv.edu

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

Carmen Vallin,
Holly Martin, Christian Ross, Ronald E. Yasbin and Eduardo A. Robleto
School of Life Sciences, University of Nevada Las Vegas, Las Vegas, NV

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 come 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.

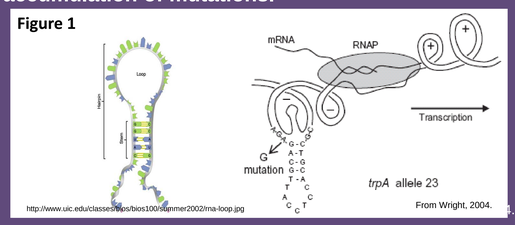
Background

- Stationary phase mutagenesis was first evidenced in the 1950s by Francis J. Ryan in a paper in Genetics: "*In the meanwhile, the fact that mutations can arise 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 Lac$^+$ 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 use 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.

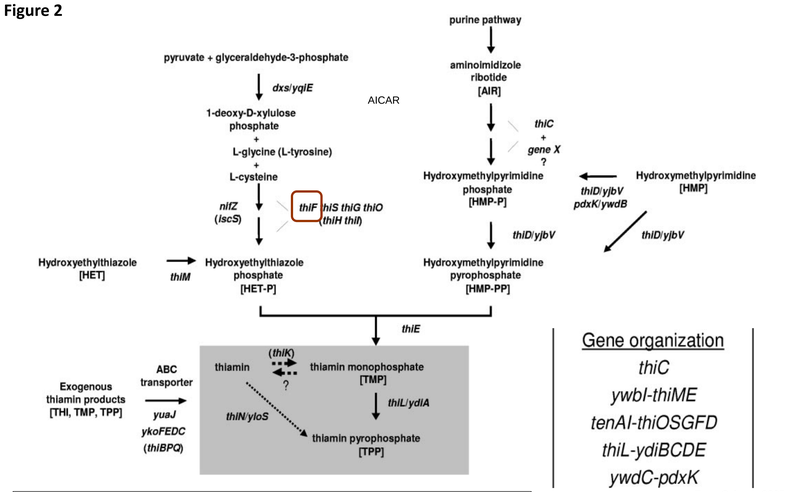
Figure 1



Methods

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

Figure 2

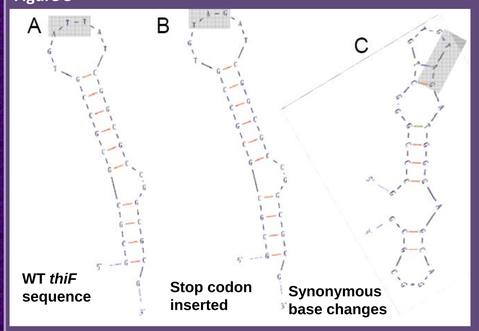


From Schyns, 2005

Once *thiF* was identified the next step is to knockout the gene to create an auxotroph.

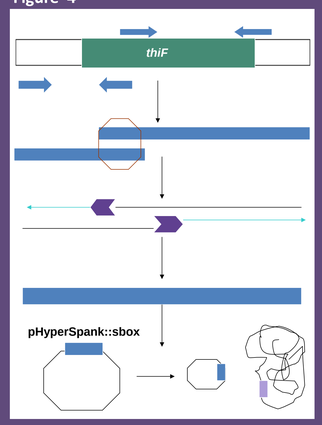
Construct alleles that differ in ability to form SLS using PCR

Figure 3



WT: Contains 30bp sequence that forms stable stem loop.
GCG-CGC-GCC-GTG-ATT ATC-GGC-GCC-GGC-GCG
Strain 1: STOP-
GCG-CGC-GCC-GTG-TAG ATC-GGC-GCC-GGC-GCG
Strain 2: STOP+UNSTABLE-
GCC CGG GC T GTG-TAG ATG GGA GCA GGC-GCG

Figure 4



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$^+$ reversions 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 2 P20 RR016463 Nevada INBRE. I would also like to acknowledge Katherine Ona and Holly Martin for her involvement in the research project.

References

Ryan, Francis J. (1955). Spontaneous Mutation in Non-Dividing Bacteria. *Genetics*.
Wright, Barbara E., et al. (2002). Hypermutable Bases in the p53 Cancer Gene Are at Vulnerable Positions in DNA Secondary Structures. *CANCER RESEARCH* 62, 5641-5644.
Wright, Barbara E., et at. (2007). Secondary Structures as predictors of mutation potential in the *lacZ* gene of *Escherichia coli*. *Microbiology*.
Schyns, Ghislain., et al. (2005). Isolation and Characterization of New Thiamine-Deregulated Mutants of *Bacillus subtilis. Journal of Bacteriology.*
Allen, Shara., et al. (2002) Metabolic Flux in Both Purine Mononucleotide and Histidine Biosynthetic Pathways Can Influence Synthesis of the Hydroxymethyl Pyrimidine Moiety of Thiamine in *Salmonella enterica*. *Journal of Bacteriology.*
Ross, Christian., et al. (2006). Novel Role of *mfd:* Effects on Stationary-Phase Mutagenesis in *Bacillus subtilis*. *Journal of Bacteriology.*
Wright, Barbara E. 2004. Stress-directed adadtive mutations and evolution. Molecular Microbiology: 52 (3); 643-650.
Sung, Huang Mo and Ronald E. Yasbin. 2002. Adaptive, or stationary-phase, mutagenesis, a component of bacterial differentiation in *Bacillus subtilis*. Journal of Bacteriology: 184; 5641–5653.
Pybus, C., et al. (2010). Transcription-associated mutation in *Bacillus subtilis* cells under stress. Journal of Bacteriology.
Cairns, J. et al (1988). The Origin of mutants. *Nature.*