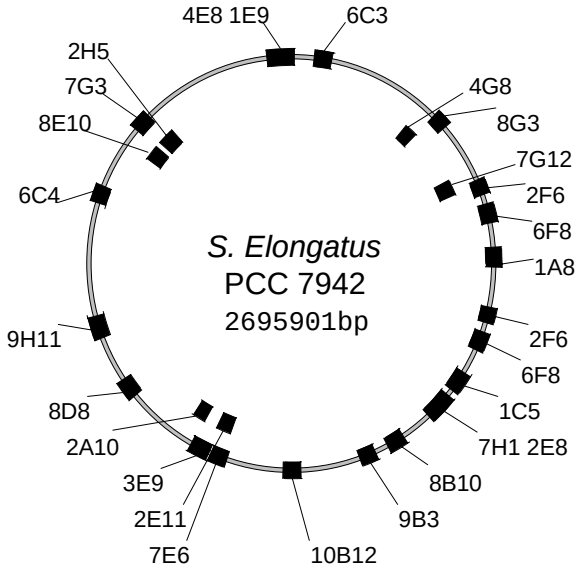
Progress Report on DE-FG03-00ER15055 (new grant DE-FG02-04ER15558)
PI: S.S. Golden
"Global functional analysis of the genome of *Synechococcus elongatus* PCC 7942"

1) Recent progress:

I am very happy to report exciting progress on this project. Some of the reviewers had expressed concern about the uncertainty of how long it would take to close the genome. The Joint Genome Institute has already closed the single circular chromosome; they are doing some 'polishing' sequences to finish the genome. With the closed contig in hand, we are able to localize our mutagenized cosmids and choose new cosmids for mutagenesis, based on their end sequences, to fill the gaps. The figure below shows the positions of cosmids for which we know the sequences at the sites of transposon insertions (Panel A). Each of them has been fully mutagenized at the density shown in Panel B. We have 18 more cosmids that have been mutagenized, and we are now getting sequence from the insertion sites. Because it is not necessary to generate the sequence of the entire cosmid from our insertions, we no longer have to work at high insertion density; in other words, we can make fewer transposon insertions—just sufficient to achieve inactivation of each gene—and require only minimal sequence in one direction from each insertion to determine its locus. We have purchased a freezer and racks to archive the clones from the JGI sequencing project, as well as our own clones, to make them available to the research community.

Several loci that affect circadian rhythms of gene expression, including some signal transduction proteins, have been identified by screening mutants in which the mutagenized cosmids have been recombined into the cyanobacterial genome. These are being retested to choose the most promising for additional genetic and biochemical analysis. Several Gateway technology vectors are under construction for the cyanobacterium that will allow facile construction of regulatable expression clones both for ORFs and for antisense RNAs.

A



B

Cosmid 4G8
31404 bp

A combination of sequence and functional genomics approaches revealed why the large plasmid of PCC 7942, pANL, is essential. It carries an addiction cassette that, when moved to the chromosome, allows the plasmid to be cured from the cell.

2) Unexpended funds: none expected.

3) Publications that acknowledge the DOE grant:

1. Thomas, C. C.R. Andersson, S.R. Canales, and S.S. Golden. 2004. PsfR, a factor that stimulates psbAI expression in the cyanobacterium *Synechococcus elongatus* PCC 7942. Microbiol. 150:1031-1040.

2. Golden, SS. 2003. Timekeeping in bacteria: the cyanobacterial circadian clock. Curr. Opinion Microbiol. 6: 535-540.

3. Golden, S.S., and S.R. Canales. 2003. Cyanobacterial circadian clocks - timing is everything. Nature Reviews Microbiol. 1:191-199.

Two papers focused on the genome project will be submitted prior to the start of the new grant. We held the global analysis for completion of the genome sequence, to allow us to provide the information shown in Panel A above. The final experiments to demonstrate curing of pANL have just been completed.