Ribosome Biogenesis Regulation by KsgA

The Use of Photocaged Proteins to Activate an Uncatalytic KsgA Mutant

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Research Proposal - Summer 2009
Bioinformatics and Bioengineering Summer Institute

I. Introduction

As bacteria that were once easily treated with common antibiotics begin to develop resistance to such treatments, new methods must be created in order for our society to continue to live with the same standard of health that we have grown accustomed to. One potential target for such a medication is the bacterial ribosome biogenesis factor universally conserved throughout evolution, KsgA. It is one enzyme of many involved in a highly regulated pathway that leads to a fully functional ribosome. The task initially attributed to the enzyme involves the dimethylation of two adjacent adenine nucleotides. However, it was recently discovered that KsgA may be involved in an essential checkpoint that can disrupt ribosome assembly and greatly affect the survival of bacteria[1].

KsgA is responsible for the dimethylation of the two adjacent A1518 and A1519 adenosines in 16S and 18s rRNA[3]. While there are many methylations within the ribosomal RNA (rRNA) of all organisms, the specific nucleotides that are methylated vary between kingdoms and species. The two aforementioned adenosines and the entire structure of the region in which they are found however, is highly conserved across prokaryotes and eukaryotes[5]. This remarkable evolutionary conservation hints at a more important role of KsgA within ribosome biogenesis. Though the specific enzyme varies across the different domains of life, the function is universally conserved and in both bacterial KsgA and the yeast orthologue Dim1p, the methyl groups are donated from S-adenosyl-L-methionine (SAM)[3,8].

In a novel experiment utilizing E. coli, the bacteria were transformed with a mutant KsgA enzyme that bound to its normal binding site on the pre-30s substrate but did not methylate the two adenosines as wild-type KsgA would[1,3]. A polysome analysis was employed in an attempt to identify how the accumulation of the different ribosomal subunits varied with differing activity of KsgA. In a normal E. coli cell, a polysome analysis generally yields a result that displays relatively low levels of the large

ribosomal subunit (LSU) and the small ribosomal subunit (SSU) and a high level of fully assembled ribosomes[1]. When using the mutant KsgA, the methylations of adenosine A1518 and A1519 were not performed, and there was an accumulation of LSUs and SSUs and a much lower level of fully assembled ribosomes. However, when KsgA was completely removed from the cells, the levels of LSUs and SSUs dropped and the level of assembled ribosomes increased until all three were nearly equal, indicating KsgA's role as an inhibitory checkpoint within ribosome biogenesis.

An open question that remains is whether or not the pre-30S particles that accumulated in the E.coli strain containing the inactive KsgA background were stalled assembly intermediates or dead end products. A method to resolve this question is to 'turn KsgA on' after the inactive KsgA/pre-30S particle has formed. To do this we will exploit an essential active site tyrosine by replacing it in vivo with a modified, photocaged tyrosine residue that can be restored to a wild-type tyrosine by shinning 365 nm light on growing cells. It is anticipated that the photocaged KsgA will be catalytically inactive, but can be fully activated. Depending on the degree of recovery that is seen in the activity of KsgA, it will explain whether or not the pre-30s subunits that accumulate can be recovered in vivo, or whether they are dead end products.

II. Materials & Methods

I: Plasmids

Initially two different plasmids, pET15b and pBAD, containing the KsgA gene will be used, the first to generate large quantities of recombinant protein for in vitro enzymatic assays and the second will be used in in vivo experiments. An additional plasmid containing a tRNA that suppresses the UAG stop codon and a complementary tRNA synthatase that recognizes nitrophenyl-tyrosine, the light activated analog of tyrosine, will be used in all experiments.

II: Mutagenesis

Via site-directed mutagenesis the UAG stop codon will be placed within the KsgA gene in both the pET15b and pBAD plasmids using standard techniques.

III: Photocaged Protein

Recombinant photocaged KsgA engineered with a poly-His tag at the N-terminal end will be made by expressing the above mutant gene from the pET15b-KsgA plasmid

in BL21 protein expression cells. Protein will be purified by affinity chromatography using Ni-NTA solid support. Inactivated and photoactivated KsgA will be assayed for methyltransferase activity using published assay conditions.

E. coli harbouring the pBAD-KsgA plasmid and helper plasmid will be tested for growth rate and pre-30S buildup before light activation and after light activation. Expression levels of mutant KsgA will be controlled by arabinose as previously described.

III. Projected Results

Through the use of a light sensitive photocaged protein which will activate an uncatalytic mutant of the Escherichia Coli KsgA enzyme, it will be determined whether the activity of the enzyme can be recovered. Initially, when the mutant KsgA is uncatalytic, it is expected that there will be an accumulation of the pre-30s subunits as KsgA will not release from the subunit until its methylation function is complete, effectively inhibiting the biogenesis pathway. This increase in 30s subunits will be confirmed though polysome analysis.

At this point in the experiment, it is expected that few functional 70s ribosomes will exist and translation levels within the cells should be depressed which will also be determined though a polysome analysis. After the application of a 365 nm light source, KsgA should no longer be uncatalytic and should soon after begin the methylation of the two adjacent adenosines within the 17s rRNA and unbind from the rRNA, allowing the biogenesis pathway to continue. This will be observed through an expected depletion in the levels of pre-30s subunits within a polysome analysis and an increase in fully assembled 70s ribosomes as seen with wild-type KsgA. There is, however, a chance that upon application of the light, the pre-30s subunits do not proceed any further along the biogenesis pathway, indicating that it may be a dead end product rather than a stalled intermediate that can be recovered simply by the addition of catalytically active KsgA.

IV. References

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