**“Characterization of tRNA Modification Patterns in a Recoded Organism”** ALEX LEATH

BNFO 300

**INTRODUCTION**

Biocontainment is a central issue in the development of industry standards and internationally accepted methods for synthetic biology pertaining to solving problems associated with the current generation of genetically modified organisms. Orthogonal systems can contribute to this end by helping to make synthetic biology safer for agricultural applications 1,2,3. Transfer ribonucleic acid (tRNA) genes and their tRNA products will be invaluable in achieving this for several reasons. One reason is that tRNA genes are inherently susceptible to horizontal gene transfer 4. Additionally, the central role of tRNA in translation, its involvement in cell stress responses, and codon recognition properties makes understanding tRNA modification patterns an inherently valuable regulatory target when designing biocontainment systems for synthetic microbes that form endosymbiotic relationships within plants 5.

The natural evolution of organelles resulted in the loss of tRNA genes and codon reassignment events 6. The evolutionary outcomes involving the translation machinery of endosymbionts has similarities to whole genome recoding methods currently under investigation as biocontainment tools for containing synthetic endosymbionts 7. When paired with the directed evolution of tRNA modifications, it may soon become possible to more effectively design biocontainment systems for the introduction of synthetic organelles capable of enhancing crop yields. This might be achieved by directing the evolution of tRNA genes and tRNA modifications that confer phenotypes for heat resistance and drought tolerance in agriculturally important crops such as wheat, maize, and rice 8.

A biocontainment strategy based on orthogonality serves as the starting point for the research proposed in the experimental section of this paper. The redundancy of the genetic code is one of the properties inherent to life that has been taken advantage of in developing orthogonal biological systems. A great variety of current research in orthogonal biology involves systems with recoded genomes. The inherent genetic isolation of a recoded genome allows a means of preventing the transfer of genetic information between engineered organisms and natural biological systems in situations where the engineered organisms are to be released into the environment or incorporated into plants that can cross pollinate with adjacent grasses 9.

Introduction of orthogonal translation components that can isolate the organism is possible to achieve with recoded genomes when all occurrences of a specific codon within the genome has been reassigned to a synonymous codon. A foreign tRNA and an associated aminoacyl tRNA synthase can then be introduced into such a recoded organism to encode a synthetic amino acid for which the organism is an auxotroph. Nucleotide modifications on tRNA, as well as changes that occur to tRNA nucleotides during cellular stress events, can help develop new tRNA based tools that act as regulatory targets in a recoded organism 10.

This proposal will address a basic question about the molecular biology of tRNA nucleotide modifications: Does the removal of a specific codon from the entirety of a bacterial genome result in correlated changes to tRNA modifications for a foreign tRNA. The experiment described below intends to answer this question about tRNA modifications by characterizing tRNA modification patterns using Liquid Chromatography-Mass Spectrometry 11.

**EXPERIMENT**

This experiment will investigate a recoded strain of E. Coli that is currently being assembled at the Department of Genetics, Harvard Medical School, Boston, MA. When complete it will be known as “rE.coli-57”. The parent strain of this recoded E. coli will serve as the control organism. It is known as E. coli MDS42 12.

The possible target tRNAs are those associated with the codons that have been replaced in rE.coli-57: AGG and AGA (Arg), AGC and AGU (Ser), UUG and UUA (Leu) 12. For the purposes of this proposal, the tRNA for the UUA codon of Leucine has been selected for investigation.

A foreign UUA Leucine tRNA gene from Arabidopsis thaliana will be introduced into the E. coli genomes. The E. coli cultures will be grown in identical conditions. The Arabidopsis tRNA will be isolated from the E. coli cultures by a pull-down assay using Dynabeads (Invitrogen) to isolate the specific tRNAs to be characterized. LC-MS methods of Chan et al will be used to characterize the tRNA modifications. The observed results will then be compared between rE.coli-57, E. coli MDS42, and available that data for tRNA modifications in Arabidopsis 13.

**Discussion**

The expected results from the experiment are that the foreign tRNA from Arabidopsis will have different nucleotide modifications in the recoded E. coli compared to the control organism. The lack of specific tRNA modification enzymes in the E. coli genome from the native Arabidopsis would account for this result. Follow up experiments might involve testing additional tRNAs beyond the UUA Leucine tRNA. Additional studies could as well insert tRNA modification proteins from Arabidopsis to see if the tRNA modifications that occur in Arabidopsis can be achieved in E. coli by inserting Arabidopsis tRNA modification genes.

Bibliography

1. Schmidt, M., & De Lorenzo, V. (2012). Synthetic constructs in/for the environment: Managing the interplay between natural and engineered Biology. *FEBS Letters*, *586*(15), 2199–2206. <https://doi.org/10.1016/j.febslet.2012.02.022>
2. Moe-Behrens, G. H. G., Davis, R., & Haynes, K. A. (2013). Preparing synthetic biology for the world. *Frontiers in Microbiology*, *4*(JAN), 1–10. https://doi.org/10.3389/fmicb.2013.00005
3. Wright, O., Stan, G. B., & Ellis, T. (2013). Building-in biosafety for synthetic biology. *Microbiology (United Kingdom)*, *159*(PART7), 1221–1235. https://doi.org/10.1099/mic.0.066308-0
4. Novoa, E. M., Pavon-Eternod, M., Pan, T., & Ribas De Pouplana, L. (2012). A role for tRNA modifications in genome structure and codon usage. *Cell*, *149*(1), 202–213. https://doi.org/10.1016/j.cell.2012.01.050
5. Lei, L., Shi, J., Chen, J., Zhang, M., Sun, S., Xie, S., … Lai, J. (2015). Ribosome profiling reveals dynamic translational landscape in maize seedlings under drought stress. *Plant Journal*, *84*(6), 1206–1208. https://doi.org/10.1111/tpj.13073
6. Salinas, T., Duby, F., Larosa, V., Coosemans, N., Bonnefoy, N., Motte, P., … Remacle, C. (2012). Co-Evolution of Mitochondrial tRNA Import and Codon Usage Determines Translational Efficiency in the Green Alga Chlamydomonas. *PLoS Genetics*, *8*(9), 1–11. https://doi.org/10.1371/journal.pgen.1002946
7. Ling, J., O’Donoghue, P., & Söll, D. (2015). Genetic code flexibility in microorganisms: novel mechanisms and impact on physiology. *Nature Reviews. Microbiology*, *13*(11), 707–21. <https://doi.org/10.1038/nrmicro3568>
8. Lei, L., Shi, J., Chen, J., Zhang, M., Sun, S., Xie, S., … Lai, J. (2015). Ribosome profiling reveals dynamic translational landscape in maize seedlings under drought stress. *Plant Journal*, *84*(6), 1206–1208. https://doi.org/10.1111/tpj.13073
9. Rovner, A. J., Haimovich, A. D., Katz, S. R., Li, Z., Grome, M. W., Gassaway, B. M., … Isaacs, F. J. (2015). Recoded organisms engineered to depend on synthetic amino acids. *Nature*, *518*(7537), 89–93. https://doi.org/10.1038/nature14095
10. Lyko, F., & Tuorto, F. (2016). Genome recoding by tRNA modifications. <https://doi.org/10.1098/rsob.160287>
11. Chan, C. T. Y., Deng, W., Li, F., Demott, M. S., Babu, I. R., Begley, T. J., & Dedon, P. C. (2015). Highly Predictive Reprogramming of tRNA Modifications Is Linked to Selective Expression of Codon-Biased Genes. *Chemical Research in Toxicology*, *28*(5), 978–988. https://doi.org/10.1021/acs.chemrestox.5b00004
12. Ostrov, N., Landon, M., Guell, M., Kuznetsov, G., Teramoto, J., Cervantes, N., … Church, G. M. (2016). Design, synthesis, and testing toward a 57-codon genome. *Science*, *353*(6301), 819–822. <https://doi.org/10.1126/science.aaf3639>
13. Chen, P., Jäger, G., & Zheng, B. (2010). Transfer RNA modifications and genes for modifying enzymes in Arabidopsis thaliana. *BMC Plant Biology*, *10*, 201. https://doi.org/10.1186/1471-2229-10-201