Dual Genetic Selection of Synthetic Riboswitches for TMAO as Genetic and Analytical Tools

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Abstract

The goal of this project is to develop synthetic riboswitches for trimethylamine N-oxide (TMAO).

A riboswitch is a non-coding RNA molecules that specifically binds to a ligand and thereby controls the expression of genes downstream. A TMAO synthetic riboswitch could be useful for regulating gene expression in response to TMAO and detecting TMAO in complex biological samples such as urine and blood. TMAO has been shown to regulate various physiological processes involved in the development of atherosclerosis.

The 17 nucleotides in the aptamer domain of a naturally occurring glycine riboswitch were randomized to generate a library containing billions of different variants. The library was placed upstream of the *cat-upp* fusion gene for a series of dual genetic selections.

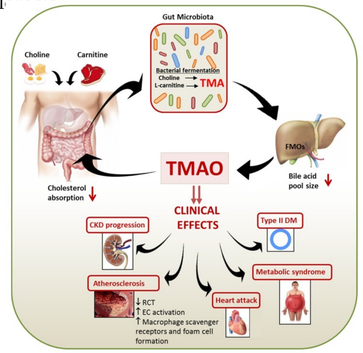
The positive selection is done in the presence of TMAO to identify functional riboswitches that make cells resistant to chloramphenicol. The negative selection is performed in the presence of 5-fluorouracil to kill the cells containing the riboswitch variants activated by any endogenous molecules.

Once identified by several rounds of dual genetic selections, the synthetic TMAO riboswitches will be tested by colorimetric and fluorescence assays.

Background

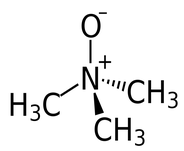
Nutrients such as phosphatidylcholine (also known as lecithin), choline, and L-carnitine are abundant in many of the animal-derived products that we often consume, such as red meat, egg yolk, and full-fat dairy products.

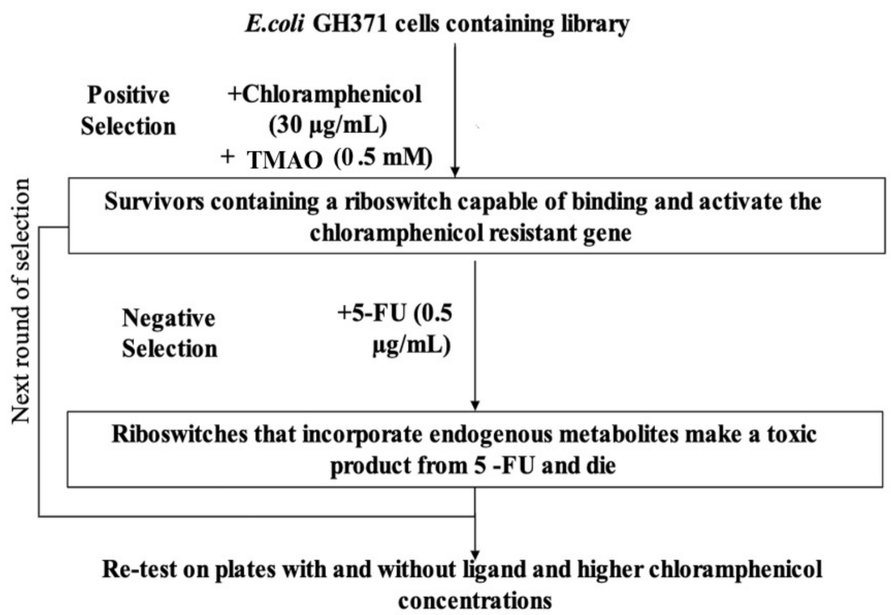
When consumed, these nutrients are processed by gut bacteria, resulting in the release of various metabolites, including TMA (trimethylamine,) into the blood, then transported to the liver, where it is converted into TMAO, which has been shown to regulate various physiological processes involved in the development of atherosclerosis.



Results

GH371 E. coli cells are currently subjected to several rounds of the positive selection on chloramphenicol in the presence of TMAO and the negative selection on 5-fluorouracil in the absence of TMAO.





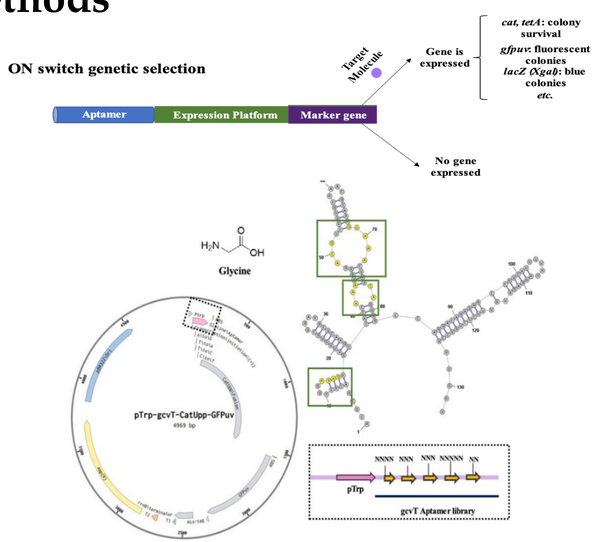
Methods

Synthetic riboswitches can be selected by the activation of a selectable gene upon binding to the target ligand in living cells.

When a library of aptamers is introduced at the upstream of selection marker genes, functional riboswitch variants can activate the genes in response of the target molecule.

Seventeen positions were randomized at the glycine binding site (gcvT) using polymerase chain reaction (PCR) to produce a gcvT aptamer library.

The library was inserted into the pTrp-gcvT-CatUpp plasmid.



Conclusions & Future Goals

- Once identified by several rounds of dual genetic selections, the synthetic TMAO riboswitches will be tested by colorimetric and fluorescence assays using the lacZ and gfp genes as the downstream genes, respectively.
- Riboswitches are highly adaptable molecules because of the specific interactions and spatial arrangement upon binding to the molecule of interest. Their gene regulation mechanisms could be used for biosensing, gene expression reprogramming, imaging, and gene therapy.

References

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