Studying the Effectiveness of RNA Riboswitches

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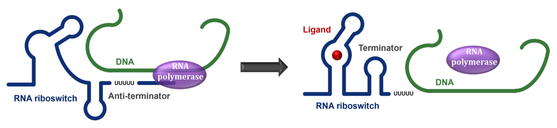
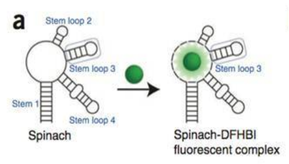
Introduction

The central dogma of biology says that DNA is transcribed into RNA and then RNA is translated into proteins. RNA's role was thought to be limited to bridging the gap between DNA and proteins. However, RNA has recently been found to be extremely functional, with different roles in the cell. One of these roles is the regulation of gene expression through noncoding segments of RNA known as riboswitches. Riboswitches serve as a genetic switch, turning gene expression on or off, depending on the presence of signaling molecules (ligands) in the cell. A riboswitch has two regions: the aptamer and the expression platform. The aptamer serves as a receptor for a specific ligand. The expression platform forms one of two mutually exclusive structures, one in the presence, the other in the absence of the ligand. The terminator structure prohibits gene expression by causing the RNA polymerase to detach prematurely. The anti-terminator structure prevents the terminator structure from forming, which allows RNA polymerase to continue transcription resulting in the gene being expressed[1].



Source: iGEM



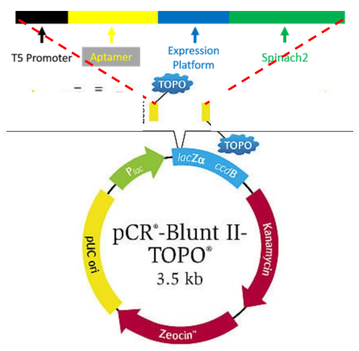
Source: MetaRNA

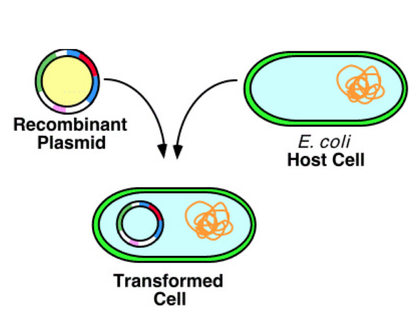
The riboswitch we're studying is the preQ$_1$ riboswitch. PreQ$_1$ is naturally found in many bacteria and is involved in some metabolic processes[1]. The gene under regulation will be the Spinach2 aptamer. The Spinach2 mRNA aptamer fluoresces in the presence of the reporting molecule DFHBI and variants of the molecule. DFBHI-1T is being used as it is reported to have a greater amount of fluorescence[3].

Experimental Methods

In order to test and quantify the effectiveness of the preQ$_1$ riboswitch *in vivo*, the riboswitch and the reporting aptamer DNA sequence must inserted into bacteria.

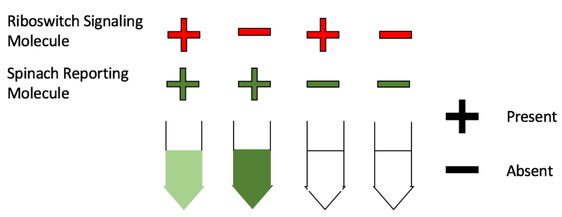
- PCR performed on preQ$_1$ riboswitch and Spinach2 DNA sequence to obtain a double stranded DNA and amplify DNA.
- A gel extraction performed to purify the PCR product
- DNA was inserted into a bacterial vector.
- *E. coli* transformed and these cells were grown on an agar plate.



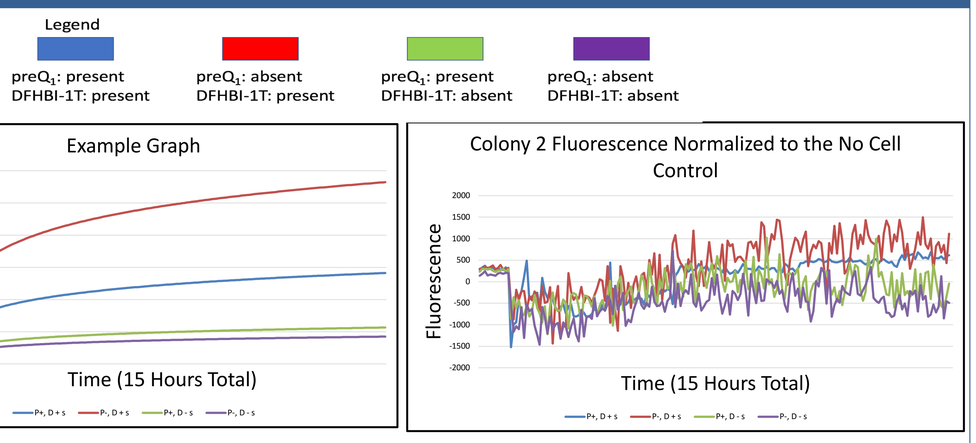


Source: Suresh Kaushik, Transformation of competent E.coli

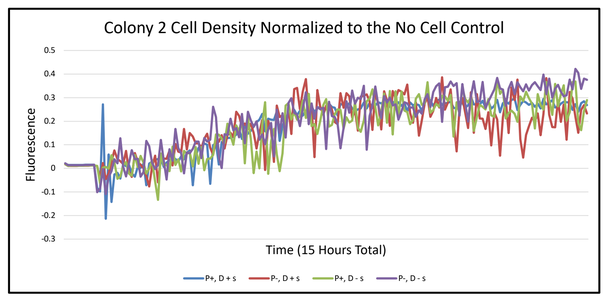
- Miniprep kit used on 4 colonies, purified plasmids sequenced to confirm that the DNA template was inserted correctly
- Fluorescence measurements and cell densities for cultures of the 4 colonies collected using the TECAN, a machine that can measure light emitted at specific wavelengths and can record the density of cells in a sample.
- Transformed bacteria placed in 96-well plate in the TECAN with varying treatments of preQ$_1$ and DFBHI-1T. Fluorescence was measured.
- Treatments:
  - PreQ1(1mM), DFHBI-1T(25μM)
  - No preQ1, DFHBI-1T(25μM)
  - PreQ1(1mM), No DFHBI-1T
  - No preQ1, No DFHBI-1T
- Measurements were taken every 5 minutes for 15 hours.



Results



- Left: predicted results for the different treatments over 15 hours
  - greatest fluorescence expected in condition where preQ$_1$ is absent due to no regulation on the Spinach2 aptamer
  - Minimal fluorescence expected when DFHBI-1T is absent
  - Right: relative fluorescence emitted by the DFHBI-1T and the Spinach2 complex under different treatments of DFBHI-1T and preQ$_1$ over 15 hours
    - The general trend shows greater fluorescence in treatment with no preQ$_1$ but with DFHBI-1T present which was what was expected.



- Graph shows cell density measurements for the different treatments normalized to cell density of the no cell condition over 15 hours. Growth rate can be inferred from the cell density values . The graph shows a similar density increase, and therefore growth rate, of cells in all conditions.

Conclusion

These results are very promising. The data showed fluorescence trends that were similar to what was expected for the different treatments. Modifying the assay can help establish a more solid measurement for the wild-type and create a more effective test for riboswitch variants. Some modifications include: testing a sequence with just the Spinach2 aptamer, and another sequence that doesn't code for any genes. This would allow for measurements of maximum fluorescence of the Spinach2 aptamer since it would not be regulated under a riboswitch. It would also allow for measurements of background fluorescence of cells without any genes under riboswitch regulation or Spinach2.

Future Applications

In the future, this assay will be used to test variants of the wild-type riboswitch. These variants may have small mutations in their sequence that can make them more effective at regulating gene control. The fluorescence in conditions where preQ$_1$ and the reporting molecule are present can be used to determine how effective the riboswitch variant is at controlling gene expression in comparison to the control. This information can help researchers optimize systems and expand the application of riboswitches. The aptamer and gene of interest can be switched to alter what triggers regulation of gene expression and the function of bacteria[3]. This may aid in engineering solutions to problems such as acting as biosensors[4] and cleaning up oil spills.

Source: Inhabitat

Source: pixnio

References

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2. Wenjiao Song, Rita L. Strack, Nina Svensen, and Samie R Jaffrey. *Plug-and-Play Fluorophores Extend the Special Properties of Spinach. (2014)*
3. Shana Topp and Justin P. Gallivan. *Emerging Applications of Riboswitches in Chemical Biology.* (2010)
4. Shana Topp and Justin P. Gallivan. *Emerging Applications of Riboswitches – From Antibacterial Targets to Molecular Tools.* (201

Funding : Simons Foundation