Quantifying Transcription-Translation Coupling in *Escherichia coli*

Elizabeth | Joseph T. Wade

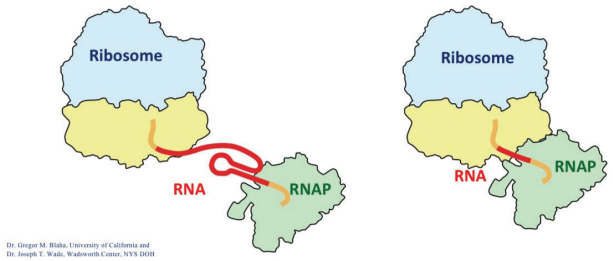
NEW YORK STATE Department of Health | Wadsworth Center

Abstract

Transcription-translation coupling is a phenomenon in bacteria in which an mRNA strand is translated as it is being transcribed. It is still largely unknown how the ribosome and RNA polymerase (RNAP) interact in this process. This work was done to develop a construct to insert two cassettes into the LacZ gene in the E.coli genome in order to better understand transcription-translation coupling. Each cassette contains ends homologous to the LacZ insertion site, either a hairpin or no hairpin sequence, nLuc, and thyA. Because one of the types of transcription termination is hairpin formation, this was used to infer how closely coupled the ribosome can be to the RNA polymerase. PCR was used to generate DNA fragments containing the cassette, which were transformed into E.Coli cells with a thyA deletion. Luciferase production was measured which indicated how closely the ribosome and RNA polymerase were coupled. It was found that the hairpin had less of an effect in the open reading frame of LacZ, while at the end the hairpin had a greater effect. The ribosome was closest to the RNA polymerase at insertion site 4.

Introduction

- **Question: How close can the ribosome get to the RNAP during transcription?**
- Transcription and translation occur simultaneously in prokaryotes.
- Transcription termination can occur by hairpin formation. This occurs after five thymines in the DNA is transcribed. The RNA base pairs to itself forming the hairpin, which causes the RNAP to fall off.
- Coupling: The ribosome and RNAP are either physically linked or working at the same rate. This prevents hairpin formation.
- This phenomenon was used to infer how close the ribosome can get to the RNAP.
- This was tested by gene insertion in LacZ in the E.Coli genome and a luciferase assay.
- Control: No hairpin sequence; RNAP transcribes nLuc.
- Experimental: Hairpin sequence; RNAP does not transcribe nLuc unless the RNAP and ribosome are coupled.



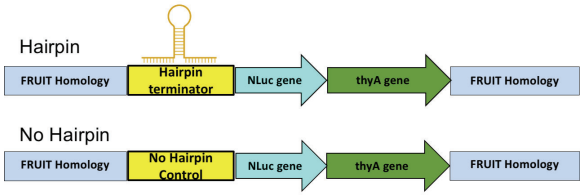
Dr. Gregor M. Blaha, University of California and Dr. Joseph T. Wade, Wadsworth Center, NYS DOH

Works Cited

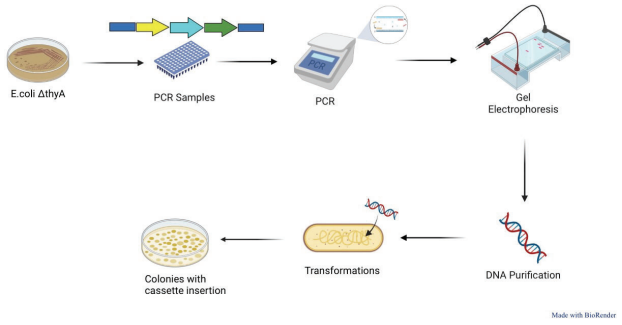
Stringer, A. M., Singh, N., Yermakova, A., Petrone, B. L., Amarasinghe, J. J., Reyes-Diaz, L., Mantis, N. J., &; Wade, J. T. (2012, September 27). Fruit, a scar-free system for targeted chromosomal mutagenesis, epitope tagging, and promoter replacement in escherichia coli and salmonella enterica. PI oS one. Retrieved August 10, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3459970/

Construct

- Selectable marker: ThyA
- Homology ends: Regions of DNA homologous to the insertion site in the E.Coli genome.
- nLuc: Luciferase gene; produces light.
- Experimental: Includes hairpin sequence.
- Control: No hairpin sequence.

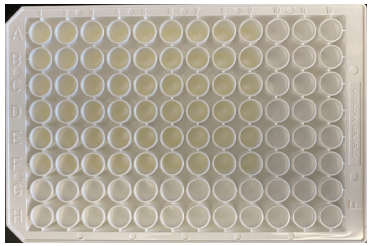


Procedure



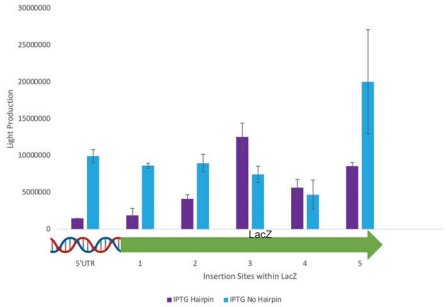
Assay

- 50:1 nano-glow substrate and Lucifrase assay buffer
- 10uL sample + 10uL reagent in each well



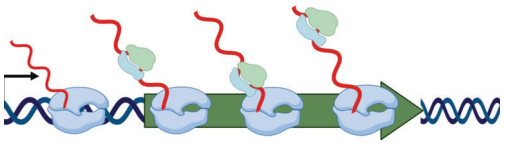
Results

- Each set of bars has different light production proportions depending on the cassette insertion site.



Interpretation of Results & Conclusions

- Upstream and at the beginning of LacZ, there is little light production in the hairpin compared to the no hairpin.
  - 5'UTR: No ribosomes can prevent hairpin formation.
- The difference in light production between 3 and 4 was less.
  - The hairpin had less of an effect towards the middle of LacZ.
- At 5, there is an increase in the no hairpin light production.
  - The ribosome seems to be farthest away at the end of LacZ.
- This distance could be due to the ribosome being stuck or the RNAP pausing less frequently.



Future Directions

- Create a mutant that causes the ribosome to stall at a codon.
  - EFP deletions

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Wadsworth Center Core Facilities