Identifying Peptide Binders Through mRNA Display

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Introduction

The Roberts' lab is developing peptide binders to specific proteins through the use of mRNA display, a technique to identify proteins and peptides that specifically bind target proteins through multiple rounds of *in vitro* selection. These peptides are useful for serving as diagnostic and therapeutic purposes for many different diseases, such as cancers and autoimmune diseases.

The research we conducted with Dr. Kaori Noridomi intends to use mRNA display *in vitro* selection to identify peptides that are non-specifically binding beads. Non-specific binders can be enriched during selection, thus reducing the process' efficiency. With four rounds of selection previously performed, we performed another round to identify non-specific binders through sequencing. Those identified peptides can be used as a blocking reagent in future selection.

Objective

The research conducted in the Roberts' lab aims to tackle the issues of affinity, specificity, structure, dynamics, and biological activity. mRNA display serves many crucial roles as one of the few functional approaches for i*n vitro* selection to isolate peptides and proteins with desired biochemical properties.

Protein Identification (i.e. cancer)

mRNA display is able to identify peptides that bind to specific proteins expressed on cancer cells, therefore, providing an effective method for potential diagnostics and treatments.

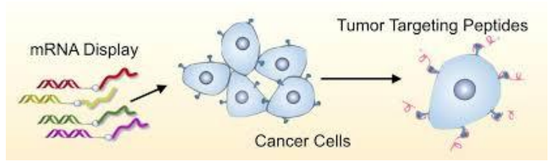

***Fig. 1**: mRNA display interacts with target proteins (PC: Liu et. al., 2017).*

Protein-Protein Interaction Inhibitor

Selected peptides are also used as a protein–protein interaction inhibitor. In autoimmune diseases, antibodies recognize proteins in our bodies as foreign, thus attacking them. mRNA display can find peptides that are able to block antibodies from attacking our bodies.

***Fig. 2**: Antibodies attacking proteins (PC: Ferrigno & McLeod, 2016)*

Methodology

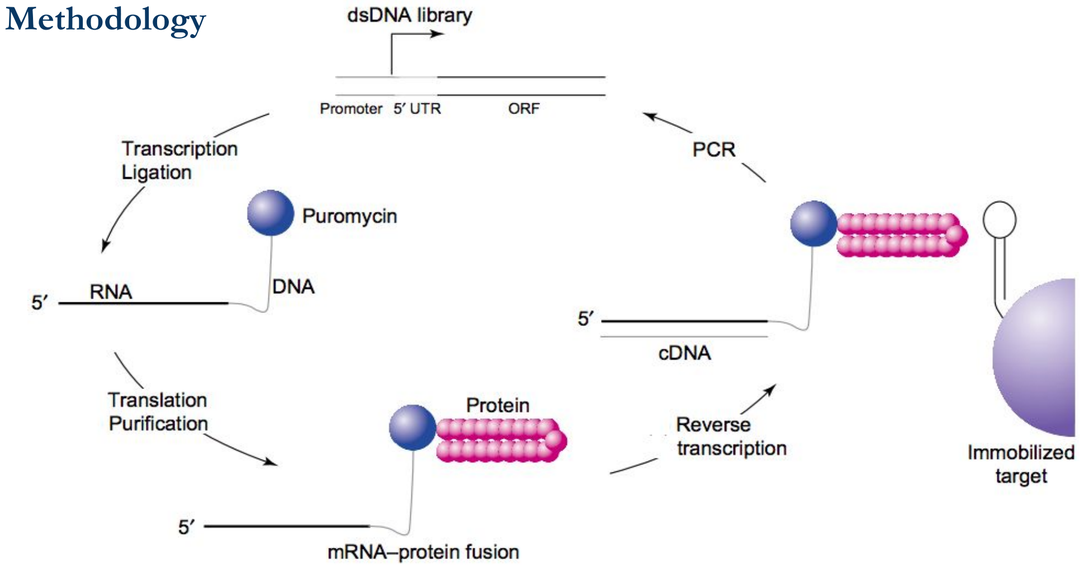

***Fig. 3**: A typical mRNA display selection cycle. (PC: Takahashi et. al., 2003)*

Polymerase Chain Reaction (PCR)

amplifies DNA through the steps of denaturation, annealing, and extension

Agarose Gel Electrophoresis

technique used to visualize DNA, often to check the results of PCR

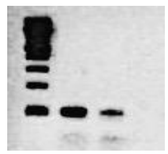

***Fig. 4**: PCR sample (PC: Kaori Noridomi)*

Transcription

converts the DNA library to an mRNA library

Ligation

the mRNA library ligates to a short piece of DNA

Urea Gel Purification

method used to purify DNA or RNA fragments

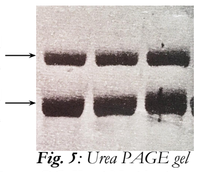

***Fig. 5**: Urea PAGE gel (PC: Kaori Noridomi)*

Translation/Fusion Formation

decodes mRNA to build a peptide containing a specific series of amino acids

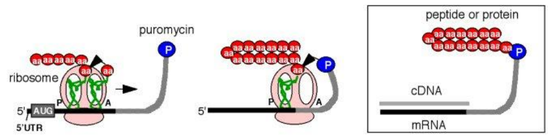

***Fig. 6**: mRNA-peptide fusion enabling polypeptide design (PC: Roberts et. al., 1997).*

dT Purification

purifies sample by selecting the fusions that have a high affinity for the target

Reverse Transcription

generates complementary DNA from an RNA template for PCR in the next round

Selection

the peptides that interact with the target protein immobilized on the beads are isolated

Next Round of Selection/PCR

the next round of selection begins from the start of the mRNA display cycle, and PCR is used to generate a new DNA library

Gel Extraction

technique used to isolate a desired fragment of DNA from an agarose gel

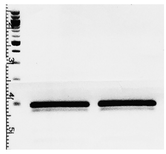

***Fig. 7**: Gel extraction (PC: Kaori Noridomi)*

TOPO Cloning

DNA fragments are cloned into specific vectors without the use of restriction enzymes

Sequence Analysis

DNA sequence is analyzed to determine the sequence of the binder peptides

Relevance to Coursework

While the work I have completed in the lab is far more different than the work I have done in school, I have noticed quite a few relations. For starters, I was surprised by how much of the material I learned in my chemistry and biology classes applied to the techniques and understanding for why we are completing each step. I expected the specific processes and concepts that were taught in these classes to be useless in the real world, but I was amazed at how my knowledge for such topics were the building blocks for understanding the bigger picture and real-world applications. The knowledge and skills I gained through working with Dr. Noridomi will definitely stick with me as I continue my scientific research journey and will be especially helpful as I begin my endeavor for my Research II course I am taking next year.

Advice

The SHINE program truly offers a one of a kind experience, and I believe that trying to embrace every moment of your time here will not fail to leave you with amazing knowledge and memories. Although the work is undoubtedly challenging, all moments of difficulty offer an opportunity to grow, and I recommend trying to view hardships in a positive light.

- Be independent, proactive, and responsible
- Have a good work ethic (be on time, be responsible, have good communication)
- Ask yourself *why* you are doing each step
- Think ahead and be ready
- Admit your mistakes instead of hiding or justifying them
- Do not be afraid to make mistakes (mistakes are great!)
- Respect the lab hierarchy

PC: Kaori Noridomi

PC: Kaori Noridomi

Acknowledgments

I would like to acknowledge Professor Richard Roberts for opening up his lab as a source of education. I would also like to thank Dr. Katie Mills and Dr. Megan Herrold for their notable dedication to organizing the SHINE program and offering endless guidance. Most importantly, I would like to extend my sincere gratitude to Dr. Kaori Noridomi and Alexandra Fidanovski for their countless tolerance, consideration, and support during the conduction of our research.

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