Identifying the RNA Binding Sites on the MeCP2 Protein by Regional Deletion

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The following is an excerpt from a longer piece. For the full text, please visit https://scholar.colorado.edu/concern/undergraduate_honors_theses/mw22v689v or scan the QR code.

Abstract

Long non-coding RNAs (LncRNAs) have been found to bind to the Methyl-CpG binding protein (MeCP2), which has been speculated to play a role in altering chromatin structure and regulating gene expression. Whether MeCP2 has other important functions related to RNA processing, the nature of the LncRNA-MeCP2 interaction and function, and how this may affect brain development is unknown. The goal of my project is to provide insight into important regions or domains of the MeCP2 protein involved in RNA binding. With this new information, a foundation for further in-depth research will be established, on the involvement of the MeCP2 protein in the pathogenesis of Rett Syndrome, through RNA binding and RNA regulation.

Redesigning Peppers RNA Imaging System: An Investigation of In Vivo and In Vitro Performance

Caitlyn Mendik

The following is an excerpt from a longer piece. For the full text, please visit https://scholar.colorado.edu/concern/undergraduate_honors_theses/ms35tb04h or scan the QR code.

Abstract

RNA is a central component of biochemistry as it carries genetic information, has enzymatic activity, mediates protein biodiversity, and supports other cellular functions. Scientists are continually developing new tools for studying RNA and applying it to fields of research such as medicine and molecular biology. RNA imaging provides one avenue for characterizing RNA by looking at trafficking, localization, and lifetime in living cells. Imaging tools have been on the rise in the past 20 years, providing innovative methods for looking at this

nucleic acid under a microscope. Peppers is one of these tools designed by Chen et al. at the East China University of Science and Technology. Peppers is an RNA aptamer engineered to bind a fluorescent probe in a stem loop. The 8Peppers aptamer is the optimized form of their probe and is not repeating units of one aptamer, but a long hairpin turn with eight binding spots for probe; this 8Peppers aptamer has been shown to have difficulties folding into native conformation. This paper aims to redesign Peppers RNA imaging system to produce an effective imaging system with enhanced folding capabilities. This paper looks at its in vitro capabilities by fluorescence assay of RNA binding to the probe, HBC620. The redesigned Peppers imaging system is further tested by live cell imaging to determine the efficacy of redesigning the structure, stem, and synonymization of non-consequential regions. The experiments did not show an enhanced fluorescence turn-on or folding capabilities of redesigned Peppers compared to Chen's original Peppers imaging system. However, the original Peppers imaging tool did not have reproducible results and illustrated the need for a comprehensive and standardized characterization system for RNA imaging tools to be dependable.

Lay Summary

Ribonucleic acid (RNA) is an integral part of any living organism, just like DNA, or your genetic code. RNA's primary function is to convey genetic information from instruction to the actual product; it acts like the recipe for cells to make proteins. It has many other functions and can contribute to disease, thus it is important to develop more informative metrics of studying RNA.

Imaging RNA involves using high powered microscopes that look at cells. The RNA can be seen by tagging it with a molecule that makes it light up under certain wavelengths of light, also known as fluorescence. This allows researchers to track where a molecule of RNA is moving within a cell, how it moves, and how long it lasts. This is invaluable for better understanding the complicated, yet essential, role RNA plays in biology.

In this experiment, I set out to optimize an RNA imaging system named Peppers by redesigning the structure of the RNA into something that forms more reliably. Peppers did not have a strong fluorescence signal and it wasn't clear if the imaging was actually showing RNA.

After testing this redesigned system in cells and in a test tube, it became clear that many RNA imaging platforms do not work as well as they boast. I found that the redesigned Peppers, original Peppers, and controls performed the same and that none of the results were statistically significant; this was consistent with other researchers' frustrations with these tools. Thus, I call to action a more standardized system for characterizing RNA imaging as the field quickly grows. Researchers should be able to make an educated choice on which tool to use and how to analyze their data accurately.