Cell-Based Assay Development Strategies for the Detection and Validation of Aberrant mRNA-Protein Interactions

Medicinal Chemistry Third Year Seminar
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Abstract:
RNA-binding proteins (RBPs) make up a class of over 2,000 proteins that bind to and regulate the diverse functions of various types of RNAs, and accordingly, are involved in controlling many cellular processes. Disruption of RNA-Protein Interactions (RPIs), consequently, has been implicated in human diseases ranging from neurodegenerative and autoimmune diseases to several human cancers. Hence, targeting RBPs and RPIs has surfaced as a new frontier in RNA-targeted drug discovery which takes advantage of the endogenous regulation of messenger RNA (mRNA). This is an alternative strategy to methods currently used in the field to target transcripts which rely on antisense oligonucleotides (ASOs) or microRNAs, resulting in the degradation and silencing of mRNA, respectively. ASO- or miRNA-mediated direct targeting of RNA transcripts poses challenges, as these compounds often possess issues with specificity, delivery, and tolerability. The aim of this work is to characterize the high-affinity interactions of RBPs with mRNA motifs through live-cell detection using an assay previously developed for the detection of pre-miRNAs and their RBP partners, RNA-interaction with Protein-mediated Complementation Assay (RiPCA, Figure 1). Our goal is to be able to expand RiPCA to allow us to study other more complex RPIs in cells composed of RNAs that are larger and more structurally diverse than pre-miRNAs and that bind to proteins which perform a variety of cellular functions. Initial studies to accomplish this included three representative examples motifs found in 3' untranslated regions (UTRs) of mRNA such as (1) expanded repeat RNA, (2) UAGUAG target sequence, and (3) AU-rich elements (AREs). My efforts towards developing these assays will be discussed, as well as future directions aimed at further improvement of this assay technology. Through RiPCA optimization, we hope to generate a platform for detecting and validating various RPIs in live cells to enable screening and drug discovery efforts.

Figure 1. Schematic representation of RiPCA.