Amplification of RNA-based device's functionality in mammalian cells via spontaneous circularization

1. Objective & Motivation

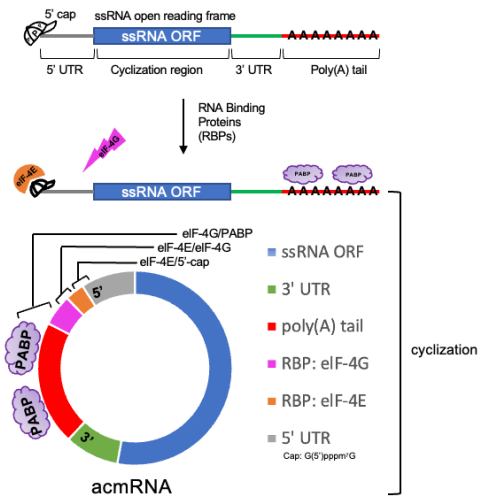
Statement of problem: RNA-based devices are potent tools in probing and programming biological systems by specifically binding to intracellular molecules or proteins. Their exceptional programmability and versatility can be widely used for cellular imaging, diagnosis, and therapies. However, a significant limitation of such RNA-based devices is that they are susceptible to RNase-mediated degradations from the free 3' or 5' end, particularly in mammalian cells. This unstable feature of RNA-based devices results in their relatively low accumulation levels in the nanomolar range and very short half-lives ranging from minutes to around one hour.

Primary Question: How to protect RNA-based devices from enzymatic degradation in mammalian cells for more efficient applications?

Proposal objective: Compared to the linear single-strand RNAs (ssRNA), their counterpart circular RNAs are more stable because of the lack of free 5' or 3' ends. Hence, we propose to develop a novel expression system where the genetically coded RNA can realize rapid circularization. The designed RNA will imitate an mRNA splicing process. Natural mRNAs will have automatical circularization, where ETS-related transcription factor eIF4 proteins and poly(A) binding proteins (PAPB) will synergistically bridge the free 5' end and 3' end, to stabilize themselves and execute translations in the cytoplasm. We expect to generate a circular and degradation-resistant RNA-based device, the fluorogenic aptamer Broccoli, based on the existing cellular machinery.

2. Hypothesis

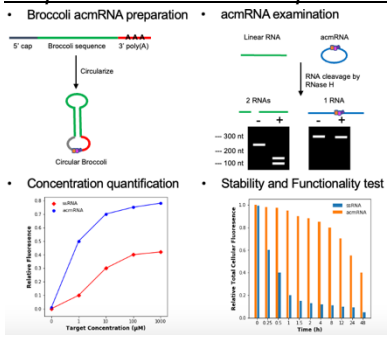
We hypothesize that the mRNA analogous circularization mechanism can be utilized to spontaneously cyclize the encoded ssRNA devices via a protein-mediated bridging mechanism. Two RNA binding protein (RBP) sites are required for bridging, where the 5' end needs to be specifically capped with a G(5')pppm^{7}G group for eIF4E's binding, and the poly(A) tail should be appended at the 3' end to be recognized by PABP. The resulting RNA-eIF4-PABP-RNA interactions will cyclize single-strand RNA in the cytoplasm to protect the designed device from enzymatic degradation. Meanwhile, the functional region on ssRNA, i.e., the protein binding region or open reading frame (ORF) that codes proteins, will be unaffected.



The designed RNA device will be called analogous circular mRNA (acmRNA).

3. Scientific/Technical Approaches

Preparation of Broccoli aptamer: The RNA aptamer will be explicitly expressed with three functional site/sequences. 1) The 5' terminal will be produced in a 5'-triphosphate form that can further be capped with a G(5')pppm^{7}G group by a set of cap-synthesizing enzymes. 2) The 3' terminal includes a poly(A) tail or the polyadenylation site (PAS) that can automatically produce the poly(A) tail triggered by polyadenylation protein (PAP). 3) The fluorogenic aptamer Broccoli sequence.



Expression system examination: After transfecting DNA into cells, RNase H will cleave the expressed RNA to test if the expression system generates circular RNA. The acmRNA is supposed have a single band solely.

Intracellular concentration characterization: acmRNA concentration can be obtained from the transfected cells by PAGE gel directly. The relative fluorescence strength can also quantify the concentration since Broccoli can bind to fluorogenic molecule, DFHBI-1T, in a dose-dependent behavior.

Stability test: The duration time of the total fluorescence activated by Broccoli's binding to DFHBI-1T will be tested to show the stability and functionality of the designed aptamer.

4. Impact Assessment

This program will extend applications of RNA-based devices for biosystems. The designed RNA device, called acmRNA, can realize spontaneous circularization after being expressed in cells. We anticipate that our work can

1. stabilize RNA-based devices in mammalian cells and keep their functions unaffected.
2. make RNA-based devices regulate intracellular interactions by specifically binding to the target proteins or small molecules because the RNA can achieve higher cellular expression levels.
3. offer an opportunity for RNA-based therapies by translating the functional proteins because the device follows an analogous mRNA mechanism. The following process for mRNA after circularization is translation.
4. provide a more durable and detectable cellular imaging technique due to the device's enhanced stability and enzymatic degradative resistance.
5. introduce minimal side effects or toxicity because no side product will be generated, and no additional drugs or proteins are required for circularization.

Reference:

[1] *Nucleic acids research*, *44*(7), pp.2987-2999.
[2] *Trends in biotechnology*, *38*(2), pp.217-230.
[3] *Nature Reviews Microbiology*, *10*(1), pp.51-65.
[4] *Nature biotechnology*, *37*(6), pp.667-675.
[5] *Biochimie*, *164*, pp.105-110.