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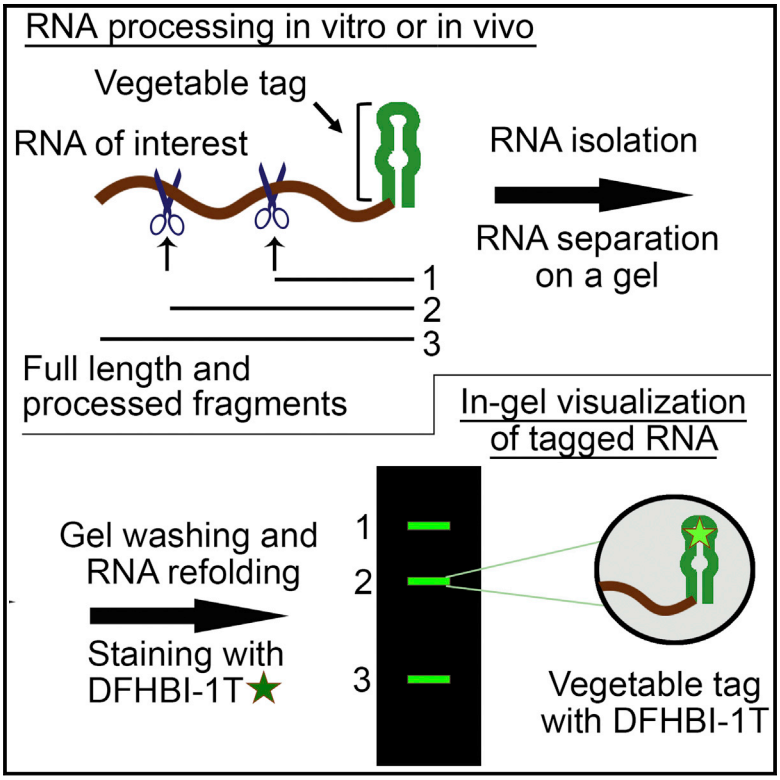
In-Gel Imaging of RNA Processing Using Broccoli Reveals Optimal Aptamer Expression Strategies

Graphical Abstract



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In Brief

Filonov et al. describe a technique for simple and sensitive detection of RNA processing by imaging Broccoli-tagged RNAs in gels. Application of this technique enabled the engineering of a three-way junction scaffold (F30) that provides robust expression of RNA aptamers in cells.

Highlights

- F30 is a highly effective RNA scaffold for expressing aptamers in cells
- Commonly used scaffolds for expressing aptamers are prone to intracellular cleavage
- Broccoli and Spinach-tagging enables RNA processing to be easily imaged in gels
- RNAs can be tagged with cassettes containing multiple Broccoli aptamers

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In-Gel Imaging of RNA Processing Using Broccoli Reveals Optimal Aptamer Expression Strategies

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SUMMARY

RNA aptamers can be expressed in cells to influence and image cellular processes. Aptamer folding is maintained by inserting the aptamers into highly structured RNA scaffolds. Here, we show that commonly used RNA scaffolds exhibit unexpected instability and cleavage in bacterial and mammalian cells. Using an in-gel staining approach for rapid and simple detection of Spinach- or Broccoli-tagged RNAs in cells, we monitored the processing of RNAs tagged with scaffolded aptamers, revealing endonucleolytic cleavage, RNA instability, and poor expression. We reengineered a natural three-way junction structure to generate an alternative scaffold that enables stable aptamer expression in cells. This scaffold was used to create cassettes containing up to four Broccoli units, markedly enhancing the brightness of mammalian cells expressing cassette-tagged RNAs. These experiments describe methods for screening RNA cleavage events in cells and identify cell-compatible scaffolds that enable efficient tagging of RNAs with aptamers for cellular expression.

INTRODUCTION

The SELEX (systematic evolution of ligands by exponential enrichment) technique is a highly effective method for producing RNA aptamers that bind diverse small molecules, proteins, and other biomolecules (Stoltenburg et al., 2007). These aptamers provide the opportunity to manipulate or investigate cellular function. For example, aptamers that bind and inhibit protein function have been developed and have the potential to serve as genetically encoded inhibitors of cellular signaling pathways (Kotula et al., 2014; Seiwert et al., 2000). Other aptamers have been developed that regulate splicing and other processes (Culler et al., 2010; Weigand and Suess, 2007). Aptamers can also be appended to RNAs to enable their purification or imaging. The Spinach, Spinach2, and Broccoli aptamers are RNA mimics of GFP and bind and switch on the fluorescence of a small molecule fluorophore that resembles the GFP fluorophore (Filonov et al., 2014; Paige et al., 2011; Strack et al., 2013). These aptamers have been expressed as fusions with other RNAs, enabling RNA imaging of various RNAs in bacterial and mammalian cells (Filonov et al., 2014; Han et al., 2013; Paige et al., 2011; Pothoulakis et al., 2014; Strack et al., 2013).

A major problem with using RNA aptamers is their poor folding in living cells. Aptamers are highly influenced by flanking sequences, which can interfere with aptamer folding (Martell et al., 2002; Strack et al., 2013). Although screening approaches have been described to improve aptamer folding (Martell et al., 2002), poor aptamer folding is a major roadblock that prevents their widespread use for diverse applications in living cells. Thus, despite their potential utility, aptamers are rarely used to influence or study intracellular processes.

To improve aptamer folding, numerous groups have developed aptamer scaffolds. These are efficiently folding RNAs that contain insertion points for introducing aptamers. The scaffold facilitates folding of the aptamer that is inserted into it. One well-known example is the tRNA scaffold. This scaffold is derived from tRNAs such as the human lysine tRNA ($tRNA^{Lys}_3$) (Ponchon and Dardel, 2007). Aptamers can be inserted into the anticodon stem of the tRNA, which improves their folding. Using this approach, aptamers can be expressed in high quantities for biochemical experiments and crystallization (Muller et al., 2011; Ponchon et al., 2013). This scaffold has also been used for heterologous expression of aptamers in living cells (Paige et al., 2011; Ponchon et al., 2013; Ponchon and Dardel, 2007).

An important feature of the scaffold is that it should be bioorthogonal. This means that the scaffold should not be recognized by intracellular nucleases and targeted for degradation. For example, tRNA precursors are recognized by dedicated RNases, resulting in cleavage near the base of the tRNA (Morl and Marchfelder, 2001). Thus, if the tRNA scaffold is appended to a target RNA, the resulting fusion RNA could be subjected to endonucleolytic cleavage. This could separate the aptamer from the RNA of interest. In addition, since cleaved RNAs are rapidly degraded, scaffold-induced RNA cleavage could reduce the stability of the fusion RNA. Thus, an important criteria when selecting an aptamer scaffold is whether it is a target for undesirable cellular processing.

The compatibility of aptamer scaffolds for eukaryotic expression has not been established. Thus, although RNAs have been expressed as fusions with aptamers scaffolded by $tRNA^{Lys}_3$, the potential cleavage and stability of these RNAs has not been addressed.

The fates of RNAs in cells are usually established by northern blotting to selectively detect specific transcripts in cells. The requirement for optimizing northern blotting conditions, as well as the large number of experiments that may be required for studying RNA cleavage reactions, makes this approach laborious.

Here, we show that RNAs that are tagged with aptamers scaffolded by $tRNA^{Lys}_3$ are targeted for endonucleolytic cleavage in

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