Tracking mRNA in living cells using Pepper RNA aptamer

Messenger RNAs (mRNAs) are a class of biomolecules that are generated in the nucleus to carry genetic information from the nucleus to different parts of the cells for making functional proteins. Because of their importance, mRNAs' trafficking and localization are highly associated with proper cell function. For example, intracellular trafficking of mRNAs plays a key role in cell division, migration, and stress response. In contrast, defective mRNA localization is a hallmark of diseases, such as musculodegenerative and neurodegenerative diseases. Therefore, being able to track the dynamics and localization of mRNA will offer great potentials to understand not only the fundamentals of how the cell works, but also the causes of many diseases.

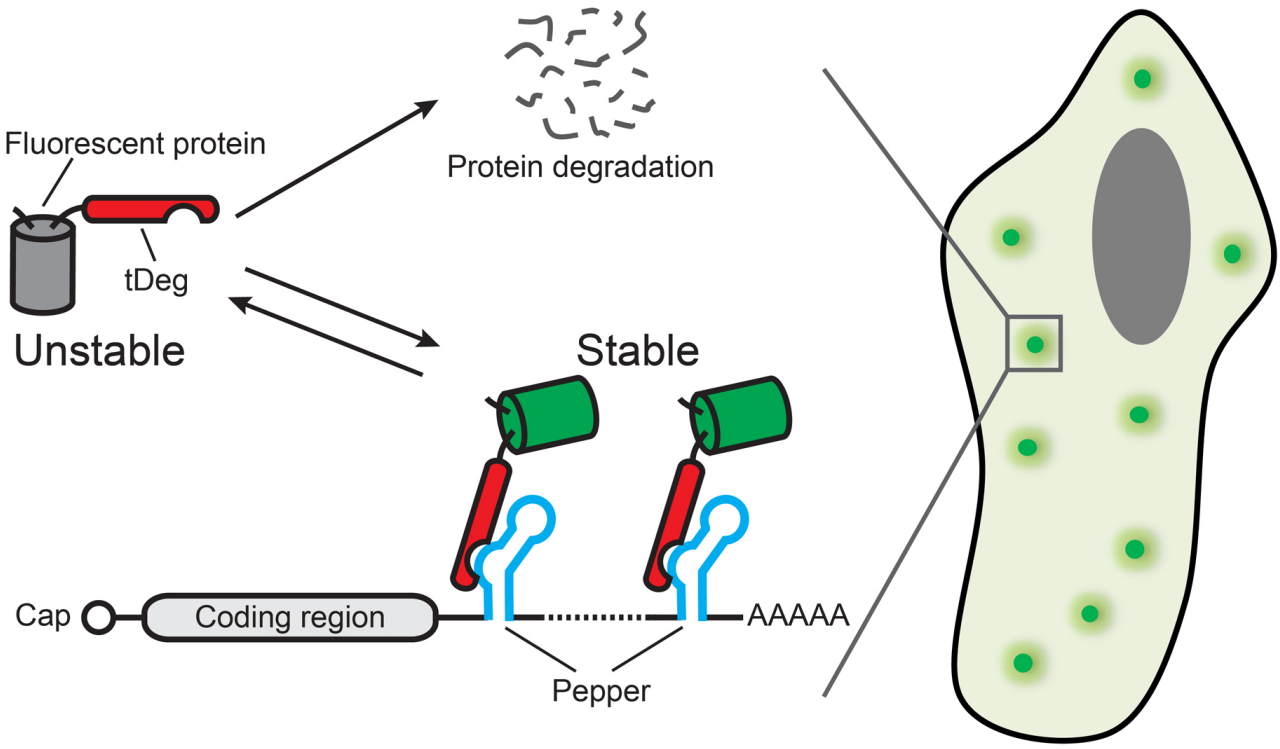

Fig. 1. Pepper-tDeg technology enables imaging an mRNA of interest in living cells.

Tracking mRNAs in living cells is a highly challenging task. One of the most robust methods for tracking biomolecules is fluorescence microscopy. In fluorescence microscopy, a fluorescent molecule (also called a dye molecule) is attached to the biomolecule of interest. Researchers can then use a microscope to track the fluorescence signals from the biomolecule of interest in the cell. To image mRNAs in living cells, traditional methods rely on a technology called fluorogenic RNA aptamers. These are RNA sequences that can bind and turn on the fluorescence of otherwise nonfluorescent dye molecules. When fused to mRNAs of interest, these fluorogenic RNA aptamers confer fluorescence signals to the mRNAs of interest under fluorescence microscopy.

However, a major problem with fluorogenic RNA aptamers is that there are a small number of fluorogenic dye molecules that can be used in this technology. Most small molecule dyes nonspecifically interact with membranes or DNA and then become fluorescent. This results in high background fluorescence signals. Another problem is that the dye molecules are not genetically encoded. Thus, the dye molecule needs to be delivered into cells, which greatly limits its utility in cultured tissues and living animals. Additionally, most dye molecules are not highly resistant to photobleaching. As a result, prolonged imaging using these dye molecules would result in a significant loss of the observed fluorescence.

To solve these problems, Wu *et al.* developed a novel fluorogenic RNA aptamer system that is fully genetically encoded. Instead of using small-molecule dyes, this system utilizes fluorescent proteins, such as green fluorescent protein or red fluorescent protein. Importantly, these fluorescent proteins have substantially higher photostability compared to the small-molecule dyes. However, since fluorescent proteins are always fluorescent, Wu *et al.* developed an approach that allows the fluorescence to primarily occur when the fluorescent protein is bound to the mRNA, minimizing the fluorescence that comes from fluorescent proteins that are not bound to the mRNA.

Wu *et al.* have developed a destabilization domain, termed tDeg, that can be added to the C-terminus of a fluorescent protein (Fig. 1). This causes the fluorescent protein to be degraded rapidly in the cell. However, Wu *et al.* also developed an RNA aptamer, termed Pepper, that binds tDeg, and prevents it from causing protein degradation, resulting in marked stabilization of the protein.

By adding Pepper sequences into the mRNA sequence, the fluorescent proteins can bind to the mRNA, causing the mRNA to be fluorescent. The fluorescent proteins are resistant to degradation while bound to the mRNA since the Pepper aptamer blocks their degradation, while unbound fluorescent proteins are degraded. This allows the mRNAs to be readily visualized as single dots using fluorescence microscopy (Fig. 1).

This work has introduced a fundamentally new tool to the fields of chemical biology and RNA synthetic biology. Overcoming the limitations of the previous methods, the Pepper-tDeg technology represents a powerful method for imaging and tracking the localization of mRNAs in living cells.

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