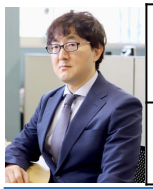
【Grant-in-Aid for Scientific Research (S)】

Molecular design and application of circular RNAs using novel translation induction techniques



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Purpose and Background of the Research

●Outline of the Research

mRNA drugs used in vaccines against new coronavirus infection have attracted much attention, but conventional mRNAs are linear and are easily degraded by intracellular enzymes. But, “circular mRNA,” is expected to become the next generation of mRNA medicine because it is less susceptible to degradation and lasts longer. However, the problem with ring-shaped mRNAs is that they are less efficient at making proteins. Until now, the introduction of a sequence called IRES was necessary, but the translation activity was still lower than that of linear mRNA (Figure 1A). On the other hand, in this study, we developed a new method to mark the inside of circular mRNA with a “cap structure” (ICIT mechanism), aiming at highly efficient protein synthesis of circular mRNA and realization of a “translation switch” that works only in diseased cells. We devised two molecular designs for the ICIT mechanism: the first is a method of covalently introducing a cap structure to circular mRNA by introducing a branching structure (Cap-circRNA) (Fig. 1B), and the second is a method of hybridization of oligoRNAs with a cap structure to circular mRNA (Cap-ASO RNA) (Fig. 1C).

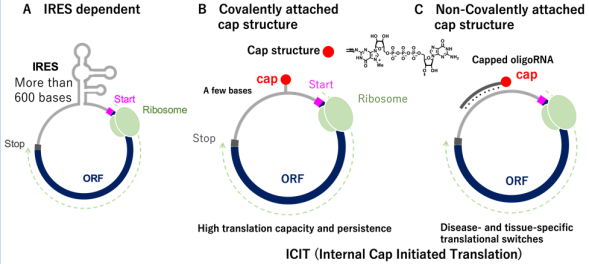

Fig. 1 Overview of IRES and ICIT mechanisms that enhance the translational ability of circular mRNA.

●Covalent type ICIT

In an experiment to evaluate luminescent protein synthesis in mice, covalent ICIT circular mRNA showed more than 200-fold higher protein synthesis compared to circular mRNA with IRES sequence (Fig. 2A). Furthermore, when the time course of protein synthesis was evaluated, ICIT circular mRNA showed its stability compared to linear mRNA, and the amount of protein synthesized was more than 10 times higher after 50 hours (Fig. 2B). As described above, covalent ICIT circular mRNA is expected to be used for mRNA medicine such as antibody therapy, genome editing, and protein replacement therapy in the future.

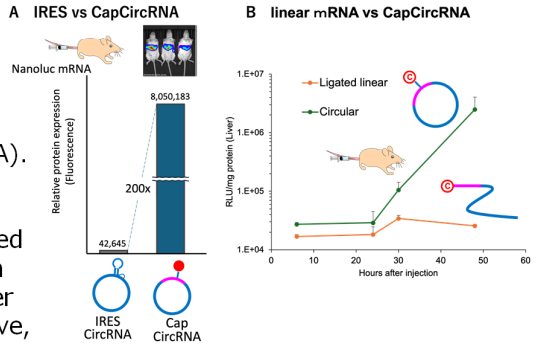

Figure 2 Highly efficient and sustained protein synthesis using covalently bound ICIT circular mRNA

●Non-covalent type ICIT

We hybridized oligoRNAs with cap structure to circular mRNA and evaluated translational activity, and found that the introduction of non-natural nucleic acids into oligoRNAs can be applied (Fig. 3A).

We further hypothesized that non-covalent ICIT could be applied to long noncoding RNAs, and by designing circular mRNA that bind in a sequence-specific manner, translation could occur in an RNA marker-dependent manner. Targeting HULC RNA, which is highly expressed in liver cancer cells, resulted in a more than 50-fold increase in protein synthesis (Figure 3B). Thus, the use of non-covalent ICIT is expected to make it possible to produce therapeutic proteins only in diseased cells and to design innovative mRNA drugs without side effects.

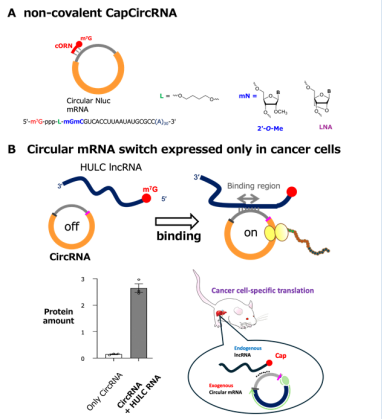

Figure 3 Cancer-selective translation switch

Expected Research Achievements

●As shown in Figure 4, this study will develop a new translation control technology based on the ICIT mechanism and clarify how this works in vivo as well. Specifically, the research will be divided into the following four pillars

1 Development of high-efficiency mRNA medicines

Using circular mRNAs with covalent cap structures, we will develop mRNA medicines with both higher stability and higher translation efficiency.

2 Improvement of translation efficiency of endogenous mRNAs

Establish a technology to artificially increase the translation efficiency of circular mRNAs by using artificial cap oligonucleic acids.

3 Establishment of disease/tissue-specific translation control

We will design “translation switches” that cause translation only in specific cells, such as cancer cells, and verify how to utilize them for disease treatment.

4 Elucidation of new translation mechanisms

Explore the possibility that the mechanism of interaction between endogenous lncRNAs and mRNAs actually functions in vivo.

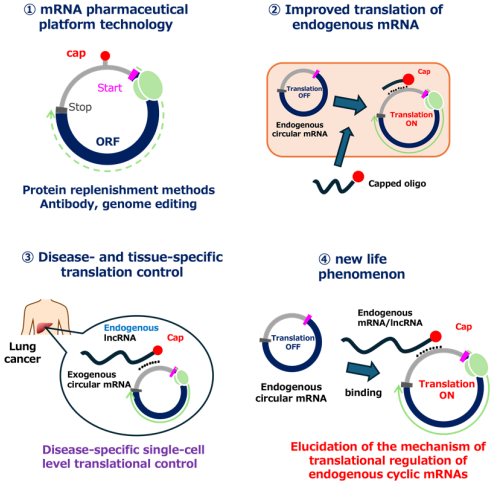

Fig. 4 New translation control technology based on ICIT and new life phenomena

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