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研究課題名（和文）RNAアプタマーにより細胞種特異的翻訳活性化機能を付与したmRNA医薬品の開発

研究課題名（英文）Development of mRNA drug with RNA type aptamer conferring cell type specific translation activation function

研究代表者
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交付決定額（研究期間全体）：（直接経費）　　4,800,000円

研究成果の概要（和文）：我々は、mRNA-アプタマーハイブリッドを開発した。アプタマーは、mRNAにハイブリダイズでき、かつテオフィリンとも結合できるよう設計した。PEG-テオフィリンコンジュゲート(PEG-t)は、ハイブリッドと混合すると、それを覆い、径約50 nmの電気的に中性なナノ粒子を形成した。一方、ハイブリッド自体は負に帯電していたことから、ハイブリッドがPEGで被覆されたことが確認された。ハイブリッドは、無細胞翻訳系において、タンパク質翻訳活性を示したが、その活性はPEG-t被覆により抑制された。そこに、テオフィリンを添加すると、タンパク質翻訳活性が回復した。

研究成果の学術的意義や社会的意義
We demonstrated the potential of our approach for developing systems triggering protein translation after sensing specific molecules. Our findings will foster novel mRNA systems triggering protein expression based on pathological signals. Further studies with aptamers for cancer markers are ongoing.

研究成果の概要（英文）：We developed mRNA-aptamer hybrids by combining mRNAs, and aptamers having a sequence for hybridization with mRNA and a binding site for theophylline. Two aptamers were constructed and their affinity to theophylline was studied by surface plasmon resonance. Then, PEG-theophylline conjugates were prepared by condensation reaction with PEG-NH2. When the polymer was mixed with the mRNA-aptamer hybrids, it coated the mRNA-aptamer hybrids, forming neutral nanoparticles of 50 nm in diameter. Conversely, mRNA-aptamer hybrids were negatively charged, which confirmed PEG coating. The nanoparticles were de-coated by adding free theophylline, which recovered the negative charge of the hybrids. mRNA-aptamers translated proteins in cell free systems, and this translation was inhibited when coated with PEG-theophylline. After adding theophylline, the protein translation was recovered, indicating that mRNA-aptamers-polymer nanoparticles can sense the surroundings.

研究分野：mRNA delivery

キーワード：mRNA　aptamer　polymer　theophyline　triggered translation

１．研究開始当初の背景

Messenger RNA (mRNA) has been considered for developing safe gene-based therapies, as it mediates the translation of genetic information to proteins in the cytosol of cells without integrating into the genome. Moreover, the ability of mRNA to induce transient protein expression in virtually all cell types is a considerable advantage for developing a broad range of gene therapies. However, the *in vivo* application of mRNA is limited by its rapid degradation by nucleases and poor access to the cytosol of cells, as well as its potential to induce immune responses. Therefore, carrier systems have been considered for overcoming the stability, safety and delivery issues of mRNA. These carriers have somewhat managed to improve the stability of mRNA, enhance the intracellular delivery and the translation efficiency, and reduce the immune responses. Nevertheless, the development of carriers capable of efficient and selective mRNA translation in desired tissues or cells in vivo remains to be addressed. Thus, besides effectively and precisely delivering the mRNA molecules, such carrier systems should be able to selectively activate the mRNA in the target cell types for achieving the appropriate therapeutic dosage.

２．研究の目的

In this study, we aim to develop safe and versatile carriers for triggered mRNA translation based on mRNA/aptamer-polymer assemblies directed to achieve high and selective protein expression after sensing specific molecules (**Figure 1**).

３．研究の方法

To prepare a pDNA template to be used for *in vitro* transcription of mRNA expressing luciferase, Gaussia luciferase (Gluc), the protein coding region of pCMV-Gluc control plasmid was incorporated into the pSP 73 vector so that it can be transcribed under promoter. For 12 × FLAG mRNA, the 12 × FLAG DNA sequence was incorporated into the pUC 57-Kan vector so that it could be transcribed under T7 promoter. Each pDNA was amplified in E. coli DH5α Competent cells and purified with NucleoBond Xtra EF. To prepare the pDNA template used for *in vitro* transcription of aptamer, aptamer sequence was incorporated into pUC 57-Kan to be transcribed under T7 promoter. pDNA was increased in E. coli competent cells, the template DNA was purified, and then *in vitro* transcription was performed. Transcripts were purified by isopropanol precipitation. The mRNA concentration was quantified by absorbance at 260 nm. The chain length of the mRNA was evaluated by an Agilent 2100 bioanalyzer electrophoresis system. To make the mRNA-aptamer hybrid FLAG RNA and aptamer were mixed at a molar ratio of 1:12 in 10 mM HEPES, 150 mM NaCl. Next, they

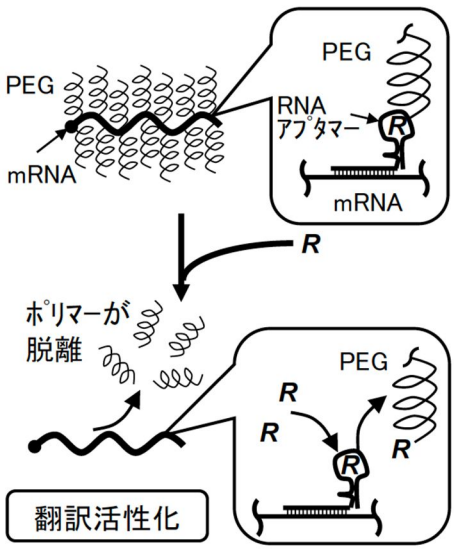

Figure 1. Scheme of proposed mRNA-aptamer-polymer system for developing environment responsive mRNA carriers. In this case, R is theophylline.

were heated by a thermal cycler at 65 °C for 5 minutes and gradually cooled to 30 °C for 90 minutes. To introduce the polymer to the hybrids. PEG-theophylline was added to a final concentration of 500 μM in the presence of 5mM $MgCl_2$. The size and the zeta-potential were measured by using Zetasizer. The ability of the systems to produce proteins was studied by using cell-free translation system.

４．研究成果

We developed mRNA-aptamer hybrids by combining the mRNAs, and the aptamers having a sequence for hybridization with mRNA and a binding site for theophylline (**Figure 1**). Two aptamers were constructed and their affinity to theophylline was studied by surface plasmon resonance. Both aptamers were able to bind to theophylline with a equilibrium constant of 0.35 μM. The aptamers were also able to bind PEG-theophylline, indicating the ability of the aptamers for proving our concept. Then, PEG-theophylline conjugates were prepared by condensation reaction with PEG-NH2 (Mw: 12,000). The conjugation rate was determined to be 88% by ^{1}H-NMR. mRNA-aptamer hybrids were made by mixing and thermal cycling. The formation of the hybrids was confirmed by western blotting. Then, PEG-theophylline or PEG-NH_2 were mixed with the mRNA-aptamer hybrids. The PEGylation of the mRNA-aptamer hybrids was follows by changes in the zeta-potential of the hybrids. Thus, mRNA-aptamer are hybrids negatively charged, and the addition of PEG-NH_2 did not change this charge. Only, PEG-theophylline make the charge of the mRNA-aptamer hybrids neutral, which confirmed the PEG coating. The PEGyalted mRNA-aptamer hybrids formed nanoparticles of 50 nm in diameter. When free theophylline was added, the charge of the nanoparticles was turned back to negative, indicating the de-PEGylation. The protein translation of the systems was studied by using cell free systems. As mRNA encodes 12 FLAG peptides, the product of the cell-free translation consists of a concatemer of 12 FLAG peptides. mRNA-aptamers translated proteins, whereas this translation was inhibited when coated with PEG-theophylline (**Figure 2**). After adding excess theophylline just for 5 minutes, the protein translation was recovered, which suggest that the de-PEGylation of the mRNA allowed the production of 12 FLAG peptides (**Figure 2**). These results indicate that the mRNA-aptamers-polymer nanoparticles can sense the surroundings and activate protein translation. Our findings prove the concept of using aptamers/polymers for controlling protein translation in a specific manner. This strategy will prompt the development of novel mRNA systems triggering protein expression based on pathological and/or exogenous signals.

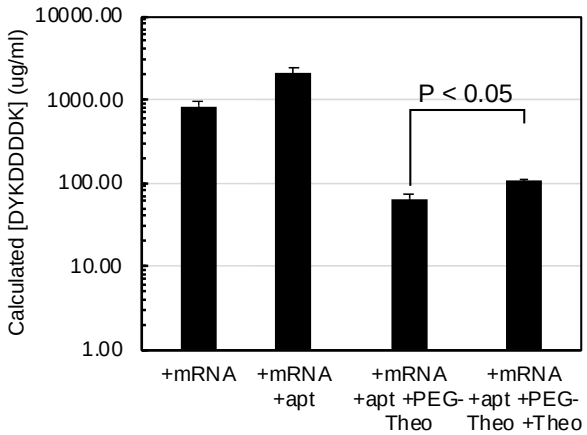

Figure 2. Expression of 12 FLAG peptides in cell-free translation system by mRNA, mRNA-aptamer hybrids, PEG-theophylline mRNA-aptamers and PEG-theophylline mRNA-aptamers plus free theophylline. Data expressed as the average ± S.D. (n = 3).

５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　2件／うち国際共著　2件／うちオープンアクセス　1件）

１．著者名 Miyazaki Takuya、Igarashi Kazunori、Matsumoto Yu、Cabral Horacio	４．巻 -
２．論文標題 One-Pot Synthesis of PEG?Poly(amino acid) Block Copolymers Assembling Polymeric Micelles with PEG-Detachable Functionality	５．発行年 2019年
３．雑誌名 ACS Biomaterials Science & Engineering	６．最初と最後の頁 -
掲載論文のDOI（デジタルオブジェクト識別子） 10.1021/acsbiomaterials.8b01549	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Kurimoto Shota、Yoshinaga Naoto、Igarashi Kazunori、Matsumoto Yu、Cabral Horacio、Uchida Satoshi	４．巻 24
２．論文標題 PEG-OligoRNA Hybridization of mRNA for Developing Sterically Stable Lipid Nanoparticles toward In Vivo Administration	５．発行年 2019年
３．雑誌名 Molecules	６．最初と最後の頁 1303～1303
掲載論文のDOI（デジタルオブジェクト識別子） 10.3390/molecules24071303	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

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２．発表標題 少数のコレステロール修飾RNAオリゴマーにより誘起されるmRNA内包高分子ミセルの構造安定化およびマウス肺局所投与への応用
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堀真緒，吉永直人、内田智士、H. Cabral、片岡一則

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Horacio Cabral

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2018年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

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