Division of Biochemistry
– Biofunctional Design-Chemistry –

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Prof
FUTAKI, Shiroh
(D Pharm Sc)

Assoc Prof
IMANISHI, Miki
(D Pharm Sc)

Assist Prof
KAWANO, Kenichi
(D Pharm Sc)

Program-Specific Assoc Prof
HIROSE, Hisaaki
(D Pharm Sc)

PD
ARAFILES, Jan Vincent V.
(D Pharm Sc)

Students

SAKAMOTO, Kentarou (D3)
SAKAI, Takayuki (D2)
HIRAI, Yusuke (D2)
IWATA, Takahiro (M2)
KUROKI, Kakeru (M2)
NAGANO, Yuki (M2)
YOSHIDA, Atsuhiro (M2)
KURIYAMA, Masashi (M1)
MICHIBATA, Junya (M1)
OKANO, Syusuke (M1)
OTONARI, Kenko (M1)
IKITOU, Asuka (UG)
NAKAGAWA, Yuna (UG)
TANAKA, Kamui (UG)

Scope of Research

The ultimate goal of our research is the regulation of cellular functions by designed peptides and proteins. Current research subjects include (1) development of novel intracellular delivery systems aiming at elucidation and control of cellular functions using designed membrane permeable peptide vectors, (2) elucidation of the DNA or RNA binding modes of nucleic acid binding proteins, and design of artificial regulators of gene expression, (3) elucidation and control of membrane curvature, and (4) design of stimulation-responsible artificial peptides and proteins.

KEYWORDS

Membrane-Permeable Peptides
Intracellular Delivery
Peptide Design
DNA/RNA Binding Protein
Membrane Curvature

Selected Publications

Arafiles, J. V. V.; Hirose, H.; Akishiba, M.; Tsuji, S.; Imanishi, M.; Futaki, S., Stimulating Macropinocytosis for Intracellular Nucleic Acid and Protein Delivery: A Combined Strategy with Membrane-Lytic Peptides to Facilitate Endosomal Escape, *Bioconjug. Chem.*, **31,** 547-553 (2020).

Masuda, T.; Hirose, H.; Baba, K.; Walrant, A.; Sagan, S.; Inagaki, N.; Fujimoto, T.; Futaki, S., An Artificial Amphiphilic Peptide Promotes Endocytic Uptake by Inducing Membrane Curvature, *Bioconjug. Chem.*, **31,** 1611-1615 (2020).

Ohtani, R.; Kawano, K.; Kinoshita, M.; Yanaka, S.; Watanabe, H.; Hirai, K.; Futaki, S.; Matsumori, N.; Uji-i, H.; Ohba, M.; Kato, K.; Hayami, S., Pseudo-Membrane Jackets: Two-Dimensional Coordination Polymers Achieving Visible Phase Separation in Cell Membrane, *Angew. Chem. Int. Ed.*, **59,** 17931-17937 (2020).

Shinoda, K.; Suda, A.; Otonari, K.; Futaki, S.; Imanishi, M., Programmable RNA Methylation and Demethylation Using PUF RNA Binding Proteins, *Chem. Commun.*, **56,** 1365-1368 (2020).

Kawano, K.; Yokoyama, F.; Kawamoto, J.; Ogawa, T.; Kurihara, T.; Futaki, S., Development of a Simple and Rapid Method for *In Situ* Vesicle Detection in Cultured Media, *J. Mol. Biol.*, **432,** 5876-5888 (2020).

Curvature-sensing Peptides to Distinguish Bacterial Extracellular Membrane Vesicles from Cells in Cultured Medium

Extracellular membrane vesicles (EMVs) are biogenic secretory lipidic vesicles that play significant roles in intercellular communication related to human diseases and bacterial pathogenesis. They are being investigated for their possible use in diagnosis, vaccines, and biotechnology. However, the existing methods suffer from a number of issues. High-speed centrifugation, a widely used method to collect EMVs, may cause structural artifacts. Immunostaining methods require several steps and thus the separation and detection of EMVs from the secretory cells is time-consuming. Furthermore, detection of EMVs using these methods requires specific and costly antibodies. To tackle these problems, development of a simple and rapid detection method for the EMVs in the cultured medium without separation from the secretory cells is a pressing task. In this study, we focused on the Gram-negative bacterium *Shewanella vesiculosa* HM13, which produces a large amount of EMVs including a cargo protein with high purity, as a model [C. Chen, *et al.*, *Front. Microbiol.,* **2020**, 10, 3001]. FAAV, a curvature-sensing peptide [K. Kawano, *et al.*, *Chem. Pharm. Bull.,* **2019**, 67, 1131], selectively binds to the EMVs even in the presence of the secretory cells in the complex cultured medium (Figure 1). FAAV can fully detect the EMVs within a few minutes, and the resistance of FAAV to proteases enables it to withstand prolonged use in the cultured medium. Fluorescence / Förster resonance energy transfer (FRET) was used to develop a method to detect changes in the amount of the EMVs with high sensitivity. Overall, our results indicate the potential applicability of FAAV for *in situ* EMV detection in cultured media.

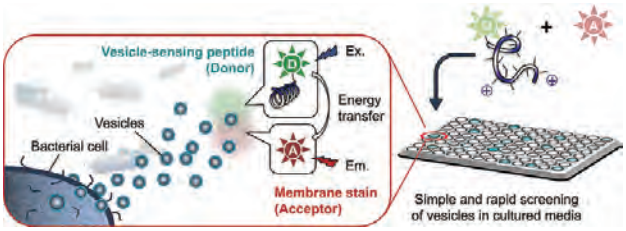

Figure 1. A method for in situ vesicle detection in cultured media.

Targeted RNA Methylation and Demethylation Using Artificial RNA Binding Proteins

*N*6-methyladenosine (^{m6}A) is the most abundant internal RNA modification in eukaryotic mRNA, regulating various aspects of mRNA metabolism, such as mRNA stability, localization, and translation, ultimately controlling gene expression. The levels of ^{m6}A methylation are controlled by ^{m6}A regulatory enzymes. METTL3 and METTL14 form a heterodimer and install the methyl group into *N*6-adenosine within the RRACH (R: A, G; H: A, C, U) sequences. FTO or ALKBH5 catalyses demethylation. Although sequencing analysis using Me-RIP-seq and down- or up-regulation of the ^{m6}A regulatory enzymes have revealed the importance of ^{m6}A in various biological phenomena, the function of individual ^{m6}A has not yet been revealed. New methods to control local RNA methylation are needed. Here fusion proteins of ^{m6}A regulatory enzymes, FTO demethylase and METTL14 methyltransferase, with a programmable RNA binding protein, PUF, were designed (Figure 2). Sequence-specific (de)methylation was demonstrated *in vitro* even in the presence of excess amount of non-specific RNA fragments derived from mammalian cells, strongly suggesting these chimeras could be used in transcriptome. This study provides a proof-of-principle that the strategy to fuse ^{m6}A-regulatory enzymes with the programmable RNA binding protein PUF is a promising way for site-specific control of RNA methylation, not only at the transcript level but also at the level of a single ^{m6}A site within a given transcript to clarify the function of individual RNA methylation.

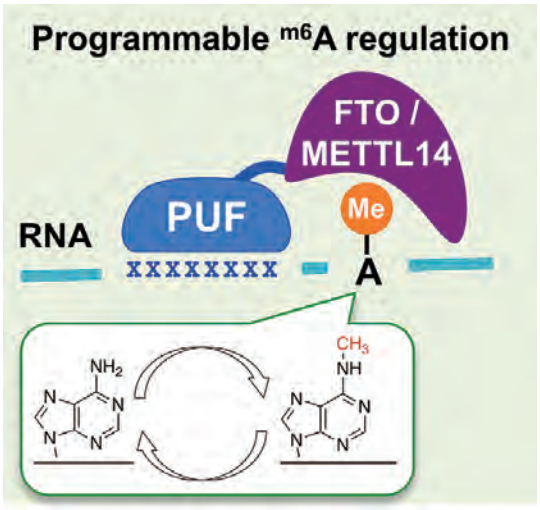

Figure 2. Targeted RNA methylation and demethylation using fusion proteins of RNA (de)methylases with programmable RNA binding protein, PUF.