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研究課題名（和文）Coenzyme engineering for the study of RNA methyltransferase

研究課題名（英文）Coenzyme engineering for the study of RNA methyltransferase

研究代表者
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研究成果の概要（和文）：本プロジェクトでは、補酵素であるS-アデノシルメチオニン（SAM）を異なる転写性基と、ヌクレオシドを持つ合成補酵素に置き換えることにより、RNAメチルトランスフェラーゼの選抜方法を開発している。また、最終的には合成補酵素を直交的に利用するRNAメチルトランスフェラーゼを開発することを目標としている。

研究成果の学術的意義や社会的意義
Herein we aimed to 1. generate an unique synthetic biology tool that sheds light on the role of mRNA in vivo 2. overcome the challenge of engineering a coenzyme binding pocket without disturb the substrate binding pocket 3. shade light on the coenzyme evolution.

研究成果の概要（英文）：In this project we were aiming to replace the coenzyme S-Adenosyl Methionine (SAM) with a synthetic coenzyme carrying a different transferable group and nucleoside ; to develop a selection method for the RNA methyltransferase and finally to engineer a RNA methyltransferase to utilize the synthetic cofactor in a orthogonal manner.

研究分野：enzyme engineering

キーワード：S-adenosyl methionine　directed evolution　Methionine adenosyltransferase　RNA methyltransferase　In-vitro　compartimentalisation　Coenzyme engineering

１．研究開始当初の背景

In literature, many studies have been reported replacing the natural coenzyme with an analogue with a different transferable group, mainly with alkyne derivatives and major effort has been done engineering in particular DNA or protein methyltransferase, to accept them (Schulz D. *et al*. Angew Chem Int Ed Engl 2013; Lukinavicius G. *et al.* Acs Chem Biol 2013). These modifications of SAM allow an easy DNA labelling but it does not guarantee orthogonality for the coenzyme. Furthermore single mutations rationally designed can be introduce in the engineering enzyme but if directed evolution is applied for the *in vivo* selection the system has to overcome a main problem: the co-existing of the synthetic SAM and natural one, the latter one in pretty high concentration (0.1mM in E.coli).

２．研究の目的

In this project we were aiming to replace the coenzyme S-Adenosyl Methionine (SAM) with a synthetic coenzyme carrying a different transferable group and nucleoside ; to develop a selection method for the RNA methyltransferase and finally to engineer a RNA methyltransferase to utilize the synthetic cofactor in a orthogonal manner.

３．研究の方法

The first step for the accomplishment of the project was the synthesis of the unnatural cofactor. Since we could not synthetically make it we decided to go for biochemical synthesis using Methionine Adenosyl Transferase (MAT), the enzyme that synthesize SAM inside in the cells. We selected three enzymes to screen for promiscuous activities for substrate analogues. We tested the E.coli MAT, M.Jannascii MAT and one human MAT (hMAT). We managed to synthesize few nucleotide analogues of SAM using the hMAT enzyme. During these attempts we realized that although the sequence similarity for the bacterial MAT and hMAT was high (70%) and the two enzymes had identical binding pocket they performed completely different for specificity towards the nucleotide base. We decided in parallel to study this interesting finding. We characterized these enzymes biochemically and structurally and we shed light on their mechanism of promiscuities (manuscript in preparation).

In the meantime, we started to set up the selection method for the RNA methyltransferase. We planned and constructed the plasmid for the in vitro selection method. The in vitro compartimentalisation (IVC) method was set up,

although we found low recovery of the RNA and we are currently working on improvement on this step.

４．研究成果

As mentioned above the promiscuities mechanism of MAT enzymes will be soon published and this will put the bases for the synthesis of synthetic SAM analogues. The structural and biochemically information that we collected will be worldwide useful since there is a major interest in synthesizing these SAM analogues. Moreover towards this study we identified a new cofactor in human cells, S guanidyl methionine.

We are setting up the IVC method and soon we will create the library and start the selection. The selection will hopefully be able to give us also more information about the function/structural relation of RNA methyltransferase -a still unexplored enzyme.

５．主な発表論文等

〔雑誌論文〕　計1件（うち査読付論文　1件／うち国際共著　0件／うちオープンアクセス　1件）

１．著者名 Chouhan Bhanu Pratap Singh、Maimaiti Shayida、Gade Madhuri、Laurino Paola	４．巻 58
２．論文標題 Rossmann-Fold Methyltransferases: Taking a “ β-Turn ” around Their Cofactor, S-Adenosylmethionine	５．発行年 2018年
３．雑誌名 Biochemistry	６．最初と最後の頁 166～170
掲載論文のDOI（デジタルオブジェクト識別子） 10.1021/acs.biochem.8b00994	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 －

〔学会発表〕　計4件（うち招待講演　1件／うち国際学会　4件）

１．発表者名 Paola Laurino, Madhuri Gade
２．発表標題 A journey from an ancient finger print of Rossmann fold enzymes to cofactor engineering
３．学会等名 Enzyme, biocatalisis and chemical biology: The new frontiers, EMBO Workshop（国際学会）
４．発表年 2018年

１．発表者名 Madhuri Gade, Paola Laurino
２．発表標題 Understanding enzymes specificities as a tool for cofactor engineering
３．学会等名 Chemical Biology workshop, EMBO 2018（国際学会）
４．発表年 2018年

１．発表者名 Madhuri Gade, Paola Laurino
２．発表標題 A journey from an ancient finger print of Rossmann fold enzymes to cofactor engineering
３．学会等名 RTG Symposium 2018（国際学会）
４．発表年 2018年

１．発表者名 Paola Laurino, Madhuri Gade
２．発表標題 A journey from an ancient finger print of Rossmann fold enzymes to cofactor engineering
３．学会等名 New Frontier in Protein Design & Engineering（招待講演）（国際学会）
４．発表年 2019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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