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研究課題名（和文）Mechanism of mRNA Recapping pathway in Trypanosome

研究課題名（英文）Mechanism of mRNA Recapping pathway in Trypanosome

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研究成果の概要（和文）：本研究ではトリパノソーマ寄生虫のmRNA再キャップ経路の特徴を解析することで新たな遺伝子発現制御機構を明らかにすることを目的としています。非キャップmRNAを細胞から同定する方法を開発し、再キャップ酵素遺伝子をノックダウンした寄生虫の遺伝子発現の変化を次世代シーケンシングで解析しました。また、５’端末のRNAメチル化によってmRNA再キャップ活性が制御されていることを新たに明らかにしました。

研究成果の学術的意義や社会的意義
mRNA再キャッピングはヒトを含む全ての真核細胞において新たな遺伝子発現制御の仕組みである可能性があります。また、トリパノソーマの再キャッピング経路を解明することによって、寄生虫に対する新しい創薬ターゲットの同定につながる可能性もあります。

研究成果の概要（英文）：In this research, we developed method to identify uncapped mRNA from the cells, performed RNA Seq analysis on recapping enzyme depleted parasite, and characterize the recapping activity to gain insight on how gene expression is regulated through cytoplasmic mRNA recapping. Results suggest mRNA recapping pathway could be involved in the expression of antigenic variation genes. We also report that hypermethylation on the 5' end of the RNA could potentially regulate the recapping activity.

研究分野：分子寄生虫学

キーワード：mRNA processing　Trypanosome　mRNA recapping　RNA ligase　gene expression

１．研究開始当初の背景

The 5′ end of all eukaryotic mRNA is modified by the addition of methylguanosine cap (m7GpppN or cap 0) to protect mRNA from degradation and enhance protein synthesis. Cap removal is part of the natural cycle of eukaryotic mRNA turnover, and the remaining 5′-monophosphorylated RNA is thought to be rapidly degraded by a 5′-to-3′ exonuclease (Xrn1/Rat1). Recent findings suggest that certain uncapped mRNAs are stored in a translationally quiescent state and could later acquire a cap in the cytoplasm to regenerate translationally active mRNAs. The mRNA recapping pathway has been an elusive phenomenon for decades in eukaryotic gene expression until our discovery of the cytoplasmic mRNA recapping enzyme in Trypanosoma brucei (TbCe1). We demonstrated that recombinant TbCe1 possesses a novel 5’-monophosphate RNA kinase activity that can convert a decapped pRNA into GpppRNA via a diphosphate RNA intermediate. TbCe1 depletion results in an accumulation of uncapped RNA in T. brucei. These findings suggest that the mRNA recapping pathway could potentially regulate the abundance and stability of translatable mRNA, thereby controlling the gene expression.

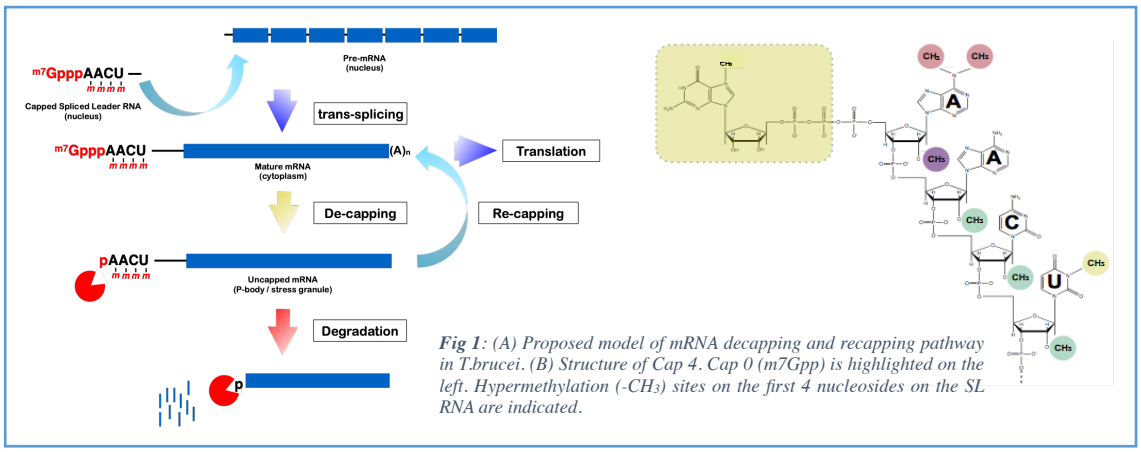

Fig 1: (A) Proposed model of mRNA decapping and recapping pathway in T.brucei. (B) Structure of Cap 4. Cap 0 (m7Gpp) is highlighted on the left. Hypermethylation (-CH3) sites on the first 4 nucleosides on the SL RNA are indicated.

２．研究の目的

In trypanosomes, genomes are organized into polycistronic clusters and therefore, post-transcriptional events have central roles in controlling gene expression. We hypothesize that certain uncapped mRNAs could be stored in a translationally quiescent state and later exit to be translationally active again by acquiring a 5′-cap structure in the cytoplasm to regenerate translatable mRNAs. The “mRNA recapping” pathway could potentially regulate the abundance and stability of selective mRNA. The key questions to be addressed in this research are: (i) What kind of transcripts are regulated by mRNA decapping and recapping pathways. Is there any specific biological process mRNA recapping control? (ii) Does differential cap 4 modification controls mRNA recapping? Our strategy is to use RNA interference to down-regulate the expression of TbCe1 to determine the targets for TbCe1. We aim to address if the methylation near the 5' end of the RNA could alter the activity of TbCe1. In addition to the study on mRNA recapping, we solved a structure of RNA Triphosphatase, an enzyme responsible for the first step of mRNA cap formation.

３．研究の方法

Our initial goal is to identify the transcript regulated by recapping. For this purpose, we developed a method to identify uncapped mRNA from the cell, performed an RNA-Seq analysis on TbCe1 silenced and unsilenced control, and evaluate the effect of RNA methylation on RNA recapping activity using a series of chemically synthesized RNA substrates, which have one or more methylations near the 5’ end.

４．研究成果

(1) **RNA ligation-mediated RT-PCR to identify uncapped mRNA from the cells** - We developed a method to identify decapped or uncapped (5' monophosphate) RNA from the cell by RNA ligation-mediated RT-PCR (1). In this procedure, polyA mRNA isolated from the cells was ligated to the anchor RNA. In this procedure, only the RNA with a 5' monophosphate can be ligated to anchor RNA, whereas mature capped RNA will not be ligated. Upon cDNA synthesis using oligo(dT) as a primer, the ligated products can be detected by PCR using oligo(dT) and a DNA primer specific to the anchor sequence. We detected PCR products in the 0.4–1.2 kb range from TbCe1 RNAi-induced cell lines but not from control cells. The procedure allows cloning of the PCR fragments using the TA-cloning method to obtain the sequence of uncapped transcripts.

(2)**RNA Seq analysis of TbCe1 knockdown** - Total mRNA derived from TbCe1 silenced and unsilenced control was subject to Illumina Sequencing to determine what kind/type of RNAs are affected in the TbCe1-knock down strain. In the TbCe1 RNAi-induced strain, the expression of several VSG (Variable Surface Glycoprotein Protein)-related transcripts were altered, which is responsible for controlling WT antigenic variation to protect the parasite against the host defense system. Nearly 40% (33 out of 121) of the genes in which the expression was increased by TbCe1 depletion were VSG-related transcripts. Out of 40 genes that showed decreased expression by TbCe1 depletion, three encodes for transcription factors that control VSG expression. These results reveal that antigenic variation expression could be regulated by mRNA recapping. TbCe1 may either directly or indirectly suppress or enhance the expression of VSG-related genes, respectively.

(3) **Effect of RNA methylation on recapping enzyme activity**- Our central hypothesis is that TbCe1 is responsible for mRNA recapping, and cap methylation plays a pivot role in selecting which transcripts are to be decapped and recapped. All mRNA undergoes trans-splicing to acquire a hypermethylated cap 4 SL RNA. The decapped mRNA should preserve hypermethylation at the 5' end.

To address whether methylation at the 5' end affects recapping, synthetic mono-phosphorylated 21-mer SL RNA with all seven methylations found in cap 4 (m6,2A1 + m3U4 + r1234) was prepared. The TbCe1 kinase activity was enhanced ~50-fold by fully methylated pRNA compared to the unmethylated control. These findings provide strong evidence that TbCe1 preferentially acts on uncapped hypermethylated mRNAs and suggest that decapped mRNA derived with a mature cap 4 is preferentially recognized by TbCe1 and is likely to be a physiological substrate for recapping. This is the first instance in which RNA methylation directly influences the catalytic activity of the enzyme.

(4) **Crystal structure of *T.brucei* RNA Triphosphatase** - RNA Triphosphatase catalyzes the first step of mRNA capping, which cleaves the terminal phosphate from the nascent triphosphate terminated RNA and is a promising anti-infective drug target against protozoan parasites because the catalytic activity and the structure of the protozoan RNA triphosphatase are completely different from the enzyme found in the metazoan host. We crystalized trypanosome RNA triphosphatase and solved the X-ray structure at 2.20–2.51 Å resolutions in complex with tripolyphosphate in the active site, which mimics a triphosphate-terminus of RNA (2). Furthermore, we identified a series of small-molecule ligands that stabilized *T.brucei* RNA Triposphatase and solved the structure with the bound ligand. We propose a putative RNA binding site based on the three sulfate ions co-crystallized with the enzyme.

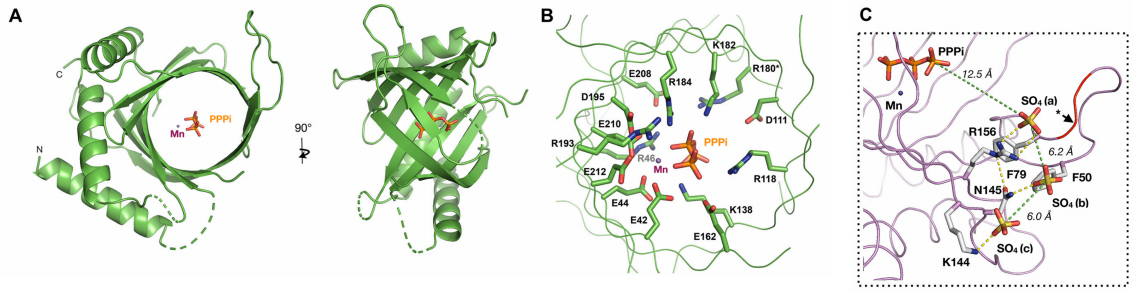

Fig 2: (A) Crystal structure of TcCet1(18–243) is depicted as a cartoon model. PPPi is shown as sticks, and manganese is shown as a magenta sphere. (B), cross-section of the triphosphate tunnel of TcCet1(18– 243). (C) PPPi and manganese were modeled on the TcCet1(18–243)·$C_{13}H_{13}NO_2$ structure from the structure of PPPi·Mn–bound complex. C, close-up view of SO_4-binding site.

(5) **Cleavage of 3′-terminal adenosine by RNA ligase** - In addition to RNA recapping, we extended our project to characterize a novel RNA modifying activity possessed by RNA ligase. The *Methanobacterium* ATP-dependent RNA ligase not only circularizes the RNA with 5'-phosphate and 3'-OH end but could cleave the adenine nucleoside from the 3'-termini of the RNA. Our findings raise possibilities for unexpected mechanisms whereby the RNA ligase could act as an RNA surveillance or editing enzyme to selectively remove the 3′-adenosine of an RNA to convert the reactive 3′-hydroxyl group into 2′,3′-cyclic phosphate and thereby regulate its metabolism.

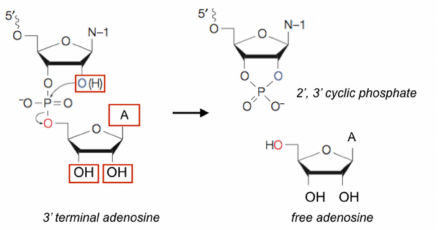

***Fig 3**: Proposed mechanism of 3′-deadenylation by archaeal ATP-dependent RNA ligase. Functional groups on the RNA that are required for the 3′-deadenylation reaction are boxed in red.*

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　2件）

１．著者名 Shigeo Yoshinari, Yancheng Liu, Paul Gollnick and C. Kiong Ho	４．巻 7
２．論文標題 Cleavage of 3′-terminal adenosine by archaeal ATP-dependent RNA ligase	５．発行年 2017年
３．雑誌名 Scientific Reports	６．最初と最後の頁 11692-11705
掲載論文のDOI（デジタルオブジェクト識別子） 10.1038/s41598-017-11693-0	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

１．著者名 Yuko Takagi, Naoyuki Kuwabara, Truong Tat Dang, Koji Furukawa, C. Kiong Ho	４．巻 295
２．論文標題 Crystal structures of the RNA triphosphatase from Trypanosoma cruzi provide insights into how it recognizes the 5'-end of the RNA substrate.	５．発行年 2020年
３．雑誌名 Journal of Biological Chemistry	６．最初と最後の頁 9076-9086
掲載論文のDOI（デジタルオブジェクト識別子） 10.1074/jbc.RA119.011811	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

〔学会発表〕　計13件（うち招待講演　1件／うち国際学会　7件）

１．発表者名 Anna V. Ignatochkina, Anilkumar R. Kore and Kiong Ho
２．発表標題 RNA methylation at the 5’-end of the mRNA stimulates Trypanosoma mRNA recapping activities
３．学会等名 Cold Spring Harbor, Translational Control Meeting（国際学会）
４．発表年 2018年

１．発表者名 Yuko Takagi, Naoyuki Kuwabar, Truong Dang, Koji Furukawa, Hitoshi Sakashita, Kiong Ho
２．発表標題 Structural and Functional Analysis of Trypanosoma RNA Triphosphatase
３．学会等名 第88回日本寄生虫学会大会
４．発表年 2018年

１．発表者名
Ho Kiong, Ignatochkina Anna, Iguchi Jesavel, Kore Anilkumar R

２．発表標題
The messenger RNA recapping pathway in Trypanosome

３．学会等名
第88回日本寄生虫学会大会

４．発表年
2018年

１．発表者名
Yuko Takagi, Naoyuki Kuwabara, Truong Dang, Koji Furukawa, Hitoshi Sakashita, Kiong Ho

２．発表標題
Structural and Functional Analysis of protozoan mRNA capping enzyme

３．学会等名
第20回日本RNA学会年会

４．発表年
2018年

１．発表者名
Kiong Ho

２．発表標題
Targeting of mRNA Cap Formation for Treatment of Parasitic Infections

３．学会等名
The Joint Symposium in Infectious Diseases（招待講演）（国際学会）

４．発表年
2019年

１．発表者名
Kiong Ho

２．発表標題
Trypanasome mRNA recapping activity is modulated by hypermethylation derived from cap 4 structure

３．学会等名
第19回日本RNA学会年会　富山

４．発表年
2017年

１．発表者名
Yancheng Liu, Yuko Takagi, Akira Hirata, Hiroyuki Hori, Katsuhiro Murakami, C. Kiong Ho

２．発表標題
Archaea RNA ligase is required for circularizing small non-coding RNA

３．学会等名
第19回日本RNA学会年会　富山

４．発表年
2017年

１．発表者名
C. Kiong Ho, Shigeo Yoshinari, Katsuhiko S. Murakami

２．発表標題
Structure and mechanism of archaeal ATP-dependent RNA ligase.

３．学会等名
Cutting Edge Developments in RNA Biology for the Control of Gene Expression.（国際学会）

４．発表年
2017年

１．発表者名
Kiong Ho

２．発表標題
Molecular Mechanism of mRNA Recapping in Trypanosome.

３．学会等名
2017年度生命科学系学会合同年次大会

４．発表年
2017年

１．発表者名
Anna V. Ignatochkina, Yuko Takagi, Jesavel Iguchi and C.Kiong Ho

２．発表標題
Identification and characterization of a novel 5'-monophosphate RNA kinase in Trypanosoma brucei

３．学会等名
21th Annual meeting of the RNA Society（国際学会）

４．発表年
2016年

１．発表者名 Ho CK, Ignatochkina AV, Takagi Y and Nagata K.
２．発表標題 Recapping the Message: The mRNA Decapping and Recapping Pathway in Trypanosomes
３．学会等名 21th Annual meeting of the RNA Society（国際学会）
４．発表年 2016年

１．発表者名 Anna V. Ignatochkina, Jesavel Iguchi, Anilkumar R. Kore and C.Kiong Ho
２．発表標題 Functional characterization of RNA re-capping enzymes in kinetoplastid parasites
３．学会等名 Gordon Research Seminar - RNA Editing（国際学会）
４．発表年 2017年

１．発表者名 Yuko Takagi, Naoyuki Kuwabar, Truong Dang, Koji Furukawa, Hitoshi Sakashita, Kiong Ho
２．発表標題 トリパノソーマRNA triphosphataseの結晶構造解析による三リン酸ならびに核酸認識機構の解明
３．学会等名 第42回日本分子生物学会（国際学会）
４．発表年 2019年

〔図書〕　計1件

１．著者名 C. Kiong Ho	４．発行年 2017年
２．出版社 Humana Press, New York, NY	５．総ページ数 9
３．書名 mRNA Processing. Methods in Molecular Biology	

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関