様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

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研究課題名（和文）Structure and biological targets of Trypanosome mRNA recapping enzyme

研究課題名（英文）Structure and biological targets of Trypanosome mRNA recapping enzyme

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交付決定額（研究期間全体）：（直接経費）　　3,300,000円

研究成果の概要（和文）：本研究は、トリパノソーマのmRNAリキャッピング酵素の反応メカニズムと生物学的機能を解明することを目的としている。Cap4と呼ばれるRNAの5'末端におけるハイパーメチル化は、トリパノソーマのmRNAリキャッピング活性を促進することを明らかにした。トリパノソーマのmRNAリキャッピング酵素の欠損は、哺乳類宿主への感染性に深刻な影響を及ぼす。 RNASeq解析から、多くの発生的に調節された遺伝子がmRNAリキャッピングによって制御されていることが示唆された。さらに、キャッピングされていない転写産物の解析から、リキャッピング酵素を欠損した寄生虫に蓄積する推定mRNA標的が同定された。

研究成果の学術的意義や社会的意義
RNA修飾は、無数の生物学的プロセスにおけるその重要な役割と、様々な疾患との関連性から、大きな注目を集めている。本研究では、トリパノソーマのmRNAキャップがメチル化されることの意義を探求し、哺乳類細胞におけるキャップメチル化の影響を理解するためのより広い示唆を得ることができた。mRNAのリキャップ経路を比較解析することで、寄生虫感染に対する新たな創薬標的が見つかる可能性がある。

研究成果の概要（英文）：This research aims to understand the mechanism and biological function of mRNA recapping enzyme in trypanosome. The hypermethylation at the 5' end of RNA called cap 4 stimulates trypanosome mRNA recapping activities, up to two orders of magnitude. Deletion of mRNA recapping enzyme in trypanosome shows severe impact on infectivity to mammalian host. Transciptosome analysis suggests that number of developmentally controlled genes are regulated by mRNA recapping. Analysis of uncapped transcripts identified putative mRNA targets, which were accumulated in parasite deleted for recapping enzyme.

研究分野：分子寄生虫学

キーワード：RNA キャッピング　トリパノソーマ　RNA プロセッシング　寄生虫

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

The 5′ end of all eukaryotic mRNA is modified by adding methylguanosine cap (m7GpppN or cap 0) to protect mRNA from degradation and enhance protein synthesis. As a part of the mRNA turnover process, the cap can be removed by decapping enzyme, and the remaining 5′-phosphorylated RNA (pRNA) is thought to be rapidly degraded by a 5′-to-3′ exonuclease (Xrn1/Rat1). However, the uncapped transcripts are detected in the cells and could later become translationally active by reacquiring the cap in the cytoplasm. In trypanosomes, cytoplasmic capping enzyme (TbCe1) possesses RNA kinase and guanylyltransferase activities that convert pRNA into GpppRNA by forming ppRNA intermediate. Depletion of TbCe1 results in the accumulation of pRNA and, therefore, has been proposed to function to regenerate translatable mRNA by converting decapped pRNA into capped mRNA.

２．研究の目的

In trypanosomes, genomes are organized into polycistronic clusters, and post-transcriptional events play central roles in controlling gene expression. The mRNA recapping pathway could potentially regulate the abundance and stability of selective mRNA. The objective of this research is to understand the mechanism of mRNA recapping and target selection. The key questions to be addressed in this research are: (i) Does differential cap 4 modification at the 5'-end of the mRNA regulate the mRNA recapping pathway? (ii) What kind of transcripts are regulated by mRNA recapping pathways? Is there any specific biological process mRNA recapping controls during the parasite life cycle?

３．研究の方法

We evaluate the effect of RNA methylation on RNA recapping activity using a series of chemically synthesized RNA substrates, which have one or more cap 4 methylations at the 5’ end. We generated a homozygous knock-out of the mRNA recapping enzyme in *T.cruzi* to characterize the expression profile of genes affected by recapping enzyme deletion. We modified to improved the RNA-ligation mediated RT-PCR method to specifically identify uncapped mRNA that has undergone trans-splicing from the parasite (see Fig 3 for details).

４．研究成果

(I) **Effect of RNA methylation on T. brucei recapping enzyme (TbCe1) activity** - All the mature mRNA in the trypanosome is capped and hypermethylated at the 5'-end, acquired through trans-splicing of an SL RNA. If TbCe1 acts as a recapping enzyme to convert decapped mRNA, a physiological substrate for the TbCe1 should preserve all the methylations present on the cap 4 structure. We demonstrated that the RNA kinase activity is stimulated by two orders of magnitude on a hypermethylated pRNA derived from cap 4 (Fig 1). The *N6, N6-2'-O* trimethyladenosine modification on the first nucleotide was primarily accountable for enhancing the RNA kinase and the guanlylyltransferase activity of TbCe1. In contrast, *N6* methyladenosine severely inhibits the guanlylyltransferase activity of the mammalian enzyme. Furthermore, we showed that TbCmt1 cap (guanine N7) methyltransferase was localized in the cytoplasm, and its activity was also stimulated by hypermethylation at 2'-O ribose, suggesting that TbCe1 and TbCmt1 act together as a recapping enzyme to regenerate translatable mRNA from decapped mRNA. These findings provide strong evidence that TbCe1 preferentially acts on hypermethylated uncapped mRNAs and is likely to be a physiological substrate for recapping. This work was published in *Nucleic Acid Research*.

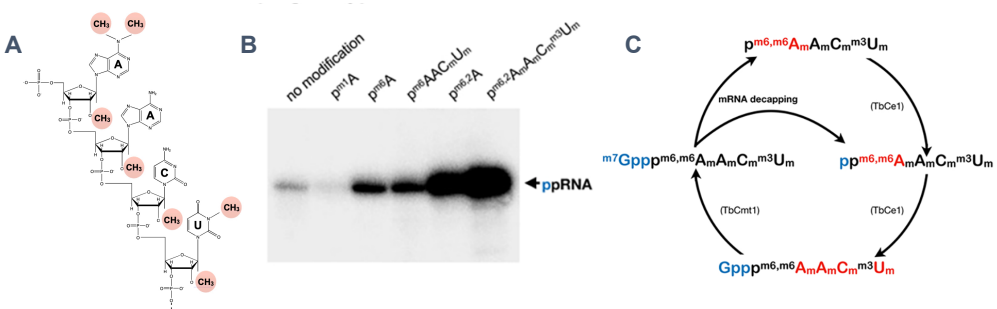

Figure 1: TbCe1 RNA kinase and guanylyltransferase activities are enhanced by hypermethylation on the 5′ end of pRNA. (A) Structure of hypermethylated pRNA (pm6,2AmAmCmm3Um) derived from decapped cap 4. (B) RNA kinase activity on modified pRNA. (C) Summary of recapping by TbCe1 and TbCmt1. Methylations highlighted in red enhance respective activities.

(II) **Analysis of *T.cruzi* recapping enzyme (TcCe1) knock-out** - We successfully generated homozygous knock-out of TcCe1. Initial characterization in epimastigote shows no change in growth phenotype. However, a severe impact on infectivity was observed when parasites were differentiated into trypomastigotes. These results suggest that TcCe1 could regulate the gene involved in parasite entry into the host or the growth of amastigote in the

mammalian host. RNA-Seq analysis shows 204 common genes were down-regulated by TcCe1 knock-out in both epimastigotes and trypomastigotes (Figure 2). There were 79 and 32 genes that were specifically down-regulated by TcCe1 deletion in epimastigotes and trypomastigotes, respectively (Fig 2). Majority of these transcripts were stage-specific genes, implies that mRNA recapping enzyme regulates expression of developmentally regulated mRNAs. Furthermore, number of these genes that are affected by TcCe1 deletion were derived from a particular chromosome locus that encodes for a polycistronic transcript, suggest that TcCe1 may affect the expression of transcription or RNA processing factors that control polycitronic transcription.

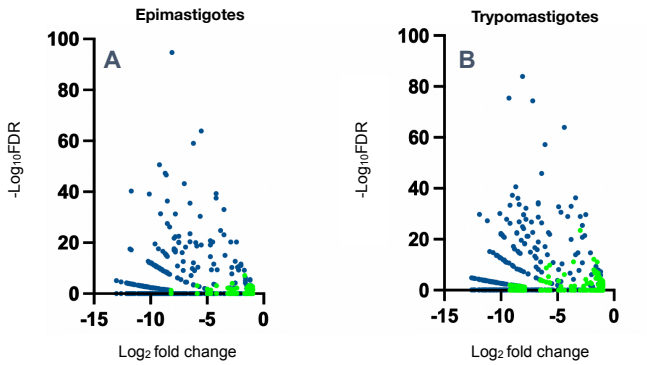

Figure 2: Effect of TbCe1 knock-out on gene expression. (A) Genes that were down-regulated by two-fold or more (FDR<0.05) in epimastigotes (left panel) and trypomastigotes (right panel). Blue dots indicate genes that were down-regulated in both epimastigotes and trypomastigotes. Green dots indicate gene that were specifically down-regulated in epimastigotes or trypomastigotes.

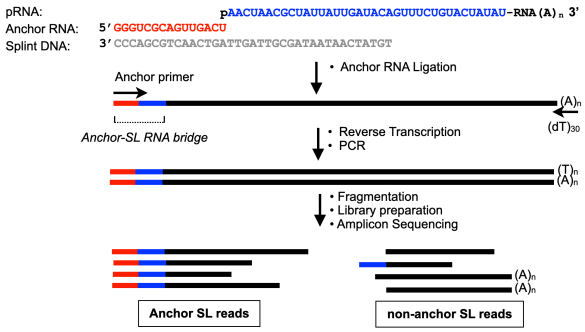

Figure 3. Schematic of RNA ligation mediated RT-PCR. The anchor RNA and splint DNA, whose 3' half is complementary to the anchor RNA sequence and whose 5' half is complementary to the SL RNA sequence are mixed with poly(A) RNA. Ligation-competent RNAs are detected by RT-PCR using DNA primers corresponding to the 5'-anchor and oligo dT30 for the PCR, and was further subject to ampicon sequenced. Reads containing anchor and spliced leader sequence were scored.

(II) **Analysis of uncapped transcript in *T.cruzi* recapping enzyme (TcCe1) knock-out.** RNA ligation-mediated RT-PCR (Fig 3) identified 27 uncapped transcripts reduced/absent in the knock-out, encoding ribosomal proteins, surface proteins, and enzymes involved in nucleic acid metabolism. Comparative analysis, together with the transcriptome data, is underway to determine how recapping pathways are regulated in kinetoplastid parasites. Progress on this work was presented in 24th RNA Society Meeting of Japan in Okinawa, and 93rd Parasitology Society of Japan Meeting in Tokyo, Japan.

(III) **Identification and characterization of a novel cap-dependent RNA methyltransferase** - To determine if cap 4 methylation is altered in trypanosome mRNA, mass spectrometry revealed additional methyl modifications on the spliced leader segment of *T. brucei* mRNA. A new cap-dependent RNA methyltransferase, responsible for these modifications, was characterized from T. brucei. This enzyme shows optimal activity when the RNA 5'-end is hypermethylated with cap 4. The presence of these modifications suggests a potential regulatory role in trans-splicing and mRNA processing in kinetoplastids. This research highlights the complexity of mRNA modification in *T. brucei* and its potential impact on gene regulation and parasite biology. This finding was presented at the 92nd Parasitology Society of Japan Meeting in Kanazawa, Japan.

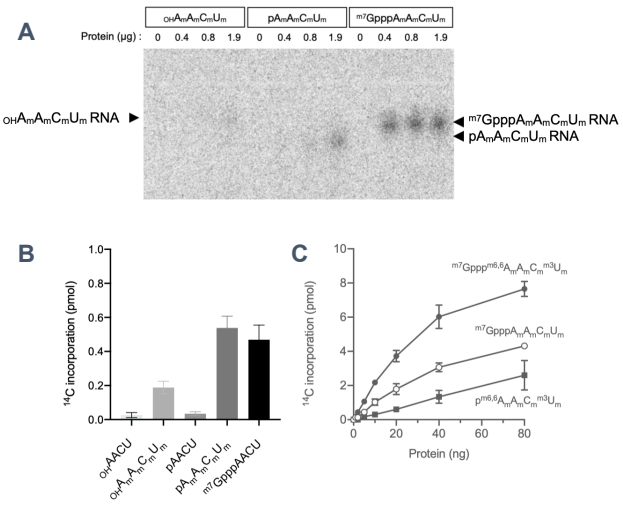

Figure 4. Characterization of T. brucei cap4 -dependent RNA methyltransferase activity (A) Reaction mixture contained[^{14}CH3] AdoMet with either hydroxyl-terminated 24-mer RNA, 5' monophosphate terminated 21-mer RNA or m7G capped 21-mer RNA with indicated amount of protein. RNA products were separated on 18% polyacrylamide gel and visualized by phosphoimager. (B) and (C) Effect of 5'-cap and ribose methylations on the RNA substrate.

(IV) **Genetic and functional analysis of Archaeal RNA ligase.** We extended research project characterized an RNA ligase function in archaea, complementing your studies on RNA recapping. can recognize pRNA. Similar to the recapping enzyme, the archaeal RNA ligase can recognize pRNA. However, instead of adding caps, it catalyzes the formation of phosphodiester bonds. This RNA ligase preferentially circularizes C/D box small nucleolar RNAs, and plays a role in ribosomal RNA processing. This work was published in Frontiers in Molecular Biosciences and 24th RNA Society Meeting of Japan in Okinawa.

５．主な発表論文等

〔雑誌論文〕　計3件（うち査読付論文　3件／うち国際共著　3件／うちオープンアクセス　3件）

１．著者名 Kiss Daniel L.、Vasudevan Deepika、Ho C. Kiong、Caliskan Neva	４．巻 9
２．論文標題 Editorial: mRNA Translational Control as a Mechanism of Post-transcriptional Gene Regulation	５．発行年 2022年
３．雑誌名 Frontiers in Molecular Biosciences	６．最初と最後の頁 Article 947516
掲載論文のDOI（デジタルオブジェクト識別子） 10.3389/fmolb.2022.947516	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 Liu Yancheng, Takagi Yuko, Sugijanto Milyadi, Nguyen Kieu Duong My, Hirata Akira, Hori Hiroyuki, Ho C. Kiong	４．巻 9
２．論文標題 Genetic and Functional Analyses of Archaeal ATP-Dependent RNA Ligase in C/D Box sRNA Circularization and Ribosomal RNA Processing	５．発行年 2022年
３．雑誌名 Frontiers in Molecular Biosciences	６．最初と最後の頁 811548-811560
掲載論文のDOI（デジタルオブジェクト識別子） 10.3389/fmolb.2022.811548	査読の有無 有
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１．著者名 Anna V. Ignatochkina, Jesavel A. Iguchi, Anilkumar R. Kore, and C. Kiong Ho	４．巻 52
２．論文標題 Trypanosome mRNA recapping is triggered by hypermethylation originating from cap 4	５．発行年 2024年
３．雑誌名 Nucleic Acid Reserach	６．最初と最後の頁 e000000000
掲載論文のDOI（デジタルオブジェクト識別子） なし	査読の有無 有
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〔学会発表〕　計6件（うち招待講演　0件／うち国際学会　0件）

１．発表者名 Kiong Ho, Anna Ignatochkina, Shinichiro Akichika, Tsutomu Suzuki
２．発表標題 Modification Beyond the Hypermethylated Cap 4 Structure in Trypanosoma Spliced Leader RNA.
３．学会等名 第92回日本寄生虫学会大会
４．発表年 2023年

１．発表者名
Kieu Duong My Nguyen, Yuko Takagi, and Kiong Ho

２．発表標題
The Identification and Characterization of RNA Recapping Targets in Trypanosomes.

３．学会等名
The 3rd University of Science- University of Tsukuba Joint Symposium in Biomedical Science

４．発表年
2022年

１．発表者名
Liu, Yancheng, Yuko Takagi, Milyadi Sugijanto, Kieu Duong My Nguyen, Akira Hirata, Hiroyuki Hori, and C. Kiong Ho.

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2022年

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Anna Ignatochkina, Shinichiro Akichika, Tsutomu Suzuki, Kiong Ho

２．発表標題
Characterization of Trypanosoma brucie cap-dependent RNA methyltransferase involved in hypermethylation of spliced leader RNA

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第23回日本RNA学会

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１．発表者名
Anna Ignatochkina

２．発表標題
Development of a Tet-inducible CRISPR-Cas9 system to elucidate the role of cap 4 methylation in Trypanosoma cruzi

３．学会等名
第90回日本寄生虫学会・第32回日本臨床寄生虫学会 合同大会

４．発表年
2021年

１．発表者名 Anna Ignatochkina
２．発表標題 Characterization of cap methyltransferase in Trypanosoma brucei
３．学会等名 Tsukuba Global Science Week Conference 2021
４．発表年 2021年

〔図書〕　計0件

〔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
研究分担者	高木　悠友子 (Takagi Yuko) (50783669)	国立研究開発法人産業技術総合研究所・生命工学領域・研究員 (82626)	

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