様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

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研究課題名（和文）Adaptive RNA editing in Cephalopods

研究課題名（英文）Adaptive RNA editing in Cephalopods

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研究成果の概要（和文）：私はイカの視葉から高品質な単一細胞の分離およびソーティングのためのプロトコルを標準化することに成功しました。また、生後1日から7日のイカにおいて、サイズと粒度が異なる独自の細胞集団を特定しました。これらの高品質な細胞のmRNAをシーケンシングすることで、特にUTR領域において、Ty3hのようなドーパミン作動性遺伝子マーカーのアイソフォーム特異的編集が明らかになりました。さらに、RNA編集が細胞型特異性の明確なパターンなしにトランスポーザブルエレメントにおいて顕著に発生することを発見しました。

研究成果の学術的意義や社会的意義
We found that dopaminergic cell types, the most abundant in the optic lobe, exhibit differential RNA-editing patterns in gene markers, particularly in regulatory regions. We expect to fully characterize cephalopod-specific and evolutionarily conserved regulatory networks that underlie RNA editing.

研究成果の概要（英文）：I successfully standardized a protocol for the isolation and sorting of high-quality single cells from the optic lobe of squid. I have also identified distinct cellular populations in squids that are 1 to 7 days old, differing in size and granularity. Sequencing the mRNA of these high-quality cells revealed isoform-specific editing in dopaminergic gene markers such as Ty3h, particularly in the UTR regions. Additionally, we found that RNA editing occurs significantly in transposable elements without a clear pattern of cell-type specificity.

研究分野：Evolutionary Biology

キーワード：RNA editing　cephalopods　single-cell mRNA　neural organs

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

Coleoid cephalopods are found in tropical, temperate, and polar regions. Because these animals are poikilotherms, they need to regulate many neurophysiological processes while adapting to the temperature of their environment. One unusual proposed adaptive mechanism is the Adenosine-to-inosine (A-to-I) messenger RNA editing, which is a post-transcriptional event catalyzed by adenosine deaminases acting on RNA. RNA editing is a fine-tuning mechanism conserved across many metazoans where adenine is converted into inosine, which is read as guanine by cellular transcriptional machinery[1]. Garrett and Rosenthal (2012)[2] showed that RNA editing produces amino acid changes in the potassium channel's pore of polar octopus, influencing the rates of repetitive firing. Such a modification, however, was not presented in the potassium channel of a related tropical octopus, suggesting that the editing mechanism in cephalopods may be a response to acclimation to different temperatures.

The availability of cephalopod genomic resources, including the カリフォルニア・ツースポットタコ *Octopus bimaculoides*[3] and ハワイ産のダンゴイカ *Euprymna scolopes*[4] genomes, and the increasing transcriptome sequencing of many species and organs has shown that mRNA editing is much more extensive in coleoid cephalopods than other animals, and is particularly enriched in the nervous system[3 - 6]. In coleoid neural systems, editing occurs in both coding and non-coding regions, with a high percentage occurring in non-synonymous sites, diversifying their proteome[5].

２．研究の目的

This KAKENHI Project 22K15085 aimed to understand how A-to-I RNA editing in neural tissues contributes to the thermal adaptability of cephalopods. I specifically aim to characterize these sites at the cellular level to understand whether this process follows a cell-type specificity pattern or not. As a model organism, I used the Japanese bobtail squid *E. berryi*, a species cultured yearly in our laboratory. I also target the optic lobe, a neural organ involved in the process of environmental information and part of the visual learning and memory circuit of cephalopods. The cellular diversity of the optic lobe was previously characterized by Gavriouchkina et al., 2022[7].

３．研究の方法

To characterize RNA editing sites at the cellular level, I initially used confocal imagining to identify the morphology and composition of cells in the optic lobe of squid. Then, I successfully established a

protocol to obtain full-length mRNA from this high-quality organ using the Fluorescence-activated Cell Sorting (FACS) device as a cell sorter. I sequenced these cells using short-read Illumina reads, enabling a preliminary characterization of RNA editing sites across distinct molecular cell types. FACS also allowed me to characterize cellular populations using size, granularity, and frequency.

４．研究成果

We identify the distinct morphology present in the optic lobe of *E. berryi*: the cortex, with the granule and plexiform layer (GL, PL); and the medulla (ME) (**Fig. 1**).

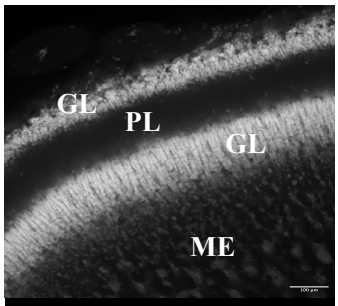

Fig. 1: Confocal image of the optic lobe showing the cortex (granule and plexiform layer (GL, PL)) and the medulla (ME).

I also found that the optic lobe of 1 to 7-day-old squids presents two distinct cell populations, differentiated primarily by their size, granularity, and frequency, as shown by the Forward Scatter Height (FSC-H) parameter. Interestingly, the larger cell population is less frequent. It undergoes a progressive reduction in abundance as the bobtail squid matures. I anticipate that these two distinct populations undergo a different regulatory process that contributes to the developmental processes of the bobtail squid's optic lobe.

Using my current sequencing reads from optic lobe single cells; I have preliminarily identified several genes under editing, particularly repetitive elements. I also found UTR-specific isoforms within dopaminergic cell types, such as the Tyrosine Hydroxylase (Ty3h) gene, which converts tyrosine to dopamine (**Fig. 2**).

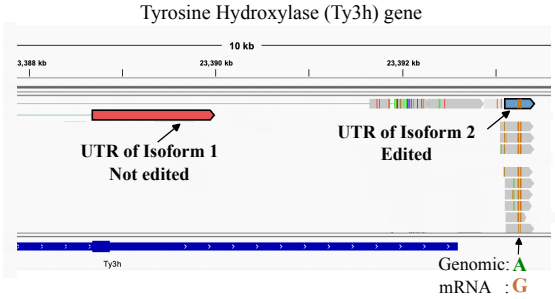

Fig. 2: Isoform-specific RNA editing in the Ty3h gene

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５．主な発表論文等

〔雑誌論文〕　計0件

〔学会発表〕　計0件

〔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

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