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研究課題名(英文) The regulatory origin of cephalopod limbs

## 研究代表者

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研究成果の概要(和文)：我々は、腕の同一性に関与する差異を予測し、新規パターンングがどのように展開されるかを理解するために、ヤリイカ胚の四肢芽の制御プロファイルを取得した。ピークコールを行い、足跡解析と連動した差分ピーク活性化解析を行った。いくつかの重要な遺伝子を同定し、in situ ハイブリダイゼーション技術に対応するプローブを注文した。

## 研究成果の学術的意義や社会的意義

Development of new cephalopod model and new molecular biology techniques. Cephalopod have a strong cultural important (especially in Japan). Better understanding of biological novelties at regulatory level

研究成果の概要(英文)：Our plan was to obtain RNA-seq and ATAC-seq library for each limb bud of a bobtail squid embryo in order to predict differences in transcription factor binding that would be involved in arm identity. We wanted to understand how a novel patterning could be deployed. We performed these experiments and sequenced corresponding libraries. We performed peak calling and differential peak activation analysis, which has been coupled with foot printing analysis. We identified some key genes and ordered corresponding probes for HCR, an advanced in situ hybridisation technique. In parallel, we optimised protocols for HCR imaging of squid embryos and fixed a lot of embryos. Some key results have been integrated in a paper about squid single-cell sequencing that will be submit in 2022.

研究分野：Genomics, evodevo

キーワード：Cephalopod limb evolution regulation

様式 C - 19、F - 19 - 1、Z - 19 (共通)

## 1 . 研究開始当初の背景

The patterning mechanisms governing the regulation of the main animal body axis originated in the last ancestor of all existing animals. Over the course of evolution, appendages or limbs evolved convergently in tetrapods, arthropods, and cephalopods. In tetrapods, the patterning of limbs follows similar regulatory principles as that of the primary body axis, particularly involving the iconic Hox genes, which is an example of the evolutionary principle of co-option which is a fundamental way in which new characters appear. However, the regulatory principles underlying the possibility to re-use genes and genetic pathways remain elusive. At the genome level, gene expression is controlled by regulatory elements, such as enhancers and promoters, and their activity shapes the three-dimensional architecture of chromatin. My previous research compared the regulatory architecture of vertebrate Hox genes, with that of amphioxus, one of their closest invertebrate counterparts, and showed that a radical change of regulatory architecture likely accompanied the novel role of Hox in limb regulation. The goal of this project was to parallel this research in a cephalopod, to determine whether common or alternative principles govern the regulation of limb patterning.

Our unit has sequenced and assembled the full genome of the Japanese bobtail squid that represents a platform for data analysis and interpretation and has carried out extensive scRNA-seq on the squid visual system (<https://www.biorxiv.org/content/10.1101/2022.05.26.490366v1>).

The goal of the present project was to show which molecular actors are involved in limb patterning in cephalopods, which new regulatory mechanisms triggered the expression of Hox genes, and whether other putative actor were utilized in limbs which varied from their ancestral role in antero-posterior patterning. More generally, the goal of this project was to inform us of the way in which cephalopods, one of the most astonishing and complex group of animals outside vertebrates regulate their genome.

## 2 . 研究の目的

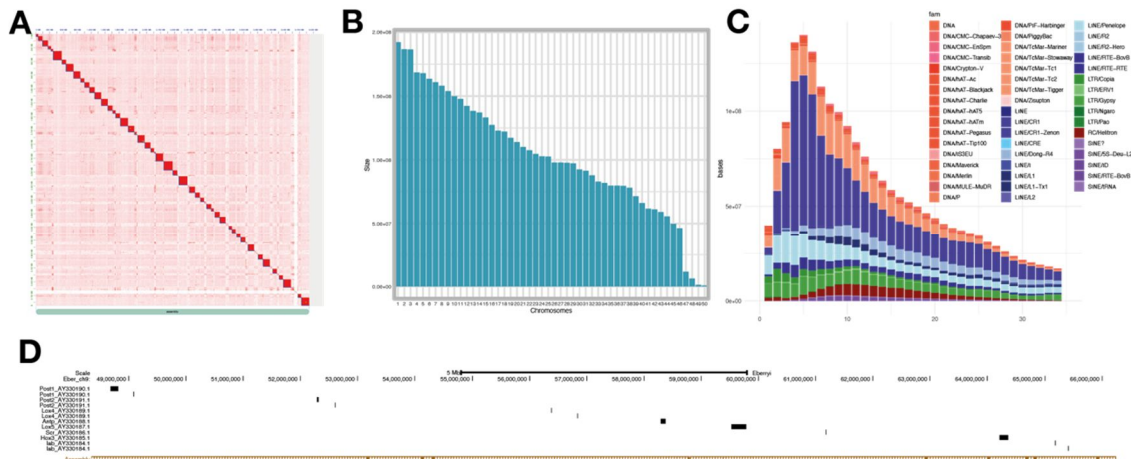
Cephalopods present an elaborate body plan among molluscs with a refined visual and sensory system, ten prehensile appendages with sophisticated motor control. They also possess a large complex genome with specific gene family expansions and a unique 'mega-Hox cluster' preserving gene order, but spanning over a much larger genomic region than the ones known in other animal lineages. Previous work showed that Hox genes play a role in controlling the identity of each cephalopod appendage. However, the diversity of molecular actors involved, as well, as the genomic elements controlling their expression are not characterised in detail. We want to use a combination of regulatory profiling techniques to further characterise these. Our lab has developed the bobtail squid *Euprymna berryi* native to Japan as a model organism for development and genomics. Preliminary work showed that limb buds at patterning (stages 18-19) could be dissected and processed individually for molecular biology. We wanted to perform RNA-seq to profile gene-expression, ATAC-seq which relies on in vitro transposition to assess open chromatin region, and Hi-Chip that uses a combination of chromatin conformation capture and Chip-seq on histone mark characteristic of activate transcription (H3K4me3) to capture the chromatin architecture around expression stages. My previous work had already demonstrated that these techniques were applicable to marine invertebrates, and I have furthermore established a computational framework for the comparative analysis. On the longer term, we wish to perform single-cell RNA-seq on each limb buds to capture the cell heterogeneity, and patterning mechanisms controlling their development.

## 3 . 研究の方法

We assembled a highly contiguous chromosome-scale reference genome for the Japanese bobtail squid *E. berryi* by combining long-read sequencing with chromatin conformation capture data. We generated 30x Pacbio data complemented with paired-end and linked-reads Illumina data starting from high molecular weight genomic DNA extracted using agarose plug. Our assembly of the *E. berryi* genome totals 5.9 Gb and captures the 46 chromosomes found in decapods in a more contiguous fashion (N50 chromosome length: 113.96 Mb, N50 contig length: 827 kb) than other available cephalopod genomes. For instance, we recovered the massive 17 Mb Hox cluster as an intact locus on chromosome 9. Different expansions of LINE transposons and other repeats may explain the difference in genome size as compared to other cephalopod species.

To support single cell and comparative analysis, we generated a combination of bulk short-

read and long-read RNA-seq (Iso-seq) from diverse neural and non-neural tissues, as well as a range of whole animal embryonic stages (Table S2). We used this transcriptome data to annotate 32,244 protein-coding genes in *E. berryi*, with detectable homology in other animals. The genome was assembled using wtdbg2 and subsequently polished using Pacbio and Illumina data as well as scaffolding using linked-reads. Finally, the genome was scaffolding using proximity ligation data (HiC) to read chromosome scale.



**Figure 1: genome assembly and annotation**

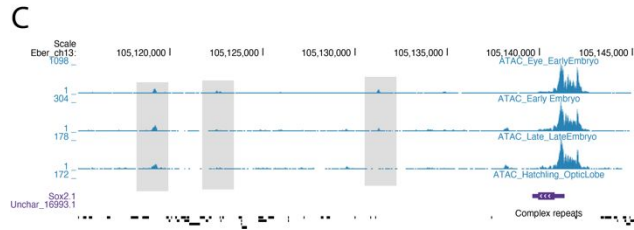
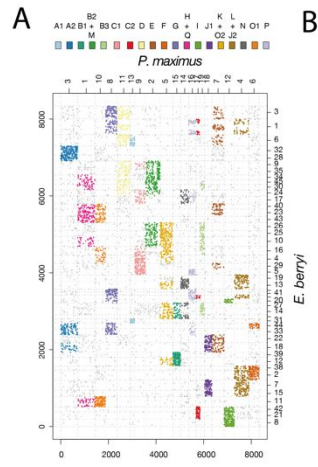
For annotation and analysis of gene expression during development, RNA was extracted from multiple tissue and organs, and processed using both RNA-seq and Isoseq approaches.

We also optimised extraction of RNA-seq from dissected limb buds and small input ATAC-seq and collected several replicates which gave successful libraries and that were sequenced. We expect these datasets to be incorporated in a publication to be finalised in 2022.

#### 4 . 研究成果

- The genome of *E. berryi* revealed a very interested pattern of genome evolution in cephalopods with a fusion of ancestral linkage groups present in other animals and well characterised, for instance in the sea scallop. This confirms that cephalopods might have undergone some regulatory rearrangements driven by these genomic changes (Figure 2a).
- We introduced a new technique of in situ hybridisation in the bobtail squid, HCR staining, which show great promise (Figure 2b).
- We generated preliminary ATAC-seq data on embryonic stages and limb binds and obtained a proof of principle that they accurately detected cis-regulatory elements (Figure 2c).
- The resources established in the course of this grant were instrumental in the interpretation and analysis of single-cell data. This that allowed to characterise the nervous and visual system of the squid *E. berryi* (see <https://www.biorxiv.org/content/10.1101/2022.05.26.490366v1>).

Figure 2. (A) Structural of *E. berryi* genome and ancestral linkage groups (B) HCR staining of the FMRF and NPY neuropeptide receptor (Fmar) gene in the bobtail squid central and peripheric (tentacle) nervous sytem (C) Genome browser scrensht in the vicinity of the gene Sox2 involved in control of embryonic development. Grey boxes indicate putative regulatory element.



## 5. 主な発表論文等

〔雑誌論文〕 計8件（うち査読付論文 0件／うち国際共著 0件／うちオープンアクセス 0件）

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3. 雑誌名 Nature Ecology & Evolution	6. 最初と最後の頁 231 ~ 242
掲載論文のDOI（デジタルオブジェクト識別子） 10.1038/s41559-020-01327-6	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 -
1. 著者名 Peijnenburg Katja T. C. A., Janssen Arie W., Wall-Palmer Deborah, Goetze Erica, Maas Amy E., Todd Jonathan A., Marletaz Ferdinand	4. 巻 117
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1. 著者名 Sanchez Gustavo, Fernandez-Ivarez Fernando., Taite Morag, Sugimoto Chikatoshi, Jolly Jeffrey, Simakov Oleg, Marletaz Ferdinand, Allcock Louise, Rokhsar Daniel S.	4. 巻 4
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1. 著者名 Xu Fei, Marletaz Ferdinand, Gavriouchkina Daria, Liu Xiao, Sauka-Spengler Tatjana, Zhang Guofan, Holland Peter W. H.	4. 巻 12
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3. 雑誌名 Genome Biology and Evolution	6. 最初と最後の頁 evab179
掲載論文のDOI (デジタルオブジェクト識別子) 10.1093/gbe/evab179	査読の有無 無
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1. 発表者名 Ferdinand Marletaz
2. 発表標題 Evolution of Novelty
3. 学会等名 University of Bristol (招待講演)
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
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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
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