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研究課題名（和文）Regulatory evolution of the turtle shell

研究課題名（英文）Regulatory evolution of the turtle shell

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研究成果の概要（和文）：形態的新機軸とは、明確な祖先的な構造がなく現れる、全く新しい、進化的新規形質のことである。カメの甲羅の起源を明らかにする研究は、研究者を１世紀以上も魅了し続けている、とても興味深く古典的な問題です。しかしカメがどのように甲羅を獲得したのかは、まだはっきりと分かっていません。
本研究では、これらを明らかにするために、最高水準のテクノロジーを利用しました。次世代シーケンス技術を用いて、ChIP-seq解析を行い、カメ特有である甲羅の発生に関わるcarapacial ridge（甲羅の隆起部）の発生をコントロールしていると思われる何百もの調節因子の候補を発見しました。

研究成果の概要（英文）：Morphological innovations are those evolutionary novelties that appear without a clear ancestral structure, totally de novo. The turtle shell is a classical morphological innovation that has mesmerized researchers over a century, and despite deep studies addressing its origin, how turtles acquire the shell remains a mistery. During my project, I have utilized state-of-the-art technologies in order to tackle this problem. By using techniques of next generation sequencing, like ChIP-seq, I have been able to establish hundreds of candidate regultatory elements that seem to be turtle-specific and linked to the carapacial ridge, the embryonic structure that controls de development of the turtle carapace.

研究分野：形態進化

キーワード：進化発生　遺伝　形態進化

１．研究開始当初の背景

(1) The evolutionary origin of morphological innovations requires the establishment of a new underlying mechanisms or the rewiring of parts of previous existing gene regulatory networks that allow the de novo invention of a previously inexistent structure. Understanding how these mechanisms are changed during evolutionary time is crucial to understand morphological evolution in general. Moreover, due to the uniqueness character of morphological innovations, their study and the genomic features coding them allow us to clearly study genotype-phenotype relationships, what eventually can help to establish general mechanisms of how genomic changes translate into phenotypic changes.

(2) The turtle shell is a genuine morphological innovation, not present in any other amniote vertebrate. The shell consists of two parts, a dorsal moiety, or carapace, and a ventral counterpart, or plastron. In this project I have studied the evolutionary development of the turtle carapace, by analyzing the genetic factors involved in the development of the carapacial ridge (CR): the embryonic structure that controls the development of the carapace.

２．研究の目的

(1) The major purpose of this project is to effectively find out which are these regulatory elements, from a genome-wide perspective, that are only present in turtles and not in other amniotes (such as the chicken and mouse), and that specifically regulate the expression of CR-specific genes.

３．研究の方法 Methods

Briefly, I have dissected hundreds of embryos of turtle and performed ChIP-seq analysis of several histone modifications: histone 3 lysine 4 tri-methylation (H3K4me3), which marks promoters; H3K27me3, which marks repressed loci; and H3K27 acetylation (H3K27ac) which marks active zones in the genome. The combination of all these marks allowed the identification of regions active in the CR and not active in other parts of the turtle embryo.

(1) Turtle and chicken eggs were obtained from local farmers and incubated until the desired stage: TK14 in the case of turtles and HH25 in the case of chicken.

(2) CRs, limbs and lateral body walls were microdissected from the above mentioned embryos in ice-cold PBS, fixed 20 min. with 1% formaldehyde and snap-frozen in liquid nitrogen.

(3) Chromosome immunoprecipitation (ChIP) of histone modifications (H3K4me3, H3K27ac and H3K27ac) were performed with commercial antibodies (abcam) according to standard protocols. The histone-bound DNA was disassociated and subjected to DNA library preparation and sequenced in HiSeq 1500 platforms in a sequencing support unit at RIKEN.

(4) ChIP-seq peaks were called by using MACS2 with default parameters, and comparisons between chicken and turtle datasets were done with BEDTOOLS.

４．研究成果 Results

(1) **Identification of DNA regions marked by histone modifications.** In general, and taking into account the thress tissues assayed in my experiments, I have found approximately an average of ~15 thousand elements marked with H3K4me3 (Fig. 1), indicating their putative role as promoters; ~40 thousand regions marked by H3K27ac (Fig. 1), indicating that these are active areas of the chromatine; and ~18 thousand of peaks called to be enriched in H3K27me3 (Fig. 1), i.e, that are repressed.

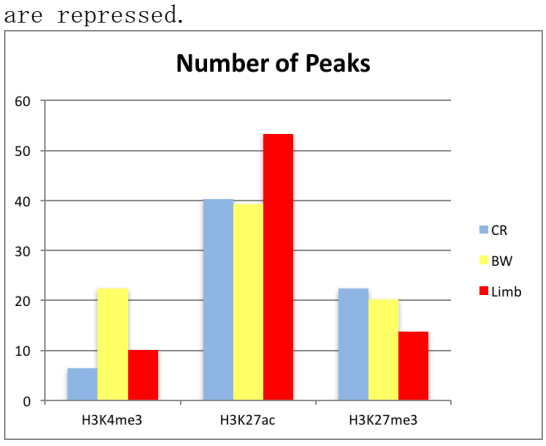

Figure 1. Number of peaks (thousands) enriched in three different histone modifications.

(2) Identification of promoters and enhancers. Next, I combined the datasets of H3K4me3 and H3K27ac in order to distinguish putative promoters from enhancers. Active promoters are generally

marked by both H3K4me3 and H3K27ac, while putative enhancers are only marked by H3K27ac, excluding H3K4me3 marks. Figure 2 shows that I have been able to identify between 40-50 thousand active regions, of which around ~6 thousand are active promoters, and the rest are enhancers. The tissue with more active regions was the limb, indicating that it develops via a more complex regulatory network.

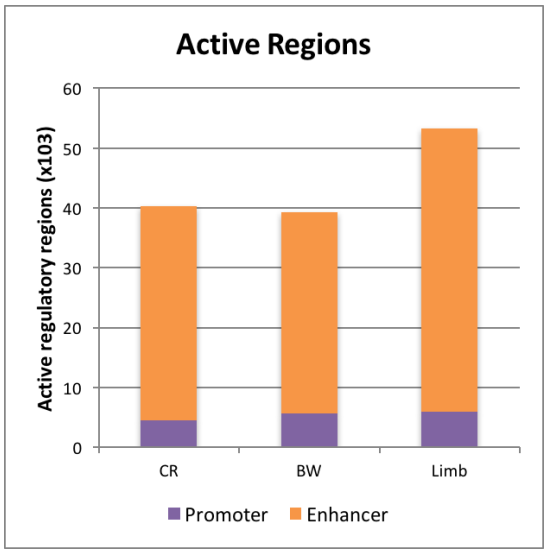

Figure 2. Number of active regions (in thousands) consisting of promoters and enhancers.

(3) CR-specific enhancers. The comparative analysis of the datasets from the three assayed tissues allowed me to identify those that are only active in the CR, not in limbs and body wall, meaning that they could have an important

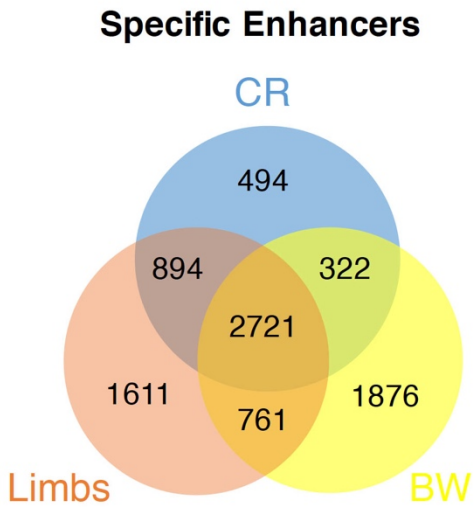

Figure 3. Venn diagram showing the enhancers identified in this study and how they are shared between tissues.

role in the formation of the carapace. Most of the active enhancers identified in this project are common in the three tissues (2721 out of 8679)(Fig. 3). The tissue with most specific enhancers was the lateral body wall, with a total of 1876 (Fig. 3). Although limbs had the majority of active regions, the proportion of those shared by other tissues was also the highest. Finally, I have been able to identify 494 enhancers that are specific to the carapacial ridge (Fig. 3).

５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計２件）

1. Hirasawa, T., Pascual-Anaya, J., Kamezaki, N., Taniguchi, M., Mine, K., Kuratani, S. (2015). The evolutionary origin of the turtle shell and its dependence on the axial arrest of the embryonic rib cage. J Exp Zool B Mol Dev Evol, 324B: 194-207. （査読あり）
Doi: 10.1002/jez.b.22579

2. Pascual-Anaya, J., Hirasawa, T., Sato, I., Kuraku, S., Kuratani, S. (2014) Comparative analysis of pleurodiran and cryptodiran turtle embryos depicts the molecular ground pattern of the turtle carapacial ridge. Int J Dev Biol, 58(10-11-12): 743-750. （査読あり）
Doi: 10.1387/ijdb.140296jp

〔学会発表〕（計５件）

1. Invited Talk at the Andalusian Center for Developmental Biology (Host: Dr. Ignacio Maeso): “Transcriptomics and epigenomics analyses of the development of the turtle shell”. April 28th 2015. Sevilla (Spain).

2. Invited Talk at the Reptile Evo-Devo Meeting (Host: Dr. Nathalie Feiner, University of Oxford) “Transcriptomics analysis of the development of the turtle shell”. April 24th 2015, Oxford, (UK).

3. [Oral] “Illuminating the evolutionary origin of the turtle shell by a comparative tissue-specific transcriptome analysis”. V Meeting of the European society

for Evolutionary Developmental biology (EED), Vienna, Austria, July 22 – 25, 2014.

4. [Poster] “Comparative analysis of embryonic tissue-specific transcriptomes of amniotes: illuminating the evolutionary origin of the turtle shell”. 2014 Annual Meeting of the Society for Molecular Biology and Evolution (SMBE), San Juan, Puerto Rico, June 8 – 12, 2014.
5. [Oral] “Evolutionary origin of the turtle shell: insights from a comparative transcriptomics analysis”. 47th Annual Meeting of the Japanese Society of Developmental Biologists (JSDB), WINC AICHI, Nagoya, Japan, May 27 – 30, 2014.

〔図書〕（計０件）

〔産業財産権〕
○出願状況（計０件）

名称：
発明者：
権利者：
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番号：
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〔その他〕
ホームページ等

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