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科学研究費助成事業　　研究成果報告書

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研究課題名（和文）Comprehensive identification of cis-regulatory elements involved in the evolutionary origin of the turtle carapace.

研究課題名（英文）Comprehensive identification of cis-regulatory elements involved in the evolutionary origin of the turtle carapace

研究代表者

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交付決定額（研究期間全体）：（直接経費）　　3,200,000円

研究成果の概要（和文）：カメの甲羅は、祖先には存在しなかった組織間相互作用により、既存の形態が変形することにより生じる新規形態物として有名である。このようなカメの甲羅の進化的起源を理解するため、甲羅の原基である甲陵（CR）発生のゲノム制御機構について解析した。ATAC-seqの結果、およそ38,000のCR特異的な制御領域を新規に特定した。また、CRで顕著に機能していることが知られるWNTシグナル経路の関連タンパク、LEF1とβカテニンの抗体を作成した。これらは、今後の解析でATAC-seqによって得られた制御領域が、WNTシグナル経路の下流であるかどうかを判定するために有用なツールとなるだろう

研究成果の概要（英文）：The turtle shell is a clear example of morphological novelty, requiring the fusion of tissues not fused before, and the repositioning of body parts to completely new locations. In order to understand the evolutionary origin of the turtle, I have analyzed the regulatory genome at the specific location of the carapacial ridge (CR), the embryonic structure that ultimately controls the formation of the shell. By means of ATAC-seq, I have been able to identify about 38,000 putative regulatory elements specific to the CR that might be related to those genes controlling the carapace formation. Last, in this project we have generated custom made antibodies specifically targeting turtle proteins Lef1 and b-catenin, which will allow us to assess which of the regulatory elements identified by ATAC-seq are downstream of the Wnt pathway, the signaling pathway at the top of the network controlling the carapace development

研究分野：　形態進化

キーワード：　比較ゲノム　進化発生　遺伝

１．研究開始当初の背景

(1) The turtle shell is a genuine morphological innovation within tetrapods. Its formation requires a complete anatomical distortion of the tetrapod body plan, resulting in an open, fan-like ribcage formed by plate-like ribs that eventually enclose the shoulder girdle (otherwise, remaining outside in the rest of amniotes). During development, the turtle shell is preceded by the formation of an ectodermal ridge, underlain by a condensed mesenchyme, running anterior-posteriorly through the dorsal flank of the body in the inter-limb region, the so-called carapacial ridge (CR). The CR is thought to control the development of the shell, but the gene regulatory network (GRN) responsable for the formation of the CR remains a mystery. In our previous project, we analyzed the transcriptomes of the CR and other structures (limbs and body walls) of the Chinese soft-shell turtle, Pelodiscus sinensis, and compare it with the transcriptomes of homologous or equivalent embryonic regions of the chicken and the mouse (dorsal flank, limbs and body walls, respectively). Our comparative analyses allowed us to identify those genes that are expressed in the CR of P. sinensis, and that might be involved in the turtle shell development. ChIP-seq analysis for histone modifications of the CR also allowed us to identify 494 enchancers specific for the CR. However, the hierarchical relationship between these regulatory sequences and the associated genes that is necessary to determine the corresponding GRN is still unkown.

２．研究の目的

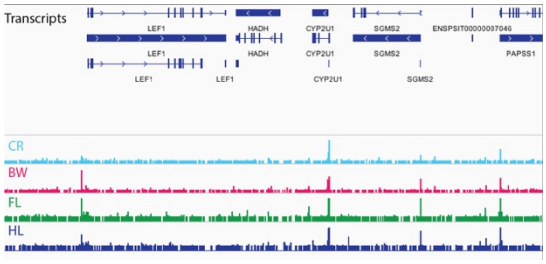
(1) The purpose of this project was to determine the hierarchical relationship between the genes specifically involved in the development of the turtle carapace. For that, we aimed at:
i) assessing the open chromatin regions of the turtle genome during CR formation;
ii) determining the subset of regulatory input that is due to Wnt pathway effectors Lef1 and b-catenin, which we know are at the top of the network.

３．研究の方法

(1) ATAC-seq: This is a very recent technique that requires a relatively low amount of cells (~50,000 cells) (Buenrostro et al., 2013). Briefly, we dissected the CR, body wall, forelimbs and hindlimbs of about 5-10 embryos, 2 biological replicates, and assayed them for ATAC and ATAC-seq libraries construction following previously published protocols (Buenrostro). 8 ATAC-libraries, 1 per tissue and per replicate, were sequenced using multiplex indexing in 2 lanes on a HiSeq 4000 platform. Reads were mapped, normalized and visualized following previous publications (Acemel et al., 2016).
(2) Lef-1 and b-catenin ChIP-seq: custom antibodies were generated by MBL(医学生物学研究所) and tested agains P. sinensis genomic DNA.

４．研究成果

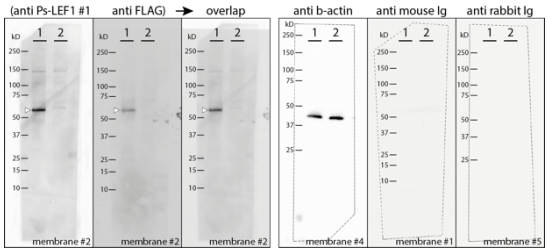
(1) ATAC-seq: I used the assay for transposase-accessible chromatin using sequencing (ATAC-seq) to determine the global epigenetic landscape of the 4 structures from the turtle embryo. In total, we found about 40-40K peaks in each of the tissue (see Fig. 1 for an example visual of the results), with a total of ~120,000 unique peaks.



Intersections of datasets form the 4 tissues showed that about ~38,000 peaks are specific for the CR.

(2) ChIP-seq: In order to determine which of the putative regulatory sequences identified by ATAC-seq and by the previous ChIP-seq against histone modifications corresponded to binding sites to Lef1, and which subset of these are actually activated by the canonical Wnt pathway, we decided to perform chromosome-immunoprecipitation followed by sequencing (ChIP-seq) against the transcription factor Lef1 and the co-factor b-catening. Wnt canonical pathway has been shown to be active during the formation of the carapace. Moreover, in my previous project, I observed that most

of the genes specifically upregulated in the CR are involved in the regulation of the Wnt pathway, both positively and negatively, so determining which regulatory regions are bound by Wnt pathway effectors, would indicate those genes that are downstream in the network.
However, one of the prerequisites to perform a high-quality ChIP-seq is to have a specific antibody. Therefore, we tested several commercially available antibodies against mouse or chicken Lef1 and b-catenin. While we found an antibody that specifically bound turtle b-catenin, none of the anti-Lef1 antibodies was able to pool down the specific turtle protein. Therefore, we decided to make our own custom antibody, made by MBL (Nagoya, Japan). We tested this custom antibody by immunoprecipitation and Western blots, as well as by proteomics analysis, and confirmed that it bound turtle Lef1 (Fig. 2).



Generating custom made antibodies has been time consuming and it has delayed the project. However, we are just at the moment proceeding with the ChIP-seq experiments and will obtain the datasets soon.

All these results, taken together, indicate that the regulatory landscape of the genes specifically active during the turtle carapace development is more complex than previously thought, and that it was the generation of these new regulatory inputs and binding events what triggered the origin of the turtle shell.

＜引用文献＞

① Buenrostro, J. D., Giresi, P. G., Zaba, L. C., Chang, H. Y., & Greenleaf, W. J. (2013). Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position. Nature Methods, 10(12), 1213-8.

② Buenrostro, J. D., Wu, B., Chang, H. Y., & Greenleaf, W. J. (2015). ATAC-seq: A Method for Assaying Chromatin Accessibility Genome-Wide. In Current Protocols in Molecular Biology (p. 21.29.1-21.29.9). Hoboken, NJ, USA: John Wiley & Sons, Inc.

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　　件）

〔学会発表〕（計１件）

1. Invited Lecture at Konan University, January 23, 2017, Kobe, Japan (Host: Takehiro Kusakabe): Juan Pascual Anaya “From amphioxus to turtles: how evo-devo and genomics studies help us to understand morphological evolution”

〔図書〕（計　　件）

〔産業財産権〕

○出願状況（計　　件）

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〔その他〕
ホームページ等

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