様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

平成　３０　年　　６　月　１２　日現在

機関番号：　８２４０１
研究種目：　若手研究(A)
研究期間：　2015～2017
課題番号：　１５Ｈ０５６２２

研究課題名（和文）Mechanisms of anhydrobiosis-associated gene regulation in the cells and tissues of chironomid Polypedilum vanderplanki.

研究課題名（英文）Mechanisms of anhydrobiosis-associated gene regulation in the cells and tissues of chironomid Polypedilum vanderplanki.

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交付決定額（研究期間全体）：（直接経費）　　18,700,000円

研究成果の概要（和文）：本研究は、複数のオミクス技術の組み合わせにより、ネムリユスリカの極限的な乾燥耐性の組織特異的な遺伝子機構を解明することを目的とした。その結果、ARIdと呼ばれる乾燥耐性関連遺伝子が集積したゲノム領域に多数のnoncoding RNA (ncRNA) が存在する事を見いだした。しかも、そのncRNAは、その近傍に存在する乾燥耐性関連遺伝子との発現相関があったことから、エンハンサーRNAである可能性がある。また、CAGE解析とRNAi実験により、熱ショック転写因子（HSF1）が、乾燥耐性特異的な転写活性化を通じて、重要な乾燥耐性機能発現調節因子であることを実証した。

研究成果の概要（英文）：The project was aimed to uncover tissue-specific genetic mechanisms of desiccation tolerance in anhydrobiotic midge Polypedilum vanderplanki by combination of several omics techniques. We defined a number of non-coding RNA , transcribed from multi-copy conservative regions of the genome, and those with the features of enhancer RNA (i.e. non-coding RNA transcribed from enhancers in the genome) with expression patterns correlated with that of anhydrobiosis-involved genes (for examples the genes located in ARIds region of the genome. We found that genetic mechanism of tissue specificity and broad expression of protective genes across tissues is the strategy of anhydrobiosis and can be used for biomimetics. Next, using CAGE data, we demonstrated that, heat shock transcription factor (HSF1) is one of the key regulators of transcriptional activation in response to dehydration in the cells of the anhydrobiotic chironomid. We further confirmed it by RNAi experiments.

研究分野：　Insect molecular biology and genomics

キーワード：　anhydrobiosis　CAGE　desiccation　chironomid　transcriptomics

１．研究開始当初の背景

Some organisms are able to maintain the viability of complete water loss, suspending their metabolism (the phenomenon of anhydrobiosis, reviewed in Watanabe, 2006). Anhydrobiosis is a model for development of dry-preservation for biomedical needs and several successful trials of improvement of resistance of mammalian cells to water loss using anhydrobioric animals-derived biomolecules were done (review in Crowe, 2014 and Loi et. al, 2013). The most evolutionary complex and the largest anhydrobiotic organism is the larva of African chironomid P. vanderplanki (Pv) (Fig.1). The larvae can withstand years of desiccation and resume the activity with 30-40 min upon rehydration.

Previously, we have completed genome project of Pv and found:

1. The chironomid genome is small (96-98 Mbp) and exclusively AT%-rich (one of the highest rates in insects)
2. There are strong evidence of differences in gene control in orthologous genes in response to desiccation in Pv and Pn (trehalose synthesis pathway and others)
3. Genome of Pv specifically contains clusters of multi-copy genes with products that act as molecular shields. In addition, the genome possesses several groups of genes with high similarity to known protective proteins, but located in distinct paralogous clusters in the genome apart from the classical orthologues. (Gusev et.al, 2014)

The genome regions containing theses Pv-specific genes, we named Anhydrobiosis-related Islands (ARId) and their number in the anhydrobiotic midge genomes is estimated to be at least 9 (Fig. 2). The major groups of genes forming ARIds are: Antioxidants, Heat shock proteins, respiratory proteins (globins), reparation enzymes (Protein L-isoaspartyl-O-methyltransferase) and several groups of Pv-specific genes, Late embryogenesis Abundant proteins (LEA) and other genes of unknown functions.

２．研究の目的

The aim of the project is to create an expression atlas of gene activity during dehydration, to identify promoters and non-coding RNAs involved in the regulation of anhydrobiosis in individual tissues, organs, and embryonic cell line of an anhydrobiotic midge. In addition, by combination of genome wide analysis of mRNA expression, Cap Analysis of Gene Expression and protein profiling to define the key sets of genes and their products required for dry preservation of alive tissues and cells.

３．研究の方法

Initially, the chironomid genome assembly to be enhanced by mate-pair sequencing using chironomid cell line Pv11 DNA. Using the improved assembly of the genome, mRNASeq-based gene expression profile will be obtained from the Pv11 cell line through the cycle of anhydrobiosis. On the next step, mRNASeq profiles will be obtained for several chironomid tissues from the larvae on different stages of the cycle of desiccation-rehydration. Further, we will employ proteomics technique to evaluate anhydrobiosis-associated changes in the protein pool in different tissues and Pv11 cell line. On the final stage of the project, we are going to use nAnT-iCAGE technique for identification of tissues and cell-specific promoters and non-coding RNA activity in the cycle of anhydrobiosis.

４．研究成果

The project was aimed to uncover tissue-specific genetic mechanisms of desiccation tolerance in anhydrobiotic midge *Polypedilum vanderplanki* by combination of several omics techniques. In initial stage of the project, we profiled expression patterns of RNA and proteins in Pv11 cell culture (obtained from embryonic cell mass of the chironomids) and tissues of the chironomids. Here, we have selected the most promising and convenient for the analysis organs (fat body, intestine and brains) of the chironomid for whole-genome and proteomic profiling. We further defined a number of non-coding RNA, transcribed from multi-copy conservative regions of the genome, and those with the features of enhancer RNA (i.e. non-coding RNA transcribed from enhancers in the genome) with expression patterns correlated with that of anhydrobiosis-involved genes (for examples the genes located in ARIds region of the genome. Next, proteomic

profile of Pv11 cell culture on different stages of anhydrobiosis was obtained. The stages were: wet control cells, cells after 48 h of incubation with trehalose, completely desiccated cells and the cells after 24 h of re-hydration. CAGE libraries from dried and normal tissues of P. vanderplanki larvae were sequenced. The clustered readings were clustered, resulting in 13,727 promoter regions, of which 9573 were associated with 7876 protein-coding genes. We found that members of this group correspond to certain "anhydrobiotic genes", like PIMT methyltransferase or Trx-oxidoreductase. Finally, we created comprehensive atlas of tissue-specific cis elements expression database for 4 main tissues of the annydrobiotic tissues. We found that genetic mechanism of tissue specificity and broad expression of protective genes across tissues is the strategy of anhydrobiosis and can be used for biomimetics. Next, using CAGE data, we demonstrated that, heat shock transcription factor (HSF1) is one of the key regulators of transcriptional activation in response to dehydration in the cells of the anhydrobiotic chironomid. We also confirmed it by RNAi experiments. Mapping the CAGE data updated genome assembly revealed new patterns of transcriptional activity in the tissues of chironomids during anhydrobiosis cycle. Most of the "anhydrobiosis-associated" were activated in dry tissues (75%), with ~ 39% of them were active in various tissues in a similar manner. We found some characteristics associated with tissue-specific activation of this group of genes: globins for the fat body, specific oxidoreductases, methyltransferase, LEA, in the brains. Final year of the current project was devoted to functional analysis of the identified regulators of anhydrobiosis. During this stage we also developed preliminary protocol of CRISPR-Cas9 knock out for anhydrobiosis-involved transcription factor gene followed by CAGE analysis to identify the regulatory network associate with anhydrobiosis. In general, our project allowed to adopt several key omics techniques to study anhydrobiosis, to develop comprehensive database of single nucleotide-resolution promoters map in three key tissues in response to desiccation, and identify tissue-specific patterns of adaptation to complete water loss on the level of transcriptional response. We also discovered that heat shock factor, undergone evolutionary changes in the anhydrobiotic midge and acts as one of the key regulators for anhydrobiosis.

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　6　件）

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6. Conbio-2017, Yokohama, Japan. (12/2017) Chromatin under extreme desiccation: 3D organization of genome of an anhydrobiotic midge *Polypedilum vanderplanki*. A. Ryabova, A.V. Cherkasov, N. Batullin, V. Fishman, V. Lukyanchikova, O.Gusev

〔図書〕（計　0　件）

〔産業財産権〕

o出願状況（計 0 件）

名称：
発明者：
権利者：
種類：
番号：
出願年月日：
国内外の別：

o取得状況（計　0　件）

名称：
発明者：
権利者：
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取得年月日：
国内外の別：

〔その他〕
ホームページ等

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