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研究課題名（和文）Cell type-specific mechanisms of desiccation tolerance in the anhydrobiotic insect

研究課題名（英文）Cell type-specific mechanisms of desiccation tolerance in the anhydrobiotic insect

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研究成果の概要（和文）：このプロジェクトは、唯一知られている無水生物昆虫であるP.vanderplanki（Pv）を使用して、完全な乾燥耐性の細胞型固有のメカニズムに関する新しい独自の情報を提供しました。ミクス分析を実施して、脳、脂肪体、腸、および胚性細胞塊由来のPv11細胞培養における水分喪失に対する分子シールドを提供する保護遺伝子の特定のセットを特定しました。 各組織には、遺伝子発現の一般的かつ固有の組織固有のシグネチャがあります。RNAiと遺伝子編集の新しいプロトコルを使用して、Hsf1を含む無水生物症の主要な転写マスターレギュレーターを特定し、乾燥耐性のゲノムワイドな調節に強く関与していることを示しました.

研究成果の学術的意義や社会的意義
The knowledge will serve for further development of new technologies of long-term preservation of different cell types under room temperature. Genome-wide studies allowed us to make a new discovery of mechanisms of appearance of evolutionary novelties in extremophilic organisms.

研究成果の概要（英文）：The project provided new unique information about cell type-specific mechanisms of complete desiccation tolerance, using the only known anhydrobiotic insect Polypedilum vanderplanki (Pv). We have conducted multi-omics analysis to identify a specific set of protective genes that provide a molecular shield against water loss in the brain, fat bodies, gut, and embryonic cell mass-derived Pv11 cell culture. Each tissue has both general and unique, tissue-specific signature of gene expression. Multiple orthologs of anhydrobiosis-related genes are linked to cell type-specific program of desiccation tolerance. We createda new, high-quality genome assembly of Pv and developed database MidgeBase 2.0, with genomic and transcriptomic data underlying anhydrobiosis. Using new protocols of RNAi and gene editing, we identified key transcriptional master regulators of anhydrobiosis, including Hsf1 and demonstrated its strong involvement in genome-wide regulation of desiccation tolerance in Pv11 cells

研究分野：昆虫生理学遺伝学

キーワード：anhydrobiosis　desiccation tolerance　gene expression　gene editing　sleeping chironomid　Polypedilum vanderplanki　scRNASeq

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

Some organisms can 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 anhydrobiotic 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**). The larvae can withstand years of desiccation and resume the activity with 30-40 min upon rehydration. Furthermore, ability of the living dissected organs and cells of Pv to be dry-preserved was experimentally conformed (reviewed in Cornette and Kikawada, 2010). Recently, Pv11 cell line, capable to induced anhydrobiosis was established (Watanabe et.al, 2016). The compact genome of Pv contains 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. 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. Our previous study showed some evidence that multiple paralogs of these protective genes can serve for different purposes (for example, has different expression profile depending on tissues, etc), but no specific cell-type oriented studies were conducted to support these observations.

２．研究の目的

Current project focused on the elucidation of several groups of key scientific questions:

1.What is a transcriptional program defining successful survival of some fraction of cells in embryo-derived Pv11 cell line during induced anhydrobiosis?

2.What is relation among success of anhydrobiosis in various cell types from the point of view of control gene expression? What would be key master regulators?

3. What are the common and unique components of mechanism of anhydrobiosis induction in the cells of different origin?

We also aimed to adapt gene editing techniques for Pv and functionally demonstrate the effect of regulatory genes on the ability of cells to tolerate complete desiccation.

３．研究の方法

Biological samples: Two main biological objects were Pv11 cell line capable to complete desiccation tolerance and the tissues from larvae of Pv from a culture provided by the collaborator (T. Kikawada, NARO). We analyzed cell culture and tissues from the larvae on the different stages of desiccation-rehydration cycles. To achieve the final goal, we used a combination of genetic and molecular biological methods.

Transcriptomics: For single cell analysis, library preparation was performed in accordance with the 10x Genomics Chromium Single Cell 3′ protocol for Reagent kits version 2. We aimed to

recover 5,000-8,000 cells with the Illumina HiSeq 2500 Sequencing System. The read setup was the standard setup recommended by 10x Genomics: read 1, 28 cycles (cell barcode and unique molecular identifier (UMI)), i7 index, 8 cycles (sample index); read 2, 91 cycles. The bulk RNAseq analysis was performed using TrueSeq kits (Illumina) followed by sequencing on Hiseq 2500 Illumina platform.

Genome Analysis: Final (chromosome level) version of Pv genome was assembled utilizing previously available (Illumina reads) and newly obtained (in the frame of the current project) PacBio, and Hi-C sequencing data from P. vanderplanki-derived Pv11 cell line.

Gene editing and silencing: Transfection into Pv11 cells was carried out using a NEPA21 Super Electroporator (Nepa Gene, Ichikawa, Chiba, Japan). Five μg each of the gRNA- (previously constructed and constructed above) and hSpCas9-expression vectors and 0.03-0.1 pmol donor vectors constructed above were transfected into cells.

4. 研究成果

Single cell data: natural anhydrobiosis (brains) vs artificial anhydrobiosis (Pv11 cell line)

Considering high cost of scRNAseq analysis, we focused on Pv11 cell line and preliminary analysis of brain of Pv. We have successfully generated 3 ' scRNASeq data from both types of samples, reflecting genome wide gene expression in approximately 900-1200 genes per cell in 5000-6000 cells for each samples. On the Figure 1 we show the four general expression clusters of genes associated with anhydrobiosis. Surprisingly, we realized that transcriptional response in case of " true " anhydrobiosis (i.e. in desiccation larvae) is generally much higher compared with that in the case of " artificial " induction of desiccation tolerance in Pv11 cell line.

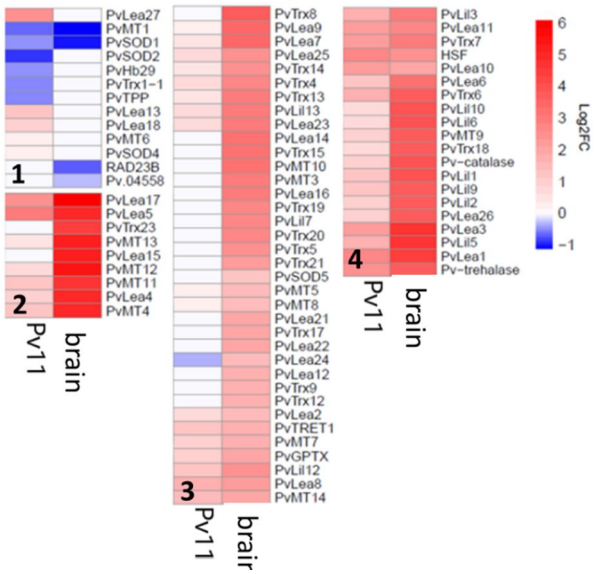

Fig. 1 Response of selected anhydrobiosis-related genes in PV11 cells and brains (scRNSeq bulk mode)

Using brain for the first ever trial to elucidate cell-type specific pattern of " true " anhydrobiosis (i.e. in the larvae) we successfully conducted series of pilot experiments. One serious challenge was to find a proper solution to identify key cell types in the nervous system of Pv. We used Drosophila data as a reference and found that while some cell types - glia (G), neurons (N) and neuronal progenitor cells(C) could be surely identified, their subtypes, and some other clusters still does not express clear markers reported for Drosophila. Thus, we assume, that there is a basic difference in cell type-composition in Pv (and, likely, chironomids in general) compared to that of Drosophila. This subject should be a topic of further, detailed investigation.

We found that the brains of Pv processes a special adaptation program toward anhydrobiosis. There was a drastic decrease of total number of neurons in dry larvae, compared to the wet, control larvae. Thus, in the dry larvae, the NPC represented most cells in the brains. Instead, upon rehydration, number of neurons increased, that is likely illustration of proliferation of NPC. Remarkably, number of glia cells did not change in the round of desiccation-rehydration. We assume, that here we observed one of the basic mechanisms of anhydrobiosis in Pv: preservation of NPC and glia cells, for post-rehydration proliferation. It is likely, that for some reason true neurons are more vulnerable to water loss.

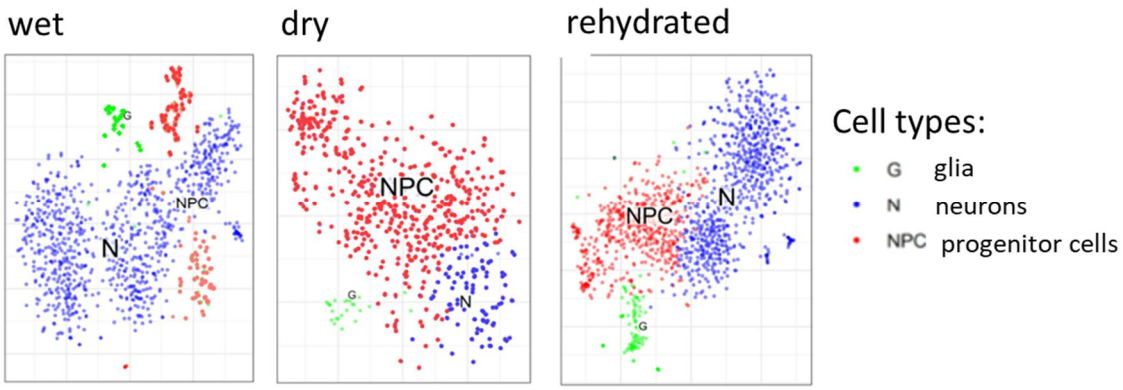

Fig - 2 Changes in contribution of different cell types in brain in the total cell number on different stages of desiccation/rehydration cycle.

Another illustration of specific fate of different cell types were the data of protective genes expression in brain cells. Figure 3 represents a dynamic of expression of anhydrobiosis-associated genes (PIMT, heat shock proteins, LeA, etc.) We see, that expression ununiformly distributed among the cell types. Among the identified cell types, glia show the highest general expression of protective genes in wet control larvae. Remarkably, PIMT (unique for Pv methyltransferases) show the highest expression in brain cells (original PIMT gene in human, for example, also shows the highest expression in brain). Upon desiccation, there is a strong over-expression in glia and NPC, but not in neurons (Fig.3, control). Counting the cells number suggest that neurons should massively die upon desiccation (Fig.2). Upon rehydration, we observe further increase of RNA of protective genes in NPC, that proliferated and result in drastic increase of neurons upon rehydration (Fig. 3, R24). Thus, our pilot study, suggest that evolution of anhydrobiosis in Pv is associated with development of specific mechanism focused on the protective of progenitor cells, rather than preserving mature neuron. Such observation opens an intriguing opportunity to further elucidate mechanisms of preservation of the cells with high proliferative potential, that is of interest for biomedical needs. Our current pilot, study, using NPC as a model, allowed to identify a set of genes in Pv that is required for “natural” preservation of this cell type.

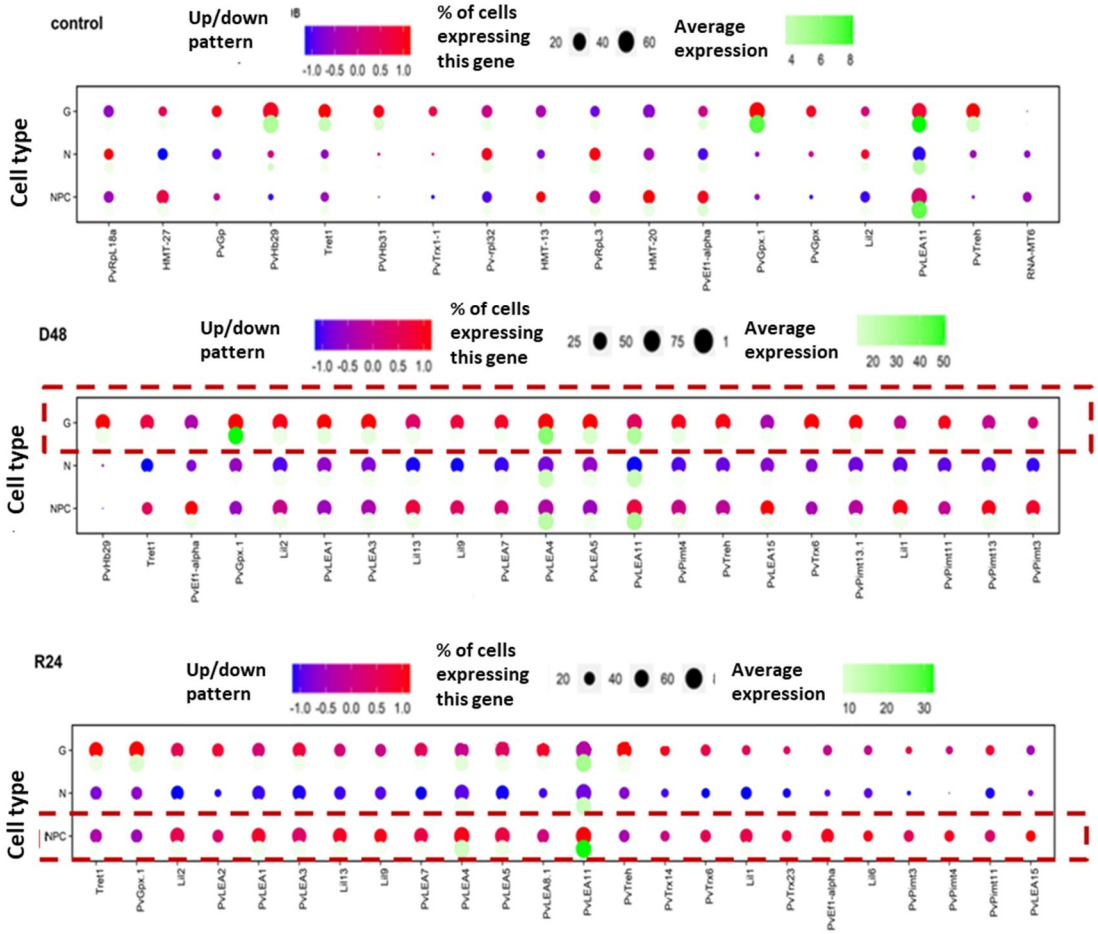

Fig. 3 Dynamic of expression of protective genes in brains of Wet (**control**), Dry (**D48**) and rehydrated (**R24**) larvae.

Genome structure of Pv11

Using a combination of previous data and high quality long reads, we used improved sequencing strategies to assemble a chromosome-level genome sequence for P. vanderplanki. We provide evidence for the specialization of chromosome 4 through extensive acquirement of novel genes. A high degree of sub-functionalization in paralogous anhydrobiosis-related genes occurs in this chromosome, as well as pseudogenization in a highly duplicated gene family. These findings suggest that the fourth chromosome in chironomids is a site of high genetic turnover, allowing it to act as a 'sandbox' for evolutionary experiments, thus facilitating the rapid adaptation of midges to harsh environments (Fig.4). We further improved and updated the first resources specifically focused on extremophilic chironomid genomics: www.midgebase.org (Yoshida et.al, submitted)

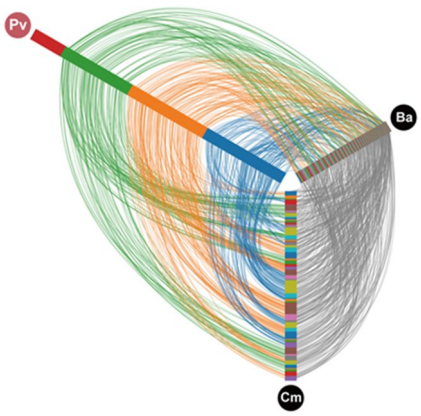

Fig. 4 Genes located on chromosome IV in Pv do not have orthologs in other midges. This is the place for "birth" of new genes for adaptation to extreme environments.

５．主な発表論文等

〔雑誌論文〕　計8件（うち査読付論文　7件／うち国際共著　8件／うちオープンアクセス　8件）

１．著者名 Takahiro G Yamada, Yusuke Hiki, Noriko F Hiroi, Elena Shagimardanova, Oleg Gusev, Richard Cornette, Takahiro Kikawada, Akira Funahashi	４．巻 15 (3)
２．論文標題 Identification of a master transcription factor and a regulatory mechanism for desiccation tolerance in the anhydrobiotic cell line Pv11	５．発行年 2020年
３．雑誌名 PLOS ONE	６．最初と最後の頁 e0230218
掲載論文のDOI（デジタルオブジェクト識別子） 10.1371/journal.pone.0230218	査読の有無 有
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１．著者名 Tokumoto Shoko、Miyata Yugo、Deviatiiarov Ruslan、Yamada Takahiro G.、Hiki Yusuke、Kozlova Olga、Yoshida Yuki、Cornette Richard、Funahashi Akira、Shagimardanova Elena、Gusev Oleg、Kikawada Takahiro	４．巻 22
２．論文標題 Genome-Wide Role of HSF1 in Transcriptional Regulation of Desiccation Tolerance in the Anhydrobiotic Cell Line, Pv11	５．発行年 2021年
３．雑誌名 International Journal of Molecular Sciences	６．最初と最後の頁 5798～5798
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２．論文標題 Development of a Tet-On Inducible Expression System for the Anhydrobiotic Cell Line, Pv11	５．発行年 2020年
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２．論文標題 New group of transmembrane proteins associated with desiccation tolerance in the anhydrobiotic midge Polypedilum vanderplanki	５．発行年 2020年
３．雑誌名 Scientific Reports	６．最初と最後の頁 N/A
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1．著者名 Ryabova Alina、Cornette Richard、Cherkasov Alexander、Watanabe Masahiko、Okuda Takashi、Shagimardanova Elena、Kikawada Takahiro、Gusev Oleg	4．巻 117
2．論文標題 Combined metabolome and transcriptome analysis reveals key components of complete desiccation tolerance in an anhydrobiotic insect	5．発行年 2020年
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2．論文標題 Cas9-mediated Genome Editing Reveals a Significant Contribution of Calcium Signaling Pathways to Anhydrobiosis in Pv11	5．発行年 2020年
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2．論文標題 Cooption of heat shock regulatory system for anhydrobiosis in the sleeping chironomid Polypedilum vanderplanki	5．発行年 2018年
3．雑誌名 Proceedings of the National Academy of Sciences	6．最初と最後の頁 E2477～E2486
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オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

〔学会発表〕　計5件（うち招待講演　4件／うち国際学会　5件）

1．発表者名
Oleg Gusev

2．発表標題
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4．発表年
2019年～2020年

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2．発表標題
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3．学会等名
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4．発表年
2019年

1．発表者名
Oleg Gusev

2．発表標題
X-midge: cell and tissue-specific genetic mechanisms to cope with complete desiccation in a unique insect

3．学会等名
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4．発表年
2018年

1．発表者名
Oleg Gusev

2．発表標題
X-midge: cell and tissue-specific mechanisms of tolerance to complete desiccation in a unique insect

3．学会等名
OIST Seminar series, OIST, Okinawa, Apr. 24, 2018（招待講演）（国際学会）

4．発表年
2018年

１．発表者名 Oleg Gusev
２．発表標題 Genome editing and anhydrobiosis
３．学会等名 CRISP-2018, Novisibirsk, Russia. Sept.10-14, 2018（招待講演）（国際学会）
４．発表年 2018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

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