Press Release

Keio University　Sanyo-Onoda City University　NARO National Agriculture and Food Research Organization

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How Cells, that Do Not Die when Desiccated, are able to Revive without Dying?

—Implications of Desiccation Tolerance and Rehydration Revival Mechanisms of Pv11 Cells—

An international research team consisting of Research Associate Takahiro Yamada and Associate Professor Akira Funahashi of the Keio University Faculty of Science and Technology, Department of Biosciences and Informatics, Dr. Ruslan Deviatiiarov and Dr. Alexander Nesmelov of Kazan Federal University (Russia), Unit Leader Oleg Gusev of RIKEN, Professor Noriko Hiroi of Sanyo-Onoda City University, and Senior Researcher Yoshitaka Suetsugu, Principal Researcher Richard Cornette, and Principal Researcher Takahiro Kikawada of NARO, focused on understanding of molecular mechanisms underlying the extreme desiccation tolerance known as anhydrobiosis in Pv11 cells, which allows the cells to survive under complete desiccation conditions with retaining proliferation potential after rehydration. The research team performed transcriptome analysis to estimate the genes involved in this mechanism. As a result, the team revealed that genes relevant to elimination of deleterious reactive oxygen species and reparation of DNA damaged due to desiccation are highly expressed during trehalose-treatment and rehydration, respectively, to achieve successful anhydrobiosis in Pv11 cells. These findings could be applied to the development of transgenic organisms with anhydrobiotic traits through the introduction of the identified gene groups to desiccation-sensitive organisms.

The results of this research was published in the online journal *Scientific Reports* on December 18, 2018 (Tues.; GMT).

1. Main Points of Research

- Transcriptome analysis was performed on Pv11 cells, cultured cells derived from the embryos of the sleeping chironomid, *Polypedilum vanderplanki*. These cells can be preserved in the dry state while maintaining viability and proliferation ability. The aim of this transcriptome analysis was to estimate the genes that possibly contribute to the mechanism of desiccation tolerance.
- Results showed that antioxidants such as thioredoxins, which eliminate the harmful influence of reactive oxygen species generated during desiccation, are highly expressed by the trehalose (*1) treatment necessary to induce desiccation tolerance in Pv11 cells.
- Results showed that genes that repair serious DNA damage are highly expressed when desiccated Pv11 cells are rehydrated.

2. Background of the Research

The sleeping chironomid, *P. vanderplanki*, is an insect native to Africa showing desiccation tolerance. Its larvae avoid death by entering into anhydrobiosis, an ametabolic state, and even if they are completely desiccated during the dry season, they return to their original life cycle when given water. Pv11 cells, which are cultured cells derived from the embryos of *P. vanderplanki*, were established in 2002. By treating Pv11 cells with a high concentration of trehalose, these cells can be desiccated and preserved at room temperature, while maintaining proliferation ability. This desiccation tolerance of Pv11 cells is achieved through the following series of processes: 1) induction of desiccation tolerance by trehalose treatment, 2) complete shutdown of metabolism because of desiccation, and 3) revival after rehydration with the culture medium. However, a comprehensive analysis of what genes are involved in these processes has not been carried out, leaving the desiccation tolerance of Pv11 cells hidden behind a veil. Therefore, the present research group obtained the expression levels of all genes during trehalose treatment, desiccation, and rehydration of Pv11 cells as time-course series CAGE-seq (*2) data, and by analyzing these, attempted to estimate the characteristic genes functioning at each stage. The expression of such specific genes is thought to change significantly due to trehalose treatment, desiccation, and rehydration. In this study, genes were identified by statistical analysis as differentially expressed genes (DEG), and based on the functions of these genes, molecular mechanisms that support specific physiological responses at each stage were suggested by analyzing which genes with what functions significantly exist at each stage.

3. Content of Research and Results

First, total RNA was collected from Pv11 cells before trehalose treatment and 48 hours after treatment, after desiccation for 3 hours and 10 days, and then 3 hours and 24 hours after rehydration. The expression levels of all genes were then quantified through a method called CAGE-seq. As a result, first, it was revealed that the expression patterns of all genes became different from those in states of normality after trehalose treatment and desiccation, and that they return to their original states after rehydration, as shown in figure 1. Subsequently, DEG during trehalose treatment, desiccation, and rehydration were detected by statistical analysis.

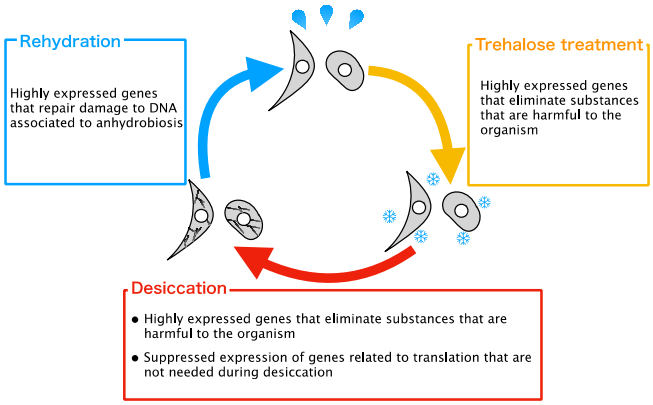

Figure 1 : Concept diagram of differential expression of genes in trehalose-treated, desiccated, and rehydrated Pv11 cells

During the trehalose treatment, many of DEG were related to antioxidant factors (such as thioredoxins) that degrade excessive oxidizing substances produced by reactive oxygen species, which are harmful to the organism. That is, it was suggested that trehalose treatment is a preparatory stage induce desiccation tolerance abilities by expressing genes that eliminate substances, which are deleterious to biological functions. Next, focusing on the DEG during desiccation, in addition to the high expression of genes that eliminate harmful substances similar to those observed during trehalose treatment, a significant reduction in the expression of ribosomal proteins involved in the translation of proteins was detected. From this, it was suggested that Pv11 cells achieve to save energy by suppressing the expression of genes that are not needed during desiccation. Moving on to the DEG during rehydration, a rise in the expression of genes that repair DNA was seen. It was suggested that

the rehydration process plays a role in restoring the functions of the cells back to their normal active state, also repairing the damages related to desiccation stress that had been accumulated by DNA.

4. Future Developments

At the present stage, it was possible to identify genes that function during trehalose treatment, desiccation, and rehydration based on an increase or a decrease of gene expression, but it is not yet clear whether they are actually essential for Pv11 cells survival after desiccation when the culture medium is added. To answer this question, it is necessary to experimentally verify whether cell division does not resume after rehydration by inactivating and activating functions of these genes through techniques such as genome editing. If genes essential for the desiccation tolerance of Pv11 cells are revealed through experiments like this, next, the possibility of creating new desiccation-tolerant cells through the introduction of such genes into different sensitive cells will increase, and ultimately, it is anticipated that this will lead to the development of new techniques of dry preservation at room temperature for biomolecules and cells that will replace, the classic methods of cold storage.

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<Details of Original Paper>

Title: Transcriptome analysis of the anhydrobiotic cell line Pv11 infers the mechanism of desiccation tolerance and recovery

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<Glossary>

*1 Trehalose
A non-reducing disaccharide made from 2 glucose molecules

*2 CAGE-seq
A technique for sensitively quantifying the expression level of a gene by selectively collecting only the sequence on the 5'-end of the RNA and performing sequencing

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