SCIENTIFIC REPORTS

OPEN

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

Received: 23 July 2018
Accepted: 15 November 2018
Published online: 18 December 2018

Takahiro G. Yamada[1], Yoshitaka Suetsugu[2], Ruslan Deviatiiarov[3], Oleg Gusev[3,4], Richard Cornette[2], Alexander Nesmelov[3], Noriko Hiroi[5], Takahiro Kikawada[2,6] & Akira Funahashi[1]

The larvae of the African midge, *Polypedilum vanderplanki*, can enter an ametabolic state called anhydrobiosis to overcome fatal desiccation stress. The Pv11 cell line, derived from *P. vanderplanki* embryo, shows desiccation tolerance when treated with trehalose before desiccation and resumes proliferation after rehydration. However, the molecular mechanisms of this desiccation tolerance remain unknown. Here, we performed high-throughput CAGE-seq of mRNA and a differentially expressed gene analysis in trehalose-treated, desiccated, and rehydrated Pv11 cells, followed by gene ontology analysis of the identified differentially expressed genes. We detected differentially expressed genes after trehalose treatment involved in various stress responses, detoxification of harmful chemicals, and regulation of oxidoreduction that were upregulated. In the desiccation phase, L-isoaspartyl methyltransferase and heat shock proteins were upregulated and ribosomal proteins were downregulated. Analysis of differentially expressed genes during rehydration supported the notion that homologous recombination, nucleotide excision repair, and non-homologous recombination were involved in the recovery process. This study provides initial insights into the molecular mechanisms underlying the extreme desiccation tolerance of Pv11 cells.

Desiccation stress, the loss of essential water, can be fatal. To tolerate desiccation stress, various organisms, such as rotifers, tardigrades, nematodes, plants, and larvae of the African midge *Polypedilum vanderplanki*, enter an ametabolic state called anhydrobiosis[1,2] and survive even if more than 99% of body water is lost[3]. According to the water replacement hypothesis, a compatible solute, such as trehalose, protects phospholipid membranes and intracellular biological molecules and ensures their preservation under desiccation[3,4]. Prolonged desiccation can lead to serious oxidative stress. For example, in the moss *Fontinalis antipyretica* an increase in the production of reactive oxygen species (ROS) is associated with dehydration[5,6]. Protein oxidation in the dehydrated cells of the yeast *Saccharomyces cerevisiae* is 10 times that of hydrated cells[5,7]. Thioredoxins (TRXs) remove harmful ROS and protect cells from ROS-induced damage[5,7]. The genome of *P. vanderplanki* has a paralogous gene cluster for TRXs[8]. These TRXs are upregulated by dehydration, and *P. vanderplanki* becomes tolerant to ROS-induced damage[8]. Upon rehydration, the anhydrobiotes return to active life.

In 2002, the Pv11 cell line was established as an embryonic cell culture from *P. vanderplanki*[9]. Desiccation tolerance of Pv11 cells is induced by treatment with culture medium containing 600 mM trehalose for 48 h. Even after dehydration in a desiccator (<10% relative humidity) for 12 days and rehydration for 1 h, trehalose-treated Pv11 cells are able to resume proliferation[10], whereas other insect cell lines (Sf9, BmN-4, AeAl-2, AnCu-35, and S2) do not. Pv11 cells are considered the only desiccation-tolerant insect cell line able to restore the regular cell

[1]Department of Biosciences and Informatics, Keio University, Yokohama, Kanagawa, 223-8522, Japan. [2]Institute of Agrobiological Sciences, National Agriculture and Food Research Organization (NARO), Tsukuba, Ibaraki, 305-8634, Japan. [3]Kazan Federal University, Kazan, Tatarstan, 420008, Russia. [4]RIKEN, Yokohama, Kanagawa, 230-0045, Japan. [5]Faculty of Pharmaceutical Science, Sanyo-Onoda City University, Sanyo-Onoda, Yamaguchi, 756-0884, Japan. [6]Graduate School of Frontier Sciences, The University of Tokyo, Kashiwa, Chiba, 277-8562, Japan. Yoshitaka Suetsugu is deceased. Correspondence and requests for materials should be addressed to T.K. (email: kikawada@affrc.go.jp) or A.F. (email: funa@bio.keio.ac.jp)