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Large-scale proteome analysis of abscisic acid and ABSCISIC ACID INSENSITIVE3-dependent proteins related to desiccation tolerance in *Physcomitrella patens*

Izumi Yotsui [a, 1], Satoshi Serada [b], Tetsuji Naka [b], Masashi Saruhashi [a], Teruaki Taji [a], Takahisa Hayashi [a], Ralph S. Quatrano [c], Yoichi Sakata [a, *]

[a] Department of BioScience, Tokyo University of Agriculture 1-1-1 Sakuragaoka, Setagayaku, Tokyo, 156-8502, Japan
[b] Laboratory of Immune Signal, National Institute of Biomedical Innovation, Health and Nutrition, 7-6-8 Saito-Asagi, Ibaraki, Osaka, 567-0085, Japan
[c] Department of Biology, Washington University in St. Louis, St. Louis, MO, 63130-4899, USA

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ABSTRACT

Desiccation tolerance is an ancestral feature of land plants and is still retained in non-vascular plants such as bryophytes and some vascular plants. However, except for seeds and spores, this trait is absent in vegetative tissues of vascular plants. Although many studies have focused on understanding the molecular basis underlying desiccation tolerance using transcriptome and proteome approaches, the critical molecular differences between desiccation tolerant plants and non-desiccation plants are still not clear. The moss *Physcomitrella patens* cannot survive rapid desiccation under laboratory conditions, but if cells of the protonemata are treated by the phytohormone abscisic acid (ABA) prior to desiccation, it can survive 24 h exposure to desiccation and regrow after rehydration. The desiccation tolerance induced by ABA (AiDT) is specific to this hormone, but also depends on a plant transcription factor ABSCISIC ACID INSENSITIVE3 (ABI3). Here we report the comparative proteomic analysis of AiDT between wild type and ABI3 deleted mutant (*Δabi3*) of *P. patens* using iTRAQ (Isobaric Tags for Relative and Absolute Quantification). From a total of 1980 unique proteins that we identified, only 16 proteins are significantly altered in *Δabi3* compared to wild type after desiccation following ABA treatment. Among this group, three of the four proteins that were severely affected in *Δabi3* tissue were Arabidopsis orthologous genes, which were expressed in maturing seeds under the regulation of ABI3. These included a Group 1 late embryogenesis abundant (LEA) protein, a short-chain dehydrogenase, and a desiccation-related protein. Our results suggest that at least three of these proteins expressed in desiccation tolerant cells of both Arabidopsis and the moss are very likely to play important roles in acquisition of desiccation tolerance in land plants. Furthermore, our results suggest that the regulatory machinery of ABA- and ABI3-mediated gene expression for desiccation tolerance might have evolved in ancestral land plants before the separation of bryophytes and vascular plants.

1. Introduction

Sessile plants developed many adaptation mechanisms to successfully colonize terrestrial habitats. Desiccation tolerance (DT) is one of such mechanisms for adaptation to a dry atmosphere. Somatic cells of non-vascular plants such as algae and bryophytes are not able to control water loss to the atmosphere but can tolerate desiccation. This vegetative DT is absent from vascular plants that evolved water conducting systems and specialized epidermal layers with cuticles and stomata to avoid water evaporation. DT is retained only in spores and seeds of most vascular plants, but certain “resurrection plants” belonging to different plant lineages, still retain the ability to survive desiccation in vegetative tissue [1]. This suggest that the DT trait is still conserved among land plants

* Corresponding author.
E-mail addresses: izumi.yotsui@riken.jp (I. Yotsui), serada@nibiohn.go.jp (S. Serada), tnaka@nibiohn.go.jp (T. Naka), s13db001@mail.saitama-u.ac.jp (M. Saruhashi), t3teruak@nodai.ac.jp (T. Taji), t4hayash@nodai.ac.jp (T. Hayashi), rsq@wustl.edu (R.S. Quatrano), sakata@nodai.ac.jp (Y. Sakata).
[1] Present address; RIKEN Center for Sustainable Resource Science, 1-7-22 Suehiro-cho Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan.

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but repressed in most vascular plants where energy resources are likely to be channeled into plant growth rather than DT.

Bryophytes such as *Tortula ruralis* possess a constitutive cellular protection mechanism and can tolerate rapid desiccation (<−500 MPa in 30 min from hydrated state) in the absence of new transcripts for proteins [2]. In contrast, some bryophytes such as *Physcomitrella patens* are desiccation sensitive and cannot tolerate rapid desiccation. However, pretreatment with the plant hormone abscisic acid (ABA) for 24 h dramatically increases the ability to survive rapid desiccation equivalent to <−273 MPa [3]. ABA is also essential for DT of angiosperm seeds, suggesting an evolutionarily conserved role of ABA in DT of plant cells. The importance of endogenous ABA in desiccation tolerance of bryophytes has been demonstrated in transgenic *P. patens* plants, which lack the zeaxanthin epoxidase gene, which is essential for epoxycarotenoid-mediated ABA synthesis. Under conditions where ABA was undetectable, reduced DT was observed [4]. Hence, this ABA-induced DT (AiDT) observed in protonemal cells of *P. patens* is an ideal model system to analyze the mechanisms for establishment of DT by comparing molecular components before and after ABA treatment. However, ABA treatment induces global changes in gene expression patterns making it difficult to clearly identify proteins essential for DT [5].

Previously we have shown that deletion of the plant-specific transcription factor *ABSCISIC ACID INSENSITIVE3* (*ABI3*) gene from *P. patens* genome (*Δabi3*) resulted in loss of AiDT [6]. Toward understanding the gene regulatory network responsible for AiDT, we took a comparative proteomic analysis of AiDT using iTRAQ (Isobaric Tags for Relative and Absolute Quantification) method. Interestingly, only 16 proteins were significantly altered in *Δabi3* and four of the 16 proteins were drastically reduced. The orthologous genes expressed in matured seeds of Arabidopsis are also under regulation of ABI3. These were a Group 1 LEA protein, a short-chain dehydrogenase, and a desiccation-related protein. Our results suggest that these proteins regulated by ABA and ABI3 both in the moss and seed plants are the candidate genes responsible in part for acquisition of desiccation tolerance in land plants.

2. Materials and methods

2.1. Plant materials and growth conditions

P. patens subspecies patens (Gransden) was used as the wild-type strain. The triple deletion mutant of *PpABI3* (*Δabi3*) was described before [6]. Protonemal tissues were grown on $PpNH_4$ medium at 25 °C under continuous light as described previously [7,8].

2.2. Sample preparation

One-week-old protonemal tissues were treated with 10 μM ABA for 24 h and subjected to desiccation for 24 h in the laminar flow as described previously [5], and then frozen by liquid nitrogen. Tissues supplied without ABA were frozen without desiccation. Detailed method of protein extraction from *P. patens* protonemal tissues was described in the Supplementary Materials and Methods section.

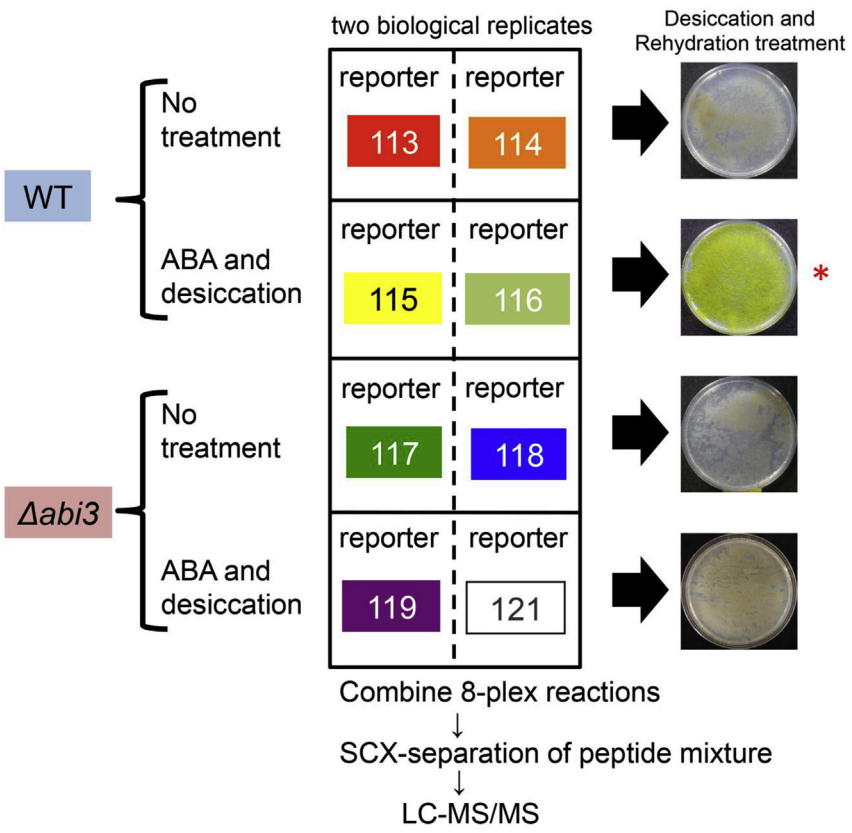

Fig. 1. Sample preparation for iTRAQ analysis. Protonemal tissues from WT and *Δabi3* were treated without or with 10 μM ABA for 24 h and followed by desiccation for 24 h. Non-treated WT was labeled with iTRAQ regent 113 and 114, while WT treated with ABA and desiccation was labeled with 115 and 116. Non-treated *Δabi3* was labeled with 117 and 118, while *Δabi3* treated with ABA and desiccation was labeled with 119 and 121. Only WT treated with ABA survived desiccation (*). Photos were taken one week after desiccation and rehydration treatment.

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