Arabidopsis poly(ADP-ribose) glycohydrolase 1 is required for drought, osmotic and oxidative stress responses

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ABSTRACT

Poly(ADP-ribosyl)ation is a post-translational protein modification that plays important roles in many cellular processes in mammalian systems. Emerging evidence indicates that poly(ADP-ribosyl)ation is also involved in plant growth, development, and stress responses. In the present study, we used genetic mutant *parg1-3* and transgenic *PARG1*-overexpressing *Arabidopsis* plants to examine the role of poly(ADP-ribose) glycohydrolase1 (PARG1) in abiotic stress resistance. Osmotic (mannitol treatment) or oxidative [methyl viologen (MV) treatment] stress reduced germination rates of the *parg1-3* seeds compared with wild type seeds. The *parg1-3* plants showed reduced tolerance to drought (withholding water), osmotic, and oxidative stress, as well as increased levels of cell damage under osmotic and oxidative stress and reduced survival under drought stress when compared with the wild type plants. Stomata of the *parg1-3* plants failed to close under drought stress conditions. The expression level of oxidative stress-related genes *AtAox1* and *AtApx2* in the *parg1-3* plants was reduced after MV treatment. However, when *PARG1* was overexpressed in the *parg1-3* mutant and the wild type Col-0 background, similar phenotypical changes to wild type were noted in response to drought, osmotic, or oxidative stress. These results suggest a function for PARG1 in abiotic stress responses in *Arabidopsis*.

1. Introduction

During their lifespan, plants encounter many unfavorable environmental conditions, such as drought, salinity, and oxidative stress, which can adversely affect their growth and development [1]. To cope with abiotic stress, plants invoke multiple complicated and precisely regulated physiological and molecular networks, which are only now becoming understood through a combination of physiological, biochemical, molecular, genetic and genomics studies [2–6]. These responses of plants to environmental stress are now recognized to occur through altered expression of many abiotic stress-related genes, many of which have great potential for crop improvement [7–11].

Recent studies have demonstrated that post-translational modifications of some regulatory proteins can modulate plant responses to abiotic stresses [12–15]. Poly(ADP-ribosyl)ation is an immediate, but transient, post-translational protein modification. This reaction is achieved by poly(ADP-ribose) polymerases (PARPs), which catalyze the transfer of ADP-ribose moieties from the substrate nicotinamide adenine dinucleotide (NAD^+) to target proteins to form poly(ADP-ribose) polymers [16,17]. By contrast, poly(ADP-ribose) glycohydrolases (PARGs) degrade poly(ADP-ribose) polymers [16,17]. Proteins modified by poly(ADP-ribosyl)ation are involved in a wide range of cellular processes in animal systems, including chromatin decondensation, centrosome duplication, and telomere integrity, as well as cell division, transcription, DNA repair, cell survival, and death [17–23].

Increasing evidence now indicates that poly(ADP-ribosyl)ation is also one of the important regulatory mechanisms that modulate plant responses to various abiotic stresses. The first line of evidence came from experiments with cultured soybean and tobacco suspension cells that were protected from programmed cell death triggered by H_2O_2 or heat shock by the addition of PARP inhibitors [24,25]. Later studies showed that DNA damage induced by ionizing radiation activates a rapid and massive expression of *PARP1* and *PARP2* genes in all Arabidopsis tissues, whereas the accumulation of *PARP2* transcripts is preferentially induced by dehydration and cadmium stress [26]. Further functional analysis revealed an inhibition of cell death and conferral of more tolerance to a broad range of abiotic stresses, such as high light intensity, drought, and heat stress, when PARP activity was reduced by means of chemical inhibitors or by gene silencing [27]. Similarly, reduction

of PARP2 levels by RNAi-mediated downregulation in transgenic Arabidopsis and oilseed rape plants resulted in greater resistance to various abiotic stresses, including drought stress, in laboratory and greenhouse experiments, but had no significant effect on growth, development, and seed production [28]. This increased stress tolerance was initially attributed to maintenance of energy homeostasis due to reduced NAD^+ consumption or increased levels of cyclic ADP-ribose, but microarray-based gene expression profiling revealed an up-regulation of a large set of abscisic acid (ABA)-responsive genes in PARP2-deficient plants [27–29]. Recent studies have also implicated PARP in plant responses to pathogen infection, as the induction of innate immune responses (e.g., callose deposition, lignin deposition, and phenylalanine ammonia lyase activity) by treatment with two well-known microbe-associated molecular patterns, flg22 and elf18, which can be blocked by PARP inhibitors [30,31].

In contrast, little is known about the functions of PARGs in plants. Recently, PARG1 (or At2g31870, also known as TEJ) was implicated as a regulator of the circadian oscillator because mutation of PARG1 in Arabidopsis affected the clock-controlled transcription of genes and altered the timing of photoperiod-dependent transition from vegetative growth to flowering [32]. Expression of putative PARG genes including PARG1 was also up-regulated in response to oxidative stress caused by methyl viologen (MV) [33]. Functional analysis using T-DNA insertion lines indicated that mutations in both PARG1 and another putative PARG gene (At2g31865) accelerated the onset of disease symptoms caused by infection with Botrytis cinerea [31]. Therefore, like PARPs, PARGs also appear to have diverse functions in plant biotic and abiotic stress responses.

In our study on the function of PARG1 in disease resistance response, we occasionally observed that plants of a parg1 mutant line suffered drought stress while the wild type plants grew normally in an accident that all Arabidopsis plants grown in a growth room were not watered for a period of 4-days. In the present study, we thus examined in detail the possible function of PARG1 in abiotic stress tolerance in Arabidopsis using genetic mutant parg1-3 and transgenic PARG1-overexpressing plants. Our results indicate that PARG1 is required for tolerance to drought, osmotic and oxidative stress in Arabidopsis and thus suggest an important role for PARGs in abiotic stress response in plants.

2. Materials and methods

2.1. Plant materials and growth conditions

Seeds of wild type (ecotypes Col-0 and Ws-0) and a T-DNA insertion line (FLAG315E11) were obtained from the Arabidopsis thaliana Resource Centre at Ohio State University, USA, and the Arabidopsis thaliana Resource Centre for Genomics at the Versailles Genetics and Plant Breeding Laboratory, France, respectively. All Arabidopsis plants were grown in soil or grown on a 1/2 Murashige and Skoog (MS) medium containing 1% sucrose and 0.8% agar in a growth room under fluorescent lighting ($150 \mu E\,m^2\,s^{-1}$) at $22 \pm 2\,°C$ with 60% relative humidity and a 12 h light/12 h dark cycle.

2.2. Identification of the parg1-3 mutant line

Homozygous plants from the FLAG315E11 line were obtained by polymerase chain reaction (PCR)-based genotyping using a pair of gene-specific primers 315E11-LP (TAC TCT CGA GCC ATC TGC TTC) and 315E11-RP (GTG AAC TCC CAA TGG AGA CTG) along with a T-DNA primer F-LB4 (CGT GTG CCA GGT GCC CAC GGA ATA GT). Seeds from homozygous plants were used for all experiments.

2.3. Generation of transgenic overexpression lines

To generate transgenic overexpression lines in parg1-3 or wild type Col-0 background, the coding region of the PARG1 gene was amplified by RT-PCR using a pair of gene-specific primers PARG1-orf-1F (ATA GAA TTC ATG GAG AAT CGC GAA GAT CT) (EcoRI site underlined) and PARG1-orf-1R (GCA GTC GAC TCA AGG CGG CTG CAT AGC TT) (SalI site underlined). The amplified coding region was cloned into pUCm-T vector by T/A cloning, yielding pUCm-PARG1-1 plasmid, confirmed by sequencing from both directions. The coding region was released from the pUCm-PARG1-1 plasmid by digestion with EcoRI/SalI and then inserted into the EcoRI/SalI sites of a binary vector pCAMBIA 99-1 under control of the cauliflower mosaic virus (CaMV) 35S promoter in the sense orientation, thus yielding pCAMBIA991-PARG1-1 plasmid. This recombinant plasmid was introduced into Agrobacterium tumefaciens strain GV3101 by electroporation using a GENE PULSER II Electroporation System (Bio-Rad Laboratories, USA).

Transformation was performed using the floral dip method as described previously [34]. Seeds from transformed plants (T0) were harvested and screened on 1/2 MS medium containing hygromycin (Hgr) at $30\,\mu g/ml$. Transformants of the T1 generation were selected and self-pollinated. The progeny of the T1 transformants were observed on selective medium and transgenic lines with 3:1 (Hgr-resistant/Hgr-sensitive) segregating ratio were selected and transferred to soil for self-pollination. Progeny of the individual T2 plants were observed on selective medium and those lines whose seedlings showed Hgr resistance were selected as homozygous lines and used for further studies.

2.4. Seed germination assays

Seeds were surface-sterilized and plated on 1/2 MS medium supplemented with 400 mM mannitol or 10 μM MV (Sigma, USA) or with same volume of water (control). The plated seeds were incubated at 4 °C for 48 h to synchronize germination and the seed germination (emergence of radicals) was scored every two days. Experiments were independently repeated at least three times.

2.5. Drought and oxidative stress treatments

Drought stress treatment was performed by withholding water for 2 weeks from soil-grown four-week-old plants. After the 2 week drought period, the plants were re-watered and the numbers of plants that continued to grow were recorded to calculate the survival rate [35].

For osmotic stress treatment, three-week-old plants grown on 1/2 MS medium were removed and transferred into water for 20 h. The plants were then transferred into solutions supplemented with 0 mM or 500 mM mannitol for 4 h. Tolerance to osmotic stress was evaluated by quantification of electrolyte leakage after stress treatment [36]. Briefly, initial conductivity of the bathing solution was measured using a DDS-IIAT type conductivity 510 meter. The samples in the bathing solutions were then boiled for 5 min and volumes of the bathing solution were brought up to the initial volumes, followed by measurement of the total conductivity. The percentage of electrolyte leakage was calculated as $100 \times$ (initial conductivity of the test samples)/(total conductivity after boiling).

For oxidative stress treatment, four-week-old plants grown in soil were treated by foliar spraying with 50 μM MV and symptoms were observed. Alternatively, leaves detached from four-week-old plants were floated on 1/2 MS liquid medium containing 1 μM MV, and oxidative stress response was evaluated by measuring the chlorophyll content of the leaf tissues according to the method described previously [37]. Chlorophyll was extracted with 95% ethanol overnight, and the content was determined spec-