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Synthesis and degradation of poly(ADP-ribose) in plants

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1. ABSTRACT

Poly(ADP-ribosylation) is a post-translational modification of proteins involved in a wide range of molecular and cellular processes in mammalian system. The main enzymes responsible for this modification are the poly(ADP-ribose) polymerases that catalyze the transfer of ADP-ribose moieties from NAD^+ to target protein acceptors, producing long and branched ADP-ribose polymers. The poly(ADP-ribosyl)ation is rapidly reverted by poly(ADP-ribose) glycohydrolase enzymes, which hydrolyzes poly(ADP-ribose) polymers, generating free ADP-ribose. So far, nine proteins with a poly(ADP-ribose) polymerase signature and two poly(ADP-ribose) glycohydrolase enzymes encoded by two adiacent genes were identified in *Arabidopsis*. The present review will describe the structures and functions of plant poly(ADP-ribose) polymerases and poly(ADP-ribose) glycohydrolases.

2. INTRODUCTION

Poly(ADP-ribosyl)ation is a covalent modification of proteins catalyzed by poly(ADP-ribose) polymerase (PARP) enzymes that use NAD^+ as substrate to transfer successive ADP-ribose (ADPR) units to glutamic or aspartic acid residues of target proteins, giving rise to long and branched ADP-ribose polymers (1-2). This modification was linked with a broad range of molecular and cellular processes, including DNA damage detection and repair, transcription and chromatin modification, cell death (2-7).

PARP enzymes are characterized by a catalytic beta-alpha-loop-beta-alpha NAD^+ fold, called PARP signature (8-9). Although PARPs were found in diverse group of eukaryotes (1, 5), these proteins were best studied in mammalian, where, 18 members of PARPs were characterized (1, 5). All members of this family share a

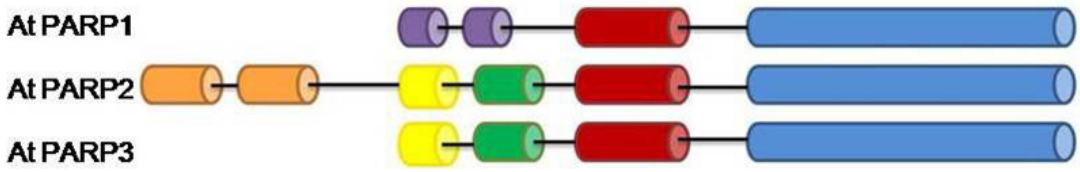

Figure 1. Conserved protein domains in plant poly(ADP-ribose) polymerase (PARPs). PARP domain (blue) = PARP catalytic domain; SAP (SAF-A/B, Acinus and PIAS) domain (purple) = putative DNA and RNA binding domain; WGR superfamily (red) = putative PARP nucleic acid binding domain; BRCT (BRCA1 CTerminal) domain (green) = protein–protein and protein–DNA break binding domain; Zinc finger (orange) = PARP-type DNA nick sensor; PADPR1 Poly(ADP-Ribose) (yellow) = unknown function, found in ADP-ribose synthetases.

PARP catalytic domain, but vary widely in other parts of the proteins (1, 5). The PARP variable domain structures could explain the different functions of mammalian enzymes. Only human PARP1 (10), and its orthologs from the other species, PARP-2, tankirase 2, vPARP show PARP activity (10-17). These proteins have a conserved catalytic glutamate residue in "HYE" catalytic triad, that seems to be important for chain elongation of ADPR. Conflicting data about human PARP3 activity were reported (18-19). Some of the recently discovered PARPs (PARP6, PARP16 and PARP10) seem be closer to (ADPribosyl)transferases (ARTs), as they catalyze mono(ADP-ribosyl)ation reactions (20), whereas PARP9 has not enzymatic activity (7).

In plants, the poly(ADP-ribosyl)ation was first described in 1970s and since than nine proteins with PARP signature were characterized. PARP enzymes in *Arabidopsis*, poplar and rice contain the conserved PARP catalytic domain and WGR nucleic acid binding domains. Based on conserved protein domain structure, plant PARPs are divided in three groups. The first contains proteins with two zinc-finger DNA binding domain at N-terminal, as human PARP1. Proteins, lacking N-terminal zinc fingers and resembling the human PARP2, belong to the second group. The third group, instead, contains only those proteins, that resemble human PARP1, but are devoid of N-terminal zinc-fingers. AtPARP2, At-PARP1-APP and AtPARP3 are the *Arabidopsis* PARPs corresponding to human PARP1, PARP2 and PARP3, respectively.

Besides human PARP1-3 counterparts, other PARP-like proteins were also found in *Arabidopsis*. These proteins include RADICAL-INDUCED CELL DEATH 1(RCD1) and SIMILAR TO RCD-ONE (SRO) 1-5 (21-22). They do not show PARP activity, but might catalyze mono(ADP-ribosyl)ation (20).

As animal PARPs, also plant proteins are associated with DNA repair (3, 23-24), transcriptional regulation (25-27) and cell death (28). In addition, plant PARPs are also implicated in response to abiotic and biotic stresses (23-24, 29), in stress tolerance (24) and in developmental processes (30).

Poly(ADP-ribosyl)ation is a transient modification. It is removed by poly(ADPR) glycohydrolase enzymes (PARGs) responsible for hydrolysis of the poly(ADP-ribose) (PAR) synthesized by PARPs (31). All known animal genomes encode a single PARG gene (32) and the protein is expressed in several isoforms (33). In *Arabidopsis* two adiacent PARG genes were identified (34). PARG enzymes were linked to DNA repair (35), circadian rhythms (36) and plant defense responses (37-39).

3. STRUCTURE AND DISTRIBUTION OF PLANT PARPs

The first evidences of plant poly(ADP-ribosyl)ation were obtained by biochemical investigations in germinating seeds (40) and cytological analysis into onion tissues (41). In 1979, Whitby *et al.* referred about an incorporation of radioactivity from NAD^+ into acid-insoluble material of wheat nuclei and showed for the first time, the modification of H1 and H2A/H2B histones by polymers consisting of about three ADPR units (42). ADP(ribosyl)ated histones were also detected in nuclei from cultured tobacco cells, where AMP and (phosphoribosyl)-AMP were the reaction products obtained by digestion of ADPR chains with snake venom phosphodiesterase (43).

Since then, a PARP family were characterized in plants, in which, human PARP1 and PARP3 orthologs were found (44). Compared to human PARP superfamily, containing 18 members divided into five subfamilies (3, 44), plants contain relative few such proteins, grouped into three categories (45-47). Based on conserved protein domain structure, in *Arabidopsis thaliana* three AtPARPs were identified: AtPARP2, which shows a high structural similarity with human PARP1 (HsPARP1), AtPARP1-APP and AtPARP3 more similar to human PARP3 (HsPARP3) (Figure 1) (47). AtPARP2, as its HsPARP1 ortholog, is localized in the nucleus, has a molecular weight of approximately 113kDa and shares a conserved domain structure with human protein (34). In line with other HsPARP1 orthologs, AtPARP2 is widely expressed (48) and in common with human PARP family presents the so called catalytic triad consisting of histidine-tyrosine-glutamic acid residues (HYE) (45). The first two residues are essential to NAD^+ binding (49), while the third is important for polymer synthesis (50).

Moreover, it is of great interest that although AtPARP2 has an organization of domains similar to that of the animal counterpart, the sequences are more similar to the mouse enzyme than to other plant PARPs (45).

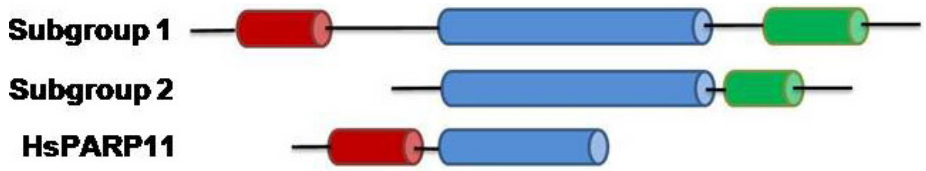

Figure 2. Structure of SRO family and Hs PARP11. The subgroup 1 contains WWE domain (red) and at C-terminal the PARP catalytic domain (blue) with RST domain (green). The subgroup 2 lacks the N-terminal region, but retains both catalytic site and RST domain.

AtPARP1-APP and AtPARP3, instead, have a molecular weight of 74kDa and 95kDa respectively (44). AtPARP1-APP differs from human PARP1, because shows a specific plants domain structure, consisting of two domains SAP at the N-terminal end (45). SAP domains are capable to bind nucleic acids (51) and essential to localize proteins to kinetochore during mitosis (52).

Opposite to AtPARP2-APP, AtPARP3 does not contain SAP domains and for this reason seems to resemble more HsPARP2 in N-Terminal domain structure (47). AtPARP3 catalytic domain has a particular triad. All members of this group have a cysteine instead of histidine at the first position and conserve glutamic acid at the third position. In seedless plants, the tyrosine remains at the second position, while the valine replaces the tyrosine in angiosperms (34).

AtPARP1 and AtPARP2 such as all plant PARP orthologs, which have the HYE catalytic triad within their PARP signature, show enzymatic activity. The substitution of the first amino acid in catalytic domain of AtPARP3, instead, might entail the elimination to NAD^+ binding site and explain the absence of enzymatic activity (44).

Orthologs of HsPARP6, 8 and 16 have been found in bryophytes (44). These proteins, as the corresponding human counterparts, seem to be unable to catalyze poly(ADP-ribosyl)ation due to the changes in catalytic domain (20).

In all land plants, a family of PARP-like proteins called SRO (Similar to RCD-ONE) has been identified too. This family includes two subgroups: the first contains an N-terminal WWE domain (44) and at C-terminal the PARP catalytic domain with an extension, which consist of RST domain (Figure 2) (53). The second subgroup, instead, lacks the N-terminal region, but retains both catalytic site and RST domain (21-22, 53).

In *A. thaliana*, SRO protein family contains six members: AtRCD1 (Radical Induced Cell Death 1) and AtSRO1 to AtSRO5. Both AtRCD1 and AtSRO1 have the same domain structure of the first subgroups of SRO family, while all other members show the same structure of the second subgroup. AtRCD1 does not show enzymatic activity (34). Although several PARPs, as HsPARP7, HsPARP12, HsPARP13 and HsPARP14 contain the WWE and PARP domain, the human PARP most similar in domain structure to *A. thaliana* RCD1 and SRO1 is HsPARP11, because it has not other conserved domains besides these two (Figure 2) (53).

4. FUNCTIONS OF PLANT PARPS

In plants, as in animals, PARPs play a relevant role in genotoxic stress response and their involvement in DNA repair (23-24), transcriptional regulation (23, 38, 54) and cell death (34) was also demonstrated. In *A. thaliana,* a massive and rapid accumulation of AtPARP1 and AtPARP2 transcripts was observed upon treatment with ionizing radiation and reactive oxigen species (ROS). The accumulation of AtPARP2 transcripts in all organs of the plant is followed by AtPARP2 protein accumulation only in tissues containing a large amount of actively dividing cells (23). AtPARP2 accumulation in response to DNA damage suggests that this protein, as animal counterpart, is a sensor of DNA damage and plays a relevant role in the maintenance of genomic integrity (23). AtPARP2 transcripts also accumulate in response to genotoxic stress in brushy 1 plants (55), while only AtPARP1expression is induced in ovules of dnalig1 mutants (56-57).

In addition to its role of sensor of DNA damage, AtPARP2 is involved in apoptosis too (58). Depending on the severity of DNA damage, plant PARP is involved in DNA repair or programmed cell death (PCD). Mild DNA damage induces PARP activation, which leads to genome repair and cell survival. At the contrary, when high levels of DNA damage occur, PARP overactivation, producing large NAD^+ consumption can cause apoptosis (58). The significant decrease in cellular NAD^+ levels measured in soybean treated with high doses of H_2O_2 demonstrated the relationship between plant PARP activation and cell death induced by oxygen radicals. The AtPARP2 overexpression, instead, seems to be indicative of its possible protective role against low H_2O_2 concentrations (58). In fact, AtPARP2 overexpression is correlated to reduction of the number of ROS-induced DNA breaks (58). On the contrary, AtPARP1 overexpression increases the number of DNA nicks (58).

PARPs is activated by several abiotic stresses, as dehydration, heat, high light and salinity (23, 56, 59-60). Plant tolerance to stresses was demonstrated by use of PARP chemical inhibitors (24, 61). In fact, PARP activity inhibition reduces cellular energy consumption, allowing to plants to became tolerant and to survival to multiple adverse environmental conditions (24). Another hypothesis to explain how the reduction of PARP activity leads to stress tolerance is that PARP is able to regulate key stress signaling pathways at transcriptional level (27) by direct control (25) or indirectly by abscissic acid (ABA) regulation (27).

AtPARP1 and AtPARP2 are also implicated in differentiation (62-63), in cell cycle (64), in mitosis (25) and in responses to biotic stress (35). In particular, in *Arabidopsis thaliana*, a poly(ADP-ribose) accumulation and changes in patterns of ADPR protein acceptors were evidenced, after exposure to bacterial infections (35, 65). Several studies, mostly based on gene expression analysis, demonstrated roles for RCD1 and SRO1 orthologs in hormone signaling, plant development and response to biotic and abiotic stresses (21, 66-73). RCD1 is considered one of the major regulators of plant ozone (O_3) tolerance (74). In RCD1 mutants, an increase of sensitivity to extracellular reactive oxygen species (ROS), a highly resistance to chloroplastic ROS formation by paraquat and ultraviolet radiation and osmotic stress (21, 75-76) were observed. Loss expression of RCD1 causes dramatic defects in plant development (21, 75, 77). In chloroplastic ascorbate peroxidase mutants, SRO2 gene is upregulated in response to high light (78). SRO5 expression is very low under normal conditions, but it is transcriptionally induced by ROS and in response to salt treatment (79). High light, instead represses its expression (80). When SRO5 is induced by salt stress, a 24-nucleotide SRO57P5CDH siRNA is formed, that cause P5CDH downregulation and accumulation of proline, which is essential for salt tolerance (79).

5. TURNOVER OF POLY(ADPR) IN PLANTS

In normal conditions, PARPs produce low basal PAR levels, which can increase dramatically in response to genotoxic stresses. In animal, as in plants, the removal of PAR by the same PARP as well as from other protein acceptors is assured by poly(ADP-ribose) glycohydrolase (PARG) (31, 81-82). Animal PARG is encoded by a single gene (32, 81-82), which is alternatively spliced to generate three different protein isoforms with different molecular weight and different cellular localization (33). Animal PARG has been reported to play a relevant role in DNA repair (83-85), cell death (86-87) and embryonic development (39).

Arabidopsis thaliana genome encodes two adiacent PARG genes, which are present due to gene duplication (At2g31870 and At2g31865) (36). *PARG1* (At2g31870, also known as TEJ) seems to be a regulator of the circadian oscillator too. Mutations of TEJ influence the clock-controlled transcription of genes and produce alteration the timing of photoperiod dependent transition from vegetative growth to flowering (36). In castor oil plant, peanut and sorghum, PARG is encoded by a single gene, whereas multiple PARG proteins are encoded in other plants, as rise, tomato, maize and poplar (36). Much less is known about the functions of plant PARG1, but enzyme activity was linked to DNA repair mechanisms and cell death too (35). In addition, like PARPs, also plant PARGs seem to be involved in both abiotic and biotic stress responses. Abiotic stimuli increase PARG2 gene expression. In fact, the infections by both virulent and avirulent *Pst* and MAMPs (65), as well as the infection with *Botrytis cinerea* produce an upregulation of PARG2 transcripts (35). PARG1 transcripts upregulation, instead, was related to tolerance to drought, osmotic and oxidative stress (39, 59, 88).

The high and toxic levels of free-ADP-ribose, produced by PARG activity, are degradated into AMP and ribose-5-phosphate by Nudix (for nucleoside diphosphates linked to some moiety X) hydrolases (59, 89-91).

In *Arabidopsis*, 27 genes encoding Nudix proteins (AtNUDX1-AtNADX27) were found (91-92). AtNUDX6 and AtNUDX7 are the most studied proteins and offer a contrasting example. In fact, contrary to AtNUDX7, showing both NADH and ADP-ribose pyrophosphatase activity (92-93), AtNUDX6 seems predominantly to be an NADH-pyrophosphatase (94). AtNUDX7 is related to immune responses to pathogens (95) and implicated in plant abiotic stress responses (47). Furthermore, AtNUDX7 is also considered as a negative regulator of plant defense responses (65, 92, 95), whereas AtNUDX6 plays a positive role in plant defences (47).

6. CONCLUSIONS

Over the past two decades, HsPARP1 orthologs and three unique subfamilies of PARP enzymes were found in land plants (44). These subfamilies include the AtPARP1 group with SAP domains, the AtPARP3, which contains unique substitutions in the catalytic domain and SRO family (21-22, 47).

The high degree of conservation at amino acid levels between *Arabidopsis* and mammalian PARPs suggests that many biological functions are conserved between plants and animals (34). In fact, PARP family and poly(ADP-ribosyl)ation are involved in a variety of biological functions in plants, including DNA repair, transcription and cell death (23-24, 34).

In addition, the well demonstrated involvement of PARPs, PARGs and SRO family in abiotic and biotic stress responses (56, 59-60) induces to hypothesize that the poly(ADP-ribosyl)ation modulation might represent a useful way to allow survival of economically valuable plant species in harsh or unpredictable environmental conditions.

Despite relevant knowledge about the structure and function of plant PARPs and PARGs, few studies have been conducted on both identification of ADPR protein acceptors and proteins interacting with them. No ADP(ribosyl)ated proteins other than histones have been identified in plants (42). The identification of new targets of poly(ADPR) will allow a better understanding of the role of PARPs and poly(ADP-ribosyl)ation in plant stress responses and development. Another fundamental question remains unresolved. It is not yet clear whether and which proteins of AtPARP3 subfamily and SRO family have poly(ADP-ribose)polymerase activity. Given the variations into the catalytic domain of plant SRO, it will be essential to determine whether some members of this family can have mono(ADP-ribose) transferase activity.

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Abbreviations: ADP: adenosine diphosphate; PARP: poly(ADPR) polymerase; NAD^+ nicotinamide adenine dinucleotide; PARG: poly (ADP-ribose) glycohydrolase; PAR: poly(ADP-ribose); ADPR: adenosine diphosphate ribose; ART: (ADPribosyl)transferase; RCD: radical-induced cell death; SRO: similar to RCD-ONE; PCD: programmed cell death; ROS: radical oxygen species; ABA: abscissic acid; MAMPS: microbe-associated molecular patterns; Pst: Pseudomonas syringae; NUDX: Nudix proteins; NADH: reduced adenine dinucleotide.

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