Review

Poly(ADP-ribosyl)ation in plants

Amy G. Briggs[1] and Andrew F. Bent[2]

[1] Program in Cellular and Molecular Biology, University of Wisconsin - Madison, Madison, WI, USA
[2] Department of Plant Pathology, University of Wisconsin - Madison, Madison, WI, USA

Poly(ADP-ribose) polymerases (PARPs) and poly(ADP-ribose) glycohydrolases (PARGs) are the main enzymes responsible for the post-translational modification known as poly(ADP-ribosyl)ation. These enzymes play important roles in genotoxic stress tolerance and DNA repair, programmed cell death, transcription, and cell cycle control in animals. Similar impacts are being discovered in plants, as well as roles in plant-specific processes. In particular, we review recent work that has revealed significant roles for poly(ADP-ribosyl)ation in plant responses to biotic and abiotic stress, as well as roles for ADP-ribose pyrophosphatases (a subset of the nucleoside diphosphate linked to some moiety-X or NUDX hydrolases). Future challenges include identification of poly(ADP-ribosyl)ation targets and interacting proteins, improved use of inhibitors and plant mutants, and field-based studies with economically valuable plant species.

Poly(ADP-ribosyl)ation

Poly(ADP-ribosyl)ation has received significant attention over the past 40 years in animal systems [1–4], but it has received surprisingly little research attention from plant scientists. Poly(ADP-ribosyl)ation is a post-translational protein modification in which poly(ADP-ribose) polymerases (PARPs, see Glossary) catalyze the transfer of ADP-ribose moieties from NAD^+ to target acceptor proteins. This modification can be reversed by poly(ADP-ribose) glycohydrolases (PARGs) that hydrolyze poly(ADP-ribose) polymers, generating free ADP-ribose. Nucleoside diphosphate linked to some moiety-X (NUDX) hydrolases with specificity for ADP-ribose, also known as ADP-ribose pyrophosphatases, can then degrade free ADP-ribose into AMP and ribose-5-phosphate (Figure 1). Excellent animal-focused reviews of poly(ADP-ribosyl)ation are available [1–4]. Auto-modified PARP and other poly(ADP-ribosyl)ated nuclear proteins can affect chromatin structure, transcription, replication, and DNA repair processes through PARP-mediated recruitment of other proteins [5–7]. Strong activation of PARP and poly(ADP-ribosyl)ation can also regulate cellular processes by consuming massive amounts of NAD^+, which can alter cellular reduction-oxidation states, impact nicotinamide levels, and induce ATP depletion [1–4,8].

In animals, poly(ADP-ribosyl)ation plays a crucial role in several cellular processes, including stress recovery, programmed cell death, DNA damage responses, chromatin structure and gene expression [1–4]. At the organism level, poly(ADP-ribosyl)ation in animals contributes to the pathology of heart attack, ischemia, Alzheimer's, and sensitivity of cancerous tissue to chemotherapeutic agents [9]. Multiple pharmaceutical companies have invested heavily in identifying medically useful PARP inhibitors (reviewed in [1–4]).

Poly(ADP-ribosyl)ation has now been implicated in several physiological processes in plants, including circadian rhythms and responses to abiotic and biotic stresses. Molecular characterization of these mechanisms has begun. Although the first documentation of an NAD^+-consuming nuclear plant protein (now known to be PARP; see Glossary) occurred three decades ago, many relevant studies have only recently been completed. The main focus of this review is on plant PARP, PARG and NUDX enzymes and on the physiological roles of poly(ADP-ribosyl)ation-related processes in plant biology, in particular during responses to biotic and abiotic stress. We also discuss current challenges in the field, including identification of poly(ADP-ribosyl)ation targets and interacting proteins, and use of mutants and inhibitors. This review is organized by enzyme type.

PARP

Eukaryotic organisms (excluding yeast) express multiple PARP proteins, all bearing a conserved C-terminal PARP catalytic domain that binds and cleaves NAD^+ into ADP-ribose and nicotinamide. The *Arabidopsis* (*Arabidopsis thaliana*) genome encodes at least three putative PARPs [At4g02390 (PARP1), At2g31320 (PARP2), and At5g22470 (PARP3)] [10,11], and maize (*Zea mays*) homologs of AtPARP1 have also been characterized [12]. There is evidence that plant PARPs are structurally homologous to mammalian PARP proteins [12–15]. The high degree of conservation at the amino acid level between *Arabidopsis*

Glossary

3AB: 3-aminobenzamide; a PARP inhibitor.
3MB: 3-methoxybenzamide; a PARP inhibitor.
ABA: abscisic acid; a plant hormone.
HR: hypersensitive response; induced plant defense response to avirulent pathogens.
MAMP: microbe-associated molecular pattern; conserved protein sequence expressed by microbial pathogens; elicits basal immune response.
Nic: nicotinamide; a PARP inhibitor.
NUDX: nucleotide diphosphate linked to some moiety-X; cleaves ADP-ribose into AMP + ribose-5-phosphate.
pADPr: poly(ADP-ribose); a nucleic acid; a post-translational modification.
PAL: phenylalanine ammonia lyase; initial enzyme in the phenylpropanoid biosynthesis pathway.
PARG: poly(ADP-ribose) glycohydrolase.
PARP: poly(ADP-ribose) polymerase.
PCD: programmed cell death.
Pst: *Pseudomonas syringae* pv *tomato*; a biotrophic bacterial plant pathogen.
ROS: reactive oxygen species.
TEJ: Sanskrit for 'bright'; also known as *Arabidopsis* PARG1.

Corresponding author: Bent, A.F. (afbent@wisc.edu)

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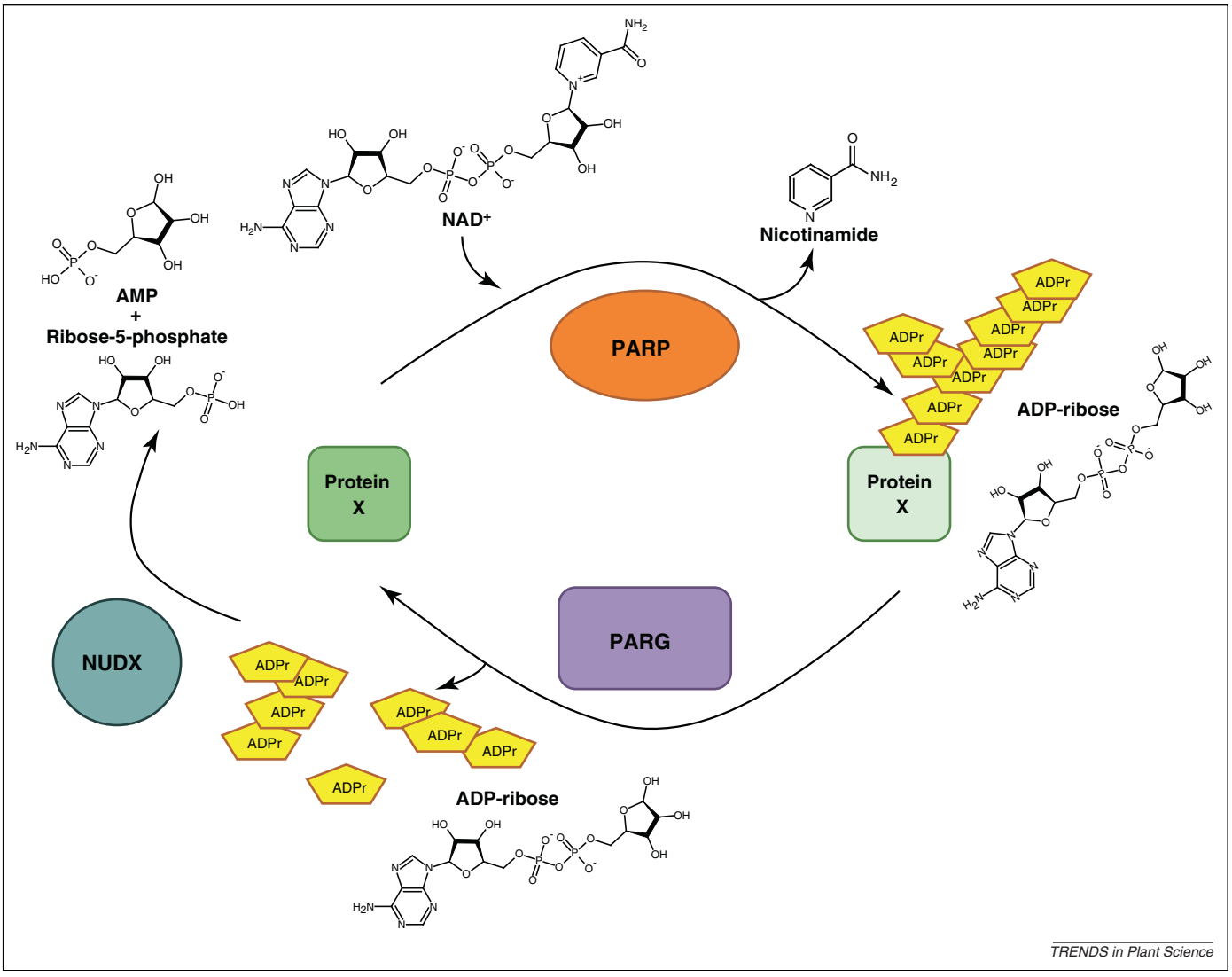

Figure 1. Poly(ADP-ribose) metabolism by poly(ADP-ribose) polymerase (PARP), poly(ADP-ribose) glycohydrolase (PARG), and nucleotide diphosphate linked to some moiety-X (NUDX) enzymes. PARP enzymes bind NAD^+ (nicotinamide adenine dinucleotide), cleave off the nicotinamide residue, and attach the remaining ADP-ribose moiety to acceptor proteins (protein X, which can include PARP itself). PARP is a polymerase that adds multiple ADP-ribose monomers as long, occasionally branched, chains up to 200 units in length (note that NAD^+ is consumed). PARG then exolytically and endolytically cleaves the ribose–ribose backbone bond of poly(ADP-ribose), releasing free ADP-ribose. ADP-ribose-specific NUDX enzymes then cleave free ADP-ribose into AMP (adenosine monophosphate) and ribose-5-phosphate.

and mammalian forms of these enzymes, shown schematically in Figure 2, suggests that PARP function is conserved between plants and animals. PARP proteins encoded by *Arabidopsis*, poplar (*Populus trichocarpa*), and rice (*Oryza sativa*) genomes all contain the conserved PARP catalytic domain and WGR nucleic acid binding domains. Based on conserved protein domain structure, plant PARP proteins can be grouped into three categories: (i) those that resemble human PARP1 with two zinc-finger DNA binding domains; (ii) those that resemble human PARP2 and lack further N-terminal domains; and (iii) those that resemble human PARP1, but lack N-terminal zinc-fingers (Figure 2). Note that the naming system for plant PARPs is not symmetric with the naming of animal PARPs.

Besides structural similarities, plant PARPs also have enzymatic activities that are functionally homologous to mammalian PARPs [12–15]. The covalent modification of plant nuclear proteins with poly(ADP-ribose) was first described as the covalent incorporation of NAD^+ into the protein fraction of tobacco (*Nicotiana tabacum*) histones H1 and H2A, and H2B [16]. In both maize [14] and *Arabidopsis* [13], PARP binding of both nicked DNA and NAD^+ has been reported.

Use of PARP inhibitors in plants

PARP inhibitors target the conserved enzymatic active site and, therefore, the use of pharmacological PARP inhibitors is a common way of overcoming the potential functional redundancy presented by the multiple PARPs encoded in any one genome. Importantly, use of PARP inhibitors also allows conditional inactivation of PARP activity. There is an extensive body of literature on the use of PARP inhibitors with animal cells or in animals, e.g. [1–4,17–20]. 3-Aminobenzamide (3AB), nicotinamide (Nic) and 3-methoxybenzamide (3MB) are all established inhibitors of animal PARPs [17–20], with benzamide structures that mimic NAD^+ and interfere with NAD^+-consuming PARP enzymes. These inhibitors have been demonstrated to work in plants as well. All three inhibitors suppress plant PARP enzyme activity in maize nuclei [14], 3MB has been

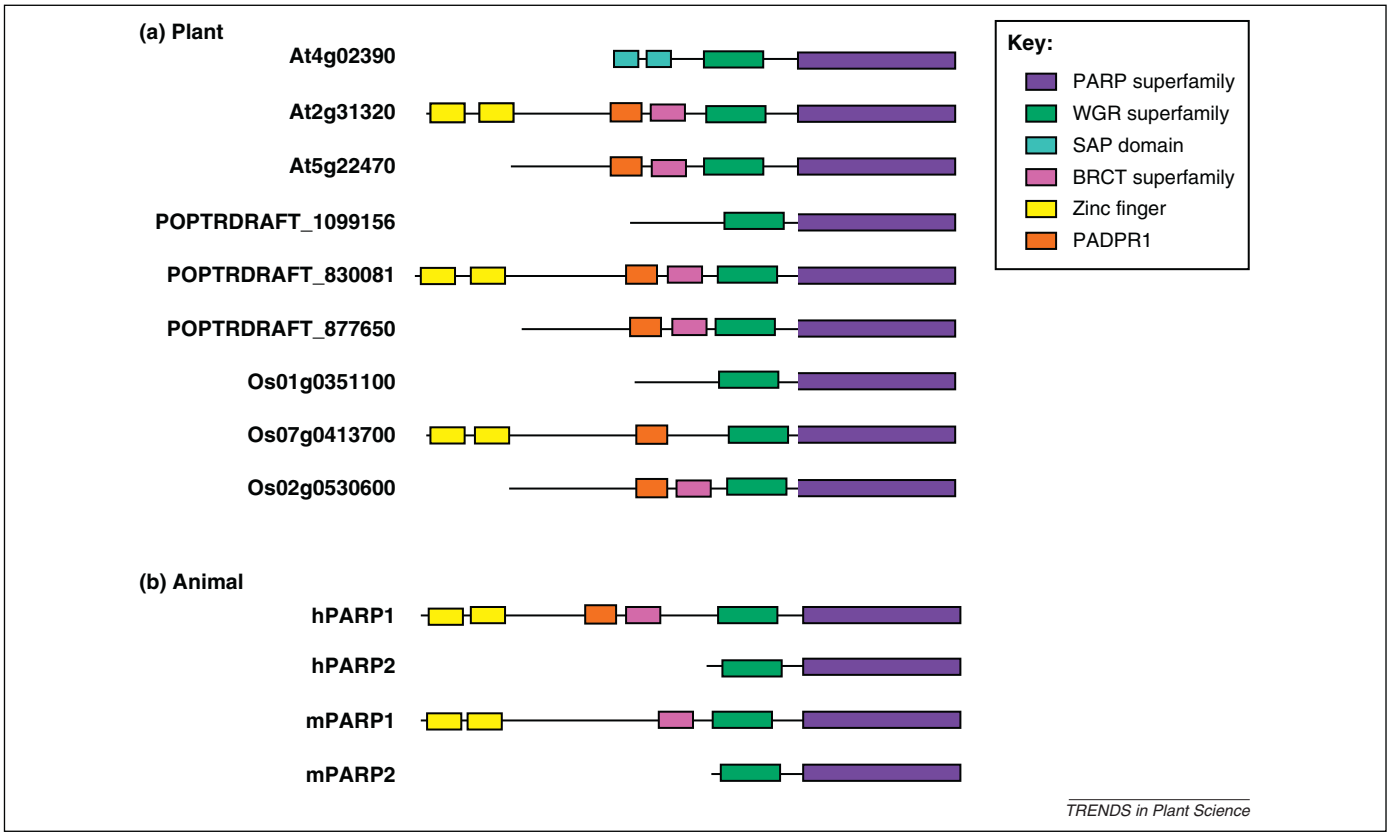

Figure 2. Comparison of conserved protein domains in **(a)** plant and **(b)** animal poly(ADP-ribose) polymerase (PARPs). Derived amino acid sequences from *Arabidopsis* (At), human (h) and mouse (m) as well as poplar (POPTDRAFT) and rice (Os) were analyzed for conserved protein domains (CDD) [69]. PARP domain = PARP catalytic domain; SAP (SAF-A/B, Acinus and PIAS) domain = putative DNA and RNA binding domain; WGR superfamily = putative PARP nucleic acid binding domain; BRCT (BRCA1 C-Terminal) domain = protein–protein and protein–DNA break binding domain; Zinc finger = PARP-type DNA nick sensor; PADPR1 Poly(ADP-Ribose) = unknown function, found in ADP-ribose synthetases.

shown to inhibit synthesis of ADP-ribose polymers by PARP2 in *Arabidopsis* [13], and 3AB prevents the accumulation of poly(ADP-ribose) polymers in *parg1* mutants [21]. Table 1 summarizes the growing body of literature regarding the use of PARP inhibitors in plants. It should, however, be noted that pharmacological inhibitor studies merit cautious interpretation. The concentrations used, tissue access, inhibitor half-life and the specificity of inhibitors for their targets must be considered. Many PARP inhibitors, for example, are light-sensitive. Recent

Table 1. PARP inhibitors in plants

PARP inhibitor	Species	Observed effect	Refs
3AB	*Arabidopsis*	Decreased *XRCC1* and *XRCC2* transcription	[34]
3AB	*Arabidopsis*	Decreased *XRCC1* and *XRCC2* transcription	[34]
3AB	*Arabidopsis*	Heat shock-induced DNA laddering inhibited	[32]
3AB	*Arabidopsis*	Increased susceptibility to paraquat-induced oxidative stress	[34]
3AB	*Arabidopsis*	MAMP-induced callose and lignin deposition blocked	[42]
3AB	*Arabidopsis*	MAMP-induced PAL enzyme activity blocked	[42]
3AB	*Arabidopsis*	MAMP-induced seedling toxicity	[41]
3AB	*Catharanthus roseus*	Oxidative stress-induced PAL activity inhibited	[40]
3AB	*Zea mays*	PARP activity inhibited	[14]
3AB	*Arabidopsis*	Prevents accumulation of ADP-ribose polymers	[21]
3AB	*Helianthus tuberosus*	Tracheary element differentiation inhibited	[37]
3AB	*Pisum sativum*	Tracheary element differentiation inhibited	[37]
3MB	*Zea mays*	PARP activity inhibited	[14]
3MB	*Arabidopsis*	PARP2 activity inhibited	[13]
3MB	*Arabidopsis*	Increased basal somatic homologous recombination rates	[33]
3MB	Tobacco	Increased basal somatic homologous recombination rates	[33]
3MB	*Brassica napus*	Increased resistance to oxidative stress	[30]
Nic	*Zea mays*	PARP activity inhibited	[14]
Nic	*Arabidopsis*	Heat shock-induced DNA laddering inhibited	[32]

Abbreviations: 3AB, 3-aminobenzamide; 3MB, 3-methoxybenzamide; MAMP, microbe-associated molecular pattern; Nic, nicotinamide; PAL, phenylalanine ammonia-lyase; PARP, poly(ADP-ribose) polymerase; XRCC, X-ray repair cross-complementing.

Table 2. Poly(ADP-ribosyl)ation-related genotypes and corresponding phenotypes in plants

Genotype	Species	Phenotype	Refs
AtPARP2 overexpression	*Glycine max*	Increased DNA nicks with high H_2O_2 concentrations	[31]
AtPARP2 overexpression	*Glycine max*	Reduced DNA nicks with low H_2O_2 concentrations	[31]
Bovine *PARP2* overexpression	*Vigna unguiculata*	Hypersensitive response-induced PARP cleavage	[70]
bru1-1	*Arabidopsis*	AtPARP2 transcript accumulates	[30]
dnalig1	*Arabidopsis*	AtPARP1 transcript accumulates	[30]
nic2-1	*Arabidopsis*	Elevated NAD^+	[11]
nic2-1	*Arabidopsis*	Reduced pADPr polymers	[11]
nudt7 knockdown	*Arabidopsis*	NADH accumulates	[65]
nudt7 knockdown	*Arabidopsis*	ADP-ribose polymers accumulate	[34]
nudt7 knockdown	*Arabidopsis*	Constitutive expression of PR1 and PR2 genes	[65,67]
nudt7 knockdown	*Arabidopsis*	Increased pathogen-induced salicylic acid accumulation	[66]
nudt7 knockdown	*Arabidopsis*	Increased resistance to *Hyaloperonospora parasitica*	[66]
nudt7 knockdown	*Arabidopsis*	Increased resistance to virulent and avirulent *Pst*	[41,65,67]
nudt7 knockdown	*Arabidopsis*	Increased susceptibility to paraquat-induced oxidative stress	[34,65]
nudt7 knockdown	*Arabidopsis*	Necrotic lesions and accumulation of ROS	[65,67]
nudt7 knockdown	*Arabidopsis*	Reduced hypersensitive response	[41]
nudt7 knockdown	*Arabidopsis*	Stunted growth	[41,65,67]
NUDT2 overexpression	*Arabidopsis*	Increased resistance to paraquat-induced oxidative stress	[62]
NUDT7 overexpression	*Arabidopsis*	Increased resistance to paraquat-induced oxidative stress	[34]
NUDT7 overexpression	*Arabidopsis*	Reduced ADP-ribose accumulation	[34]
parg1	*Arabidopsis*	ADP-ribose polymers over-accumulate (25-fold increase)	[21]
parg1	*Arabidopsis*	Increased leaf movement, early flowering (short and long day), longer circadian period length	[21]
parg1 knockdown	*Arabidopsis*	Increased susceptibility to *Botrytis cinerea* infection	[42]
parg1 knockdown	*Arabidopsis*	Increased susceptibility to mitomycin-C treatment	[42]
parg1 knockdown	*Arabidopsis*	MAMP-induced phenylpropanoid pigment accumulation exacerbated	[42]
parg1 knockdown	*Arabidopsis*	MAMP-induced seedling toxicity	[42]
parg1 knockdown	*Arabidopsis*	Reduced tolerance to osmotic stress	[57]
parg1 knockdown	*Arabidopsis*	Reduced tolerance to methylviologen-induced oxidative stress	[57]
parg1 knockdown	*Arabidopsis*	Stomatal apertures remain open during and after drought stress	[57]
parg1 knockdown	*Arabidopsis*	Reduced expression of oxidative stress response genes *Aox1* and *Apx2*	[57]
parg2 knockdown	*Arabidopsis*	Increased susceptibility to *Botrytis cinerea* infection	[42]
parp1 knockdown	*Arabidopsis*	Increased H_2O_2-induced DNA nicks	[31]
parp1/parp2 knockdown	*Arabidopsis*	High light stress gene induction is inhibited	[39]
parp1/parp2 knockdown	*Arabidopsis*	High light stress-induced loss of NAD^+ is inhibited	[30]
parp1/parp2 knockdown	*Arabidopsis*	Increase in ABA, dehydration, and cold stress-related transcripts	[39]
parp1/parp2 knockdown	*Arabidopsis*	Increased resistance to DNase1-induced DNA breaks	[30]
parp1/parp2 knockdown	*Arabidopsis*	Increased resistance to drought, methyl viologen, acetylsalicylic acid, and high light stress	[30]
parp1/parp2 knockdown	*Arabidopsis*	No oxidative stress-induced increase in ROS	[30]
PARP2 overexpression	*Arabidopsis*	Reduced incidence of H_2O_2-induced DNA nicks	[31]

Abbreviations: ABA, abscisic acid; Aox1, alternative oxidase 1; Apx2, ascorbate peroxidase 2; H_2O_2, hydrogen peroxide; NAD^+/NADH, nicotinamide adenine dinucleotide; NUDT, nucleotide disphosphate linked to some moiety-X; PARG, poly(ADP-ribose) glycohydrolase; PARP, poly(ADP-ribose) polymerase; PR1 and PR2, pathogenesis-related; ROS, reactive oxygen species.

mammalian work has led to the engineering and discovery of many PARP inhibitors with greater specificity than 3MB, 3AB and Nic [3,22], but most of these chemicals have yet to be tested in plants.

Related work with *Arabidopsis nic2* (nicotinamidase mutant) seeds, which accumulate high levels of PARP-inhibiting nicotinamide, revealed reduced poly(ADP-ribose) levels and elevated NAD^+ levels compared with wild-type seeds, suggesting that PARP activity is impaired [23]. Poly(ADP-ribose) levels also correlated with nicotinamidase activities and treatment with the DNA-damaging agent methyl methanesulfonate, indicating a link between plant PARP enzyme activity and nicotinamide levels [24]. These data suggest not only that plant PARPs have homologous enzyme activities to their mammalian counterparts, but also that several published PARP inhibitors can be confidently used to inhibit plant PARP activity.

Genetic mutations that disrupt poly(ADP-ribosyl)ation activities have been finding increasing use in plant research as an alternative to pharmacological inhibitors, and Table 2 presents a brief summary of some of the phenotypes that have been associated with altered poly(ADP-ribosyl)ation-related plant genotypes. The potential impacts of full knockout of *PARP* genes has led to the use of gene silencing technologies to genetically reduce PARP enzyme levels. However, the level of silencing can be variable. In addition, the potential pleiotropic effects of chronic deficiencies in PARP leave PARP inhibitors as an important alternative to mutants or gene-silenced plant lines. Inducible or tissue-specific gene silencing may offer valuable improvements in the future.

PARP and plant abiotic stress responses

As a DNA nick-activated enzyme, PARP is one of the first responders to sites of DNA breaks. PARP acts as a DNA-break sensor and a DNA repair-signaling molecule, with a caretaker role that can lead cells either toward repair or toward programmed cell death, depending on the severity of the damage and amplitude of PARP activation (reviewed in [25]). Human PARP-1 and PARP-2 bind to and are activated by nicked DNA, undergo auto-modification (self-poly(ADP-ribosyl)ation), and thereupon recruit other DNA repair proteins [26,27]. Plant PARPs play a similar role in genotoxic stress responses. *AtPARP1* and *AtPARP2* mRNAs accumulate rapidly upon treatment with gamma radiation and reactive oxygen species (ROS) [13]. In *brushy1* (*bru1-1*) plants that show constitutive genotoxic stress response activation, *AtPARP2* transcript also accumulated [28]. AtPARP1 and AtPARP2 proteins localize to the nucleus [29], and *AtPARP1* expression was induced in the ovules of *dnalig1* mutants suffering from impaired DNA repair machinery [30]; see also [12]. Overexpression of *AtPARP2* allowed fewer ROS-induced DNA nicks, whereas knocking down *AtPARP1* expression increased the number of DNA nicks [31]. However, somewhat counter-intuitively, silencing both *AtPARP1* and *AtPARP2* increased resistance to DNAse1-induced DNA breaks [30], which might have been due to some compensatory response to a chronic PARP deficit. However, overall, the above results suggest that in plants, as in animals, PARP enzymes can act to protect against or aggravate DNA damage, depending upon the severity of the injury. Particularly in light of the findings reported below on poly(ADP-ribosyl)ation in plant biotic as well as abiotic stress, this will be an interesting area for further research.

There are other contexts in which disruption of plant PARP expression has been either protective against DNA damage or harmful, again suggesting a complex role for PARPs in genotoxic stress responses. PARP inhibitor treatment blocked heat shock-induced DNA breakage and genomic laddering [32], while also increasing basal rates of homologous recombination in *Arabidopsis* and tobacco plants [33]. In 3AB-treated *Arabidopsis* plants, several DNA repair genes were activated under both normal and oxidative stress-inducing conditions compared with wild-type plants [34]. These studies suggest a requirement in plant cells for PARP to maintain genome integrity, as both a negative and positive regulator.

Owing to its role in DNA damage sensing, PARP also plays a role in apoptosis in animals. Low levels of DNA damage lead to some PARP activation, which generally leads to successful genome repair. However, if high levels of DNA damage occur, PARP enzymes become overactivated and consume large amounts of NAD^+. This can cause respiratory stress and initiate apoptosis [35,36]. A similar role for PARP has been at least partially established in plant programmed cell death. Overexpressing *AtPARP2* in cultured soybean (*Glycine max*) cells was protective against low ROS concentrations, but exacerbated cell death at high ROS concentrations [31]. In addition, PARP inhibitors protect soybean and tobacco cell suspensions from oxidative- and heat shock-induced programmed cell death [31,32], suggesting that PARP can contribute significantly to apoptosis in plant cells. These studies also suggest that poly(ADP-ribose) homeostasis must be tightly regulated in plants, given that low levels of activation lend protection, whereas overactivation leads to cell death.

Plant PARPs have also been implicated in differentiation and cell cycle control. 3AB treatment abolished tracheary element development in artichoke (*Cynara cardunculus*) tubers and pea (*Pisum sativum*) roots [37]. Recently, increases in cellular glutathione pools, *AtPARP1* and *AtPARP2* gene expression, and PARP enzyme activity were all observed during the exponential growth phase of *Arabidopsis* tissue culture cells, suggesting a link between redox homeostasis regulation, poly(ADP-ribosyl)ation, and cell cycle control or cell growth and differentiation [38].

Can less PARP lead to improved whole-plant abiotic stress tolerance?

Studies have shown that poly(ADP-ribosyl)ation plays a significant role in the organism-level plant response to abiotic stress. For example, silencing of *AtPARP1* or *AtPARP2* in *Arabidopsis*, or *PARP* silencing in oilseed rape (*Brassica napus*) plants, enhanced plant tolerance of drought, high light and heat stress [30]. This study showed that plants under such stresses activate PARPs, leading to more NAD^+ and ATP consumption, and to accumulation of poly(ADP-ribose). The finding provides support for the mechanistic hypothesis that plants with reduced poly(ADP-ribosyl)ation activity consume less NAD^+ in stressful environments and thereby reduce over-intense mitochondrial respiration, lower ROS production, and improve energy use efficiency [30]. These concepts are consistent with physiological observations made in studies of animal poly(ADP-ribosyl)ation. No obvious deleterious impacts of reduced PARP expression on overall plant growth or appearance were observed [30], although this requires extensive testing in economically valuable plants grown in field environments, which is underway [39]. The residual PARP activity present in partially *PARP*-silenced plants might be sufficient to carry out DNA repair and other essential roles.

The full basis of the above stress tolerance is not yet understood. It is of interest, among other findings, that *B. napus* hypocotyl explants treated with acetylsalicylic acid showed large increases in H_2O_2 production, whereas *PARP*-silenced lines showed almost no increase in ROS [30]. 3MB treatment also increased the resistance of *B. napus* callus tissue to oxidative stress. High energy status, efficient cellular respiration, and low free-radical production in *parp*-silenced lines indicated that they have low energy consumption (and lower respiration rate) when under stress [30]. The hypothesis of increased energy efficiency when PARP activity is reduced gained further support when *PARP*-silencing conferred tolerance to growth on a suboptimal carbon source and inhibited high light stress-induced losses of NAD^+ [30]. Oxidative-stress-dependent gene expression (*HSPs*, *DNAJ*, *RBOHC* and glutaredoxins) is also attenuated in high light-stressed *parp1*/*parp2* silenced plants [39]. See [8] for a recent review on NAD^+ and plant stress responses.

Further hypotheses for the stress tolerance observed in PARP-silenced plants have more recently gained correlative support, as additions to (and not mutually exclusive

with) the hypothesis of improved energy homeostasis. Abscisic acid (ABA)-, dehydration- and cold-related genes were upregulated in *PARP* silenced plants [39]. Greater activity of ABA signaling pathways could play a significant role in the stress tolerance of PARP-deficient plants, and it is speculated that cyclic ADP-ribose (cADPR) might participate in this regulation [39].

In other work, it was previously shown that 3AB inhibited oxidative stress-induced phenylalanine ammonia lyase (PAL) activity in *Catharanthus roseus* tissue culture [40]. However, it was recently reported that 3AB treatment rendered *Arabidopsis* plants more susceptible to paraquat-induced oxidative stress, apparently by reducing ADP-ribose accumulation and increasing NAD^+ concentrations [34]. This again highlights the complex role of ADP-ribose and PARP homeostasis in abiotic stress resistance in plants, and the need for further research.

Plant PARPs and biotic stress responses

It is now known that poly(ADP-ribosyl)ation also has significant impacts on plant responses to pathogens. Gene expression profiling data has shown that certain PARG and NUDX genes are among the most reliably upregulated genes in the defense responses mediated by different *R* genes [41]. In *Arabidopsis*, accumulation of poly(ADP-ribose) polymer during bacterial infection and altered patterns of poly(ADP-ribosyl)ated proteins during fungal infection have been documented [42]. More significantly, the PARP inhibitor 3AB blocks a distinct subset of the responses characteristic of the plant basal defense responses to microbe-associated molecular patterns (MAMPs) such as bacterial flagellin or EF-Tu epitopes. Early responses such as the ROS burst and early MAMP-responsive gene expression occur despite the presence of a PARP inhibitor, but other responses are greatly curtailed including cell wall reinforcement with callose and lignin, phenylpropanoid pigment accumulation, and PAL activity [42]. Plants responding to MAMPs in the presence of a PARP inhibitor also exhibit a much greater decrease in health than plants exposed only to the MAMP or the PARP inhibitor, suggesting that aspects of the normally productive defense response become toxic in the absence of PARP activity [41,42]. A leading hypothesis is that plant PARPs help to ameliorate the cellular stresses associated with expression of antimicrobial defenses (e.g. the effects of elevated ROS levels). Additional recent findings regarding poly(ADP-ribosyl)ation and pathogen-induced plant stress are discussed below in the sections on PARG and NUDX enzymes.

RCDs and other PARP-like proteins

Before turning from PARP to other poly(ADP-ribosyl)ation-associated enzymes, the subject of other possible PARPs merits brief mention. *Arabidopsis* and other plant genomes encode more than just three protein types that contain a conserved PARP catalytic domain. Besides PARP1, 2 and 3, these proteins notably include RADICAL-INDUCED CELL DEATH 1 (RCD1) and SIMILAR TO RCD-ONE (SRO) 1–5. Despite the presence of this conserved catalytic domain, RCD1 does not appear to bind NAD^+ or have any detectable poly(ADP-ribosyl)ation activity, and the other SRO enzymes are also predicted to lack poly(ADP-ribosyl)ation activity [43]. It is possible that RCD1 and the SROs possess mono(ADP-ribosyl)-transferase activity, similar to human PARP10 [44]. In the future, more plant proteins might be added to the PARP pantheon, but this will require confirmation beyond bioinformatic findings.

PARG

Poly(ADP-ribosyl)ation is not irreversible. PARG enzymes hydrolyze the ADP-ribose polymers synthesized by PARP [45], de-modifying target proteins and in that way counteracting PARP activity. However, PARG does not restore the large amounts of NAD^+ that can be consumed through PARP activity, and PARG activity can increase cellular pools of free ADP-ribose, a known cell death signal in mammalian cells [46]. PARG activity might also free target proteins for further poly(ADP-ribosyl)ation. Hence, PARG can be thought to either counteract or further contribute to the impacts of PARP activation, depending on cellular context. Partly because of the lethality of PARG knockouts in animals, PARG has not received nearly the same level of characterization as its counterpart, PARP, and much about its functions remains unknown. However, it has been established that, owing to its roles in poly(ADP-ribose) homeostasis, PARG in animals plays crucial roles in embryonic development [47], cell death [48,49] and DNA repair [50–52].

Known animal genomes, from mouse to *Drosophila*, human or cow, encode a single *PARG* gene [53]. Knocking out *PARG* leads to accumulation of toxic ADP-ribose polymers and is lethal in mice and *Drosophila* [47,54]. However, *Arabidopsis* encodes two adjacent *PARG* genes, which are present due to gene duplication (At2g31865 and At2g31870, as well as the pseudogene At2g31860). Several other plant species are also predicted to encode multiple PARG proteins, including rice, poplar, tomato (*Solanum lycopersicum*) and maize, whereas some other plants are predicted to encode only a single PARG gene, including the castor oil plant (*Ricinus communis*), peanut (*Arachis hypogaea*) and sorghum (*Sorghum bicolor*). The presence of multiple PARGs might enable a level of genetic and molecular investigation not available in animal models of poly(ADP-ribosyl)ation. In animals, PARG is expressed in several isoforms encoded by the same open reading frame [55], but this has not been reported for plants. The two *Arabidopsis* PARGs each contain the conserved PARG protein family domain, but share only 52% amino acid identity with 68% similarity. Thus far, PARG enzyme activity in plants has been mainly inferred from one study in which ADP-ribose polymer concentrations were 25-fold higher in *parg1* versus wild-type plants, suggesting that *Arabidopsis* PARG1 does have poly(ADP-ribose) glycohydrolase enzyme activity [21].

Much less is known about the functional role of PARG than PARP in plants, but it has been shown that PARG1 plays a role in regulating circadian rhythms in *Arabidopsis* [21]. *PARG1* (*TEJ*) was originally identified as a circadian rhythm regulator. The *parg1* mutation increased leaf movement, caused early flowering under both short and long days, and lengthened the period length of all known

circadian clock-controlled genes [21]. This initial study suggested that poly(ADP-ribosyl)ation of a regulator protein could contribute to setting the period length of the *Arabidopsis* central oscillator, but there has been little follow-up work that successfully integrates PARG1 with other aspects of current models for control of circadian rhythms.

As was noted above, *PARG2* gene expression is elevated in multiple *R*-gene-mediated interactions between *Arabidopsis* and the bacterial pathogen *Pseudomonas syringae* pv. *tomato* (*Pst*). *PARG2* transcripts were significantly upregulated by both virulent and avirulent *Pst*, as well as by MAMPs [41], by infection with the necrotrophic fungus *Botrytis cinerea*, by the constitutive defense mutations *cpr1 and nudt7* [42], and in cucumber mosaic virus-resistant plants [56]. Upregulation by such a wide variety of biotic stimuli suggests that *PARG2* transcriptional activation is a general component of induced plant defenses (note that *PARG2* was not represented on the widely used Affymetrix 'whole genome' chip, limiting further data availability). *Arabidopsis parg1* and *parg2* knockouts exhibit a partial increase in susceptibility to the necrotrophic (feeds on dead tissue) pathogen *B. cinerea* [42], suggesting a possible connection between plant PARG and programmed cell death. *Arabidopsis parg1* (but not *parg2*) mutants were also more responsive to basal defense elicitation, which was characterized by exacerbated MAMP-induced seedling growth inhibition and enhanced phenylpropanoid pigment accumulation [42].

To date, plant PARGs have been linked to DNA repair mechanisms only preliminarily, when it was recently demonstrated that *parg1* knockouts are hypersensitive to the DNA damaging agent mitomycin-C [42]. This is an area that merits further investigation, along with further work explaining the impacts of PARG on circadian rhythms and plant defense responses, and on any possible ties between its roles in DNA repair and necrotroph infection. There is also a need to determine whether or not PARGs are a regulator of other cellular pathways (such as abiotic stress tolerance) for which involvement by plant PARPs has already been established. An important advance in this area was very recently achieved with the report that knocking out *PARG1* expression results in dramatically reduced tolerance to drought, osmotic and oxidative stress [57].

ADP-ribose pyrophosphatase NUDX proteins

Free ADP-ribose is highly reactive and will non-enzymatically mono(ADP-ribosyl)ate proteins, altering or eliminating their functions [58,59]. It is therefore important for cells to have a mechanism in place for removing the ADP-ribose generated by PARG enzyme activity. ADP-ribose-specific NUDX hydrolases reduce the high levels of toxic free ADP-ribose, re-establish energy levels by supplying a source for ATP, and contribute to NAD^+ maintenance by degrading ADP-ribose into AMP and ribose-5-phosphate [34,60–62].

There are 27 *Arabidopsis* genes that encode proteins with a NUDX box domain ($GX_5EX_7REUXEEXGU$); these were formerly termed AtNUDT proteins but are now called AtNUDX1–AtNUDX27, with the same gene and protein numbering used in both systems [61,63]. *AtDCP2* also encodes a NUDX hydrolase [64]. The plant NUDX domain shares a low level of homology with *Escherichia coli* MutT (a nucleoside triphosphate pyrophosphohydrolase) and human NTHL1 (a DNA *N*-glycosylase) proteins [61]. Different plant NUDX proteins show targeting to the cytosol (AtNUDX1–AtNUDX11), mitochondria (AtNUDX12–AtNUDX18) or chloroplasts (AtNUDX19–AtNUDX24, and possibly AtNUDX26 and AtNUDX27) [61,63]. Of the two dozen *Arabidopsis* NUDX proteins characterized, the cytosolic AtNUDX2, AtNUDX6, AtNUDX7 and AtNUDX10 products hydrolyze both ADP-ribose and NADH to AMP *in vitro*, with high affinity for ADP-ribose, while other members of this protein family hydrolyze other substrates such as 8-oxo-dGTP, dNTPs, NADH, CoA and FAD [61]. When expressed in *E. coli*, AtNUDX7 showed preferential *in vitro* activity for both ADP-ribose and NADH [65]. AtNUDX7 has further been proposed as the predominant NADH and ADP-ribose pyrophosphatase in *Arabidopsis* cells [34,65].

So far, AtNUDX7 is the only NUDX protein shown to have functional ADP-ribose pyrophosphatase activity *in planta*. At*NUDX7* gene expression is upregulated by virulent and avirulent pathogens [41,65,66], and several laboratories have shown that AtNUDX7 (formerly NUDT7) is a negative regulator of plant defense responses [41,65–67]. Knocking out *AtNUDX7* expression can reduce the hypersensitive response (HR) to an avirulent pathogen [41,66], and mutant *nudx7* plants are more resistant to virulent and avirulent *Pst* [41,65,67] and *Hyaloperonospora parasitica* [66].

NUDX7 has also been implicated in plant abiotic stress responses. This gene has been found in many stress-responsive *Arabidopsis* cDNA libraries [67]. Owing to excess sensitivity to environmental stresses, *nudx7* mutants are stunted [41,65,67] and show microscopic necrotic lesions and the accumulation of ROS [65,67]. These knockout plants were more susceptible to paraquat-induced oxidative stress [41,65] and accumulated more ADP-ribose polymer. Conversely, overexpressing *AtNUDX7* increased oxidative stress tolerance and lowered ADP-ribose levels [34]. These data again implicate AtNUDX7 as an ADP-ribose pyrophosphatase.

Arabidopsis AtNUDX6 offers a contrasting example. Despite showing a preference *in vitro* for ADP-ribose as a substrate [61], AtNUDX6 might predominantly be an NADH-pyrophosphatase [68]. AtNUDX6 does play a role in plant defenses as a positive regulator of NPR1-dependent salicylic acid defense signaling pathways, but this activity was more attributable to NADH degradation than to ADP-ribose degradation. This observation serves as a reminder that many aspects of cellular physiology can and do impact plant defense systems. It is important to note (and investigate) that even those biotic or abiotic stress alterations that arise through alteration of confirmed poly(ADP-ribosyl)ation machinery might not arise directly from protein post-translational modification of 'defense proteins' via poly(ADP-ribosyl)ation. Some alterations might instead arise from other downstream outcomes of the alteration of poly(ADP-ribosyl)ation processes, such as altered redox homeostasis and NAD^+ pools, which could be affected not

Box 1. Outstanding questions

- What are the protein targets of poly(ADP-ribosyl)ation in plants? Histones or other nuclear proteins?
- Which plant proteins interact with poly(ADP-ribose) and poly(-ADP-ribosyl)ated proteins?
- Do plant PARPs have functions independent of their poly(ADP-ribosyl)ation activity?
- What are the roles of poly(ADP-ribosyl)ation in plant programmed cell death and circadian rhythms?
- In what way does disruption of PARP activity disrupt MAMP-induced cell wall reinforcement?
- What are the roles of NUDX ADP-ribose pyrophosphatases in oxidative stress responses in plants?
- What deleterious effects accompany the apparent enhancement of abiotic stress tolerance in plants with reduced PARP levels? Can poly(ADP-ribosyl)ation be manipulated to engineer useful improved stress tolerance in economically valuable plant species?
- Are plant PARGs also involved in the physiological responses for which a role for PARP has been established?

only by PARP and PARG enzymes but also, for example, by AtNUDX6 activity.

Concluding thoughts

Over the past two decades, it has been established that poly(ADP-ribosyl)ation and the enzymes for its synthesis and degradation not only exist in plants, but also can perform similar roles to their mammalian counterparts. Plant responses to DNA damage, oxidative and other abiotic stresses, and pathogen attack have all been found to require at least some of the components that modulate this post-translational modification. These studies provide further evidence that poly(ADP-ribosyl)ation plays significant, diverse roles in the coordination of plant responses to a variety of environmental stresses. *Arabidopsis* continues to offer excellent experimental advantages and, conveniently, the presence of two expressed PARG enzymes in many plants is a tool that could allow deeper characterization of this less-understood poly(ADP-ribosyl)ation enzyme. However, it is hoped that future studies will also be carried out using economically valuable plant species, and include peer-reviewed publications on field-based studies of the impacts of altered poly(ADP-ribosyl)ation on multiple plant performance characteristics during abiotic and biotic stress. Owing to its roles in circadian rhythms as well as abiotic and biotic stress responses, manipulation of plant poly(ADP-ribosyl)ation might aid in the cultivation of more robust and dependable sources of food, even in harsh or unpredictable environmental conditions.

Recent studies have re-invigorated the field of plant poly(ADP-ribosyl)ation, and have raised new challenges. In studies of plant responses to any particular stress, areas that require further study (Box 1) include the identification of plant proteins that become poly(ADP-ribosyl)ated, whether they be PARPs, core histones or other proteins. The proteins that interact with PARP, PARG and ADP-ribose also remain to be identified. Lastly, due to the inherent limitations of both mutants (chronic effects, pleiotropy and lethality) and pharmacological inhibitors (specificity, physiological ranges and chemical stability), future studies are likely to benefit from more advanced use of genetic alterations or improved PARP inhibitors. With attention to these areas, further important insights should be forthcoming regarding the molecular roles of poly(ADP-ribosylation) post-translational modifications in plants.

Note added in proof

A recent study provides significant new insights into the evolutionary history and structural/functional diversification of PARPs and PARP-like proteins [71].

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References

1 Rouleau, M. *et al.* (2010) PARP inhibition: PARP1 and beyond. *Nat. Rev. Cancer* 10, 293–301
2 Hassa, P.O. (2009) The molecular "Jekyll and Hyde" duality of PARP1 in cell death and cell survival. *Front Biosci.* 14, 72–111
3 Virag, L. and Szabo, C. (2002) The therapeutic potential of poly(ADP-ribose) polymerase inhibitors. *Pharmacol. Rev.* 54, 375–429
4 Kirkland, J.B. (2010) Poly ADP-ribose polymerase-1 and health. *Exp. Biol. Med. (Maywood)* 235, 561–568
5 Ahel, D. *et al.* (2009) Poly(ADP-ribose)-dependent regulation of DNA repair by the chromatin remodeling enzyme ALC1. *Science* 325, 1240–1243
6 Simbulan-Rosenthal, C.M. *et al.* (1999) Poly(ADP-ribosyl)ation of p53 during apoptosis in human osteosarcoma cells. *Cancer Res.* 59, 2190–2194
7 Masson, M. *et al.* (1998) XRCC1 is specifically associated with poly(ADP-ribose) polymerase and negatively regulates its activity following DNA damage. *Mol. Cell Biol.* 18, 3563–3571
8 Hashida, S.N. *et al.* (2009) The role of NAD biosynthesis in plant development and stress responses. *Ann. Bot. (Lond.)* 103, 819–824
9 Strosznajder, R.P. *et al.* (2005) Poly(ADP-ribose) polymerase: the nuclear target in signal transduction and its role in brain ischemia-reperfusion injury. *Mol. Neurobiol.* 31, 149–167
10 Otto, H. *et al.* (2005) In silico characterization of the family of PARP-like poly(ADP-ribosyl)transferases (pARTs). *BMC Genomics* 6, 139
11 Hunt, L. *et al.* (2004) NAD-new roles in signalling and gene regulation in plants. *New Phytologist* 163, 31–44
12 Babiychuk, E. *et al.* (1998) Higher plants possess two structurally different poly(ADP-ribose) polymerases. *Plant J.* 15, 635–645
13 Doucet-Chabeaud, G. *et al.* (2001) Ionising radiation induces the expression of PARP-1 and PARP-2 genes in Arabidopsis. *Mol. Genet. Genomics* 265, 954–963
14 Chen, Y.M. *et al.* (1994) Poly(ADP-ribose) polymerase in plant nuclei. *Eur. J. Biochem.* 224, 135–142
15 O'Farrell, M. (1995) ADP-ribosylation reactions in plants. *Biochimie* 77, 486–491
16 Willmitzer, L. (1979) Demonstration of in vitro covalent modification of chromosomal proteins by poly (ADP) ribosylation in plant nuclei. *FEBS Lett.* 108, 13
17 Bryant, H.E. *et al.* (2005) Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. *Nature* 434, 913–917
18 Beauchamp, M.C. *et al.* (2009) BMS-536924 sensitizes human epithelial ovarian cancer cells to the PARP inhibitor, 3-aminobenzamide. *Gynecol. Oncol.* 115, 193–198
19 Ding, W. *et al.* (2009) Inhibition of poly(ADP-ribose) polymerase-1 by arsenite interferes with repair of oxidative DNA damage. *J. Biol. Chem.* 284, 6809–6817
20 Hernandez, A.I. *et al.* (2009) Poly-(ADP-ribose) polymerase-1 is necessary for long-term facilitation in Aplysia. *J. Neurosci.* 29, 9553–9562
21 Panda, S. *et al.* (2002) *tej* defines a role for poly(ADP-ribosyl)ation in establishing period length of the *Arabidopsis* circadian oscillator. *Dev. Cell* 3, 51–61
22 Penning, T. (2010) Small-molecule PARP modulators-current status and future therapeutic potential. *Curr. Opin. Drug Discov. Devel.* 13, 577
23 Hunt, L. *et al.* (2007) Nicotinamidase activity is important for germination. *Plant J.* 51, 341–351

24 Hunt, L. and Gray, J. (2009) The relationship between pyridine nucleotides and seed dormancy. *New Phytologist* 181, 62–70
25 Huber, A. *et al.* (2004) PARP-1, PARP-2 and ATM in the DNA damage response: functional synergy in mouse development. *DNA Repair (Amst)* 3, 1103–1108
26 Molinete, M. *et al.* (1993) Overproduction of the poly(ADP-ribose) polymerase DNA-binding domain blocks alkylation-induced DNA repair synthesis in mammalian cells. *EMBO J.* 12, 2109–2117
27 Wang, M. *et al.* (2006) PARP-1 and Ku compete for repair of DNA double strand breaks by distinct NHEJ pathways. *Nucleic Acids Res.* 34, 6170–6182
28 Takeda, K. and Akira, S. (2005) Toll-like receptors in innate immunity. *Int. Immunol.* 17, 1–14
29 Babiychuk, E. *et al.* (2001) N terminal domains of plant poly (ADP ribose) polymerases define their association with mitotic chromosomes. *Plant J.* 28, 245–255
30 De Block, M. *et al.* (2005) Poly(ADP-ribose) polymerase in plants affects energy homeostasis, cell death and stress tolerance. *Plant J.* 41, 95–106
31 Amor, Y. *et al.* (1998) The involvement of poly(ADP-ribose) polymerase in the oxidative stress responses in plants. *FEBS Lett.* 440, 1–7
32 Tian, R. *et al.* (2000) Involvement of poly(ADP-ribose) polymerase and activation of caspase-3-like protease in heat shock-induced apoptosis in tobacco suspension cells. *FEBS Lett.* 474, 11–15
33 Puchta, H. *et al.* (1995) Induction of intrachromosomal homologous recombination in whole plants. *Plant J.* 7, 203–210
34 Ishikawa, K. *et al.* (2009) Modulation of the poly(ADP-ribosyl)ation reaction via the Arabidopsis ADP-ribose/NADH pyrophosphohydrolase, AtNUDX7, is involved in the response to oxidative stress. *Plant Physiol.* 151, 741–754
35 Du, L. *et al.* (2003) Intra-mitochondrial poly(ADP-ribosylation) contributes to NAD^+ depletion and cell death induced by oxidative stress. *J. Biol. Chem.* 278, 18426–18433
36 Chiarugi, A. (2002) Poly (ADP-ribose) polymerase: killer or conspirator? The 'suicide hypothesis' revisited. *Trends Pharmacol. Sci.* 23, 122–129
37 Phillips, R. and Hawkins, S.W. (1985) Characteristics of the inhibition of induced tracheary element differentiation by 3-aminobenzamide and related compounds. *J. Exp. Bot.* 36, 119–128
38 Pellny, T.K. *et al.* (2009) Pyridine nucleotide cycling and control of intracellular redox state in relation to poly (ADP-ribose) polymerase activity and nuclear localization of glutathione during exponential growth of Arabidopsis cells in culture. *Mol. Plant* 2, 442
39 Vanderauwera, S. *et al.* (2007) Silencing of poly(ADP-ribose) polymerase in plants alters abiotic stress signal transduction. *Proc. Natl. Acad. Sci. U.S.A.* 104, 15150–15155
40 Berglund, T. *et al.* (1996) UV-B- and oxidative stress-induced increase in nicotinamide and trigonelline and inhibition of defensive metabolism induction by poly(ADP-ribose)polymerase inhibitor in plant tissue. *FEBS Lett.* 380, 188–193
41 Adams-Phillips, L. *et al.* (2008) Discovery of ADP-ribosylation and other plant defense pathway elements through expression profiling of four different Arabidopsis-Pseudomonas R-avr interactions. *Mol. Plant Microbe Interact.* 21, 646–657
42 Adams-Phillips, L. *et al.* (2010) Disruption of poly (ADP-ribosyl) ation mechanisms alters responses of Arabidopsis to biotic stress. *Plant Physiol.* 152, 267
43 Jaspers, P. *et al.* (2010) The RST and PARP-like domain containing SRO protein family: analysis of protein structure, function and conservation in land plants. *BMC Genomics* 11, 170
44 Kleine, H. *et al.* (2008) Substrate-assisted catalysis by PARP10 limits its activity to mono-ADP-ribosylation. *Mol. Cell* 32, 57–69
45 Davidovic, L. *et al.* (2001) Importance of poly(ADP-ribose) glycohydrolase in the control of poly(ADP-ribose) metabolism. *Exp. Cell Res.* 268, 7–13
46 Andrabi, S.A. *et al.* (2006) Poly(ADP-ribose) (PAR) polymer is a death signal. *Proc. Natl. Acad. Sci. U.S.A.* 103, 18308–18313
47 Hanai, S. *et al.* (2004) Loss of poly(ADP-ribose) glycohydrolase causes progressive neurodegeneration in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U.S.A.* 101, 82–86
48 Erdelyi, K. *et al.* (2009) Dual role of poly (ADP-ribose) glycohydrolase in the regulation of cell death in oxidatively stressed A549 cells. *FASEB J.* 23, 3553
49 Formentini, L. *et al.* (2008) Mono galloyl glucose derivatives are potent poly (ADP ribose) glycohydrolase (PARG) inhibitors and partially reduce PARP 1 dependent cell death. *Br. J. Pharmacol.* 155, 1235–1249
50 Fujihara, H. *et al.* (2009) Poly (ADP-ribose) Glycohydrolase deficiency sensitizes mouse ES cells to DNA damaging agents. *Curr. Cancer Drug Targets* 9, 953–962
51 Fisher, A. *et al.* (2007) Poly (ADP-ribose) polymerase 1 accelerates single-strand break repair in concert with poly (ADP-ribose) glycohydrolase. *Mol. Cell. Biol.* 27, 5597
52 Gao, H. *et al.* (2007) Altered poly (ADP-ribose) metabolism impairs cellular responses to genotoxic stress in a hypomorphic mutant of poly (ADP-ribose) glycohydrolase. *Exp. Cell Res.* 313, 984–996
53 Ame, J.C. *et al.* (1999) Assignment of the poly(ADP-ribose) glycohydrolase gene (PARG) to human chromosome 10q11.23 and mouse chromosome 14B by in situ hybridization. *Cytogenet. Cell Genet.* 85, 269–270
54 Koh, D.W. *et al.* (2004) Failure to degrade poly(ADP-ribose) causes increased sensitivity to cytotoxicity and early embryonic lethality. *Proc. Natl. Acad. Sci. U.S.A.* 101, 17699–17704
55 Meyer-Ficca, M. *et al.* (2004) Human poly (ADP-ribose) glycohydrolase is expressed in alternative splice variants yielding isoforms that localize to different cell compartments. *Exp. Cell Res.* 297, 521–532
56 Sekine, K. *et al.* (2008) High level expression of a virus resistance gene, RCY1, confers extreme resistance to cucumber mosaic virus in *Arabidopsis thaliana*. *Mol. Plant-Microbe Interact.* 21, 1398–1407
57 Li, G. *et al.* (2011) Arabidopsis poly (ADP-ribose) glycohydrolase 1 is required for drought; osmotic and oxidative stress responses. *Plant Sci.* 180, 283–291
58 Jacobson, E. *et al.* (1994) Glycation of proteins by ADP-ribose. *Mol. Cell. Biochem.* 138, 207–212
59 Cervantes-Laurean, D. *et al.* (1996) Glycation and glycoxidation of histones by ADP-ribose. *J. Biol. Chem.* 271, 10461
60 Rossi, R. *et al.* (2002) DNA ligase I is dephosphorylated during the execution step of etoposide-induced apoptosis. *Cell Death Differ.* 9, 89–90
61 Ogawa, T. *et al.* (2005) Comprehensive analysis of cytosolic Nudix hydrolases in Arabidopsis thaliana. *J. Biol. Chem.* 280, 25277–25283
62 Ogawa, T. *et al.* (2009) Overexpression of an ADP-ribose pyrophosphatase, AtNUDX2, confers enhanced tolerance to oxidative stress in Arabidopsis plants. *Plant J.* 57, 289–301
63 Ogawa, T. *et al.* (2008) Molecular characterization of organelle-type Nudix hydrolases in Arabidopsis. *Plant Physiol.* 148, 1412–1424
64 Gunawardana, D. *et al.* (2008) Identification of functional domains in *Arabidopsis thaliana* mRNA decapping enzyme (AtDcp2). *Nucleic Acids Res.* 36, 203–216
65 Ge, X. *et al.* (2007) AtNUDT7, a negative regulator of basal immunity in Arabidopsis, modulates two distinct defense response pathways and is involved in maintaining redox homeostasis. *Plant Physiol.* 145, 204–215
66 Bartsch, M. *et al.* (2006) Salicylic acid-independent ENHANCED DISEASE SUSCEPTIBILITY1 signaling in Arabidopsis immunity and cell death is regulated by the monooxygenase FMO1 and the Nudix hydrolase NUDT7. *Plant Cell* 18, 1038–1051
67 Jambunathan, N. and Mahalingam, R. (2006) Analysis of Arabidopsis growth factor gene 1 (GFG1) encoding a nudix hydrolase during oxidative signaling. *Planta* 224, 1–11
68 Ishikawa, K. *et al.* (2010) Distinct regulation of Arabidopsis ADP-ribose/NADH pyrophosphohydrolases, AtNUDX6 and 7, in biotic and abiotic stress responses. *Plant Signal. Behav.* 5
69 Marchler-Bauer, A. *et al.* (2009) CDD: specific functional annotation with the Conserved Domain Database. *Nucleic Acids Res.* 37, D205
70 D'Silva, I. *et al.* (1998) Activation of cysteine proteases in cowpea plants during the hypersensitive response–a form of programmed cell death. *Exp. Cell Res.* 245, 389–399
71 Citarelli, M. *et al.* (2010) Evolutionary history of the poly(ADP-ribose) polymerase gene family in eukaryotes. *BMC Evol Biol.* 10, 308